



PROTEUM3 Software

- User Manual

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This document covers the PROTEUM3 software suite.

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1 About this User Manual

1.1 PROTEUM3 for Protein Crystallography

The PROTEUM3 data collection and processing suite, specially designed for the macromolecular crystallographer, represents a significant departure from the SAINT data processing system developed primarily for small molecule applications. A significant advantage of the PROTEUM3 suite is that intelligent defaults and crystallographic experience have been coded into the software wherever possible. Therefore, data processed using the supplied defaults should in most cases produce data of excellent quality.

A graphical user interface (GUI) guides the operator through the steps of data collection and processing using a series of specialized plug-ins. The Suite includes:

- Automatic structure determination with XPRESSO;
- Automatic phasing with the Determine Phases plug-in;
- A powerful data collection strategy tool; and
- An innovative profile analysis and refinement engine for integration.

The software runs under Microsoft Windows 7/10 and Red Hat Fedora, and has been designed to support a number of detectors and goniometer combinations. The use of secure data sockets allows data collection from another properly-configured computer connected to the network.

1.2 Terms and Conventions

1.2.1 Typographical Conventions

Table 1.1 shows typographical conventions used to help you quickly locate and identify information in this document.

Table 1.1 — Typographical conventions

Convention	Usage
boldface	Software user interface controls (such as icons, menu items, and buttons) to be selected as part of the current procedure.
<i>italics</i>	New terms and words requiring emphasis.
monospace	Information read from or entered into a field or command prompt.
>	Navigation through a hierarchical menu. For example, "Choose Start > Programs > Bruker AXS > APEX3 " describes navigating Windows' menus from Start to Programs to Bruker AXS to APEX3 .
[square brackets]	Keyboard input.

1.2.2 Equivalent Terms

1.2.2.1 Frame/Image

In 2-dimensional X-ray diffraction, the terms *frame* and *image* refer to the same 2-dimensional view of the diffraction pattern.

1.2.2.2 Greek and Roman Text


This document uses scientific terminology that may be rendered in Greek text. However, this document follows a convention of using Roman text to the greatest extent possible.

Table 1.2 — Greek and Roman Text


Greek	Roman
θ	theta
ω	omega
φ	phi
χ	chi
κ	kappa

1.2.3 Notices: Danger, Caution, and Note

This document contains notices that must be observed to ensure personal safety, as well as to protect the product and connected equipment. These notices are highlighted as follows according to the level of danger.

 **DANGER**

The word “DANGER” alerts you to an immediate or potential hazard that can result in death, severe personal injury, or substantial property damage.

 **CAUTION**

The word “CAUTION” alerts you to a potential practice or condition that could result in minor personal injury or damage to the product or property.

NOTE: The word “NOTE” in bold capital letters draws your attention to particularly important information on the product or handling of the product, or to a particular part of the product documentation.

1.3 Referenced Documentation

The following Table contains a list of documentation referenced by this document. It is necessary to have this additional documentation available as you work with this document. In the documents' part numbers, a variable revision number is given immediately after the part number. Always use the most current revisions available.

All of the documents shown may be found at Bruker's comprehensive support site www.bruker.com/support, or on the Online Documentation CD-ROM that accompanies the shipment.

Table 1.3 — Referenced documentation

Part Number	Title
DOC-269-0175XX	SAINT Software Reference Manual
DOC-M86-EXX024	Microscope Focus and Sample Alignment
DOC-M86-EXX184	D8 QUEST and D8 VENTURE Video Microscope Alignment Procedure
DOC-M88-EXX099	License Manager User Manual
DOC-M88-EXX190	DIFFRAC.SUITE Installation Guide

1.4 X-ray Safety



DANGER

X-ray equipment produces potentially harmful radiation and can be dangerous to anyone in the equipment's vicinity unless safety precautions are completely understood and implemented. All persons designated to operate or perform maintenance on this instrument must be fully trained on the nature of radiation, X-ray generating equipment, and radiation safety. **All users** of the X-ray equipment may be required to accurately monitor their exposure to X-rays by proper use of X-ray dosimeters.

For safety issues related to operation and maintenance of your particular X-ray generator, diffractometer, and shield enclosure, please refer to the manufacturer's operation manuals or to your Radiation Safety Officer. The user is responsible for compliance with local safety regulations.

1.5 License Considerations

You need a software license to activate your purchased software packages, options, and/or features.

Your Suite program (e.g., PROTEUM3) requires a valid license file to operate. This file, "bn-license.dat", must be present in the root directory of drive C:\ for the software to start properly.

If the license file is not present or has expired, an error window will appear when you try to start your Suite program. This window prompts you to run the License Manager application to obtain a valid license for the Suite.

For more information on licenses and License Manager, refer to DOC-M88-EXX099 License Manager User Manual.

Inquiries about licenses and Bruker licensing may be directed to **licenses.BAXS@bruker.com**.

1.6 Help and Technical Support

1.6.1 The “What’s This?” Help Function

Context-based help is available at any time within the program by simply clicking the “What’s This?” help icon:

Figure 1.1 — “What’s This?” help icon



After clicking the icon, click on any window or field to obtain detailed help in a pop-up window.

The “What’s This?” help function can also be used at any time by pressing [Shift]+[F1].

1.6.2 Technical Support from Bruker Service Centers

You are invited to contact Bruker whenever there are problems or questions related to the system. Before you contact Bruker, please:

- Have the system’s serial number available;
- Determine the system’s software version (if you suspect a software problem);
- Record any error messages that appear; and
- Determine steps and conditions that recreate the problem (if possible).



CAUTION

Failure to refer instrument servicing to qualified Bruker personnel may result in injury or property damage!

1.6.2.1 Finding Your Local Bruker Service Center

A complete list of all Bruker Service Centers worldwide is available at:

<https://www.bruker.com/about-us/offices/offices/bruker-axs/xrd-sc-xrd-xrf-offices.html>

1.6.2.2 Brukersupport.com: Bruker’s Comprehensive Service Website

Registration with Bruker’s comprehensive support website, www.brukersupport.com, provides you with complete and up-to-date information for all of your owned equipment, including:

- All User Manuals, Procedures, FAQs, and other documentation;
- Software updates;
- Remote backup of your equipment settings and configurations; and
- Bruker official training and certification options.

When a new document or software update becomes available, you are immediately alerted via email.

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2 PROTEUM3 Software Overview

From a software and operational viewpoint, the PROTEUM3 software controls all operations—from crystal screening to report generation—for a typical crystallography study.

2.1 Server Programs

2.1.1 The Measurement Server

Bruker D8 QUEST and D8 VENTURE instruments are delivered with DIFFRAC.SUITE, a set of software applications used to create and start measurements, and perform other measurement-related tasks on the instrument.


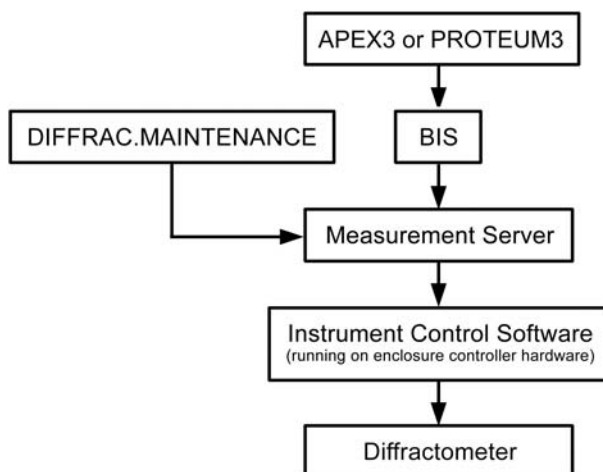
Communication with the instrument takes place via a program called the *Measurement Server*, which is part of DIFFRAC.SUITE. The Measurement Server is started via a Desktop icon , and information about the Measurement Server is shown in the Windows Taskbar's hidden icons.

Figure 2.1 — Software relationships



Before working with PROTEUM3, you must establish a connection between the Measurement Server and the instrument, and then give the Measurement Server control of the instrument. This is done via the Measurement Server's "Status Window" window.

Once the Measurement Server is connected to the instrument, you can start BIS, which interprets commands sent by PROTEUM3. Then, in PROTEUM3, you can connect to BIS.

2.1.2 BIS

BIS (Bruker Instrument Server) is the software layer between the Suite program (i.e., PROTEUM3) and the Measurement Server.

In the case of pre-D8 VENTURE (i.e., D8 Series I and II) instruments, BIS (also called "BIS Classic") is all that is required; the Measurement Server is not used.

2.2 Client Programs

2.2.1 PROTEUM3 GUI and Plug-ins

PROTEUM3 is the main program that controls experiments and analyzes data. The user works with PROTEUM3 through a variety of software modules, or *plug-ins*, for different aspects of an experiment. The Suite also includes a Sample Database that stores relevant data from each step in the experiment.

2.2.2 Online and Offline Operation

The Suite can operate in *online* or *offline* mode.

In online mode:

- DAVINCI.DESIGN instruments use BIS Server to communicate with the instrument for performing experiments. To work in online mode, you must start Measurement Server, BIS Server, and your Suite program (i.e., PROTEUM3).
- Pre-DAVINCI.DESIGN (i.e., D8 Series I or II) instruments use BIS Classic to communicate with the instrument. To work in online mode, you must start BIS. BIS Classic will also start VIDEO when needed.

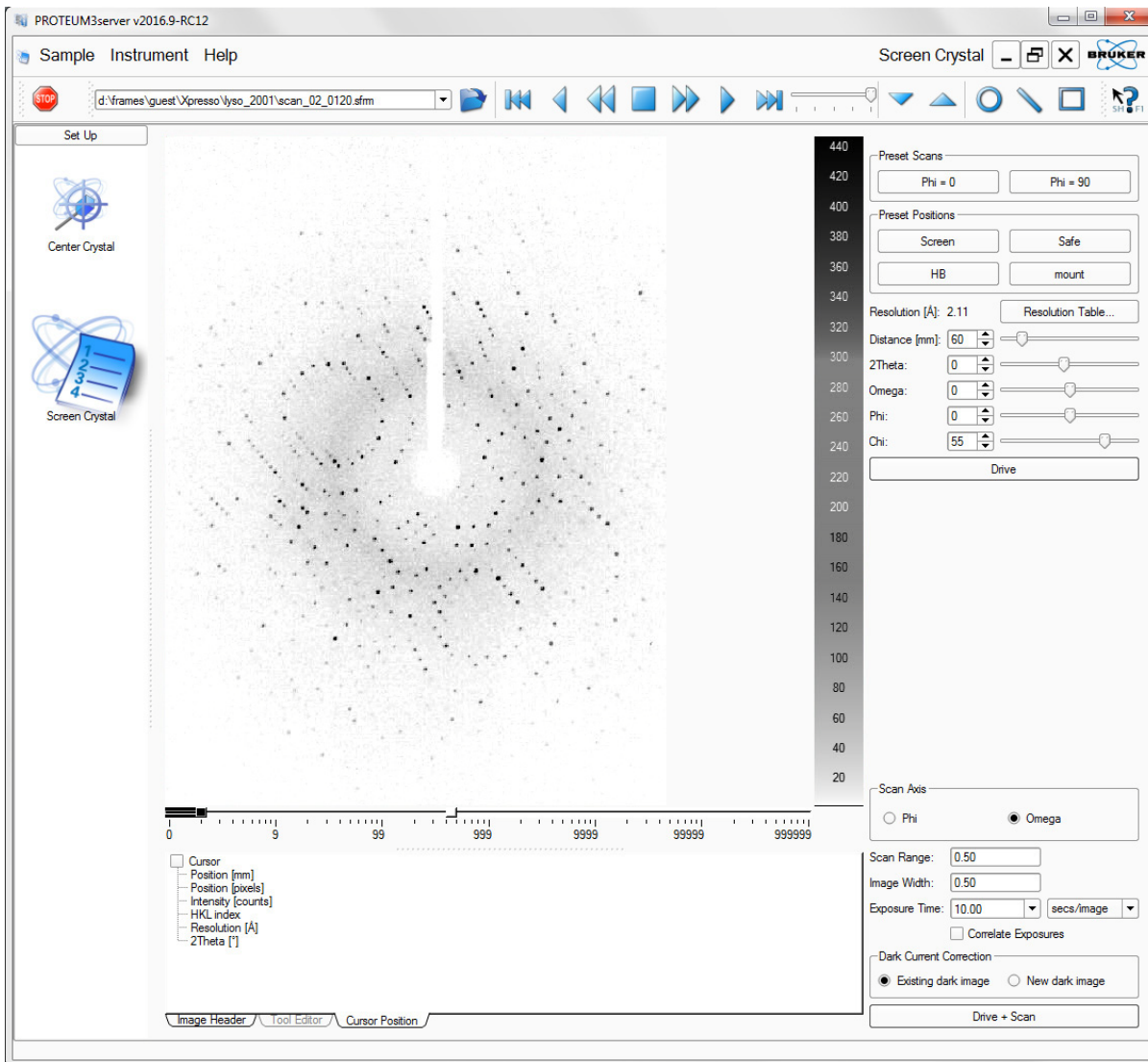
In offline mode, the Suite program is not connected to the instrument, but is still able to analyze and interpret existing data (plug-ins requiring communication with the instrument will prompt you to connect). To work in offline mode, you only need to start your Suite program.

2.2.3 PROTEUM3server

PROTEUM3Server is operated on the computer that controls the instrument. It provides functionality for quickly centering a crystal and determining its diffraction quality.

PROTEUM3server contains two main plug-ins: “Center Crystal” and “Screen Crystal” (see Figure 2.2).

Figure 2.2 — PROTEUM3server’s “Screen Crystal” plug-in



2.3 The Sample Database

The Sample Database is used for the storage of data generated by the Suite. It is designed to transparently handle data from all of the Suite’s plug-ins without user intervention.

The Sample Database is used internally by the Suite and is not available for user customization or manipulation. It is automatically started when the computer starts up, and it is automatically stopped before the computer shuts down.

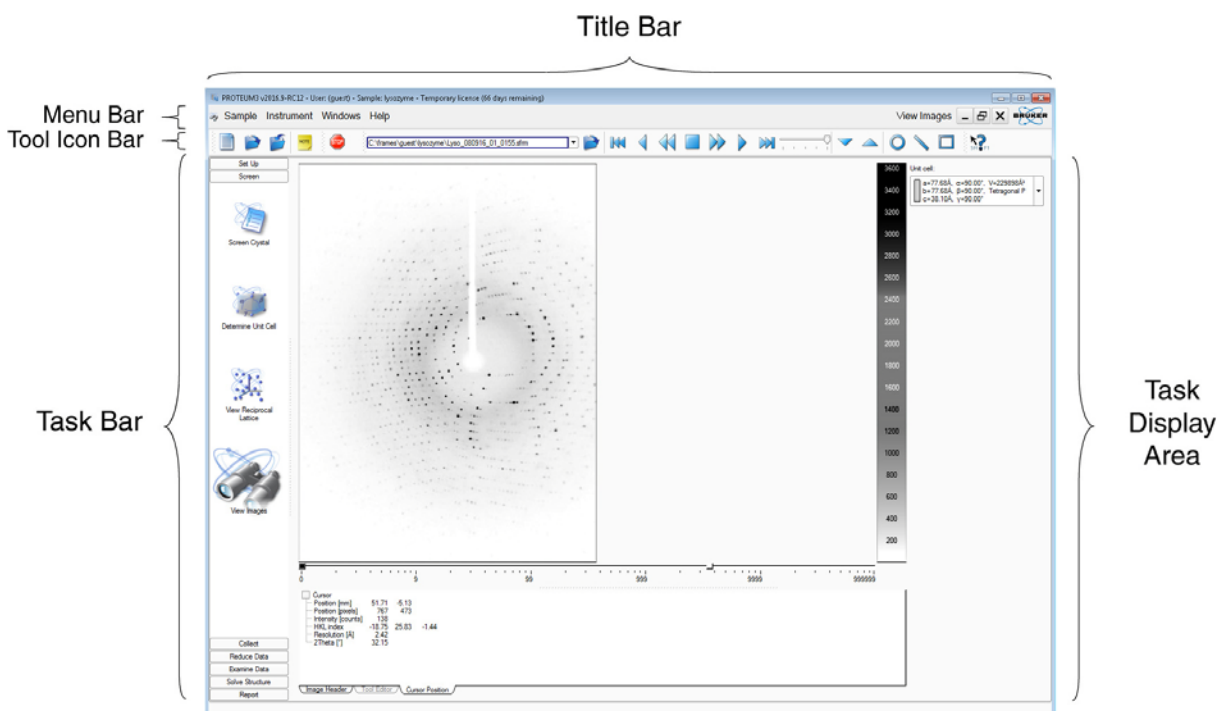
2.4 The Main Window

The PROTEUM3 graphical user interface (GUI) has one main window (Figure 2.3).

The main window is divided into five sections:


- Title Bar
- Menu Bar
- Tool Icon Bar
- Task Bar
- Task Display Area

Figure 2.3 — Main window diagram



NOTE: Throughout the interface, input fields with pink backgrounds indicate invalid entries. Disabled (i.e., grayed-out) fields indicate that a feature is not available (i.e., not supported or dependent upon the instrument configuration).

2.4.1 The Title Bar

The Title Bar displays the software name and version, user name, sample, license type, and active plug-in. The right-hand edge of the Title Bar also contains the three Windows buttons  for minimizing, restoring, and closing the main window.

2.4.2 The Menu Bar

The Menu Bar provides drop-down menus for a variety of file operations, image tools, and help files.

An icon for the currently-active plug-in appears on the left-hand side of the Menu Bar. Clicking on this icon allows you to minimize, restore, resize, close, or move the currently-active window.

2.4.2.1 The Sample Menu

Figure 2.4 — Sample menu

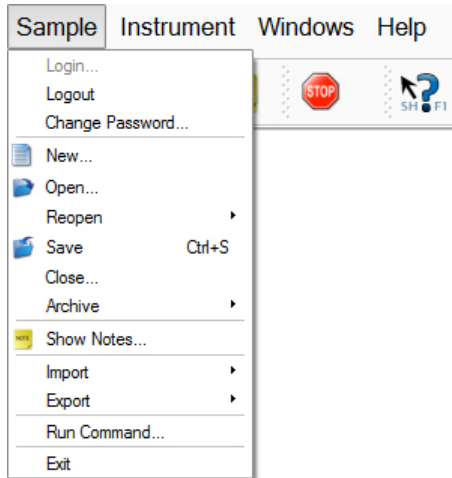



Table 2.1 — Sample menu commands

Sample menu command		Description
Login...		Opens a window for logging into the Sample Database using your username and password.
Logout		Logs out of the Sample Database.
Change Password...		Opens a dialog for changing your Sample Database password.
New...		Opens a dialog for creating a new sample in the Sample Database.
Open...		Opens a previously-saved sample.
Reopen		Reopens a recently-opened sample.
Save		Saves the current sample.
Close...		Closes the current sample.
Archive	Restore Archive...	Restores an archived sample from a .zip file or CD/DVD.
	Archive Sample...	Archives the current sample to a .zip file or CD/DVD.
	Delete Samples...	Deletes samples from the Sample Database.
Show Notes...		Opens the “Sample Notes” window.
Import		Allows you to import crystal information contained in a .p4p or .spin file into the Sample Database.
Export		Allows you to export crystal data for the current sample as an .xml or .p4p file.
Run Command...		Opens a command prompt window.
Exit		Exits the application. This menu item has the same function as the  button.

2.4.2.2 The Instrument Menu

Figure 2.5 — Instrument menu

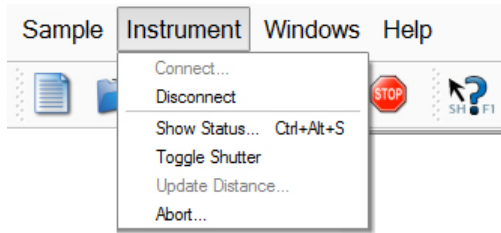


Table 2.2 — Instrument menu commands

Instrument menu command	Description
Connect...	Opens a connection to BIS Server.
Disconnect	If connected, disconnects from BIS Server.
Show Status...	Opens a window showing a summary of the current status of the instrument and the program's connection to it.
Toggle Shutter	Instructs BIS Server to send a shutter open or close command to the instrument. If all interlocks and safety circuits are satisfied, the shutter will open and close.
Update Distance...	For manual detector tracks, allows you to update the detector distance if it disagrees with the value shown in the software.
Abort...	Immediately aborts all pending instrument operations.

2.4.2.3 The Windows Menu

Figure 2.6 — Windows menu

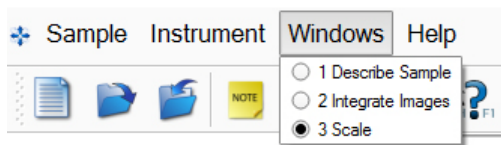


Table 2.3 — Windows menu command

Windows menu command	Description
Window selection	Displays a list of active windows. Any of these windows may be brought to the front by clicking its title.

2.4.2.4 The Help Menu

Figure 2.7 — Help menu

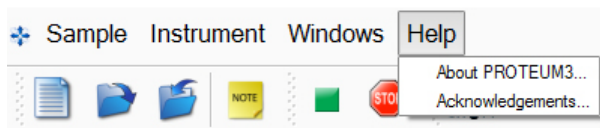








Table 2.4 — Help menu command

Help menu command	Description
About PROTEUM3...	Displays PROTEUM3's version and copyright information.
Acknowledgements...	Displays third-party software contributions to APEX3.

2.4.3 The Tool Icon Bar










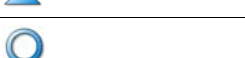





Some icons on the Tool Icon Bar provide shortcuts to the options available through the Menu Bar.

Table 2.5 — Tool Icon Bar: menu bar shortcuts

Icon	Description
	Creates a new entry in the Sample Database.
	Opens an existing entry in the Sample Database.
	Saves the current information to the Sample Database.
	Shows the “Sample Notes” window
	Stops any instrument activity.
	“What’s this?” Context-sensitive help.

Other icons on the Tool Icon Bar are visible only when a frame is displayed.

Table 2.6 — Tool Icon Bar: displayed frame controls

Icon	Description
	Shows the currently-displayed frame’s filename. Click the drop-down triangle to choose previously-displayed frames.
	Opens a frame using a browse dialog.
	Displays the first frame in a run.
	Displays previous frame ([Alt + left arrow]).
	Sequentially displays frames in reverse as a movie.
	If displaying frames as a movie, stops the movie.
	Sequentially displays frames as a movie.
	Displays the next frame ([Alt + right arrow]).
	Displays the last frame in a run.
	Adjusts the rate of display when viewing frames as a movie.
	Displays frames from the next lower-numbered run ([Alt + down arrow]).
	Displays frames from the next higher-numbered run ([Alt + up arrow]).
	Draws a resolution circle.
	Draws a plotting line. The plot appears below the image.
	Selects a region of the frame.

2.4.4 The Task Bar

The Task Bar gives you access to all of the plug-ins available in the Suite.

NOTE: If the Task Bar is not visible, there is no open sample. Choose **Sample > New...** or **Sample > Open...** to open or create a new sample.

Table 2.7 — “Set Up” category




Icon	Name	Description
	Describe Sample	Specify crystal size, color, shape, etc.
	Center Crystal	Crystal centering for manual (and, if applicable, motorized) goniometer heads.
	XPRESSO	Automatic structure determination.

Table 2.8 — “Screen” category





Icon	Name	Description
	Screen Crystal	Simple scans.
	Determine Unit Cell	Determine unit cell constants and Bravais lattice type.
	View Reciprocal Lattice	3D visualization of the lattice projected in reciprocal space.
	View Images	View and analyze diffraction images.

Table 2.9 — “Collect” category




Icon	Name	Description
	Calculate Strategy	Calculation and customization of data collection strategies.
	Run Experiment	Edit, start, and monitor data collection experiments.
	Orient Crystal	Measure diffraction images with the crystal aligned along specified axes.

Table 2.10 — “Reduce Data” category




Icon	Name	Description
	Integrate Images	Integrate diffraction data.
	Scale	Scale intensities and perform absorption correction.
	Unwarp and Convert Images	Convert frames between formats.

Table 2.11 — “Examine Data” category






Icon	Name	Description
	Determine Space Group	Dataset analysis and manipulation.
	Analyze Data	Run XPREP.
	Compare Unit Cells	Compare unit cells to find twin laws, etc.
	Synthesize Precession Images	Create precession images based on measured frames.
	Find a Reflection	Find a reflection in measured frames.

Table 2.12 — “Solve Structure” category



Icon	Name	Description
	Determine Phases	Macro structure solutions.

Table 2.13 — “Report” category

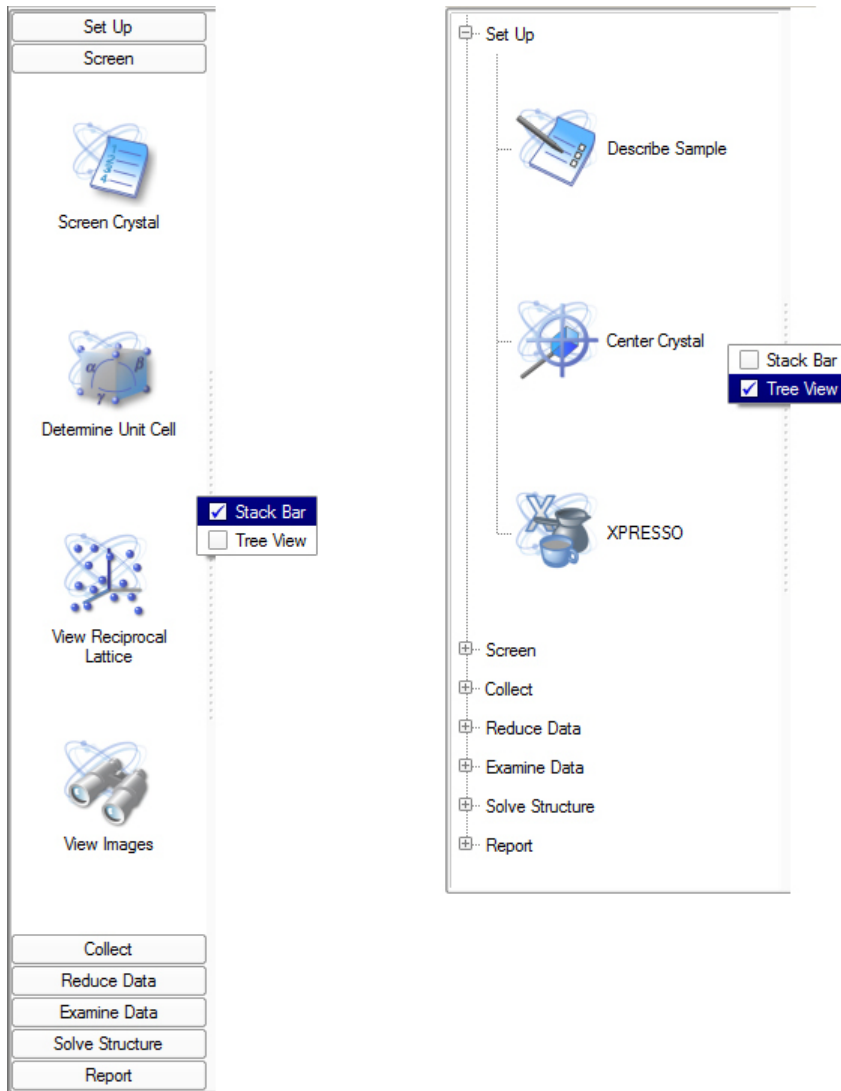
Icon	Name	Description
	Generate Report	Use Bruker’s automatic report generation system.

Right-click in the Task Bar to choose one of two views: Stack Bar or Tree View.

In Stack Bar view, click one of the Task Bar categories to display only the icons belonging to that category. All other Task Bar icons will be hidden.

In Tree View, categories may be expanded to display their Task Bar icons by clicking the plus or minus sign next to the category name.

Figure 2.8 — Stack Bar vs. Tree View

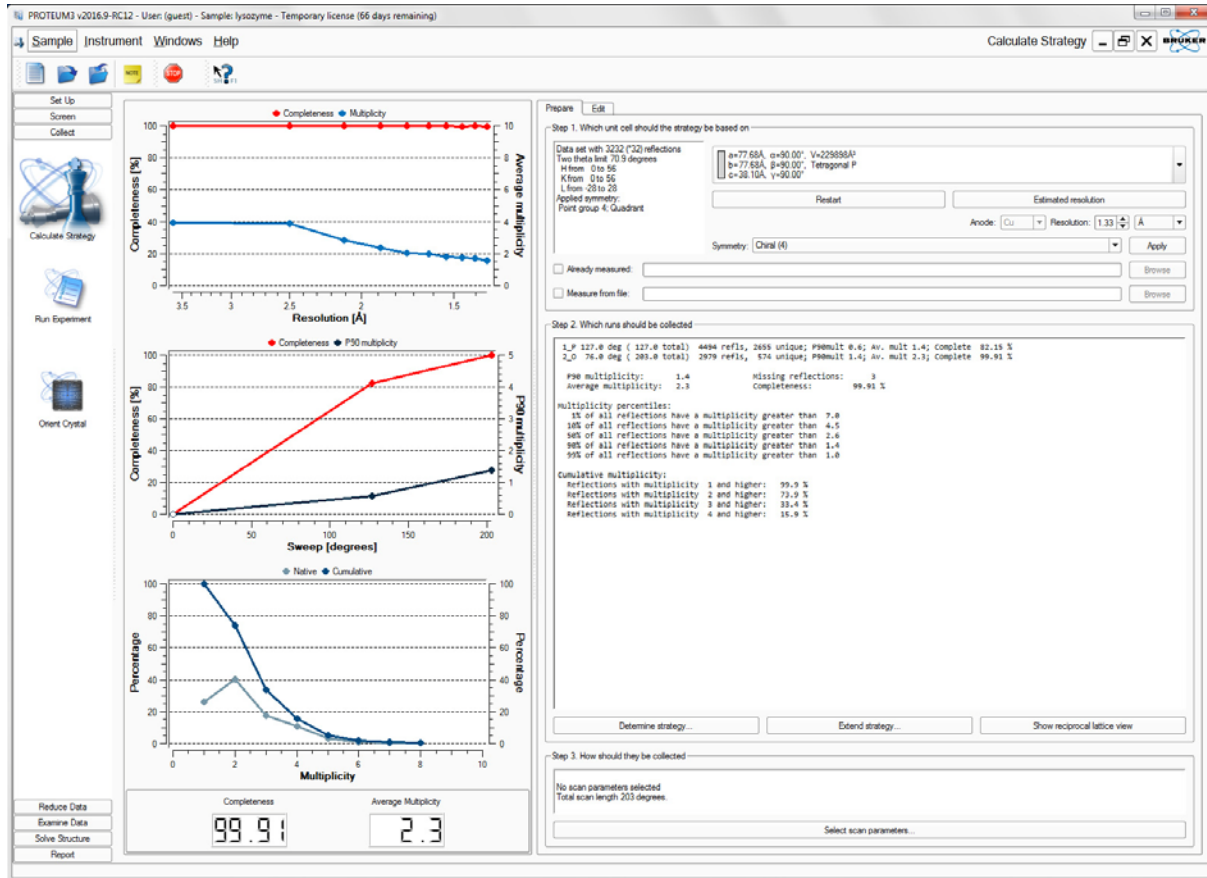


2.4.5 The Task Display Area

The Task Display Area (Figure 2.9) is the main area for tasks, user input, and graphical output (PROTEUM3 also produces files as output, such as *.hkl files).

This area displays images and other output from the plug-ins.

Figure 2.9 — Task Display Area showing the Calculate Strategy plug-in



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3 Getting Started with PROTEUM3

Before you begin using PROTEUM3, it is necessary to start the program, log in, and create a sample in the Sample Database. All files (e.g., frames, *.p4p files, etc.) will be contained in a directory that you specify when you create the sample.

3.1 User Access

Bruker software allows different user accounts to have different levels of access to the instrument:

- Service
- Security
- Administrator
- User
- Non-user

You need “Bruker Instrument Administrator” rights to run DIFFRAC.CONFIGURATION and “Bruker Instrument User” rights to run programs from the Suite. Typically, your System Administrator will already have your user account configured or you will be using the default accounts as provided by Bruker.

3.2 Start PROTEUM3

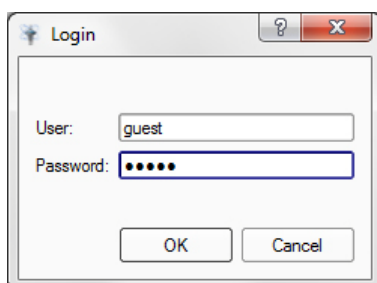
Double-click the PROTEUM3 icon on the Desktop (or choose **Start > All Programs > Bruker AXS > PROTEUM3** if the license is properly installed).

PROTEUM3’s main window opens.

3.3 Log In

At the “Login” dialog, enter your user name and password (as shipped by Bruker, the default account is `guest` with password `guest`). Click **OK**.

Figure 3.1 — “Login” dialog



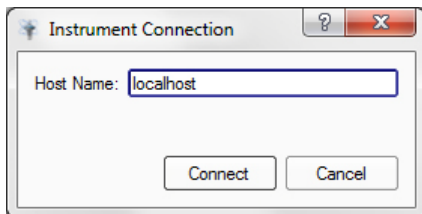
NOTE: If your setup does not require individual login accounts, you can configure the software to automatically log into a specified account by setting the shortcut’s target to e.g.,
“C:\bn\src\scripts\proteum.b
at user:guest
password:guest”.

3.4 Connect to BIS (or BIS Classic)

NOTE: This step is optional; plug-ins will request a connection when needed.

1. To use the software in online mode, choose **Instrument > Connect...**
The “Instrument Connection” dialog appears.
2. Enter the name (or IP address) of the computer running BIS or BIS Classic (the dialog shows the default host; if BIS or BIS Classic is running on the same computer as PROTEUM3, the host name is `localhost`). Click **Connect**.

Figure 3.2 — “Instrument Connection” dialog

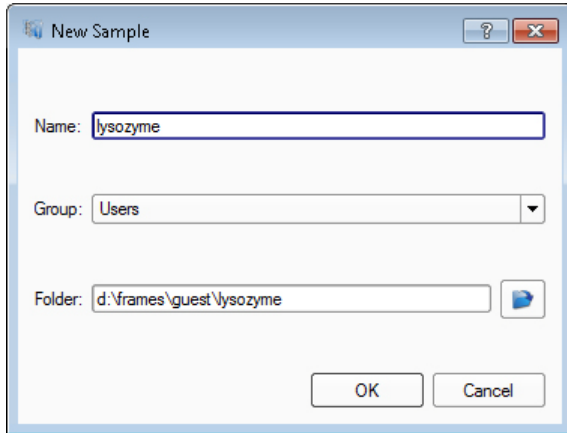


3.5 Create a New Sample

1. Choose **Sample > New...**

The “New Sample” dialog opens.

Figure 3.3 — “New Sample” dialog



2. In the “New Sample” dialog, enter the sample name and choose an access group for your sample (if desired). The Suite will automatically create a directory for data storage.

NOTE: You can only use a sample name once per database. If the name is already taken, the program suggests an alternative sample name.

NOTE: The sample name and sample folder do not need to match. Even though the folder name is automatically filled in by the software, the user can redirect it to any folder on the computer (or network).

3. Click **OK**.

The Task Bar appears with the “Set Up” category open.

3.6 Describe the Sample with the Describe Sample Plug-in




1. In the Task Bar's "Set Up" category, click the **Describe Sample** icon .
The Describe Sample plug-in opens.
2. Fill in the fields.

Figure 3.4 — Describe Sample plug-in (filled in)

Name:	<input type="text" value="lysozyme"/>					
Source:	<input type="text" value="hen egg white"/>					
Compound:	<input type="text" value="lysozyme"/>					
Sequence:	<input type="text" value="/WCNDGRTPGSRNLCNIPCSALLSSDITASVNC AKKIVSDGNGMNAWVAWRNRCKGTDVQAWIRGCLR"/>	<input type="text" value="protein (single-letter code)"/>				
Crystallization:	<input type="text"/>					
Crystal Color:	<input type="text" value="colourless"/>					
Crystal Dimensions:	<input type="text" value="0.300"/>	x	<input type="text" value="0.300"/>	x	<input type="text" value="0.300"/>	[mm]
Crystal Shape:	<input type="text" value="rectangular"/>					

The "Sequence:" field may be used to store a protein's or nucleic acid's one-letter sequence code in the database. This can be used by the "Generate Report" plug-in to determine the solvent content.

3. Close the Describe Sample plug-in by clicking the  button on the right-hand side of the Menu Bar.

The data is automatically saved to the Sample Database.

4 Crystal Centering and Screening

4.1 Centering the Crystal

To obtain accurate unit cell dimensions and collect high-quality data, it is necessary to position the crystal in the center of the X-ray beam and maintain this position for the entire experiment.

Your video microscope should be aligned so that its reticle coincides with the goniometer center. If the reticle does not coincide with the goniometer center, you can still center the crystal. A successfully-centered crystal stays in the same place in the microscope's field of view in all orientations.

Also, the centering process can be greatly helped by lighting both the sample and background for maximum contrast.

NOTE: To make adjustments easier, the goniometer head's X, Y, and Z locks may be loosened (with the thin end of the wrench—the other end of the wrench is used to move the adjustment sleds). Locking after centering needs only a very slight touch; do not overtighten the locks.

NOTE: Centering is often easier if the crystal is rotated to give a good view before the actual centering process is started (e.g., down an edge for a plate). To do this, simply manually rotate the crystal on its magnetic mount to a suitable position.

4.1.1 Start the Center Crystal Plug-in

Under the "Set Up" category in the Task Bar, click the **Center Crystal** icon



The Center Crystal plug-in opens.

4.1.2 Mount the Goniometer Head

1. Open the enclosure door(s).
2. Carefully remove the goniometer head containing the sample from its case.
3. Place the goniometer head onto its base on the phi drive. Line up the appropriate slot on the bottom of the goniometer head with the pin on the mounting base (Figure 4.1).

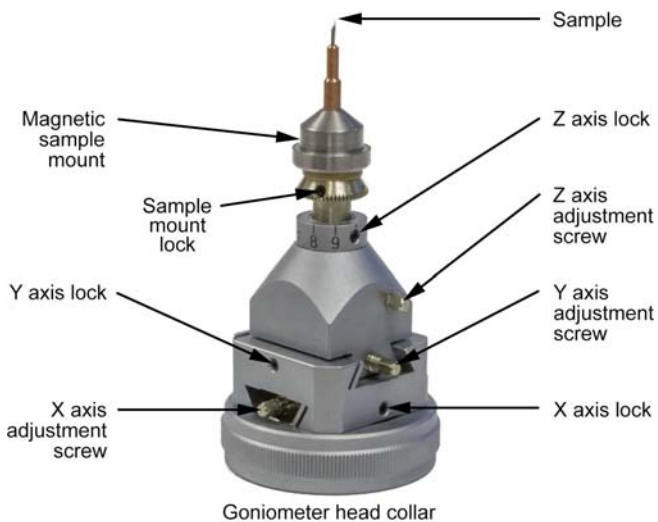
NOTE: Modern goniometer heads have two slots, but only one fits the goniometer pin. In the default center position, the Z axis adjustment screw will face you.

Figure 4.1 — Goniometer head bottom




4. Screw the goniometer head's collar to the base so that the head does not move. Do not overtighten it (finger-tighten only).

Figure 4.2 — Huber goniometer head




4.1.3 Center the Crystal on the Goniometer

NOTE: This procedure is applicable to both FIXED-CHI and KAPPA goniometers.

1. To show the video image, click the **Start Grab** button  .

NOTE: On pre-D8 VENTURE systems, the video will appear in a separate window.

2. Choose the desired reticle for centering by clicking the appropriate button (this example uses the crosshair reticle  .
3. Click the **Center** button.

The goniometer head drives so that its Y and Z translation axes are positioned perpendicular to the microscope.

Figure 4.3 — Center position, Y- and Z-axis adjustment screws

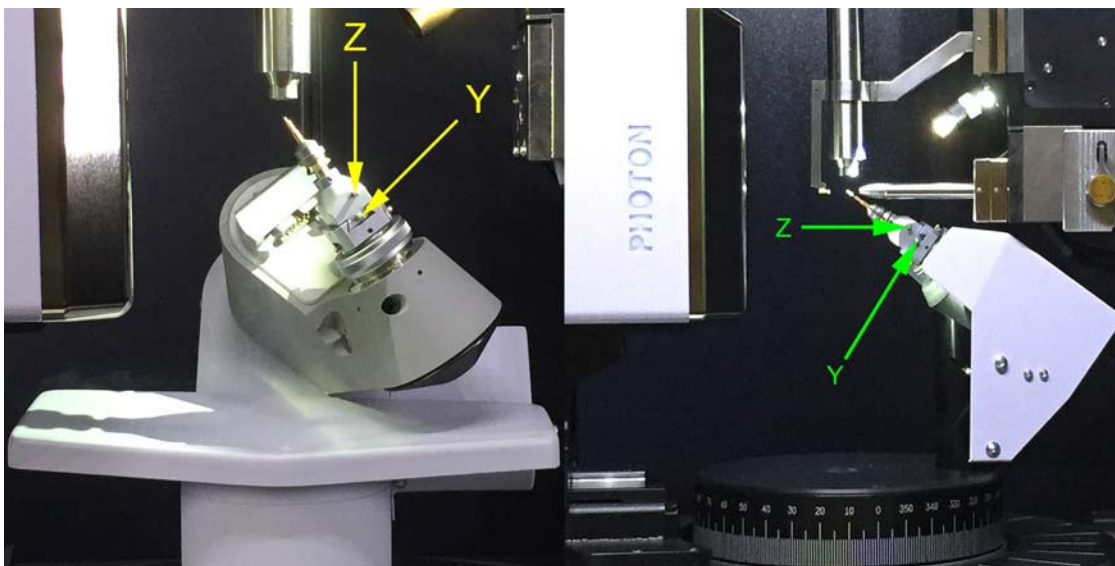
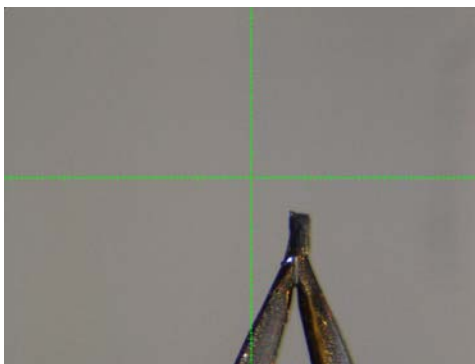
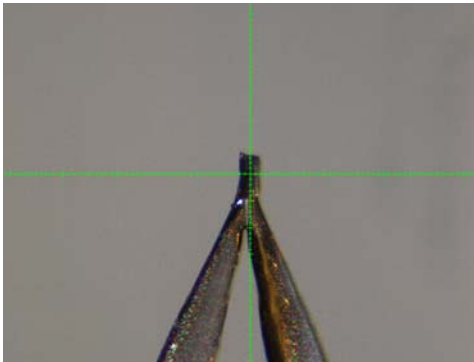


Figure 4.4 — Uncentered crystal as initially mounted



- Center the crystal in the video microscope reticle by making adjustments to the Y- and Z-axis adjustment screws.

Figure 4.5 — Centered crystal after Y- and Z-axis adjustments



- Click **Spin Phi 90**.

Phi rotates so that the X-axis adjustment is facing the front of the diffractometer. Any error in the crystal's position along the X axis will now appear in the microscope.

Figure 4.6 — Center position, X-axis adjustment screw

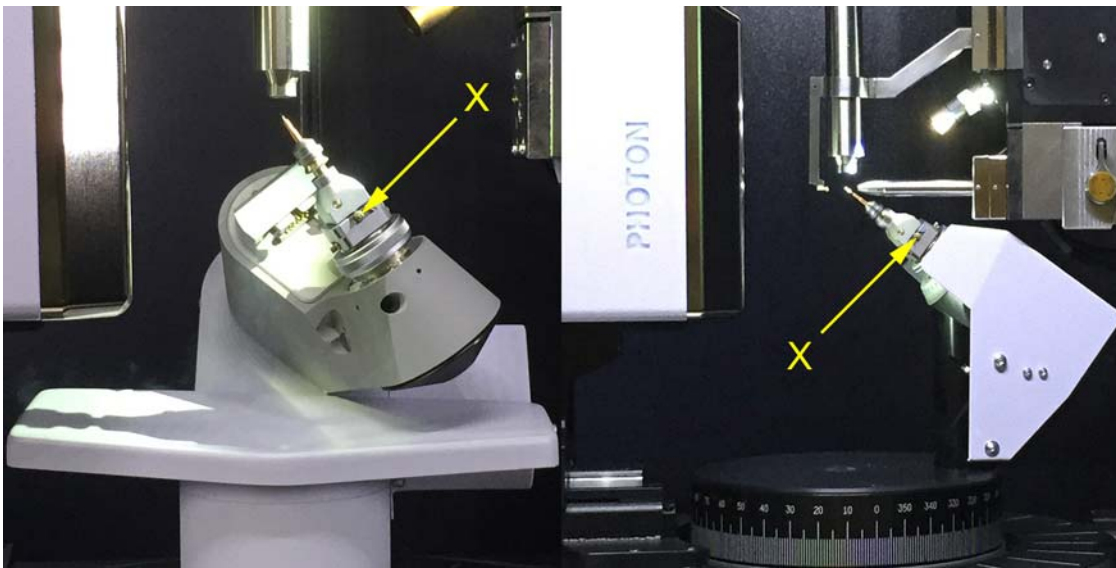
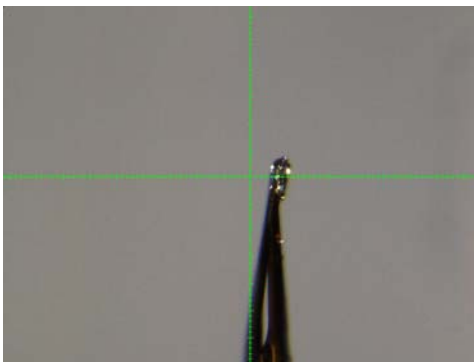
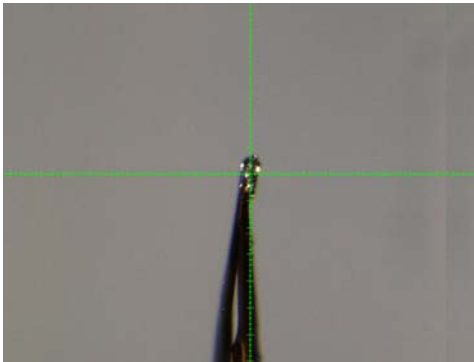


Figure 4.7 — X-axis error after spinning phi 90°



- Center the crystal in the video microscope reticle by making adjustments to the X-axis adjustment screw.

Figure 4.8 — Centered crystal after X-axis adjustments



- Click **Spin Phi 180** and check that, after rotating 180°, the crystal appears in the same position in the microscope reticle. If the crystal does not appear in the same position:
 - Use the adjustment screw facing you to remove half of the difference shown in the reticle (Figure 4.9 and Figure 4.10).
 - Click **Spin Phi 180**, and check that the crystal appears in the same position in the microscope reticle.
 - Repeat the preceding two steps until you are satisfied that the crystal remains in the same position in the microscope reticle when rotated by 180°.

Figure 4.9 — Example: error in Y axis

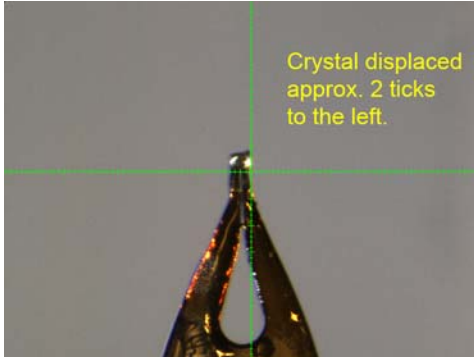
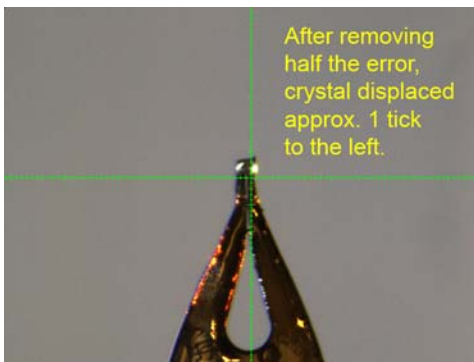


Figure 4.10 — Example: error removed by half



8. Alternately click **Spin Phi 90** and **Spin Phi 180** to verify that the crystal stays in the same place in the microscope reticle through all motions of phi. If the crystal fails to stay in the same position as phi is rotated, make adjustments to the axes by repeatedly removing half the error (always using the adjustment screw facing you) as in step 7.

If the crystal does not remain centered, go back to step 3.

NOTE: If the crystal consistently fails to appear in the same position when rotated by 180° , the position of the microscope reticle may need adjustment. For more information, refer to DOC-M86-EXX184 D8 QUEST and D8 VENTURE Video Microscope Alignment Procedure or DOC-M86-EXX024 Microscope Focus and Sample Alignment Procedure, as appropriate.

The crystal is now centered on the goniometer.

4.1.3.1 Additional Verification Positions: Left, Right, and Top

In addition to the **Center** position, three other positions are available for verification of the microscope and crystal alignment: **Left**, **Right**, and **Top**.

These additional positions are typically only used for verifying that the microscope reticle is pointing at the goniometer center. If the crystal moves more than 10 microns (i.e., one-half reticle “tick”) between positions, the microscope may need alignment; see DOC-M86-EXX184 D8 QUEST and D8 VENTURE Video Microscope Alignment Procedure or DOC-M86-EXX024 Microscope Focus and Sample Alignment Procedure, as appropriate.

1. Click the **Right** button.

The goniometer drives to place the spindle horizontal and to the right.

NOTE: On a KAPPA goniometer, this position has an increased risk of ice formation if a low-temperature device is being used.

Figure 4.11 — Right position

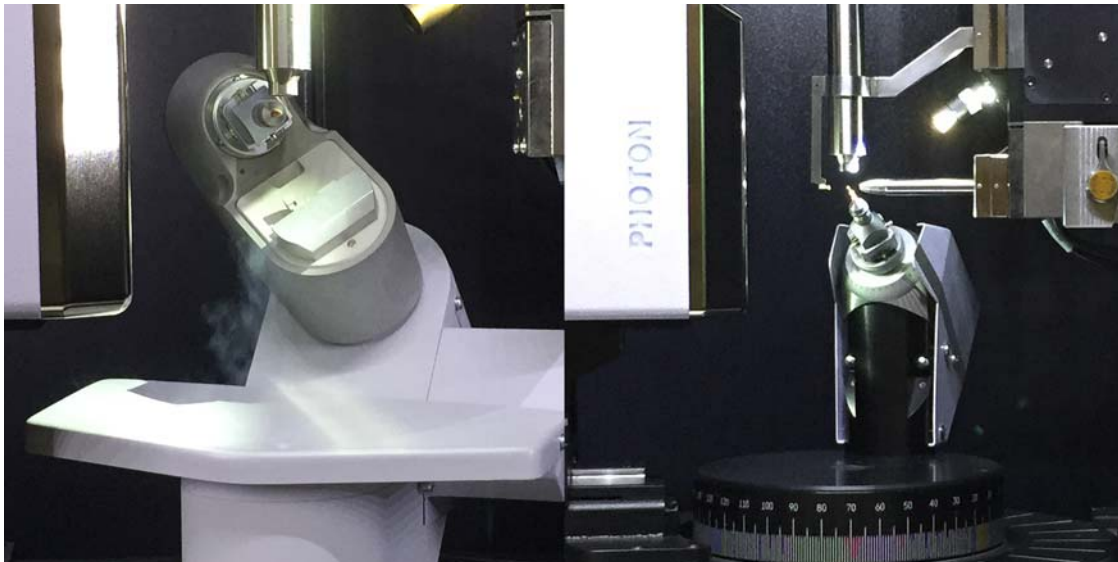
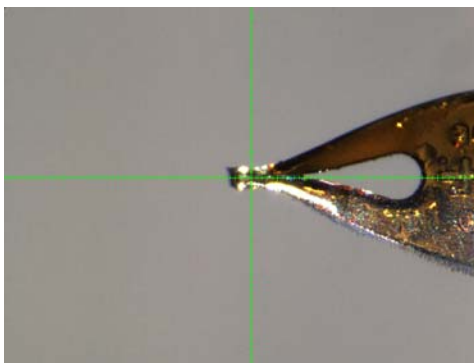


Figure 4.12 — Crystal in right position



2. Click the **Left** button.

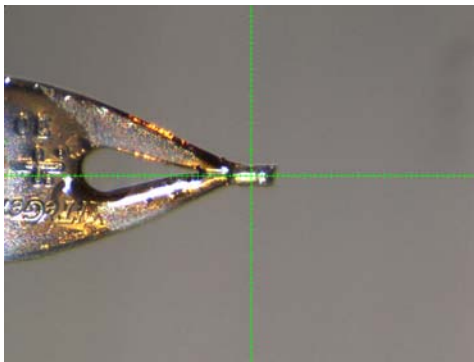
The goniometer drives to place the spindle horizontal and to the left. Note the height of the crystal in the video microscope reticle.

NOTE: On a KAPPA goniometer, this position has an increased risk of ice formation if a low-temperature device is being used.

Figure 4.13 —Left position



Figure 4.14 —Crystal in left position



3. Check that the crystal height is the same as the height you noted in step 4.1.3.
 - 3.1. If the height is the same, you may be confident that the crystal is in the center of the instrument.
 - 3.2. If the height is not in the same place, adjust to remove half of the difference, click **Right**, and repeat step 4.1.3 and step 3.

4.2 Checking Crystal Quality

A number of tools provide information about the current image. These tools, present throughout the Suite, can help determine the diffraction characteristics of a crystal.

4.2.1 Start the Screen Crystal Plug-in

In the Task Bar's "Screen" category, click the **Screen Crystal** icon



The Screen Crystal plug-in opens.

4.2.2 Set up the Scan

Figure 4.15 —Screen Crystal menu

The screenshot shows the 'Screen Crystal' menu interface. It is organized into several sections:

- Preset Scans:** Two buttons labeled 'Phi = 0' and 'Phi = 90'.
- Preset Positions:** Four buttons labeled 'User 1', 'User 2', 'User 3', and 'User 4'.
- Resolution [Å]:** A text field showing '2.34' and a 'Resolution Table...' button.
- Distance [mm]:** A text field with '70' and a slider.
- 2Theta:** A text field with '0' and a slider.
- Omega:** A text field with '0' and a slider.
- Phi:** A text field with '0' and a slider.
- Chi:** A text field with '55' and a slider.
- Drive:** A large button.
- Scan Axis:** Radio buttons for 'Phi' and 'Omega', with 'Omega' selected.
- Scan Range:** A text field with '0.50'.
- Image Width:** A text field with '0.50'.
- Exposure Time:** A text field with '10.00' and a dropdown menu set to 'secs/image'.
- Correlate Exposures:** An unchecked checkbox.
- Dark Current Correction:** Radio buttons for 'Existing dark image' (selected) and 'New dark image'.
- Drive + Scan:** A large button at the bottom.

1. If you have a dual-source system, set the system to collect the scan at the desired wavelength.

- Set the goniometer to the desired position either by using the preset position buttons, adjusting the sliders, or by manually editing the goniometer angle values.

Table 4.1 — Presets

Preset		Description
Preset Scans	Phi = 0	Sets 2theta, omega, and phi to 0° and chi to 55°. The DX is not set.
	Phi = 90	Sets phi to 90°. The DX is not set.
Preset Positions	User 1 - User 4	The "User" positions may be defined in the bn-config.py file.

NOTE: If the **Drive** button is gray, the requested position violates the software limits.

- The "Resolution [Å]" value shows the maximum resolution at the edge of the detector (it will update as the 2theta and Distance values are changed). If desired, examine the resolution table by clicking the **Resolution Table...** button.
The table shows the 2theta value required for a desired resolution at the edge of the detector.
- Click **Drive** to initiate the requested movement.

NOTE: You must click the **Drive** button to move the goniometer to the new position.

- Choose whether to use the existing dark current correction or a new one by clicking the appropriate radio button (not applicable for shutterless data collection).
- Click **Drive + Scan** to start the scan.

4.2.2.1 Collect a Still Image

1. Click the **Still** button.

A value of 0.00 is displayed in both the "Scan Range:" and "Scan Width:" fields.

2. Enter an exposure time.

You can select the units to be either seconds/frame or seconds/degree.

3. To start the scan, click the **Drive+Scan** button.

An example of starting goniometer positions for sample screening is shown in Table 4.1. If the reflections are overlapping, increase the detector distance. If there are reflections that extend out to the edge of the image, increase the 2theta angle to determine how well the crystals diffract (10° intervals work well).

NOTE: If the low-temperature device's nozzle is perpendicular to the goniometer base and chi is near zero for a long period of time, ice may form on the goniometer head. Whenever BIS Server becomes idle (and if the de-icing option is activated), it will automatically drive the crystal to chi = 45° to eliminate icing.

4.2.2.2 Collect a Rotation Scan

1. Set the scan range and image width for the rotation scan.

The scan range determines the angular range for the scan, and the image width is the rotation of each image.

For example, a scan range of 3.0° and an image width of 0.5° will collect 6 images.

2. Adjust the exposure time as you would for a still image.
3. Click the **Drive + Scan** button.

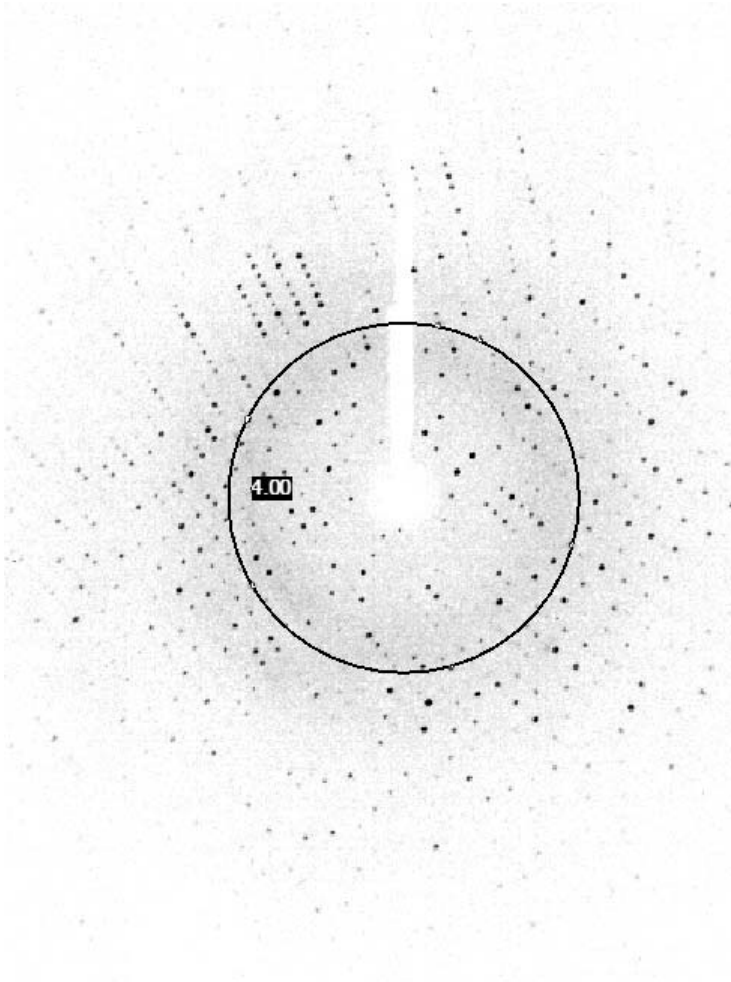
If the **Correlate Exposures** checkbox is activated, the image will be taken in two parts and added together. If the **New dark image** radio button is activated, the system will generate a new dark image with a duration equal to the current exposure time.

4.2.3 Use the Resolution Circle Tool

1. Click the Resolution Circle tool  in the Tool Icon Bar to activate it.
2. Click the left mouse button and drag to draw the resolution circle.

The circle will extend outward from the beam center and display the resolution at the circle edge (the circle is always drawn from the direct beam position).

Figure 4.16 —Drawing a resolution circle




3. To hold the position, release the button.
4. To deactivate the Resolution Circle tool, click its icon in the Tool Icon Bar.

The Resolution Circle tool also provides a quick way to check the current beam center position. Shrinking the circle so that it is very small will indicate where the program thinks the beam center is located. If it is not behind the beamstop shadow, it is likely not correct.

4.2.4 Use the Line Tool

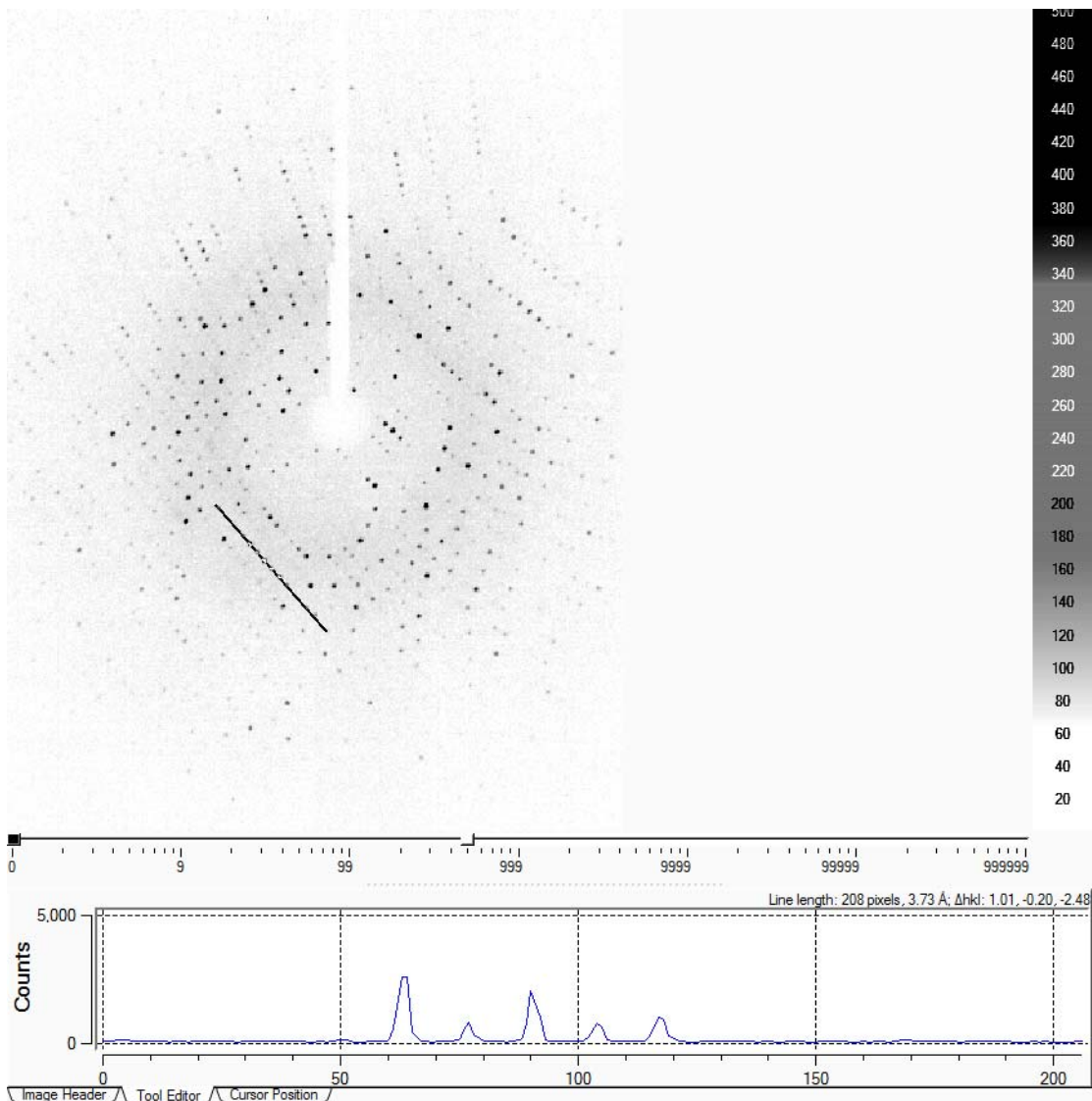
A quick way to check whether reflections are overlapping is to use the Line Tool.

A chart showing the counts for each peak under the line is displayed at the bottom. The length of the line in pixels and angstroms is also shown.

1. Click the Line tool  in the Tool Icon Bar to activate it.
2. To draw the line, click and drag the mouse.

When you release the button, the length of the line remains fixed and it can be placed anywhere on the image.


Figure 4.17 —Drawing a line



3. To deactivate the Line tool, click its icon in the Tool Icon Bar.

4.2.5 Plot a Rocking Curve

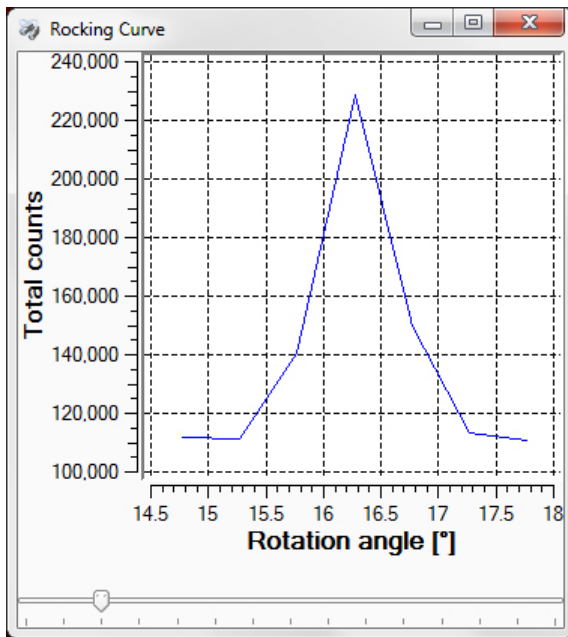
The Box tool allows the rocking curve determination of a spot. The full-width half maximum for a peak's rocking curve is a good estimate of the peak's mosaic spread. Looking at a few peaks away from the high-Lorentz-factor region or "cusp" gives a rough idea of the overall mosaicity.


1. Click the Box tool  in the Tool Icon Bar to activate it.
2. Click and drag to draw the box.
If necessary, zoom into the selected area by right-clicking and choosing **Zoom+**.
3. Draw a box around a single reflection, right-click, and choose **Rocking Curve**.

A graph appears displaying the total counts as a function of the rotation angle.


You typically need around 1.5° of data on either side of a scan to sample several peaks. If the peak begins at and returns to the background level, the full-width half-maximum value will be displayed. If additional scans are available, the slider bar at the bottom of the graph allows the angular range to be increased.

Figure 4.18 —Rocking curve



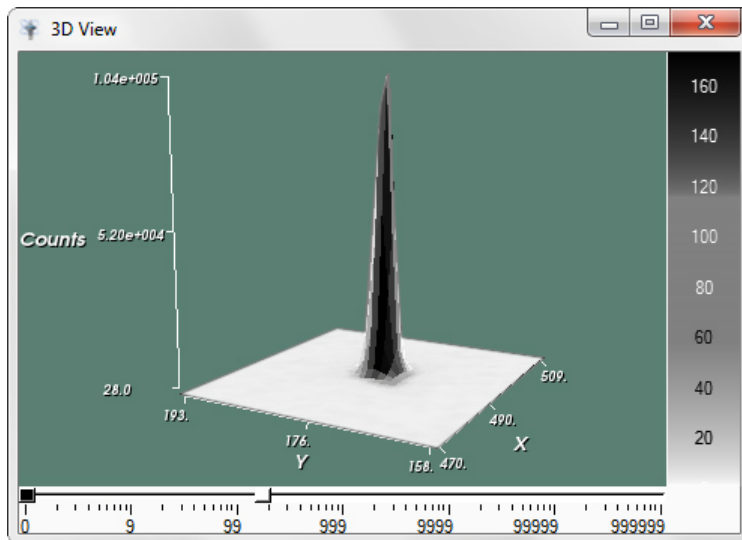
4. To close the "Rocking Curve"  window, click the button.
5. To deactivate the Box tool, click its icon in the Tool Icon Bar.


4.2.6 View Peak Profiles in 3D

1. Click the Box tool  in the Tool Icon Bar to activate it.
2. To draw the box, hold the left mouse button down and drag the mouse.
If necessary, zoom into the selected area by right-clicking and choosing **Zoom+**.
3. Draw a box around a peak or group of peaks, right-click, and choose **3D View**.
A 3D profile of the box region appears.

The profile can be rotated by holding down the left mouse button and dragging the mouse. the display colors can be changed by right-clicking the contrast scale.

Figure 4.19 —3D View



4. To close the "3D View" window, click the  button.
5. To deactivate the Box tool, click its icon in the Tool Icon Bar.

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5 Using the XPRESSO Plug-in

5.1 About XPRESSO

The XPRESSO plug-in is a fully-automatic data collection and reduction plug-in, operating entirely within PROTEUM3.

The XPRESSO plug-in enables the instrument to automatically carry out **all** steps of the crystal structure analysis—through report generation—without further user intervention.

5.2 Before Using XPRESSO

Before using the XPRESSO plug-in, it is necessary to:

1. Create a new sample in PROTEUM3 (Section 3.5);
2. Describe the sample with the Describe Sample plug-in (Section 3.6); and
3. Center the crystal on the goniometer (Section 4.1).

5.3 Start the XPRESSO Plug-in

In the Task Bar's "Set Up" category, click the **XPRESSO** icon



The XPRESSO plug-in opens.

The XPRESSO plug-in's main window contains three sections:

- An "information" section on the left that contains threetabs: "Images", "Report", and "Output".
- A "parameters" section on the right that contains sections for image data, in addition to unit cell information.
- The **Start** and **Stop** buttons.

5.3.1 Information Section

5.3.1.1 “Images” Tab

This tab contains the same image browsing and analysis tools as PROTEUM3's View Images plug-in.

Figure 5.1 — “Images” tab

Unit Cell Determination

Exposure Time [s]	
Distance [mm]	

Data Set

Target Resolution [Å]	
Target Multiplicity	
Output CBF	No

Stop After Crystal Characterization

Results:

Crystal Characteristics

Mosaicity [°]	0.61
---------------	------

Data Set Characteristics

Resolution [Å]	1.69
I/σ(I) in Outer Shell	3.01
Multiplicity	8.28

Unit Cell

Space Group	P 43 21 2
a [Å]	77.83
b [Å]	77.83
c [Å]	37.92
α [°]	90.00
β [°]	90.00
γ [°]	90.00
Volume [Å ³]	229704

Cursor

Position [mm]	39.83	57.11	
Position [pixels]	679	934	
Intensity [counts]	78		
HKL index	-28.67	13.23	-9.42
Resolution [Å]	2.10		
2Theta [°]	43.03		

Image Header / Tool Editor / Cursor Position

Start Stop

5.3.1.2 “Report” Tab

After XPRESSO has collected and reduced the data, this tab will display a report in HTML format.

Figure 5.2 — “Report” tab

lysozyme report

Compound: *lysozyme*
 Source: *hen egg white*
 Sequence: 129 AA; 14313 Mw

KVFFGRCLAA AMKRHGLDNY RGYSLGNWVC AAKFESFPNT QATNRNTDGS TDYCIILQINS
 RHWKNDGRTP GSRNLNCIDP SALLSSDITA SVNCAKRIYS DGMGMAMVA WRBRCRGTDV
 QAWIRGCRL

Crystal characteristics
 A colorless rectangular crystal with approximate dimensions of 0.300 x 0.300 x 0.300 mm was used for data collection.
 Unit cell constants: a=77.76, b=77.76, c=37.91 Å, α=90, β=90, γ=90°
 Lattice type: Primitive
 Laue group: 4/mmm
 Space group: P4(3)2(1)2
 Diffraction limit: 1.8 Å
 Matthews Coefficient:

Number (mol)	Overall Probability	V _m	Solvent content (%)
1	0.998	2.0	39
2	0.002	1.0	0

Data Collection:
 The sample temperature was 100 K. The total data consists of 1 scan containing 150.0 degrees of data (table 1). The data collection time was 00:25 hh:mm.

Table 1: Data collection strategy

Axis	dx/mm	2θ/°	ω/°	φ/°	χ/°	Width (°)	Sweep (°)	Frames	Time (s)
Phi	49.877	0.00	372.00	-150.00	23.00	0.50	150.00	300	5.00

The frames were integrated and scaled using SAINT¹ and SADABS. The relevant scaling statistics are listed in table 2.

Table 2: Data statistics

Resolution	% Complete	Redundancy	Mean I/σ	R(int)
20.63 - 7.62	86.6	5.73	49.95	0.0251
7.62 - 5.08	98.5	7.89	45.55	0.0319
5.08 - 4.00	100.0	8.37	48.72	0.0333
4.00 - 3.47	100.0	8.57	46.91	0.0372
3.47 - 3.14	100.0	8.61	40.17	0.0425
3.14 - 2.91	100.0	8.62	32.78	0.0480
2.91 - 2.73	100.0	8.65	26.52	0.0591
2.73 - 2.59	100.0	8.58	23.80	0.0629
2.59 - 2.47	100.0	8.66	21.01	0.0753
2.47 - 2.37	100.0	8.62	19.17	0.0844
2.37 - 2.29	100.0	8.76	16.99	0.0895
2.29 - 2.21	100.0	8.44	16.10	0.0995
2.21 - 2.15	100.0	8.44	13.52	0.1235
2.15 - 2.08	100.0	8.43	12.53	0.1333

Unit Cell Determination

Exposure Time [s]
 Distance [mm]

Data Set

Target Resolution [Å]
 Target Multiplicity
 Output CBF No

Stop After Crystal Characterization

Results:

Crystal Characteristics

Mosaicity [°] 0.61

Data Set Characteristics

Resolution [Å] 1.69
 I/σ(I) in Outer Shell 3.01
 Multiplicity 8.28

Unit Cell

Space Group P 43 21 2
 a [Å] 77.83
 b [Å] 77.83
 c [Å] 37.92
 α [°] 90.00
 β [°] 90.00
 γ [°] 90.00
 Volume [Å³] 229704

Start Stop

5.3.2 Parameters Section

5.3.2.1 Unit Cell and Data Set Parameters

This window contains information about the unit cell and data set being collected by XPRESSO. It continuously updates as better data is obtained.

If the Target Resolution is left blank, XPRESSO estimates the diffraction limit and finds a strategy and exposure time for that resolution range. If a value is input for Target Resolution, XPRESSO will determine the appropriate strategy and exposure time for that resolution limit. If the diffraction limit does not meet the target resolution, XPRESSO will terminate the experiment.

If you want to forego the full data set and stop after the crystal has had its basic characteristics determined, activate the **Stop After Crystal Characterization** checkbox.

Figure 5.3 — Unit cell and data set parameters

Unit Cell Determination	
Exposure Time [s]	
Distance [mm]	
Data Set	
Target Resolution [Å]	
Target Multiplicity	
Output CBF	No
<input type="checkbox"/> Stop After Crystal Characterization	

5.3.2.2 Results Display

As the analysis proceeds, the unit cell, data set characteristics, and mosaicity will appear in the “Results:” area.

Figure 5.4 — Results display

Results:

Crystal Characteristics	
Mosaicity [°]	0.63
Data Set Characteristics	
Resolution [Å]	2.23
I/σ(I) in Outer Shell	6.19
Multiplicity	7.40
Unit Cell	
Space Group	P 43 21 2
a [Å]	79.37
b [Å]	79.37
c [Å]	38.13
α [°]	90.00
β [°]	90.00
γ [°]	90.00
Volume [Å ³]	240222

5.4 Start Data Collection and/or Structure Solution

Click **Start** to begin execution of the XPRESSO plug-in.

As the analysis proceeds, each step in the process will be given a quality score (with 1.00 being perfect). In addition, the percent completion, elapsed time, and remaining time will be displayed in BIS.

Figure 5.5 — “Results” area

Results:

Crystal Characteristics	
Mosaicity [°]	0.63
Data Set Characteristics	
Resolution [Å]	2.23
I/σ(I) in Outer Shell	6.19
Multiplicity	7.40
Unit Cell	
Space Group	P 43 21 2
a [Å]	79.37
b [Å]	79.37
c [Å]	38.13
α [°]	90.00
β [°]	90.00
γ [°]	90.00
Volume [Å ³]	240222

5.4.1 XPRESSO’s Data Collection and/or Structure Solution Process

The XPRESSO plug-in is completely automated, from the time the **Start** button is clicked through report generation. User intervention is not permitted. A **Stop** button is provided for the user to completely terminate the execution of the XPRESSO plug-in.

Once **Start** has been clicked, XPRESSO will acquire a set of preliminary images (named “reso_0n_000x.sfrm”) to determine the diffraction limit and data signal-to-noise for a set of pre-configured exposure times. Based on the initial images, matrix runs are collected to determine the preliminary unit cell constants and orientation matrix, and to evaluate the crystal quality.

After these preliminary scans, the plug-in optimizes the data collection parameters, taking into account such factors as crystal diffracting power and mosaicity, and begins collecting the dataset (named “scan_0n_0xxx.sfrm”). The plug-in also provides an initial estimate of the time required to collect the complete dataset. Following the first scan, the space group is determined and the data collection strategy reevaluated.

After the data is collected and reduced, the XPRESSO plug-in will automatically generate a report in HTML format and output a reflection file in both SHELX HKLF4 and Scalepack format.

5.5 Close the XPRESSO Plug-in

Click the **Close** button in the Menu Bar (not in the Title Bar).

The plug-in closes.

5.6 Using XPRESSO in Conjunction with PROTEUM3

You may use PROTEUM3's other plug-ins to manually evaluate the completed dataset.

You also have the option of repeating the entire analysis in a conventional, stepwise manner beginning with the data collection images (i.e., "scan_0n_0xx.sfrm"). This option may be required if the XPRESSO plug-in fails to solve the structure.

6 Data Collection

In the data collection example that follows, we will use a lysozyme crystal with the sample name `lysozyme` (entered in Section 3.5).

6.1 Determining the Unit Cell

Unit cell determination is performed in several steps:

1. Data collection
2. Harvesting
3. Indexing the reduced triclinic cell
4. Refinement of the triclinic cell
5. Bravais lattice
6. Final refinement

These steps are integrated in the Determine Unit Cell plug-in, which also contains a one-click solution for fully-automated unit cell determination.

The upper right-hand corner of the Determine Unit Cell plug-in contains two sections:

- **Automatic Mode:** for fully-automated unit cell determination (Section 6.1.2); and
- **Manual Mode:** for unit cell determination with user-defined parameters (Section 6.1.3).

Figure 6.1 — Determine Unit Cell plug-in (initial view)

The interface is divided into several sections:

- Automatic Mode:** Includes dropdown menus for 'Start at:' (set to 'Collect Data') and 'Stop after:' (set to 'Refine'), and a 'Run' button.
- Manual Mode:** A vertical stack of radio buttons for 'Collect Data', 'Harvest Spots', 'Index', 'Bravais', and 'Refine'. 'Collect Data' is selected.
- Unit cells:** An empty list box with 'Edit...', 'Delete', and 'Delete All' buttons to its right.
- Reflections:** An empty list box with 'Edit...', 'Delete', and 'Delete All' buttons to its right.
- Expected resolution:** A table with columns for 'Exposure time [s/°]' and 'Resolution [Å]'. The table contains four rows of data.
- Crystal Mosaicity [°]:** A label with the value '0.40'.

	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

6.1.1 Start the Determine Unit Cell Plug-in



In the Task Bar's "Screen" category, click the **Determine Unit Cell** icon

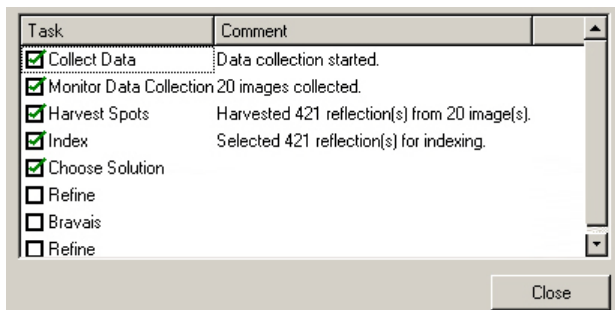
The Determine Unit Cell plug-in opens.

6.1.2 Determine the Unit Cell in Automatic Mode

1. From the **Start At:** pull-down menu, select the step at which you want to begin automatic unit cell determination (useful if you want to find a unit cell from frames that have already been collected).
2. From the **Stop After:** pull-down menu, select the step at which you want to stop automatic unit cell determination.
3. Click **Run**.

Automatic data collection begins, and collected frames appear in the Information Display Area (you can abort the determination procedure at any time by clicking **Abort**.). The area containing the Automatic and Manual mode options changes to a list showing the system's progress through the steps of unit cell determination.

Figure 6.2 — Automatic Mode progress list



When automatic unit cell determination is complete, the progress list will show a check mark and comments for each completed step.

4. Click the **Close** button to close the progress list and return to the main Determine Unit Cell window.

The window's right-hand side displays the unit cell parameters, along with options to edit or delete the unit cell and reflection list if you wish to perform additional operations in Manual Mode.

If desired, you can set up your own runs for the Determine Unit Cell plug-in by editing the bn-config.py configuration file (or, for D8 Classic instruments, using BCP's **Single Crystal** function).

6.1.3 Manual Mode

The Determine Unit Cell plug-in's Manual Mode functions allow you a great degree of control over unit cell determination. Manual Mode consists of five sections, each with its own menu, corresponding to the five stages of unit cell determination:

1. Collect Data
2. Harvest Spots
3. Index
4. Refine
5. Bravais
6. Final refinement

6.1.3.1 Collect Data

Figure 6.3 — Collect data menu

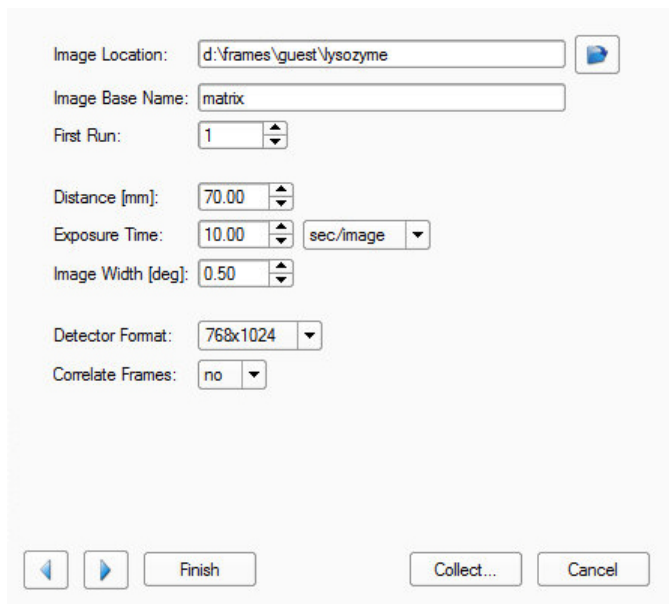


Table 6.1 — Collect Data menu items

Menu Item	Function
Image Location:	Location where collected frames will be saved.
Image Base Name:	Text string that is appended to each frame's filename and is used to identify frames by their filenames.
First Run:	Choose which run number will be the first run; useful for adding runs without overwriting previous ones.
Distance [mm]:	Detector distance.
Exposure Time:	Duration, in seconds, of each frame.
Image width [deg]:	Rotation, in degrees, that the scan axis travels over the course of a single exposure.
Detector Format:	Resolution of the frames collected by the detector.
Correlate Frames:	Two frames are taken (each with half the duration of the overall Exposure Time), to correct for spurious events appearing on individual frames (not available in shutterless mode).
Anode:	For dual-source systems, choose the wavelength for unit cell determination.
[left arrow]	Go to the previous step in the unit cell determination process.
[right arrow]	Go to the next step in the unit cell determination process.
Finish	Finish the sequence of steps in the unit cell determination process, beginning with the current step. Perform any tasks that are necessary.
Collect...	Proceed with data collection according to the options set in the preceding menu items.
Cancel	Leave Manual Mode and return to the initial Unit Cell Determination page, performing no operations and ignoring any changes made to menu items.

6.1.3.2 Harvest Spots

Figure 6.4 — Harvest Spots menu

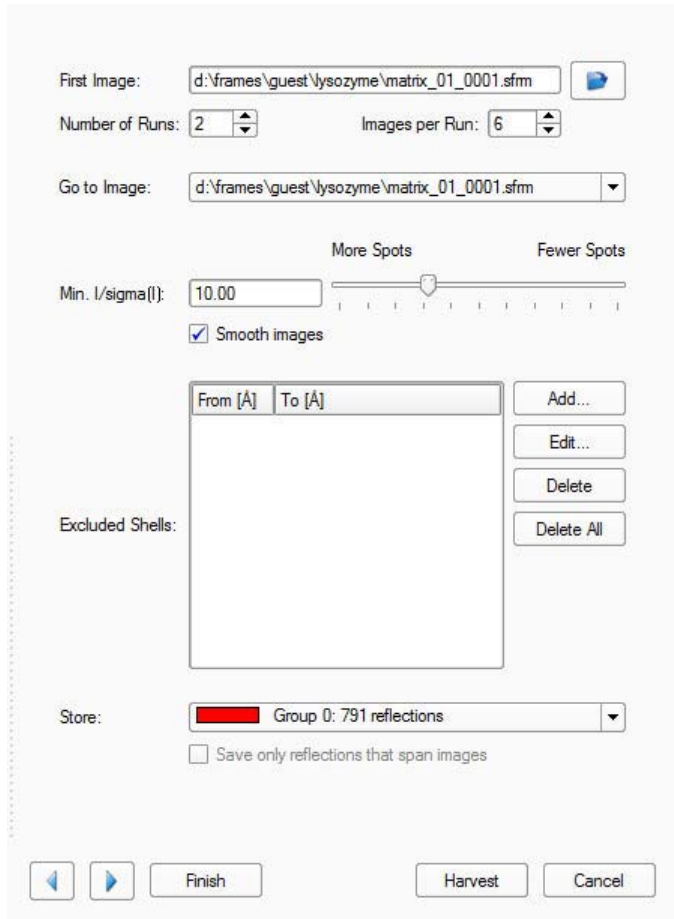




Table 6.2 — Harvest Spots menu items

Menu Item	Function
First Image:	Select the first image in the group of images to be examined for spots.
Number of Runs:	Number of runs to be examined.
Images Per Run:	Number of images to be examined in each run.
Go to Image:	Select a frame filename from this drop-down menu to display the frame in the Image Information Area.
Min. $I/\sigma(I)$:	Adjust the criterion for harvesting a spot based on its pixels' intensity versus their standard deviation. Slide the slider between "More Spots" and "Fewer Spots" to vary the minimum $I/\sigma(I)$.
Smooth images	A Gaussian filter is applied to the frames prior to harvesting, which reduces the noise and eliminates falsely harvested pixels. For very weak data, however, the "Smooth images" function can interfere with successful harvesting.
Store (Reflection Group Combo Box):	The Reflection Group Combo Box shows a list of reflection groups to choose from. For each group, the group name and number of reflections in the group are displayed. If, instead of the number of reflections, an entry is labeled empty, this indicates an unassigned entry. Choosing the empty entry creates a new group instead of appending to the current one. A color is associated with each reflection group. The color is displayed in the box to the left of the group name. The color helps in recognizing a group while navigating through the software.
Save only reflections that span images	With this checkbox enabled, a spot is only harvested if it is found on multiple contiguous frames.
 [left arrow]	Go to the previous step in the unit cell determination process.
 [right arrow]	Go to the next step in the unit cell determination process.
Finish	Finish the sequence of steps in the unit cell determination process, beginning with the current step. Perform any tasks that are necessary.
Harvest	Proceed with harvesting according to the options set in the preceding menu items.
Cancel	Leave Manual Mode and return to the initial Unit Cell Determination page, performing no operations and ignoring any changes made to menu items.

6.1.3.3 Index

Figure 6.5 — Index menu

Reflections: █ Group 0: 1582 reflections

Go to Image: d:\frames\guest\lysozyme\matrix_01_0001.sfm

Min. I/sigma(I): 10.00 More Reflections Fewer Reflections

Resolution [Å]: 9999.00 - 2.43

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

1582 Reflections selected for Indexing

Store: █ a=38.21Å, α=89.97°, V=240554Å³
b=79.33Å, β=90.00°
c=79.35Å, γ=89.91°



Corrections: From store From last harvest Manual

Distance [mm]: 0.00 Pitch [°]: -0.01
X Beam Center [mm]: -0.29 Roll [°]: 0.52
Y Beam Center [mm]: -0.53 Yaw [°]: 0.39

Methods: Difference Vectors
 Fast Fourier Transform
 Least Squares

Navigation: ◀ ▶ Finish Index... Cancel

Table 6.3 — Index menu items

Menu Item	Function
Reflections:	Opens the Reflection Group Combo Box to allow you to select a certain group of reflections for indexing.
Go to Image:	Select a frame filename from this drop-down menu to display the frame in the Image Display Area.
Min. $I/\sigma(I)$:	Adjust the criterion for indexing a reflection based on its integrated intensity versus its standard deviation. Slide the slider between "More Spots" and "Fewer Spots" to vary the minimum $I/\sigma(I)$ needed for a reflection to qualify for indexing.
Resolution (Å):	Set the resolution range from which spots will be used to index the unit cell.
Reflections must be isolated	If this checkbox is activated, overlapping spots will not be used for indexing.
Reflections must span images	If this checkbox is activated, only spots which registered on at least two adjacent images will be used for indexing.
Reflections must be whole	If this checkbox is activated, spots that have only partially registered on the first and last images of the range from which they are harvested will not be used for indexing.
Store (Unit Cell Combo Box):	The Unit Cell Combo Box shows a list of unit cells to choose from. For each unit cell, the unit cell parameters are displayed, as is the unit cell volume and, optionally, the Bravais lattice type. A color is associated with each unit cell. The color is displayed in the box to the left of the unit cell. The color helps in recognizing a unit cell while navigating through the software. It is also used in the Image Display Area to color the overlay and indicate the unit cell that was used to calculate the overlay. The order and types of colors can be changed by modifying the <code>groupcolors</code> configuration variable in the <code>bn-config.py</code> file.
Corrections:	Select corrections to detector parameters. Corrections are available from the stored parameters (i.e., from the Sample Database), from the last harvest, or from manual input.
Methods:	Select methods employed in indexing: difference vectors, fast fourier transform, or least squares.
 [left arrow]	Go to the previous step in the unit cell determination process.
 [right arrow]	Go to the next step in the unit cell determination process.
Finish	Finish the sequence of steps in the unit cell determination process, beginning with the current step. Perform any tasks that are necessary.
Index...	Proceed with indexing according to the options set in the preceding menu items.
Cancel	Leave Manual Mode and return to the initial Unit Cell Determination page, performing no operations and ignoring any changes made to menu items.

6.1.3.4 Bravais

Figure 6.6 — Bravais menu

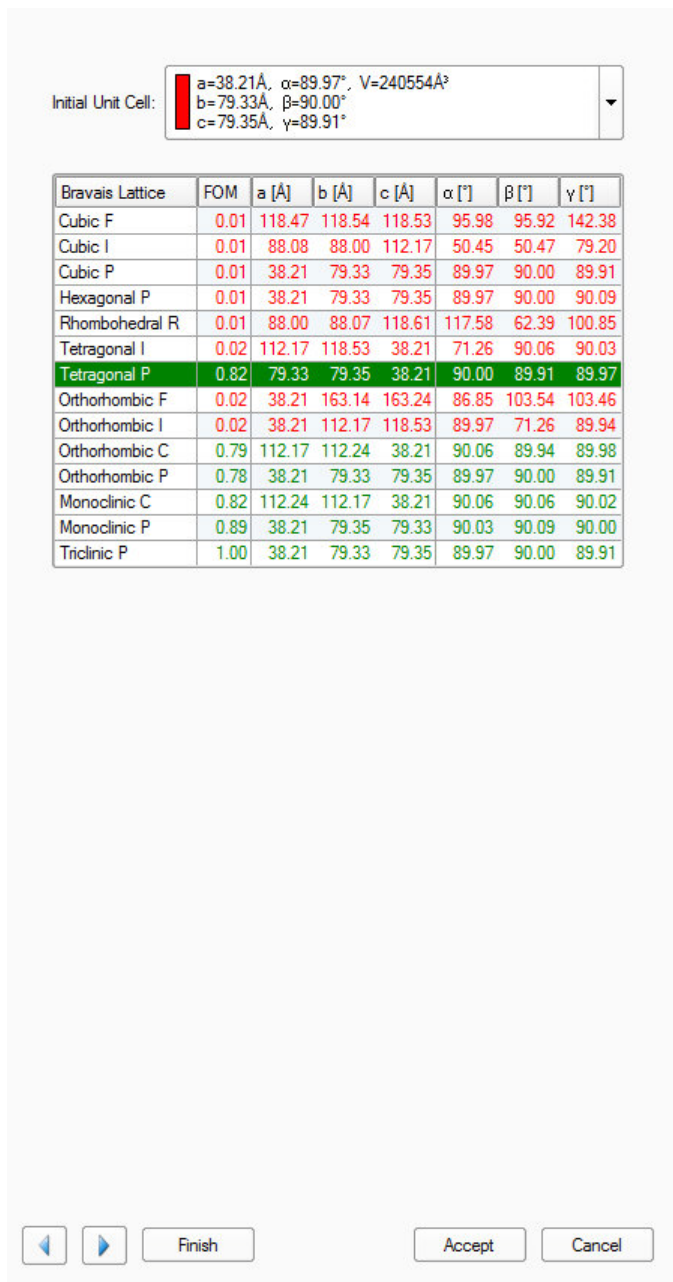


Table 6.4 — Bravais menu items

Menu Item	Function
Bravais Lattice:	This list shows the 14 Bravais lattice types for the selected unit cell parameters. For each entry, the list contains (from left to right) the Bravais lattice type, the figure of merit (ranging from 0.0 to 1.0, with 1.0 being perfect agreement), and the six unconstrained unit cell parameters for that Bravais lattice type. Bravais lattices that are in agreement with the unit cell are displayed in green, while those that do not are displayed in red. The most likely Bravais lattice type is chosen automatically. If necessary, you may override the program's decision by clicking on a different entry.

6.1.3.5 Refine

Figure 6.7 — Refine menu

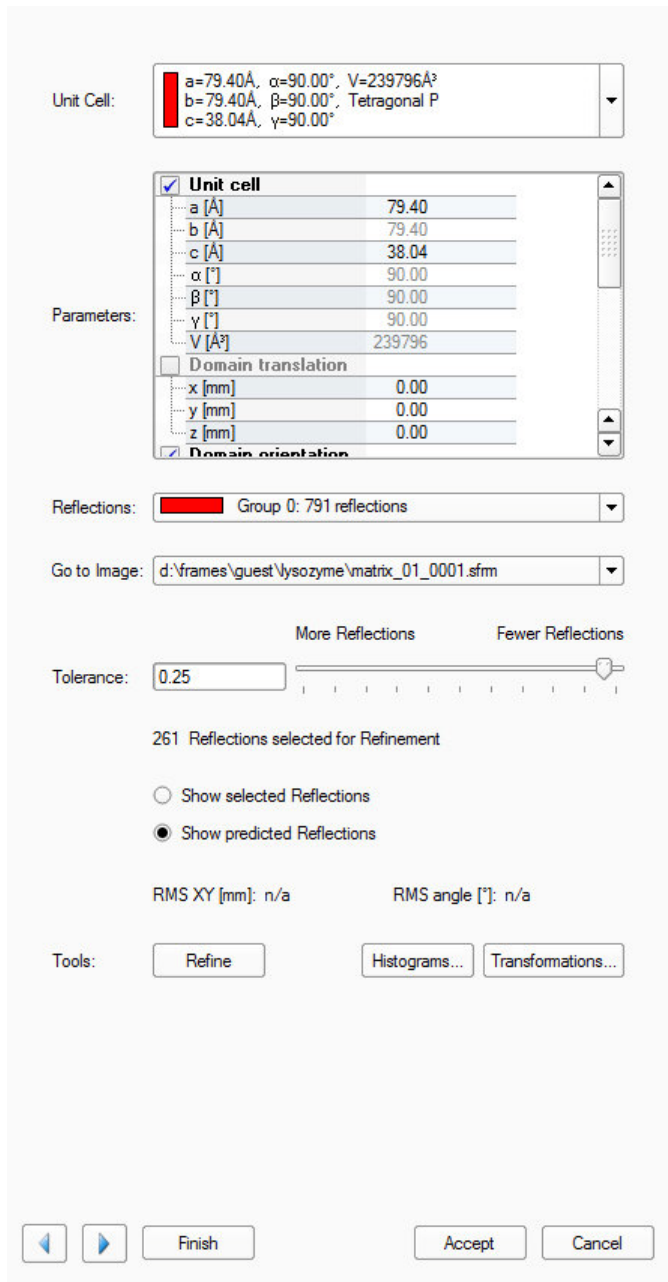


Table 6.5 — Refine menu items

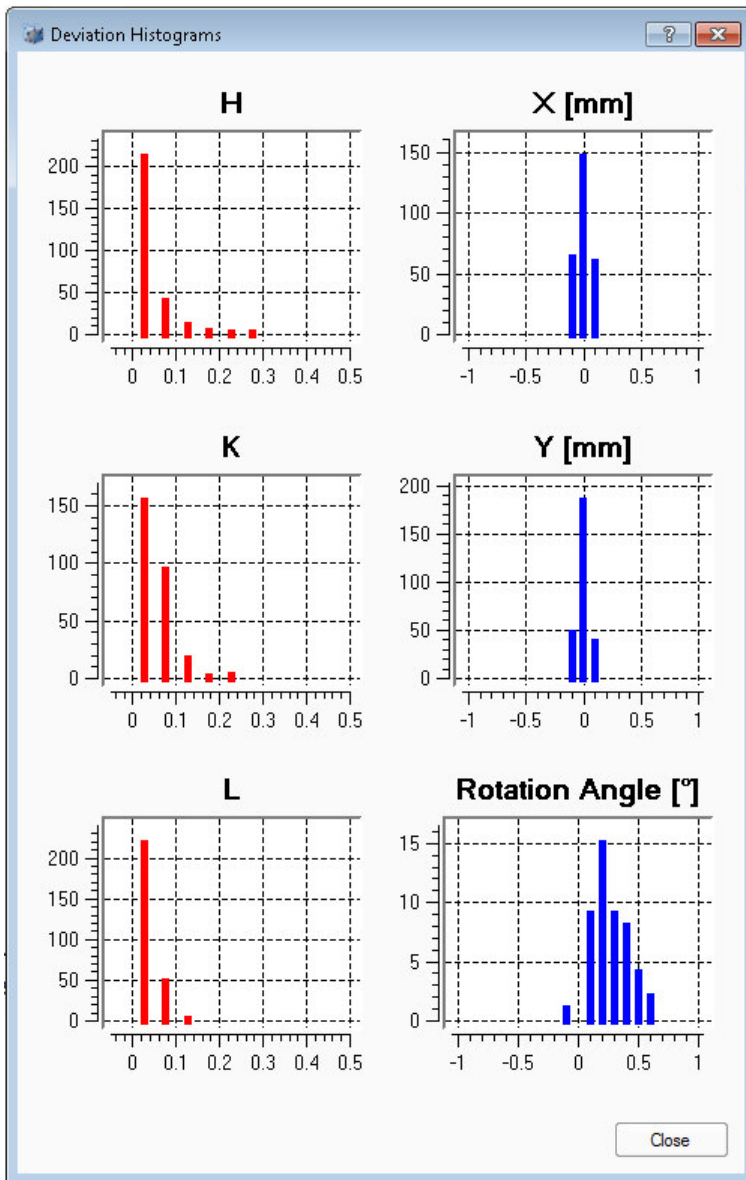
Menu Item	Function
Tolerance:	Upon startup, the Refine dialog determines a useful value for the tolerance. This may take a few seconds depending on the number of reflections.
RMS XY [mm], angle [°]:	The root mean square of the deviation between observed and predicted spot positions in the XY image plane and along the trajectory of the spot while it passed through the Ewald sphere.

Histograms

Clicking **Histograms...** in the “Refine” step opens a window showing the reflections’ distribution of deviances from the calculated orientation matrix. The criteria include:

- H, K, and L;
- X and Y displacement in (mm); and
- Rotation angle in degrees.

Figure 6.8 — “Deviation Histograms” window



Additionally, the user can click on individual bars in the histogram to display the corresponding reflections in a list view.

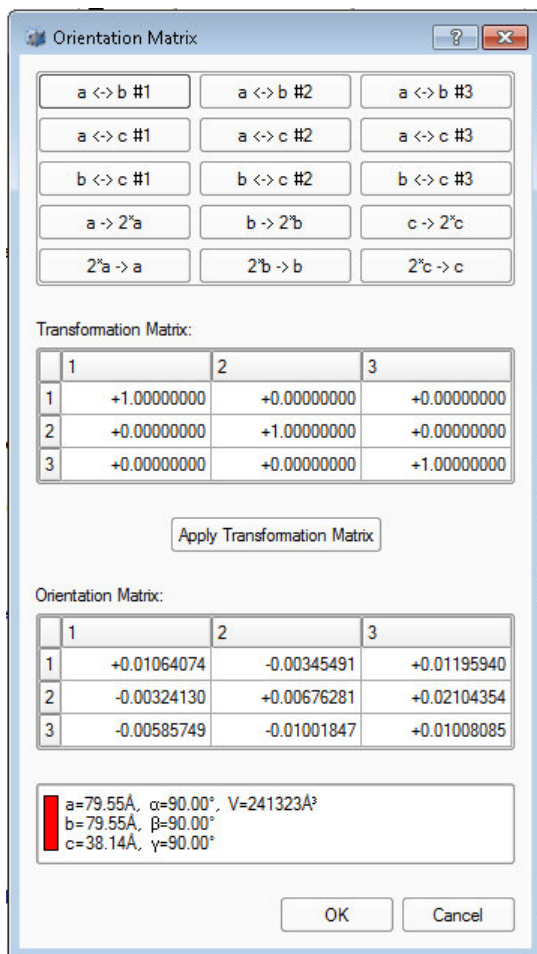
Orientation Matrix Transformations

Clicking **Transformations...** in the “Refine” step opens a window giving manual control over the orientation matrix.

A transformation matrix is created using the buttons at the top of the window (and the transformation matrix’s values can be manually edited by double-clicking the values). Clicking the **Apply Transformation Matrix** button results in a new unit cell, which is shown at the bottom.

This new unit cell may be committed by clicking **OK**, or discarded by clicking **Cancel**.

Figure 6.9 — “Orientation Matrix” window



6.2 Calculate a Data Collection Strategy

PROTEUM3 includes powerful algorithms for determining flexible, efficient data collection strategies for your instrument.

6.2.1 Start the Calculate Strategy Plug-in

In the Task Bar's "Collect" category, click the **Calculate Strategy** icon



The Calculate Strategy plug-in opens.

6.2.2 Set Strategy Parameters Based on the Unit Cell

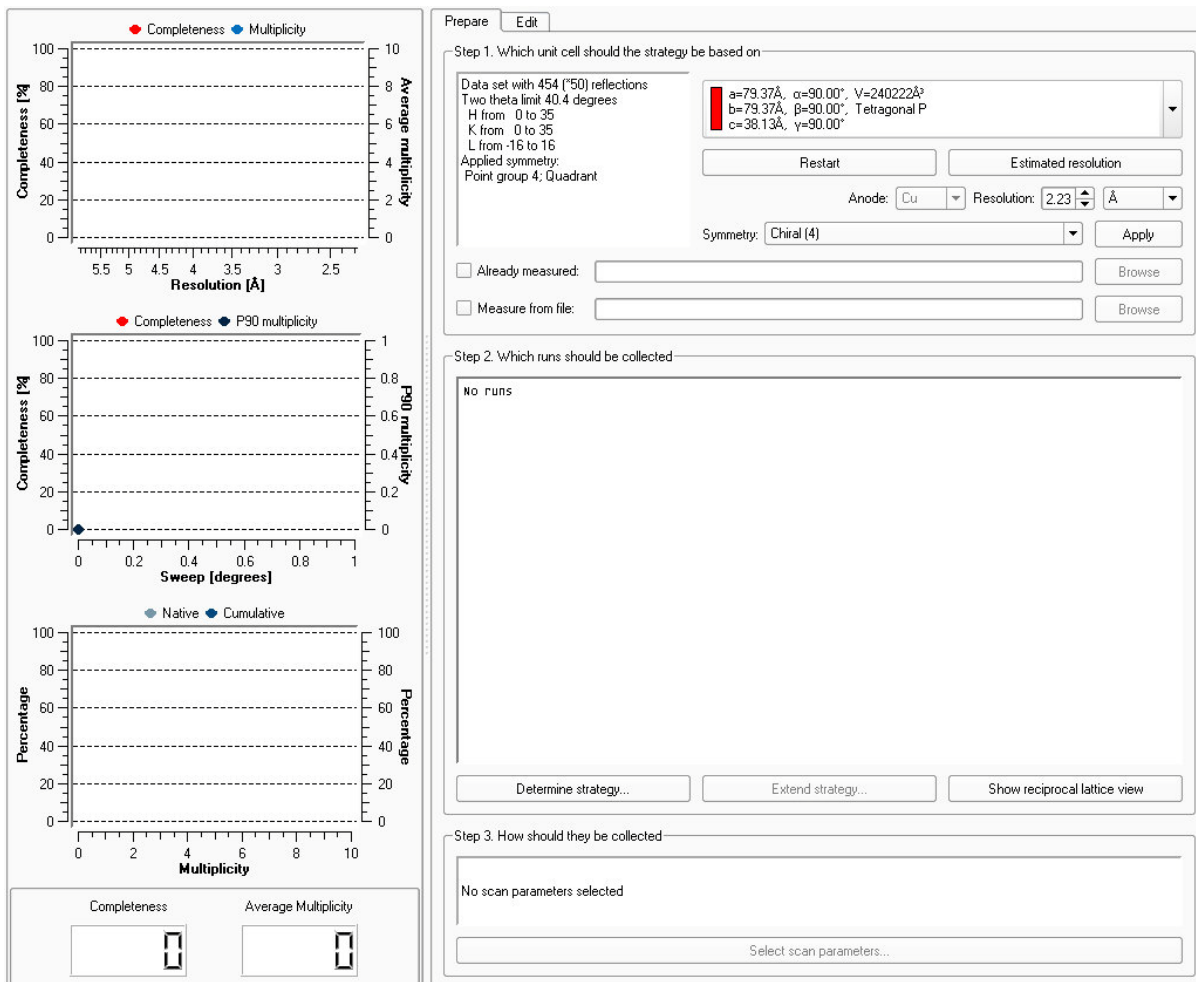
1. In the "Prepare" tab's "Step 1" area (in the upper right-hand part of the screen), choose the desired unit cell from the drop-down menu.
2. In the case of dual-source systems, choose the desired anode with the **Anode:** drop-down menu.
3. Set the appropriate resolution limit for the desired strategy using the **Resolution:** field and menu.

NOTE: Check the default resolution (that is estimated by the Determine Unit Cell plug-in) before proceeding.

4. Use the "Symmetry:" drop-down menu and **Apply** button to choose the symmetry. Set the symmetry to treat Bijvoet pairs as inequivalent (**Chiral**), or choose a specific point-group symmetry based on the metric symmetry of the unit cell. A full sphere may be collected using a setting of **1**.

NOTE: Although it is not necessary to click **Apply** if the symmetry was changed, it is very important to click **Apply** if the resolution limit was changed.

Figure 6.10 —Initial view with unit cell and symmetry chosen



- If you have already measured a group of reflections for this sample and do not wish to do so again, activate the **Already Measured:** checkbox and use the **Browse** button to select a *.raw or *.hkl file specifying the reflections.

These reflections are removed from the unique reflections list, and a strategy to record missing reflections will be optimized.

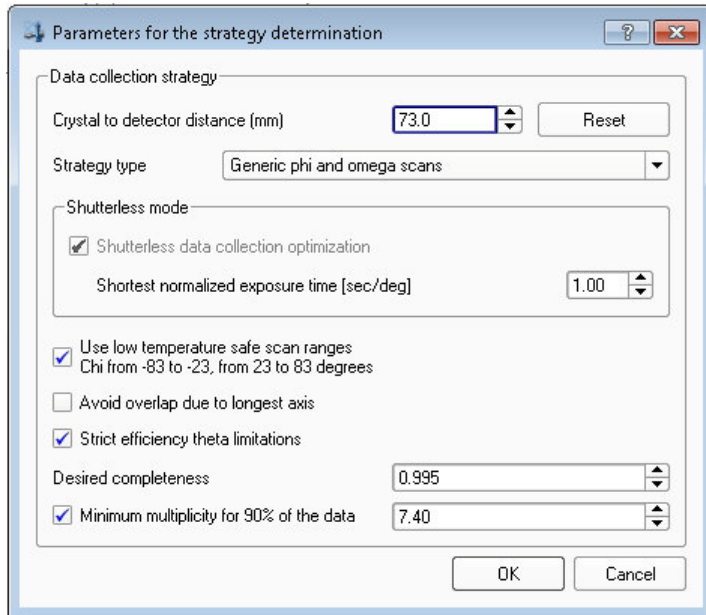
NOTE: It is important to make sure that the *.raw or *.hkl file is consistently indexed to the unit cell chosen in step 1.

6.2.3 Determine the Runs to be Collected

1. In the “Step 2” area, click the **Determine Strategy...** button.

The “Parameters for the strategy determination” window opens.

Figure 6.11 — “Parameters for the strategy determination” window



2. Choose the strategy determination options:

- 2.1. Set the crystal-to-detector distance.

The strategy optimizer suggests the minimum possible detector distance that can still resolve the reflections (based on the unit cell and crystal mosaicity). Any distance greater than the suggested value should work. Please note that centered lattices are intentionally treated as primitive in case of a mis-assignment or a supercell.

- 2.2. Choose the strategy type (phi and omega scans, or simply omega scans).

KAPPA goniometers also offer options for collecting Friedel pairs in the same frame (with omega scans for those that cannot be collected on the same frame) and for two 360° phi scans in reverse-beam geometry.

The shortest detector distance is suggested for which no reflection overlaps are expected based on the unit cell and crystal mosaicity.

- 2.3. For shutterless data collection, activate the **Shutterless data collection optimization** checkbox and set the shortest desired exposure time.
- 2.4. If you have a KAPPA goniometer and a low-temperature device, use the **Use low temperature safe scan ranges** checkbox to avoid chi angles that are prone to ice formation and to avoid centering problems due to thermal compression of the goniometer head.

- 2.5. Activate the **Strict efficiency theta limitations** checkbox to limit the swing of 2theta to improve efficiency (this may limit the ability to acquire all data for triclinic settings).

This option can only be set when the full theta range is available and a sufficiently large omega scan can be performed. Depending on the hardware configuration, this is at a slightly further distance than the smallest DX.

This option limits the maximum 2 theta swing. The specified resolution will be found on either side of the detector, and therefore you will get the maximum redundancy. However, this may block some goniometer positions and the optimizer will automatically disable this option if it is impossible to achieve a sufficiently high completeness.

- 2.6. Set the desired completeness and multiplicity (i.e., redundancy).

The checkbox for minimum multiplicity includes the specified minimum multiplicity in the strategy calculation. By default, 90% of the data will have at least the specified multiplicity.

For a regular data collection optimization, a multiplicity of 1 is normally sufficient. If you want to reduce the resulting redundancy, deactivate the checkbox; the optimizer then tries to give complete data with as few frames as possible.

3. Click **OK**.

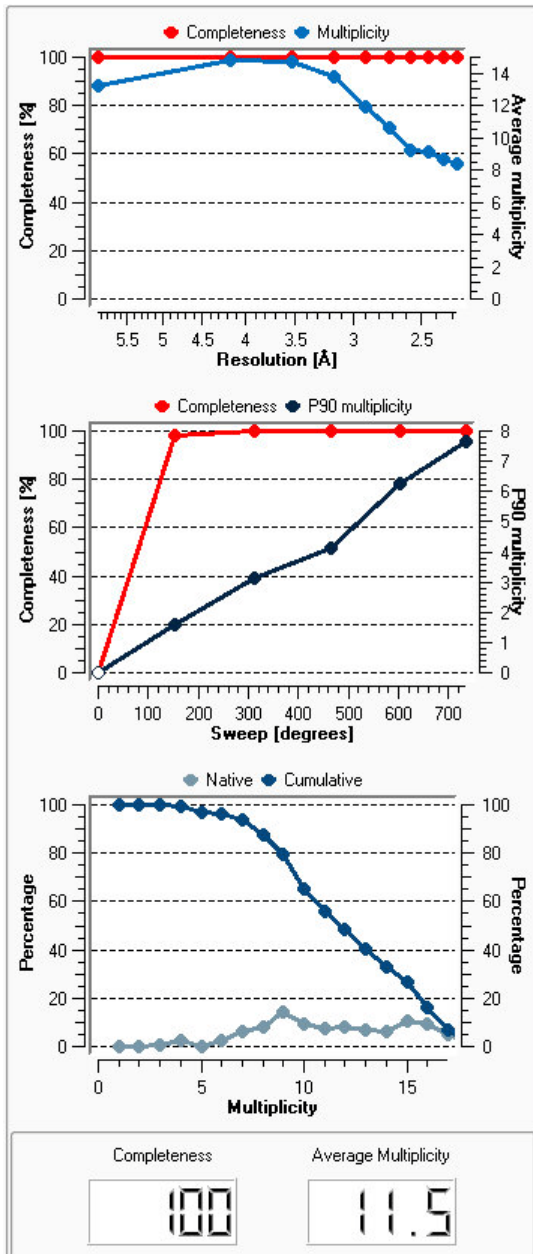
The plug-in computes the data collection strategy.

6.2.3.1 The Calculated Strategy Summary

After the calculation, a summary appears showing graphs of the calculated strategy along with a summary of the strategy's runs.

The graphs on the left-hand side of the Task Display Area show relationships between completeness, resolution, multiplicity, and time, along with overall completeness and average multiplicity at the bottom.

Figure 6.12 —Strategy summary graphs



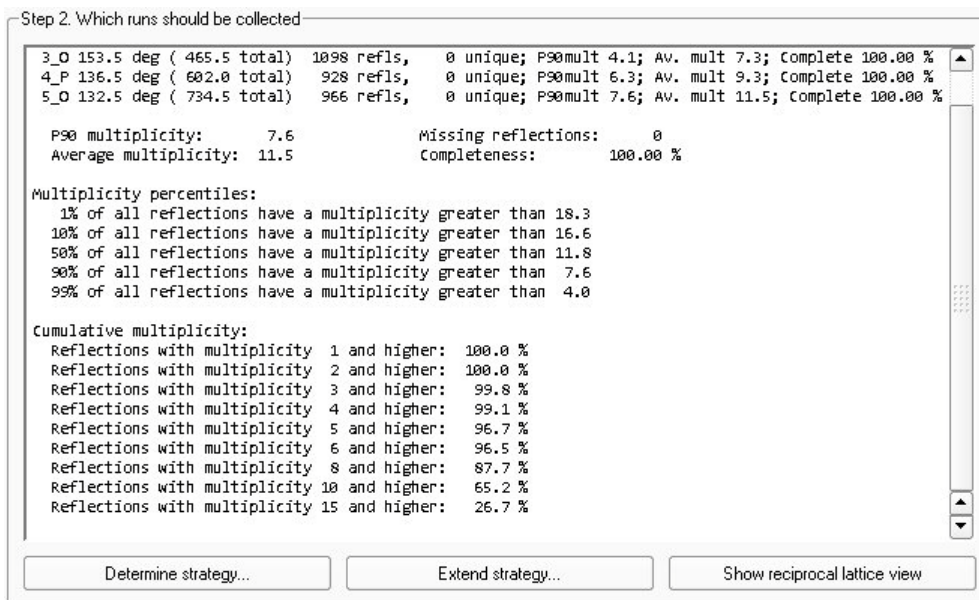
The “Step 2” area of the plug-in shows a text summary of the runs including, for each run:

- overall sweep;
- number of reflections to be collected;
- number of unique reflections to be collected;
- (cumulative) multiplicity achieved;
- average multiplicity; and
- completeness.

Following this is a breakdown of the reflection distribution with respect to multiplicity. For example, multiplicity at 90% is the lowest multiplicity measured for 90% of the unique reflections.

At the end of the summary is the average multiplicity and the overall completeness (including missing reflections). If no exposure time is specified in step 3, this area shows the number of degrees scanned. If step 3 was done, you get the run time and the estimated completion time.

Figure 6.13 —Text summary

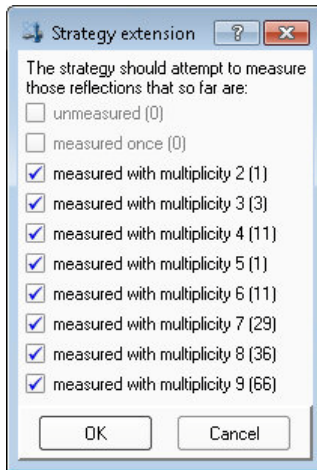


6.2.3.2 Extending the Strategy

If desired, click **Extend strategy...** to specify collection of additional data based on multiplicity.

NOTE: Extending the strategy will increase data collection time. It is also necessary to increase the desired multiplicity above the already-achieved one; otherwise, the program will not add additional runs.

Figure 6.14 — “Strategy extension” window



If desired, click the “Edit” tab to manually change the calculated runs’ scan parameters.

The fields may be edited by double-clicking.

The sort option is an ideal tool for rearranging runs to achieve completeness as quickly as possible for a certain higher symmetry (e.g., if the strategy was calculated for a non-centrosymmetric point group, but there is a fair chance that the crystal symmetry is centrosymmetric).

Figure 6.15 —“Edit” tab

Prepare Edit

	Operation	Active	Distance [mm]	2Theta [deg]	Omega [deg]	Phi [deg]	Chi [deg]	Time [sec]	Width [deg]	Sweep [deg]	Direction
1	Omega Scan	Yes	73.000	6.428	-28.681	-106.791	-27.733	default	default	153.000	positive
2	Omega Scan	Yes	73.000	6.428	-36.303	-26.562	-24.132	default	default	159.000	positive
3	Omega Scan	Yes	73.000	-6.428	-42.163	159.892	-27.457	default	default	153.500	positive
4	Phi Scan	Yes	73.000	5.325	264.621	-48.225	23.000	default	default	136.500	positive
5	Omega Scan	Yes	73.000	6.428	-100.559	20.398	37.115	default	default	132.500	positive
6	No Operation	Yes									
7	No Operation	Yes									
8	No Operation	Yes									
9	No Operation	Yes									
10	No Operation	Yes									
11	No Operation	Yes									
12	No Operation	Yes									
13	No Operation	Yes									
14	No Operation	Yes									
15	No Operation	Yes									
16	No Operation	Yes									
17	No Operation	Yes									
18	No Operation	Yes									

Edit options

Sort Apply

6.2.3.3 Viewing the Strategy in Reciprocal Space

If desired, click **Show reciprocal lattice view** to display the strategy in reciprocal space.

Click a radio button in the “Show reflections” area to view unique reflections, all of the symmetric equivalents, or the missing reflections.

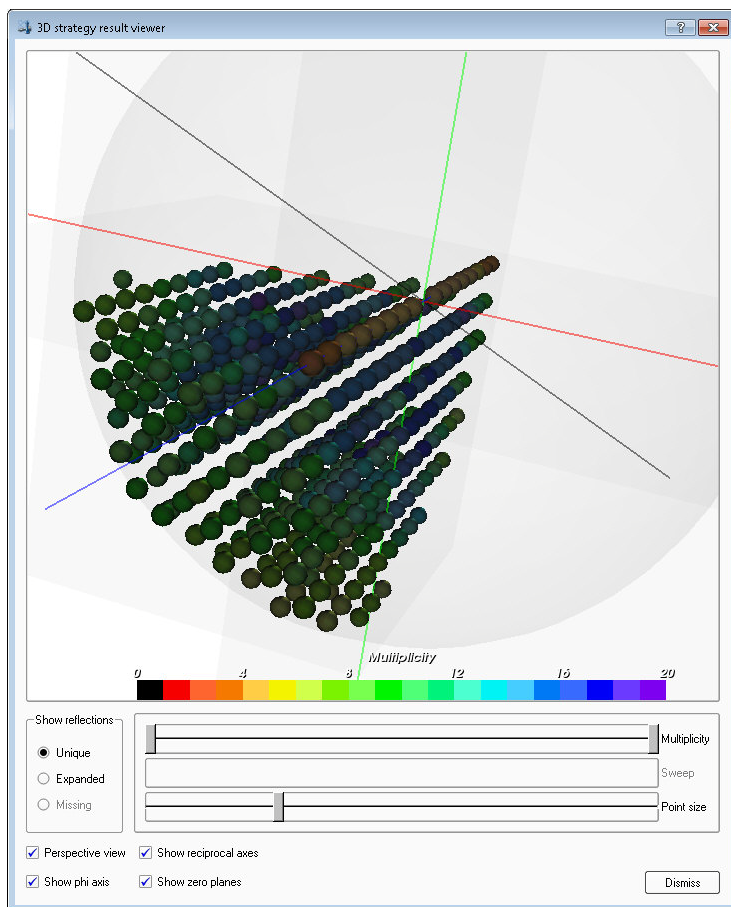
NOTE: If missing reflections and the black phi axis coincide, it is likely that the initial crystal orientation on the goniometer head is preventing a 100% complete data set (see Section 4.1.2).

The sliders to the right adjust the visualization of the displayed reflections:

- The **Multiplicity** slider applies a lower-redundancy cutoff (left slider), or a higher-redundancy cutoff (right slider) to help visualize the data collection.
- The **Sweep** slider can be used to examine the data collection coverage with respect to experiment time.
- The **Point size** slider sets the size of the reflections in the visualization.

Additionally, checkboxes are available to activate and deactivate perspective view, reciprocal axes, the phi axis, and the HKL zero planes. Right-clicking in the window shows a menu with additional keyboard commands (e.g., alignment of the view along reciprocal axes).

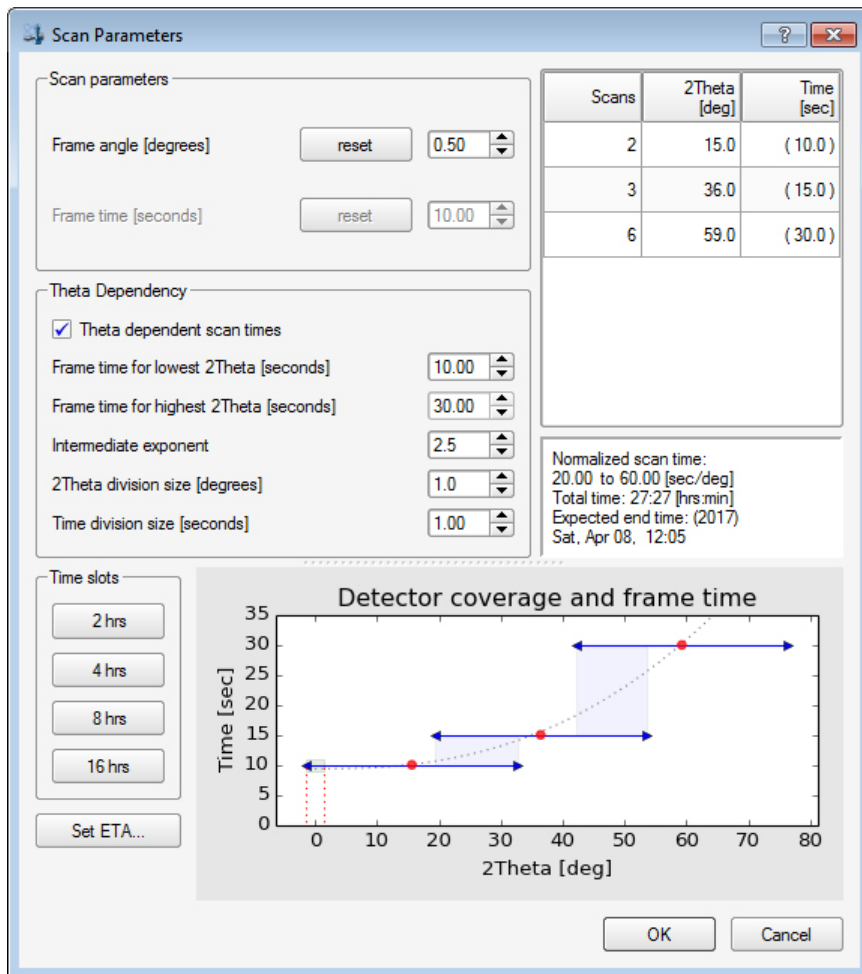
Figure 6.16 — “3D strategy result viewer” window



6.2.4 Set the Scan Parameters

- At the bottom of the “Step 3” area, click the **Select scan parameters...** button.
The “Scan Parameters” window opens.

Figure 6.17 —“Scan Parameters” window



2. Set the scan parameters:
 - 2.1. Set the desired frame angle (i.e., scan width) based on the crystal's mosaicity.

The strategy optimizer will suggest the widest possible scan width (the default range is between 0.3° and 2.0°, but this range can be edited via the bn-config.py file).

The suggested frame angle is calculated to avoid overlaps in the rotation direction, based on the current cell parameters and the observed mosaicity. The **reset** button repeats the calculation.
 - 2.2. Set the frame time in seconds.

The initial suggested frame time should be checked and eventually modified by the user.

The frame time is initially derived from the statistics of the intensity-versus-resolution table created during peak harvesting (this calculation is repeated when clicking the **reset** button). Use this value as an indication only; the value may be derived from poor statistics, particularly when the table does not fully cover the intended data resolution.
 - 2.3. Optionally, use one of the "Time slots" buttons to quickly fit the measurement in the indicated number of hours. If desired, click the **Set ETA...** button to open a dialog that allows you to set the finish time (this adjusts the measuring time to best fill the time allowed).
3. The **Theta dependent scan times** checkbox allows you to adjust the time you want to spend on a particular range of 2theta. Adjustments to the parameters in this area appear as graphical feedback in the "Detector coverage and frame time" graph:
 - In the summary table, the calculated scan times are shown in parentheses (these are also visible in the plug-in's "Edit" tab). Manually setting a scan's time in the "Edit" tab will cause the corresponding red dot in the graph to turn gray, indicating that the time is not calculated for that scan.
 - The arrows in the graph show the 2theta span between the centers of the vertical edges of the detector (calculated using the effective DX).
 - The theta dependency inputs give you control over timing (i.e., the dotted green line in Figure 6.17). The slope is defined by the lowest and highest point. If you change the end points, you change the slope and the curve moves along (including the intermediate points). Changing the **Intermediate exponent** value changes the curve but not the end points, so only intermediate points move up or down.
 - Transparent rectangles are used to highlight the overlap areas. The rectangles get darker when more scans are involved.
 - Around 2theta=0, there are green enclosing areas with a small height to indicate possible overlap of positive and negative theta in the same scan.

NOTE: Additional parameters may be set in the bn-config.py file (see Section 12.5.1).

4. When you are satisfied with the scan parameters, click **OK**.

The "Scan Parameters" window closes. Note that the elapsed time and expected end time have been added to the "Step 3" area.

The strategy calculation is now complete. The plug-in may be closed, and the runs may be used in the experiment by clicking the **Append Strategy** button in the Experiment plug-in.

6.3 Run the Experiment



1. In the Task Bar's "Collect" category, click the **Run Experiment** icon .
The Run Experiment plug-in opens, showing a table of operations to be performed.
2. If the list of operations contains orientation matrix runs, select and delete them by clicking (or [Shift] + clicking) the desired rows in the table and then right-clicking on the row numbers at the left and choosing **Delete**.

Figure 6.18 —Deleting matrix runs

	Operation	Active	Distance [mm]
1	Anode	Yes	Anode:
2	Phi Scan	Yes	40.000
3	Phi Scan	Yes	40.000
4		Yes	40.000
5		Yes	
6		Yes	
7		Yes	
8		Yes	
9	No Operation	Yes	

3. Click **Append Strategy**.
The runs found by the Calculate Strategy plug-in appear in the list of operations.
The software changes the filename to the name of the current sample.

Figure 6.19 —List of operations with strategy appended

Set Up Experiment
Monitor Experiment

Image location:

Filename or prefix:

First run:

Exposures:

Retake if topped

Generate new dark images

Unwarp images

Default time: [sec/image]

Default width: [degrees]

Detector format:

Deicing:

	Operation	Active	Distance [mm]	2Theta [deg]	Omega [deg]	Phi [deg]	Chi [deg]	Time [sec]	Width [deg]	Sweep [deg]	Direction
1	Omega Scan	Yes	73.000	6.428	-28.981	-106.791	-27.733	1.500	0.300	153.300	positive
2	Omega Scan	Yes	73.000	6.428	-36.603	-26.562	-24.132	1.500	0.300	159.300	positive
3	Omega Scan	Yes	73.000	-6.428	-42.263	159.892	-27.457	1.500	0.300	153.600	positive
4	Phi Scan	Yes	73.000	5.325	264.621	-48.225	23.000	1.500	0.300	136.500	positive
5	Omega Scan	Yes	73.000	6.428	-100.659	20.398	37.115	1.500	0.300	132.600	positive
6	No Operation	Yes									
7	No Operation	Yes									
8	No Operation	Yes									
9	No Operation	Yes									
10	No Operation	Yes									
11	No Operation	Yes									
12	No Operation	Yes									
13	No Operation	Yes									
14	No Operation	Yes									

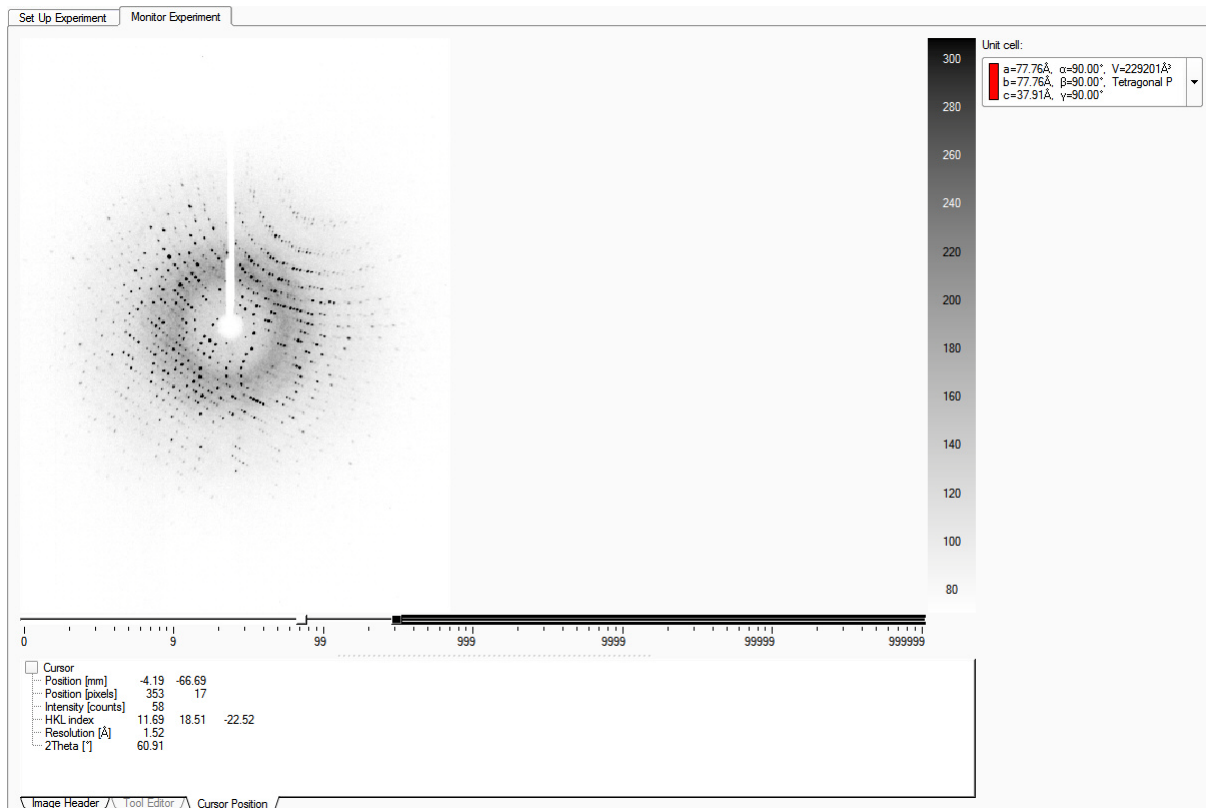
Append Strategy
Append Matrix Strategy
Load Table...
Save Table...
Validate
Resume
Execute

- To begin the experiment, click **Execute**.

The “Monitor Experiment” tab opens, and diffraction images begin to appear. This may take a minute or two if new dark current corrections are being collected, or if the generator is being ramped up from a low-power state.

NOTE: If resuming after a data collection interruption, the software will look for the first missing frame, and collect everything after that frame (even if it overwrites existing frames). The software will prompt you to overwrite any existing frames.

Figure 6.20 — “Monitor Experiment” tab



6.3.1 User-Defined Runs and Settings

You can load and save your own experiments (in *.exp format) by using the **Load Table...** and **Save Table...** buttons at the bottom of the “Set Up Experiment” tab.

NOTE: Only allowed runs are imported when selecting a *.exp file. All runs that would lead to a collision are skipped without further notice.

Data collection controls are shown at the top of the “Set Up Experiment” tab. Usually, the default values are correct.

You can define the data collection mode of your detector with the **Exposures** option.

Automatic mode, typically the best option, decides which mode to use based on the experiment.

Automatic mode will record uncorrelated images for exposure times shorter than 10 seconds (if shuttered data collection is available). However, for applicable detectors, you can also force uncorrelated or correlated frames. Exposures of 3 or above correspond to multiple correlated images (for data collection times of less than 5 seconds, correlation can usually be turned off).

If new dark frames are required (for applicable detectors), BIS will automatically collect them. Activating the **Generate new dark images** checkbox forces the collection of a new dark frame for each exposure time used in the experiment (not before every run, though this can be specified in the bn-config.py file if necessary).

Retake if topped: For shuttered data collection, the instrument will automatically use your instrument’s overflow protection routine:

- D8 Series II instruments perform a fast re-scan. For correlated images, the time is $\frac{1}{8}$ of the original exposure time (but no shorter than 0.5 second). For uncorrelated images, the time is 0.5 second.
- DAVINCI.DESIGN instruments engage the attenuator.

Intensities of the saturated pixels will be scaled and replaced in the original frame.

Unwarp images: For detectors with spatial distortion (e.g., detectors using a demagnifying fiber-optic taper), the image can be stored fully corrected (i.e., “unwarped”).

If the experiment table shows “default” in the “Time” or “Width” column, the default exposure time or image width is used. You can set this option in the table by entering 0.

The de-icing feature will interrupt the data collection after the specified time and rotate phi by 180°. Afterwards, it continues automatically. Ice is normally blown off the sample holder by this routine. If you are using BIS, the system tries to keep your crystal ice-free even after the data collection is finished when the system is idle.

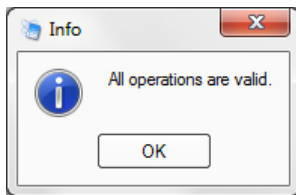
You can influence the naming of your run with the “Filename” field and the “First run” number (e.g., the first active data collection run can get run no. 3).

In Figure 6.19, the time and width are explicitly set for each run, so changing the default width and time will have no effect. If the explicit time or width for a run is deleted so that the box is empty, the word `default` appears and the default values at the top right will be used.

The software tests the runs for illegal goniometer movements before the runs are executed. Therefore, it is not necessary to explicitly click **Validate**. However, it is useful if you are looking for the maximum allowed omega scan for a certain goniometer position without the risk of starting the data collection once you have found an allowed range.

If you click **Validate** and all movements are valid, the “All operations are valid” window will appear (Figure 6.21).

Figure 6.21 —“All operations are valid” window




6.3.2 Closing PROTEUM3 or the Project

If desired, you can exit PROTEUM3 after you begin data collection:

- In a single-computer setup, BIS Server and the Measurement Server must be left running (or, in the case of D8 Series II instruments, BIS Classic).
- In a dual-computer setup, the Server computer must be left on. If communications are lost between the Client and the Server, frames will be stored on the Server. Typically, they will be in the directory “C:\frames\”. They should be copied into the correct project directory before proceeding.

6.3.3 Abort the Experiment if Necessary

To abort the data collection, choose **Instrument > Abort...** or click the **Stop** button  in the Tool Icon Bar.

NOTE: You are not prompted to verify that you wish to abort the data collection.

7 Data Integration and Scaling

Before the data can be used to solve and refine the crystal structure, it is necessary to convert the information recorded on the frames to a set of integrated and scaled intensities.

When setting up the integration, the Integrate Images plug-in creates a subdirectory named “\work” and assigns an output filename for each run’s integration results (“.raw” for single-crystal data, “.mul” for twins, and “.ram” for modulated samples—plus associated output files). These files are in ASCII form, which can be read by the Scale or Determine Space Group plug-in.

The SAINT Software Reference Manual DOC-269-0175XX provides a detailed description of the process of integration along with the various parameters involved, along with the SAINT file format.

It is possible to integrate while collecting data.

The Scale plug-in corrects for absorption effects in the data set and puts all of the measured data on the same scale. It uses data redundancy to achieve this, and tables and charts of diagnostics are presented throughout the process. Furthermore, Scale is able to correct for crystal volume variations, beam inhomogeneities, and crystal decay.

7.1 Integrating Data

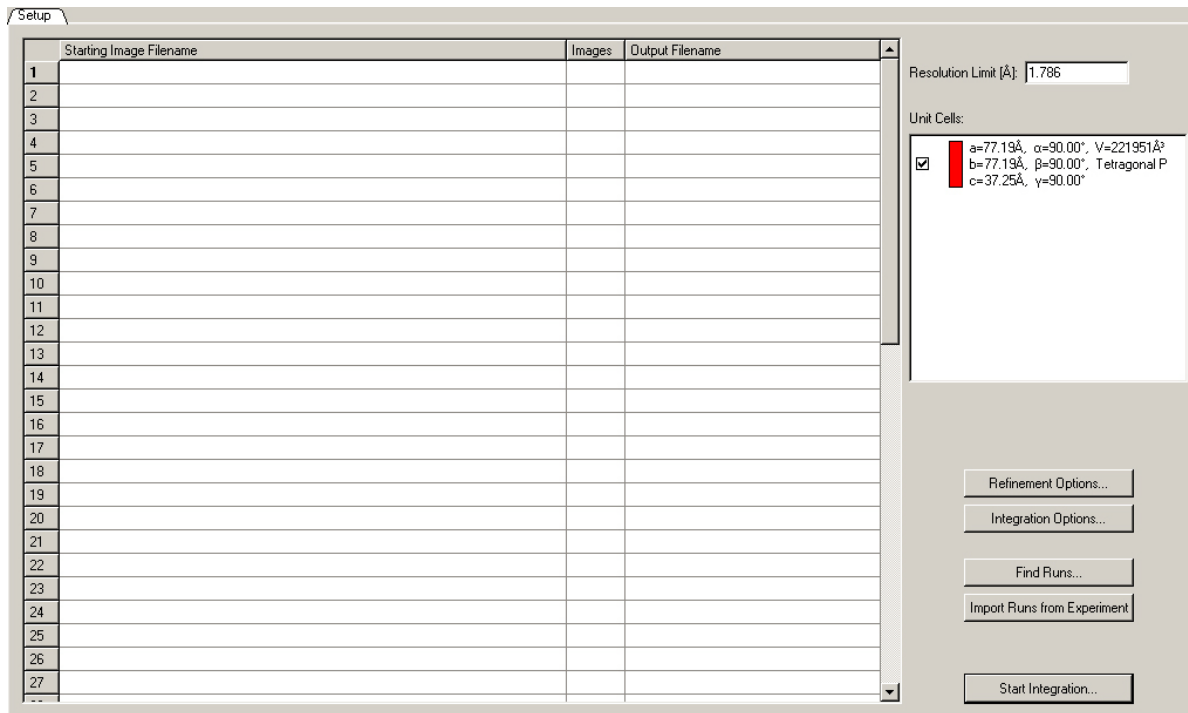
7.1.1 Start the Integrate Images Plug-in



In the Task Bar's "Reduce Data" category, click the **Integrate Images** icon

The Integrate Images plug-in opens (Figure 7.1), and the orientation matrix and Bravais information are loaded from the database.

Figure 7.1 — Integrate Images plug-in: initial view

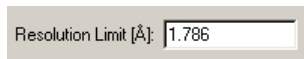


7.1.2 Set the Resolution Limit

The "Resolution Limit (Å):" field (in the plug-in's upper right-hand corner) sets the upper (i.e., better) resolution limit to which the data will be integrated. The plug-in suggests a resolution limit, but this value can be edited.

Check the suggested resolution limit. If you want a limit other than that suggested, enter it in the field.

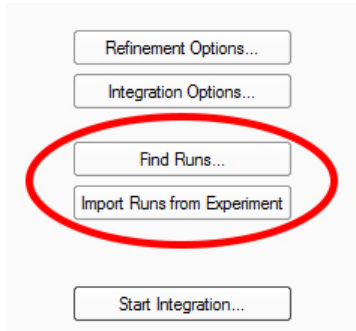
Figure 7.2 — "Resolution Limit (Å):" field



7.1.3 Select Runs for Integration

The plug-in's right-hand side has two buttons for defining the data collection runs to be integrated: **Find Runs...** and **Import Runs from Experiment**.

Figure 7.3 — **Find Runs...** and **Import Runs from Experiment** buttons



The **Find Runs...** button is used to browse to the set of runs to be integrated. This button is normally used when the data collection is finished.

1. Click **Find Runs...**

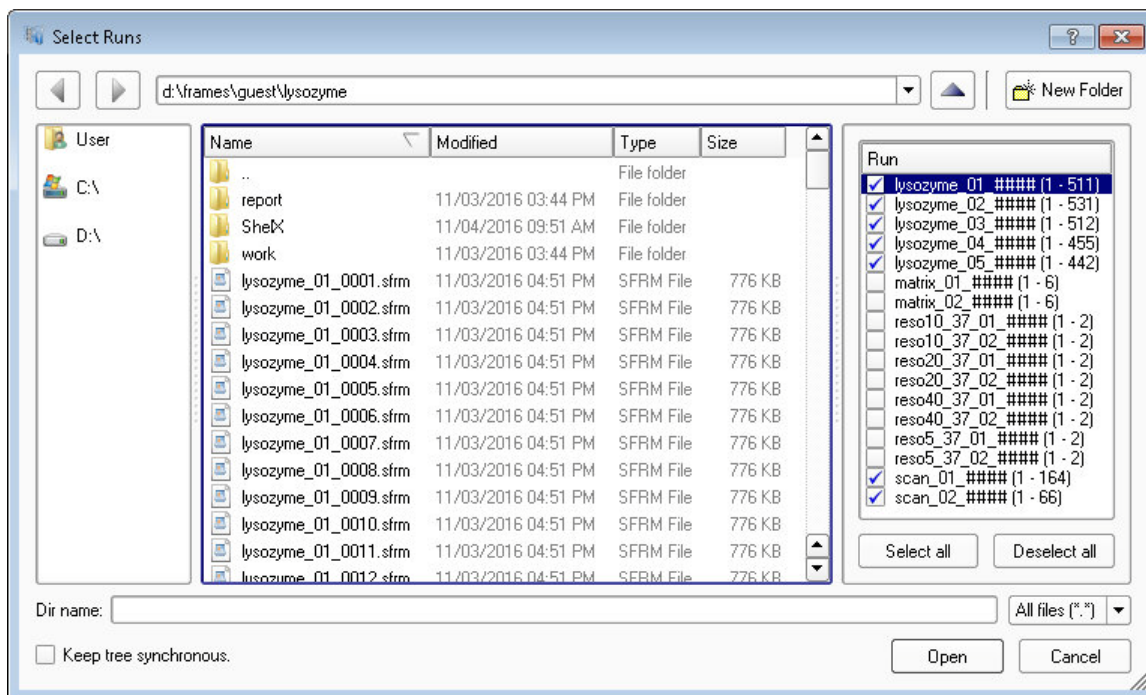
The "Select Runs" window (Figure 7.4) opens.

2. Usually, the "Select Runs" window has the correct directory and base name as the defaults. If these are not correct, browse to the runs for the experiment of interest.

NOTE: To integrate images from a CD or DVD, browse to the device and find the runs to be integrated. The plug-in automatically writes the result in the "work" directory for the current project.

- The window pane on the right-hand side displays all of the runs available in the current directory. Any combination of runs may be selected by simply activating the checkboxes next to the groups.

Figure 7.4 — “Select Runs” window



- Click **Choose**.

The plug-in populates the list of runs to be integrated with the selected runs.

- Alternatively, you can click **Import Runs from Experiment**.

The **Import Runs from Experiment** button determines the runs to be integrated from the experiment that has just been submitted. Using this button allows you to start integration while the data is still being collected.

NOTE: When integrating while collecting data, the Integrate Images plug-in will integrate all of the data currently measured and then wait for the next image, integrate that image, wait for the next, etc.

The plug-in populates the list of runs to be integrated with the runs from the experiment.

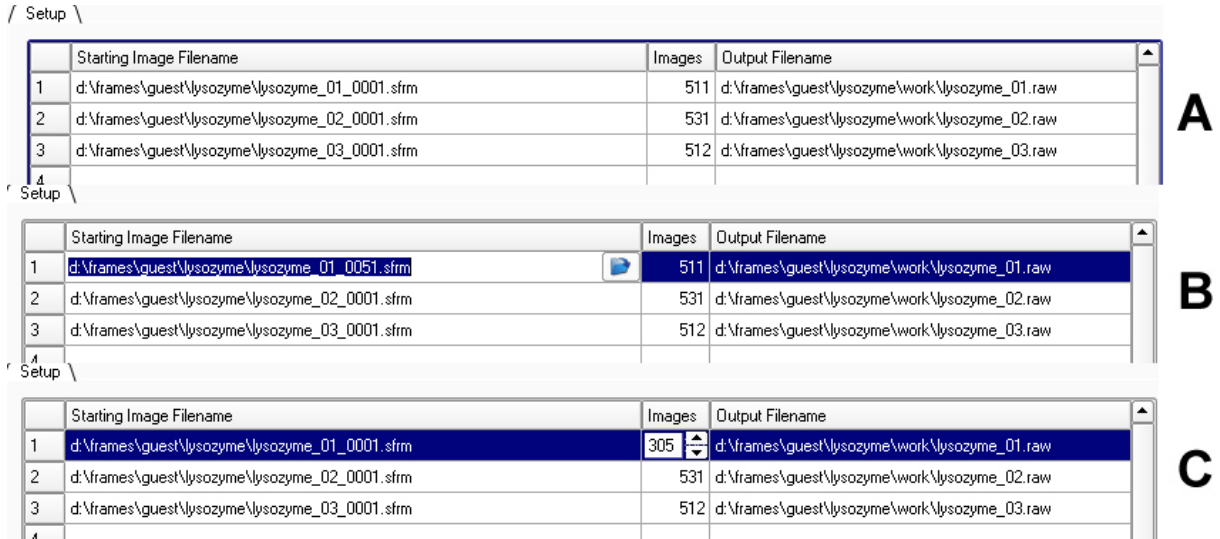
7.1.3.1 Modifying Integration Run Lists

By editing the fields in the run list, the starting image name and output filename can be changed. You can use standard clipboard tools (e.g., **Copy, Paste**) by selecting a run entry and right-clicking on the entry number. In this way, it is also possible to count the number of frames available for each run.

Figure 7.5 shows a run list entry being modified. (A) shows the original run information. (B) shows the run with the starting image number changed to 51. (C) shows the number of frames to process changed to 363.

Double-click any of the fields in this list to open the value for editing.

Figure 7.5 — Example: manually editing a run list

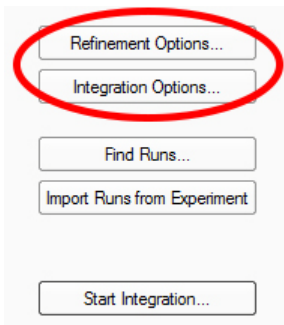


7.1.4 Refinement Options

Although the “Refinement Options” and “Integration Options” windows’ default values are generally very good, it may be necessary to adjust the integration box size and other parameters.

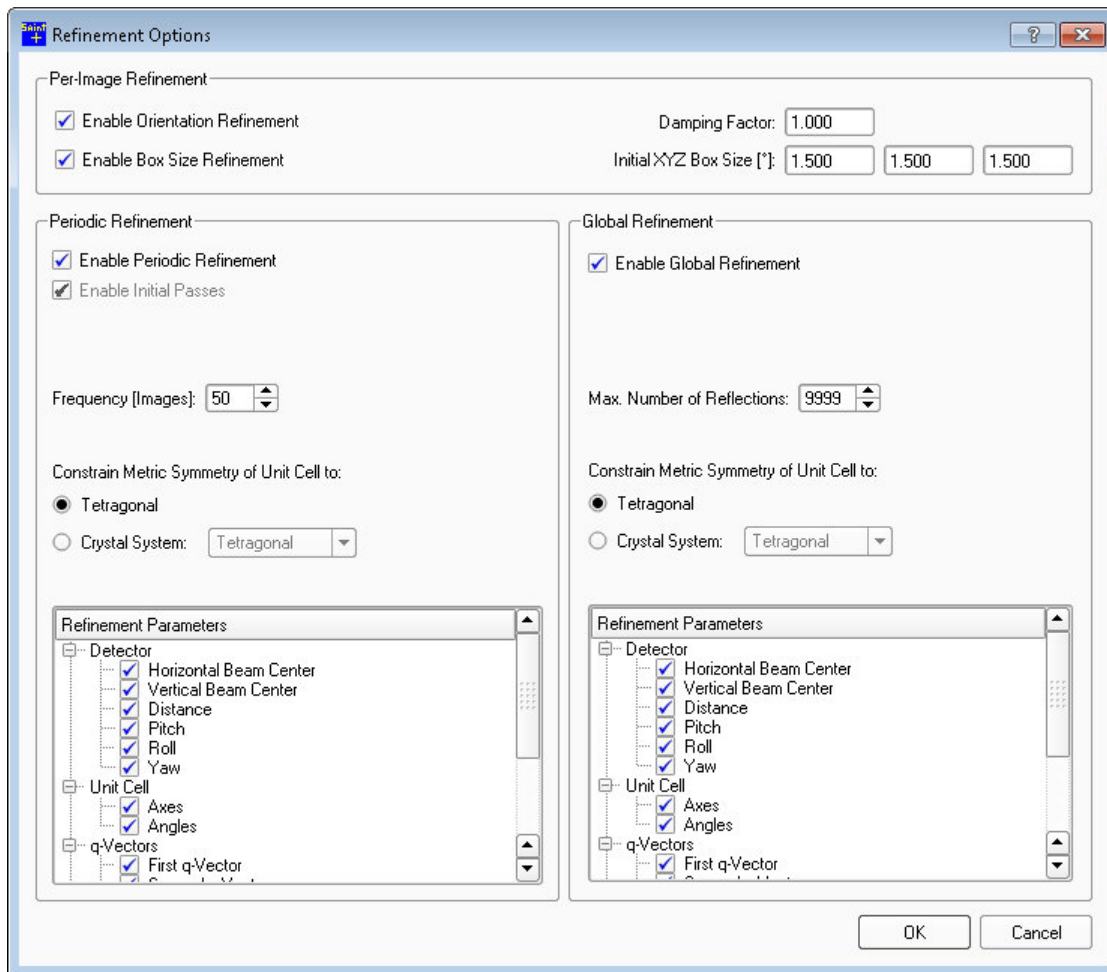
1. Click the **Refinement Options...** button in the lower right-hand corner of the plug-in (Figure 7.6).

Figure 7.6 — Refinement and Integration Options buttons



2. If necessary, adjust the refinement options (Figure 7.7).

Figure 7.7 — “Refinement Options” window



NOTE: To change the default values that appear in the “Refinement Options” window, create a text file C:\saint.ini. Copy the sections and parameters you want to change from C:\bn\src\interface\saint.ini into C:\saint.ini (do not change C:\bn\src\interface\saint.ini).

Table 7.1 — Refinement options

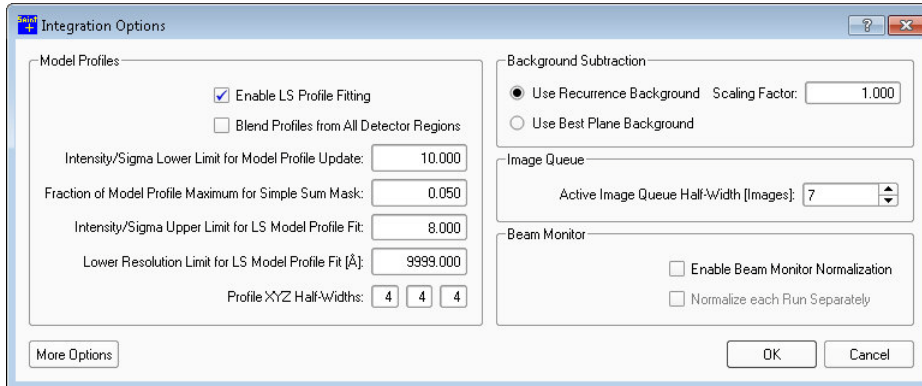
Option		Usage
Per-image refinement	Enable Box Size Refinement	If this checkbox is activated, SAINT will attempt to refine the initial box size estimates (i.e., the size of the integration box) to optimize the angular range of the volume used to store the model spot profile and the profile of each reflection integrated. It is sometimes necessary to set the integration box to compact values (in “Initial XYZ Box Size”) in order to avoid reflection rejections (e.g., exceeding the frame queue) or too many twin overlaps.
	Initial XYZ Box Size	The plug-in’s estimate of the spot size in degrees. The program tries to come up with a good starting value, but sometimes the box is too small. This value is refined at the beginning of integration, so it usually does not need to be changed unless instructed by the plug-in.
Periodic refinement	Frequency (Images)	This parameter determines after how many frames the periodic least squares refinement will be carried out during the integration. This has no effect on the least squares refinement taking place throughout the initial passes prior to integration. Consider reducing the default value for wide frames.

3. Click **OK** to close the window.

7.1.5 Integration Options

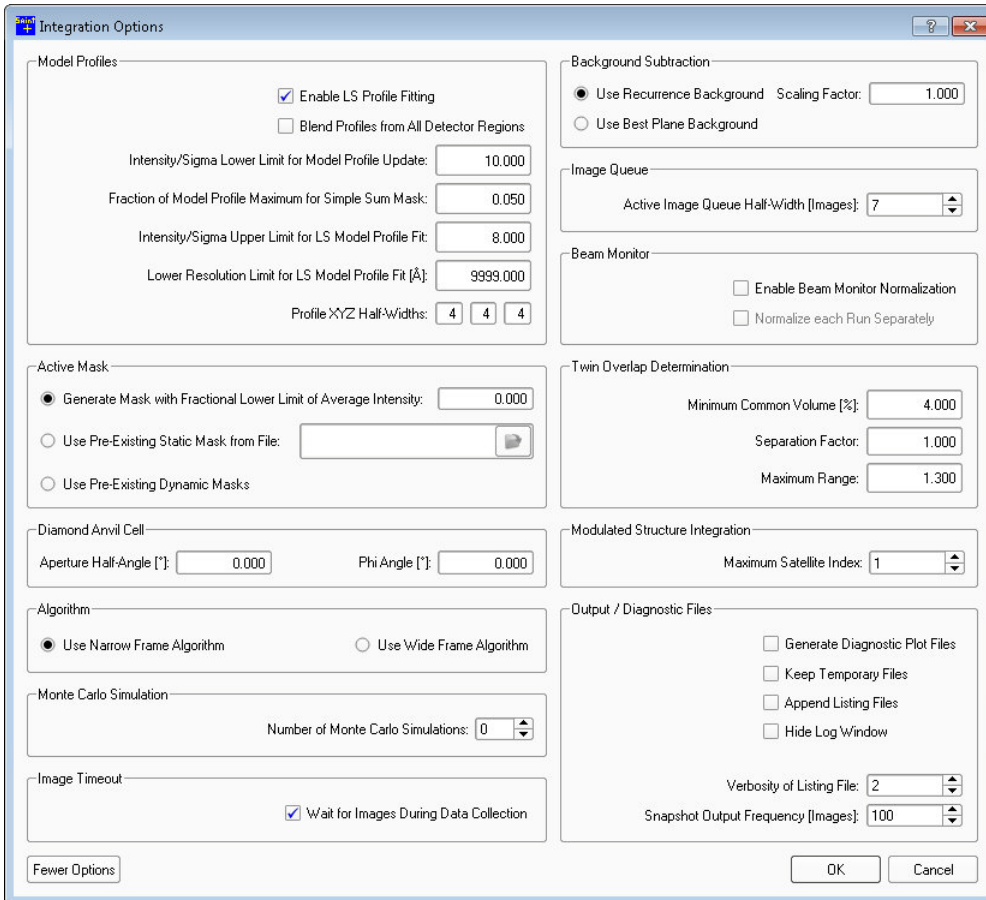
1. Click **Integration Options...** on the plug-in's right-hand side.
The "Integration Options" window opens (Figure 7.8).

Figure 7.8 — "Integration Options" window



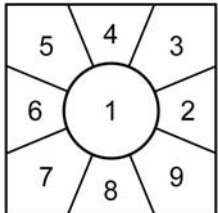
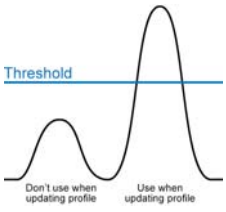
2. Click the **More Options** button to expand the window (see Figure 7.9).

Figure 7.9 — "Integration Options" window (expanded)



3. Set the options as necessary for your data.

Table 7.2 — Integration options

Option		Usage	
Model Profiles	Blend Profiles from All Detector Regions	During the integration, the model profile shape is determined separately for nine regions of the detector (see picture). Blending the model profiles results in less variation across the detector area, and may provide better statistics for regions where the reflections are very weak.	
	Intensity/Sigma Lower Limit for Model Profile Update:	<p>Prior to and during integration, a model of the average reflection profile shape is determined and updated. This profile is used to get a better estimate for the true intensity of weak reflections.</p> <p>This parameter sets an Intensity/Sigma cutoff which determines the reflections that will be used to update the model profile. All reflections with an Intensity/Sigma value lower than this threshold are ignored.</p> <p>It may be necessary to lower this value for frames with only weak reflections (typically in conjunction with activating the Blend Profile option).</p>	
Active Mask	Fractional Lower Limit of Average Intensity:	<p>(This option is useful for CCD cameras, but should not be used for PHOTON detectors.) To compute an active pixel mask, the software has to determine which pixels are active. This decision is made by comparing the counts that were registered by each pixel with the average counts on the image. A pixel is defined as “active” if it contains more counts than the fraction of average intensity which is specified here.</p> <p>A value of 0.5 normally works fine for Mo data. A lower value is normally better for Cu data (~ 0.35). The resulting mask (“_am_XX_XXXX.sfrm”) should be checked in the “/work” directory. If the value is set too high, it is possible to mask large areas of your detector unknowingly.</p> <p>PHOTON instruments use computed occlusion mask files “_xa_XX_XXXX.sfrm”, which include the bad pixel and beamstop shadow information, as integration masks.</p>	
Algorithm	Use Narrow Frame Algorithm/ Use Wide Frame Algorithm	<p>The wide-frame algorithm differs from the narrow-frame algorithm as follows:</p> <ul style="list-style-type: none"> • Spot sizes in Z are estimated based on partiality statistics accumulated for those spots which span frames. • Orientation least squares uses both “fulls” and “partials”, with “fulls” given zero weight in Z. • Reflection profiles are collapsed in Z for purposes of correlation with model profiles and LS profile fitting. • Spots that collide in Z (i.e., spots at similar X and Y with overlapping intensity on a common frame) are recognized and excluded from output. Their Z centroids may occur on the same frame, or on different (and not necessarily adjacent) frames. <p>Integration of wide-frame twin and modulated-structure data are both supported although not recommended, since problems with spot overlap will tend to increase. The wide-frame algorithm will usually give better results in cases where the frame width is substantially greater than the spot width. Acquisition of wide frames often degrades the signal-to-noise ratio, especially for weak reflections, and results in a higher number of overlapping spots. Narrow-frame data collection is therefore recommended, and will usually result in higher data quality.</p>	
Image Queue	Active Image Queue Half-Width (images)	<p>The number of frames in the in-memory frame queue will be $2N + 1$, where N is the value entered here. The default is $N = 7$, corresponding to a 15-frame queue, which should be fine for most purposes. The accepted range of N is 2 - 32, producing queue sizes from 5 to 65 frames.</p> <p>It is recommended to reduce the number for wide frames.</p>	

4. Click **OK**.

7.1.6 Start the Integration

Click the **Start Integration...** button on the lower right-hand side of the plug-in.

Integration begins, and the “Integration” tab opens showing the SaintChart images for monitoring the integration.

A .raw file (containing the unmerged raw intensities) is written for each run. If multiple runs are integrated, a merged intensity file is written as well. The filename of the merged file contains a “0m” for the run number.

At the end of the integration run, the plug-in carries out a global refinement of crystal and detector parameters, followed by a statistical analysis of the integrated data set. The results are reported in the text display at the bottom of the “Integration” tab.

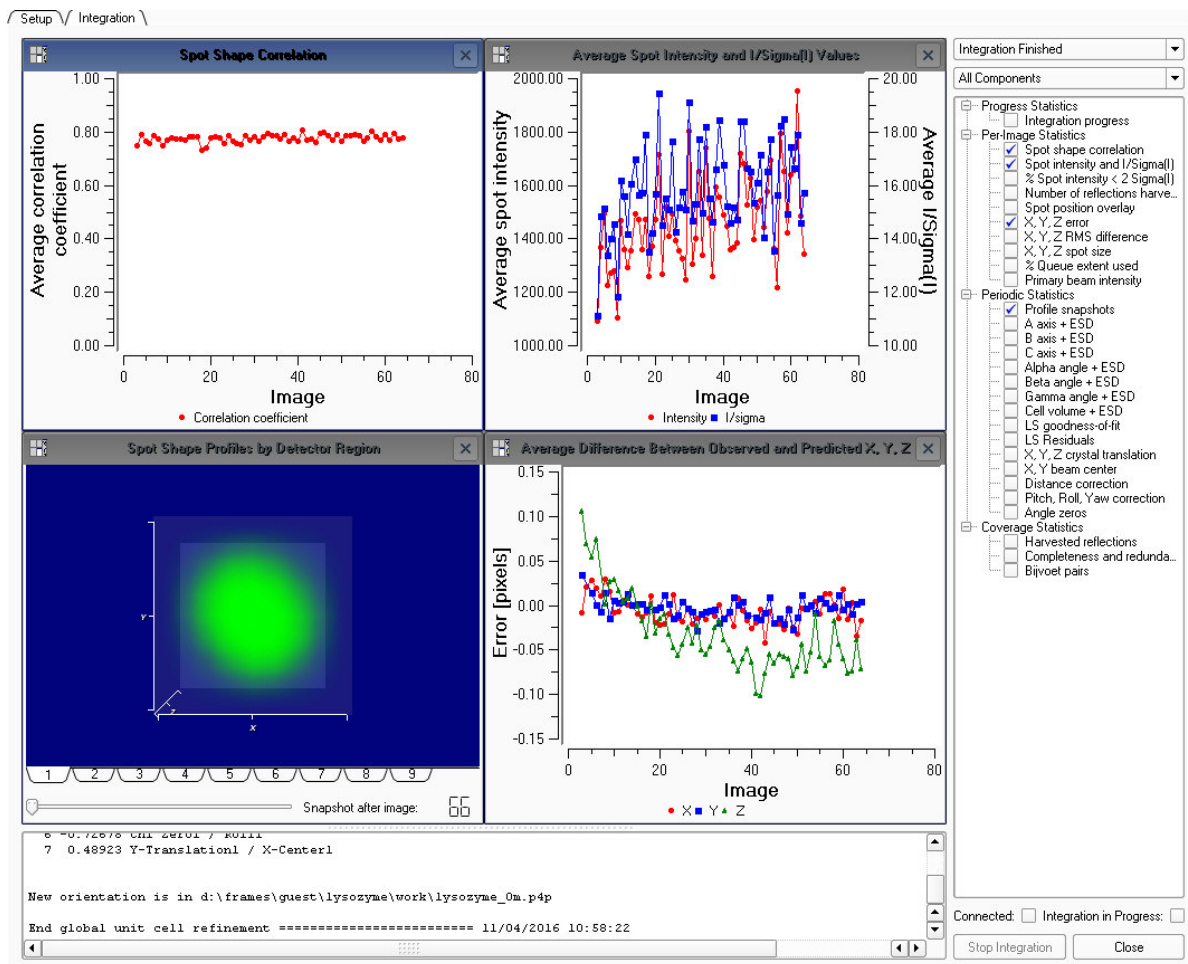
7.1.6.1 Monitoring Integration Progress with SaintChart

SaintChart is a powerful tool for monitoring the progress of the integration process and for graphically presenting the results of the integration process (Figure 7.10).

To the right of the image area are a series of checkboxes that select the displays. Activate or deactivate the checkboxes to add or remove displays.

Change the text area by clicking and dragging on the line between the text and image areas. You can also expand the windows to fill the available image area by clicking **Chart > Tile** in the Menu Bar.

Figure 7.10 —SaintChart view



For a full description of SaintChart’s windows and functions, refer to Section 12.3.

Chart Files

After integration, the results are reported in the text display and a chart file (*.cht) is written to the work directory. You can save and open chart files using the commands under **Chart** in the Menu Bar.

7.2 Scaling Data

The Scale plug-in's underlying engine is either SADABS for single crystals or TWINABS for twinned samples. The only required inputs for the Scale plug-in are:

- the integration results from the Integrate Images plug-in; and
- the Laue group.

For a more detailed description of the plug-in's parameters, refer to the SADABS documentation file located at "C:\bn\SXTL\help".

7.2.1 Start the Scale Plug-in

In the Task Bar's "Reduce Data" category, click the **Scale** icon



The Scale plug-in opens.

7.2.2 Set Up the Scale Operation

Figure 7.11 shows the right-hand portion of the plug-in's "Setup" tab.

Figure 7.11 — "Setup" tab

Setup

Input Folder: d:\frames\guest\lysozyme\work

Input File(s)

- lysozyme_0m.raw

Select All Deselect All

Laue Group: 4/m

Point Group: 4/m

Additional Spherical Absorption Correction

Mu_r of Equivalent Sphere: 0.20


Absorption Correction

- Multi-Scan
- Numerical Mu Calculated
- Numerical Mu From Formula

Advanced Setup

Start Over Start Finish

1. Check that the defaults are correct.
2. If necessary, read in the runs to be scaled:

Click the “Input Folder:” field’s **Browse** button . A window appears showing the reflection files available for scaling. If the files are not visible, select the correct folder. A single run can be selected by highlighting that run and clicking **Open**. All runs with the same base name can be chosen by highlighting any of the runs in that series and clicking **Open**. The runs with the selected base name appear in the “Input File(s)” area, and they can then be selected individually with checkboxes. Selecting a merged file (i.e., the one ending in “m” rather than a run number) will use all of the runs that were integrated during the same integration job. The program will assign filenames for the output files. To change these names, edit the “Output HKL File Name” field under the “Advanced Setup” tab.

3. Choose the preferred Laue group and point group.
4. If necessary, adjust **Additional Spherical Absorption Correction** (it is not necessary for a numerical absorption correction, but it can still be used).

It is possible to apply an additional absorption correction assuming a spherical crystal with given μr , where μ is the linear absorption coefficient (in mm^{-1}) and r is the radius of the equivalent sphere (in mm). This correction is included because the theta-dependent part of the absorption cannot be modeled well by comparing equivalent reflections for the empirical absorption correction (because these invariably have the same 2θ values). The main effect of applying it will be to increase the equivalent isotropic displacement parameters in the resulting refinement.

NOTE: It is strongly recommended to set this parameter correctly for large, strongly-absorbing crystals. Otherwise, your data will be biased.

5. If necessary, set additional parameters under the “Advanced Setup” tab.

For most in-house data collection with frozen crystals, no correction is necessary. In that case, choose **None**. If conducting a long SAD experiment, the crystal may suffer radiation damage to some extent. It may be worthwhile to try the linear dependence correction.

These include:

- Fast scan resolution cutoff
- Allow for crystal decomposition by B-value Refinement
- Lambda correction

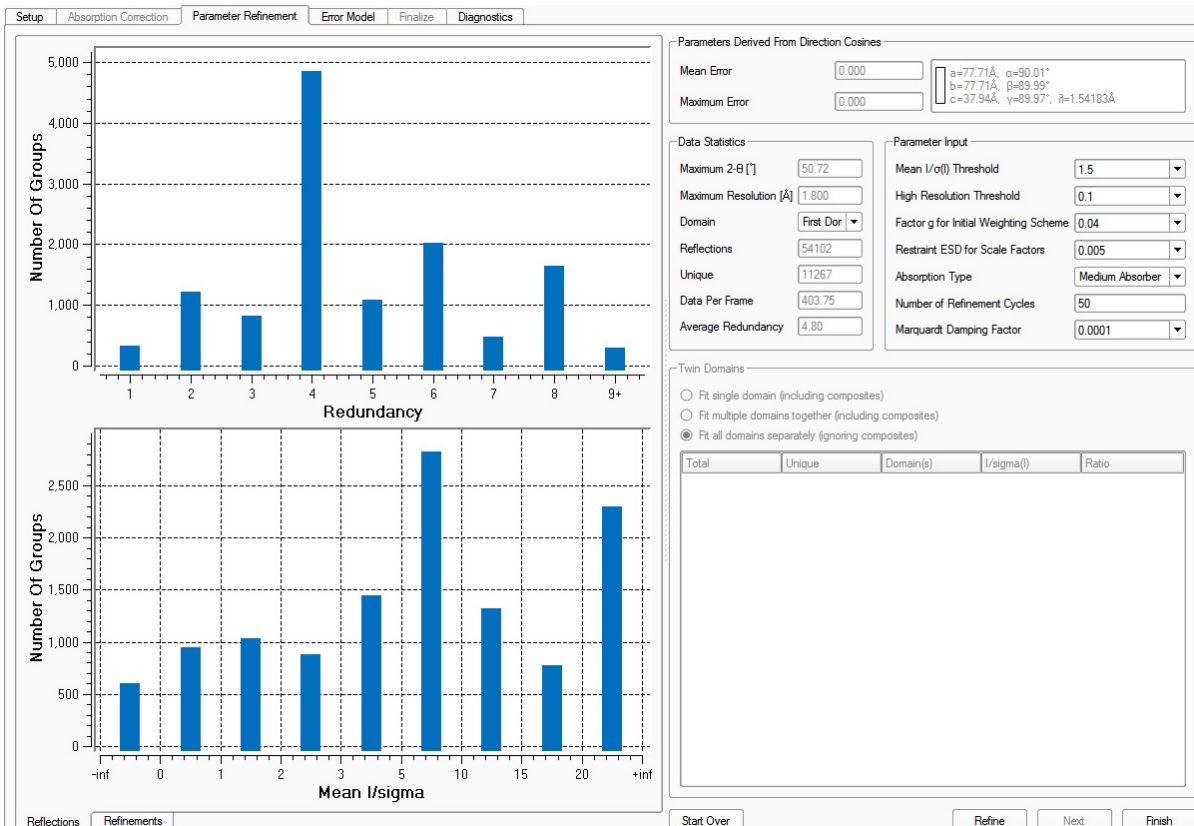
6. Click **Start**.

The plug-in proceeds to the “Parameter Refinement” tab.

7.2.3 Refine the Parameters

The “Parameter Refinement” tab shows the reflection statistics before refinement, under the “Reflections” tab (Figure 7.12).

Figure 7.12 —“Parameter Refinement” tab before refinement



1. If necessary, set the refinement parameters (the defaults work well in most cases).

Two parameters deserve particular attention:

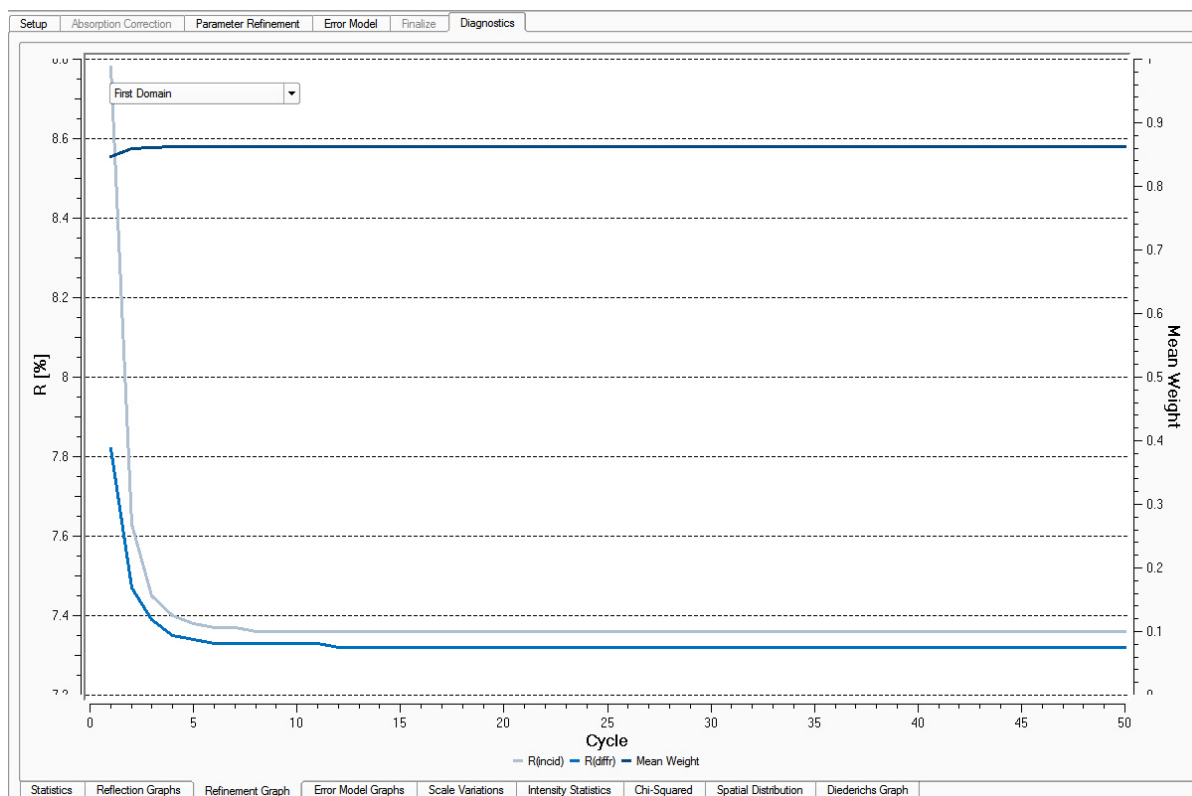
- **Restraint ESD for Scale Factors:** Restrains the scale factors from shifting too much during refinement. Relaxing the restraints a bit may help in some cases (e.g., for heavy absorbers or crystals larger than the beam), but be careful not to over-fit the data.
- **Absorption Type:** Sets the order of spherical harmonics used for the absorption correction. Medium is fine for all data sets.

2. Click **Refine**.

The plug-in proceeds to the “Refinements” tab.

Figure 7.13 shows a typical refinement result. The R-values should decrease quickly and converge to similar values. The Mean Weight should increase and converge. The mean weight gives feedback about the data quality or the correct Laue group assignment. Values below 0.7 are suspicious, and the data should be carefully checked.

Figure 7.13 —“Refinement Graphs” tab after refinement



3. If necessary, make changes to the refinement parameters and click **Refine** to refine the data set again.

4. When you are satisfied with the refinement, click **Next**.

The plug-in proceeds to the “Error Model” tab.

7.2.4 Refine the Error Model

Initially, the graphs in the “Error Model” area show an initial error model based on the default values in the “Outlier Rejection” area.

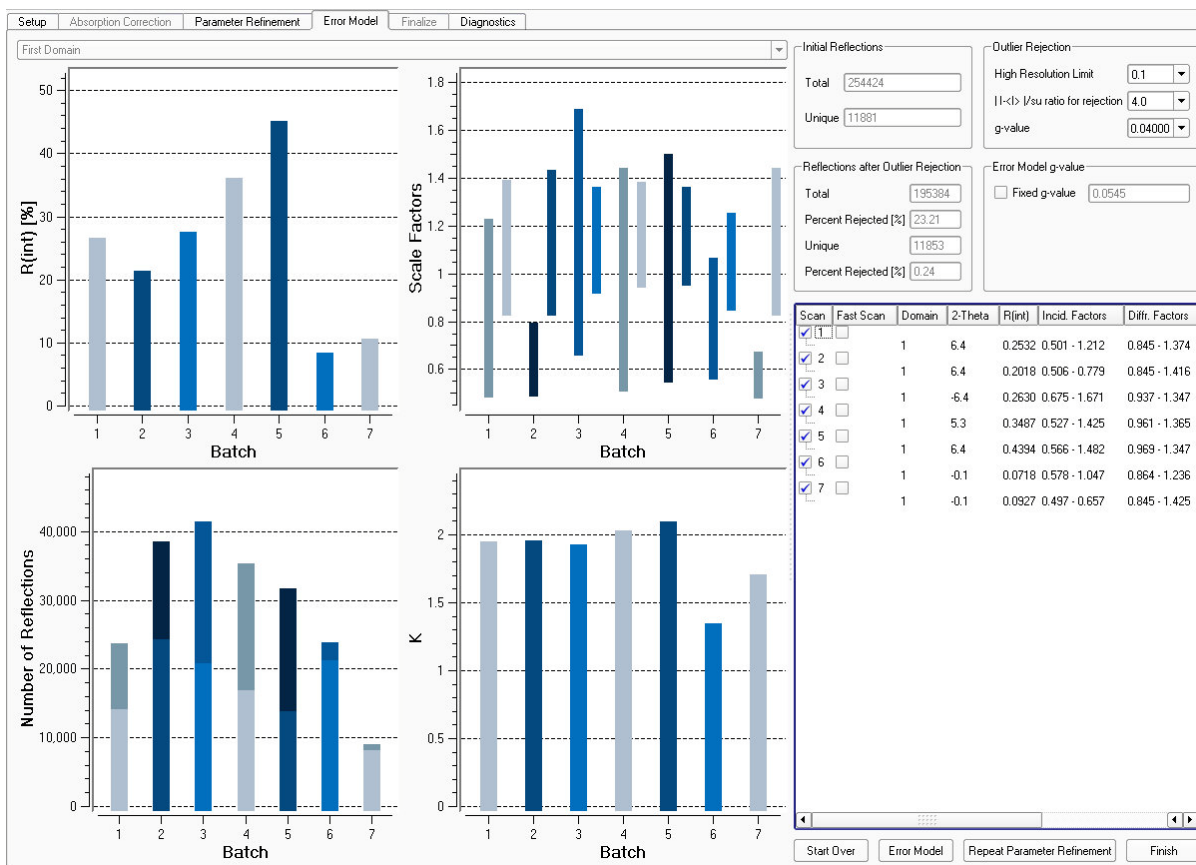
1. If necessary, adjust the parameters in the “Outlier Rejection” area.

The table contains a “Fast Scan” column. Runs below 10° 2theta can be selected as “Fast Scans”. Fast scans are recorded for shutterless data collections to prevent missing strong reflections in the data set because of detector saturation in the main runs. The “Fast Scan” is only used to fill in missing reflections in the main data set.

2. Click **Error Model**.

Figure 7.14 shows typical Error Model results.

Figure 7.14 —Error Model results



3. Examine the error model for problems:

- **R(int):** Agreement factor of the reflection intensities of the individual runs compared to the mean intensities of symmetry-independent reflections based on the Laue/point group.
- **Number of Reflections:** The column height displays the total number of reflections per run. The darker part of the column represents the number of reflections below 2 I/sigma(I).
- **K:** Scale factor for the standard uncertainties.

4. If necessary, adjust the error model and click **Error Model** again.

For example, to reject fewer reflections, increase the “ $|I|/su$ ratio for rejection” parameter in the “Outlier Rejection” area.

NOTE: If the R-values in one run are significantly higher than the others, you can repeat the entire scaling calculation with that run omitted. Deactivate the checkbox next to the “bad” run, and click **Repeat Parameter Refinement**.

5. When you are satisfied with the error model, click **Finish**.
The plug-in proceeds to the “Diagnostics” tab.

7.2.5 Examine the Diagnostic Plots

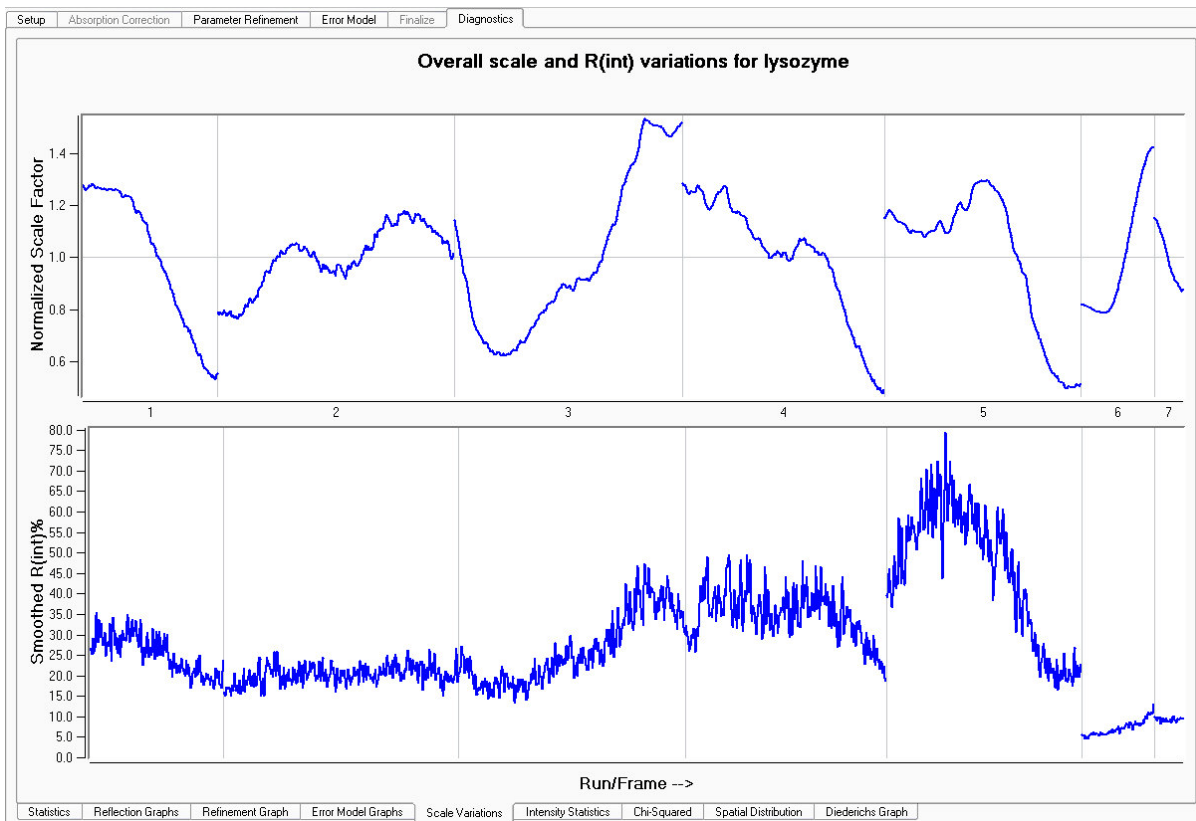
Diagnostic plots provide valuable insight into the quality of the data and possible problems with the data.

Click the tabs at the bottom of the plug-in to view the diagnostic data (some of these plots are reviews of plots that have already been displayed).

7.2.5.1 Scale Variations

The Scale Variations plot (Figure 7.15) shows the overall variation in Scale and R(int) for the individual frames. The Scale plot should be flat (for strongly-absorbing, irregularly-shaped crystals it will probably be sinusoidal), and the R(int) plot should not show large variations (more than 2%).

Figure 7.15 —Overall Scale and R(int) variations

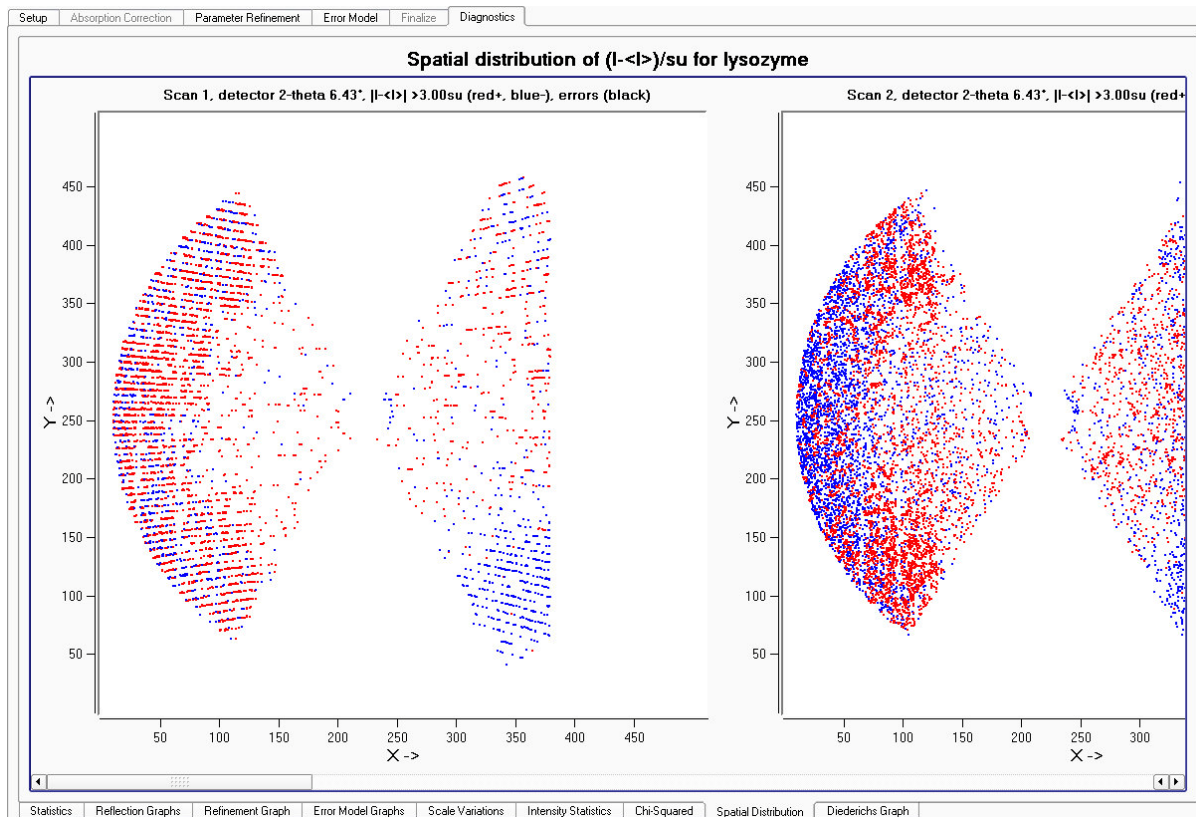


7.2.5.2 Spatial Distribution Plots

Spatial Distribution plots are generated for each data collection run. They indicate spots that were either stronger or weaker than expected with a deviation from the mean intensity larger than three standard uncertainties.

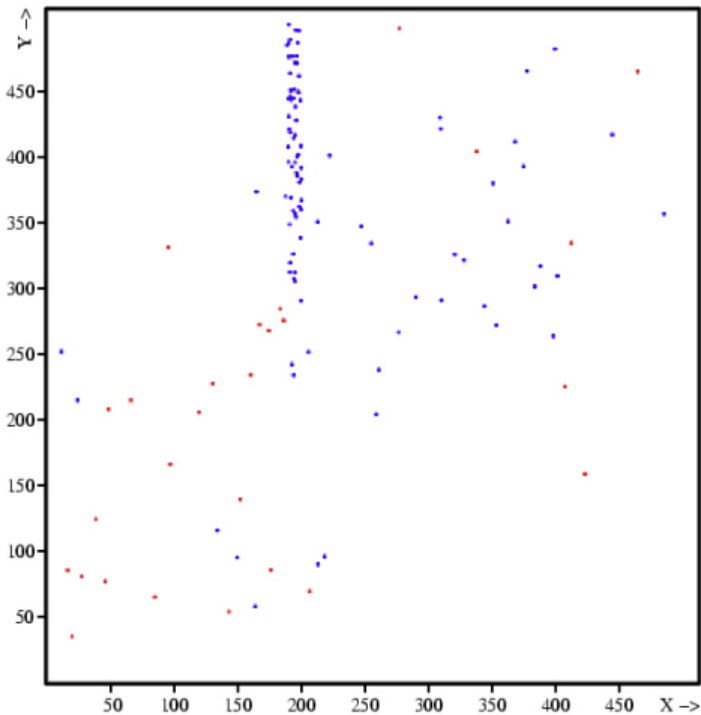
Typically, the points of disagreement are spread fairly evenly over the detector face (Figure 7.16).

Figure 7.16 —Spatial Distribution plots



If the points are clustered in a single area or if there are significantly more spots of one color than another, the data should be examined critically. Figure 7.17 is from a data set in which the reflections collected in a certain area are consistently weaker than expected (possibly because the beamstop is not centered in the direct beam, or because the “<name>_xa_<run>_<frame>.sfrm” files were not present during integration). This kind of problem may affect the final results.

Figure 7.17 —Spatial Distribution plot showing a problem area



7.2.6 Close the Scale Plug-in

Click the **Close** button  in the upper right-hand corner of the plug-in.

The final hkl is generated, and the plug-in closes.

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8 Examining Data

After integrating and scaling the data, the crystal's space group is determined and optional simulated precession photographs are calculated to further evaluate the overall quality of the data. Five plug-ins are available for this:

- Determine Space Group (based on XPREP);
- Analyze Data (which opens the command-line version of XPREP);
- Synthesize Precession Images (for looking at undistorted slices of reciprocal space);
- Find a Reflection (for finding a reflection in the acquired images).

8.1 Using the Determine Space Group Plug-in

8.1.1 Start the Determine Space Group Plug-in

In the Task Bar's "Examine Data" category, click the **Determine Space Group** icon



The plug-in opens with the "Setup" tab active.

8.1.2 Set Up the Determination Parameters

1. In the plug-in's "Setup" tab, check that the default-loaded files are correct.

NOTE: Typically, the files to use for space group determination are the "/work" directory's *.p4p file (containing the final unit cell parameters from integration) and *.hkl file (containing the corrected intensities). You can browse to choose other files if desired.

Figure 8.1 — "Setup" tab

Setup | Lattice Exceptions | Space Group Determination | Statistics | Cell Information | Diagnostics

Input Files

hkl file: lysozyme_0m.hkl

p4p file: lysozyme_0m.p4p

Output Files

output.hkl file: lysozyme_0m.hkl

ppp file: lysozyme_0m.ppp

output.sca file:

Unit Cell

	a	b	c	alpha	beta	gamma
cell	79.350	79.350	38.159	90	90	90
cell esds	0.006	0.000	0.003	0	0	0

Experimental Parameters

Must be chiral

Radiation Type: Cu

Next

Finish Start Over Exit

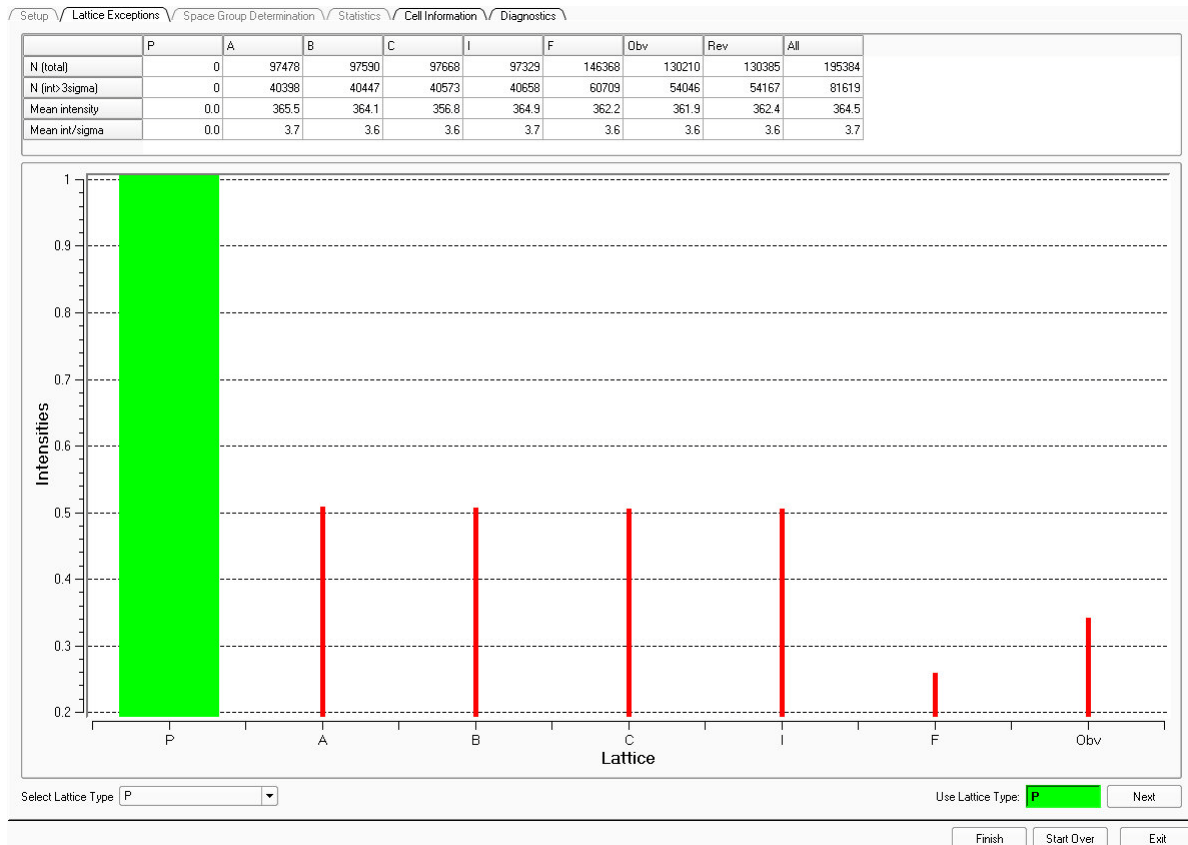
2. Click **Next**.

The plug-in proceeds to the "Lattice Exceptions" tab.

8.1.3 Examine the Lattice Exceptions

The plug-in evaluates the data and suggests a lattice type based on the mean intensities and the mean int/sigma.

Figure 8.2 — “Lattice Exceptions” tab



1. If you know that your sample has a different lattice type than that suggested by the plug-in, you can choose it from the **Select Lattice Type** pull-down menu.
2. Click **Next**.
The plug-in proceeds to the “Space Group Determination” tab.

8.1.4 Choose a Space Group

The plug-in determines the reduced unit cell based on the chosen lattice type.

Figure 8.3 — Space group determination

The screenshot shows the 'Space Group Determination' dialog box. At the top, there are tabs for 'Setup', 'Lattice Exceptions', 'Space Group Determination', 'Statistics', 'Cell Information', and 'Diagnostics'. The 'Space Group Determination' tab is active.

Bravais Lattices: A table with columns: Option, A, B, C, Alpha, Beta, Gamma, Volume, R(sym). The 'TETRAGONAL P-lattice' is selected.

Option	A	B	C	Alpha	Beta	Gamma	Volume	R(sym)
<input checked="" type="radio"/> TETRAGONAL P-lattice	79.350	79.350	38.159	90.00	90.00	90.00	240263.38	0.135
<input type="radio"/> ORTHORHOMBIC P-lattice	38.159	79.350	79.350	90.00	90.00	90.00	240263.38	0.170
<input type="radio"/> MONOCLINIC C-lattice	112.217	112.217	38.159	90.00	90.00	90.00	480526.75	0.195
<input type="radio"/> MONOCLINIC P-lattice	38.159	79.350	79.350	90.00	90.00	90.00	240263.38	0.149
<input type="radio"/> MONOCLINIC P-lattice	38.159	79.350	79.350	90.00	90.00	90.00	240263.38	0.149
<input type="radio"/> MONOCLINIC P-lattice	79.350	38.159	79.350	90.00	90.00	90.00	240263.38	0.112
<input type="radio"/> Retain Original Cell								

Systematic absence exceptions: A table with columns: N, 41/43, 42, n-, -b-, -c-, -n, -21-, -c.

	41/43	42	n-	-b-	-c-	-n	-21-	-c
N	57	38	4495	4429	4349	4384	148	3179
N > 3s	1	0	1915	1796	1940	1826	0	1432
<l>	26.3	23.3	437.1	335.3	389.0	346.3	33.3	450.5
<l/>s	0.6	0.4	3.9	3.8	4.1	3.7	0.5	4.1

E-value statistics: A slider bar with 'Non-centrosymmetric: 0.736' on the left and 'Centrosymmetric: 0.968' on the right. The 'Mean |E*E-1|' is 0.723.

Identical indices and Friedel opposites combined before calculating R(sym): A table with columns: Space Group, No., Type, Axes, CSD, R(sym), N(eq), Syst. Abs., CFDM.

Space Group	No.	Type	Axes	CSD	R(sym)	N(eq)	Syst. Abs.	CFDM
<input type="radio"/> P4(1)2(1)2	# 92	chiral	1	245	0.183	36970	0.6 / 3.7	13.60
<input checked="" type="radio"/> P4(3)2(1)2	# 96	chiral	1	245	0.183	36970	0.6 / 3.7	13.60

At the bottom, there is a checkbox 'Choose a different space group:' with a dropdown menu showing 'P1'. There are buttons for 'Repeat', 'Next', 'Finish', 'Start Over', and 'Exit'.

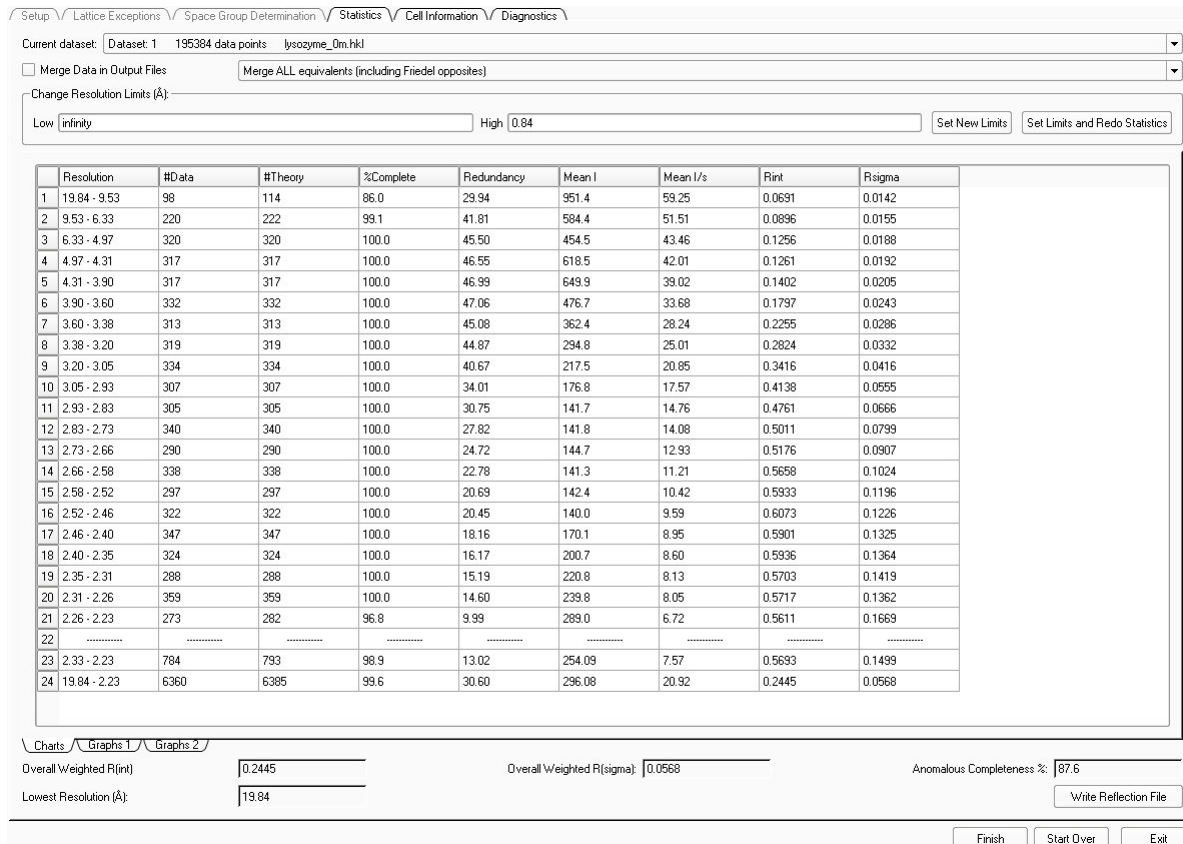
1. Check the E-value statistics to determine if the space group is centrosymmetric or non-centrosymmetric.
2. At the bottom of the Task Display Area, examine the suggested space groups and choose a space group by clicking its radio button.

NOTE: To choose a space group other than the suggested ones, click the **Choose a different space group** radio button. This opens a drop-down menu of all permissible space groups.

3. Click **Next**.
The plug-in proceeds to the "Statistics" tab.

8.1.5 Examine the Reflection Statistics

Figure 8.4 — “Statistics” tab



- Adjust the statistics parameters if desired:
 - The two pull-down menus at the top of the “Statistics” tab allow you to change the current dataset and control merging of equivalent reflections.
 - Change Resolution Limits allows you to adjust the range of resolutions for which statistics are displayed. Adjust the quantities in the fields and click **Set New Limits**.
- Examine the data presented in the table of statistics and in the “Graphs” tabs along the bottom of the screen:
 - Is the completion near 100%?
 - Are the redundancies at various resolutions close to the desired values?
 - Are R_{int} and R_{sigma} small and increasing smoothly from top to bottom (low-angle to high-angle data)?
 - Are the Overall Weighted R(int), Overall Weighted R(sigma), Lowest Resolution (Å) and Down-weighted Outliers appropriate for your experiment?

Figure 8.5 — Graphs 1

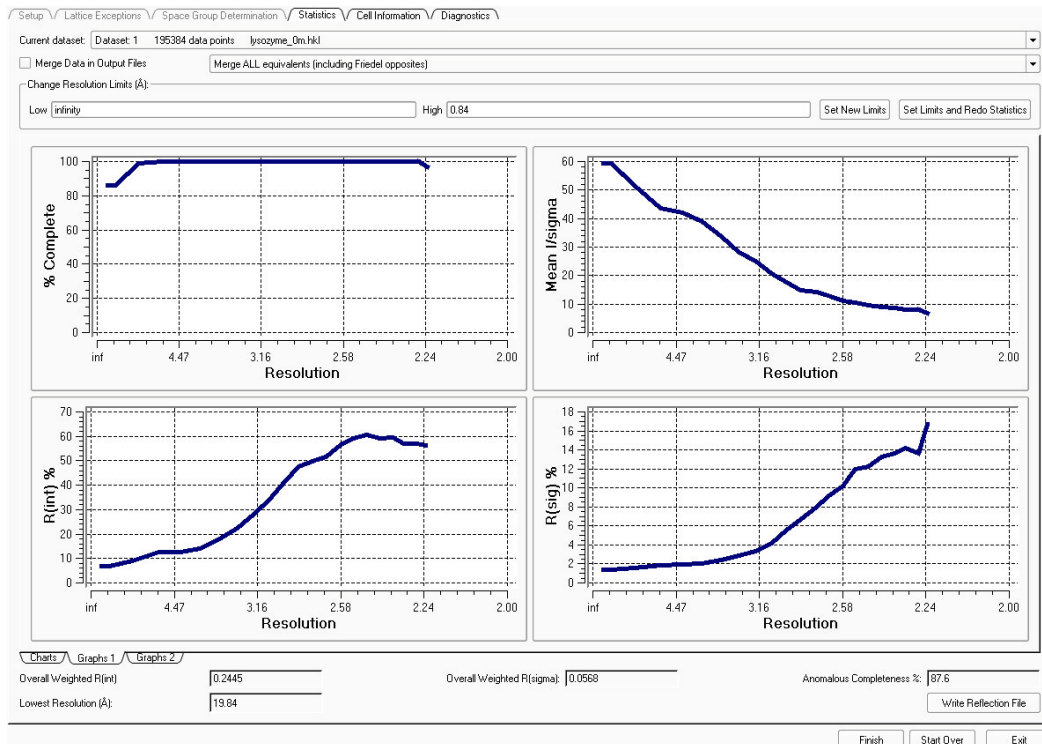
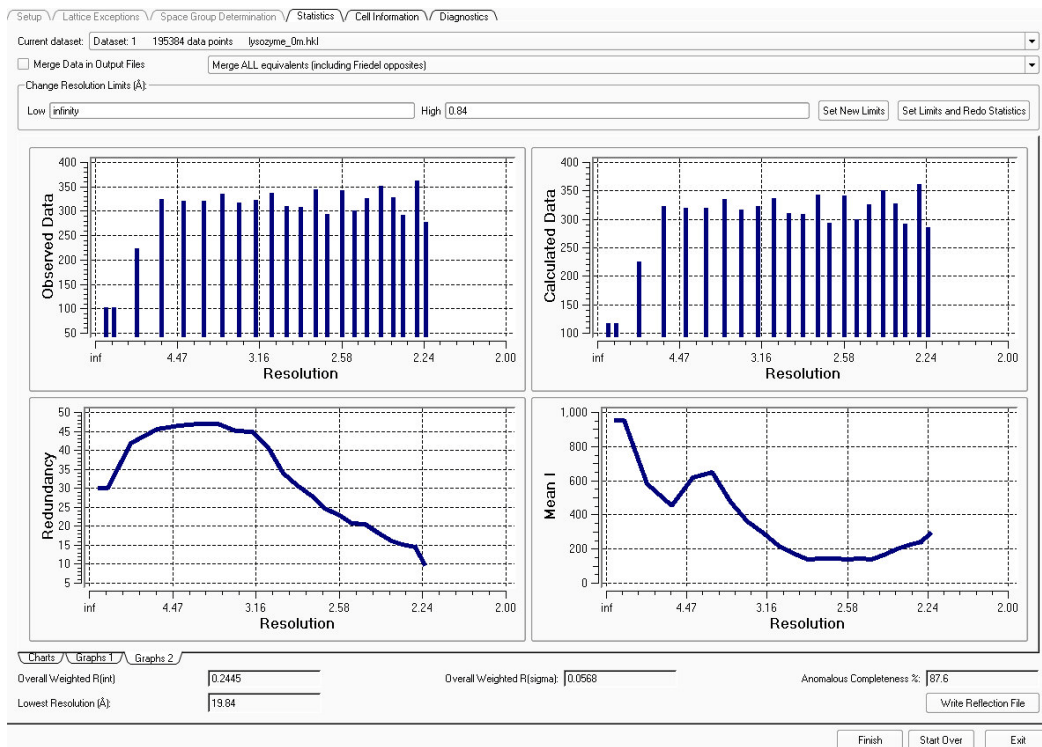


Figure 8.6 — Graphs 2



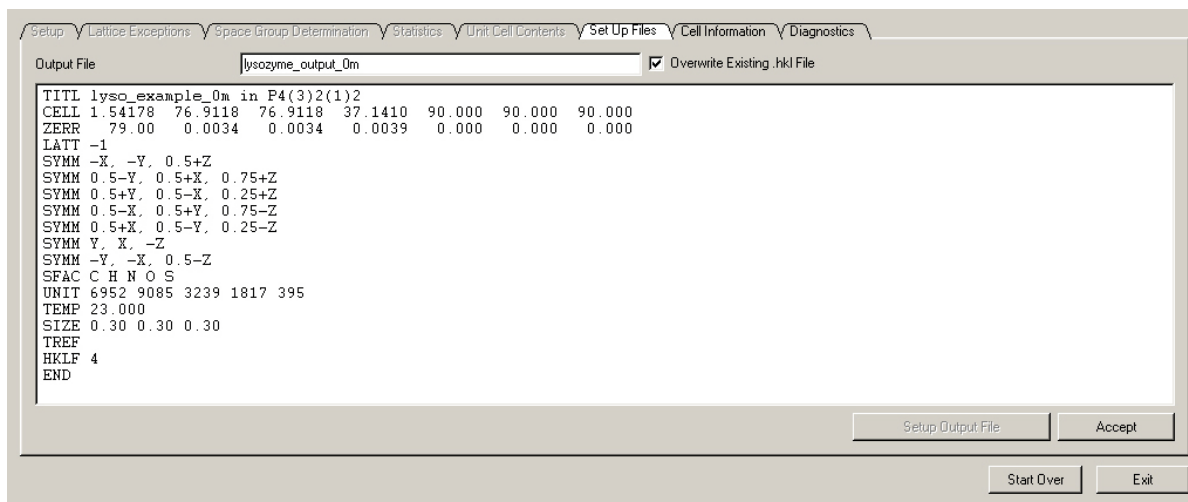
3. Click the **Next** button.
The plug-in proceeds to the “Unit Cell Contents” tab.

8.1.6 Set Up the Output File

1. If you want to change the output file name, type the new name in the “Output File” field.
2. If you want to overwrite the existing .hkl file, activate the **Overwrite Existing .hkl File** checkbox.
3. Click **Setup Output File**.

The plug-in fills in the appropriate commands for the .ins file.

Figure 8.7 — “Set Up Files” tab (filled in)

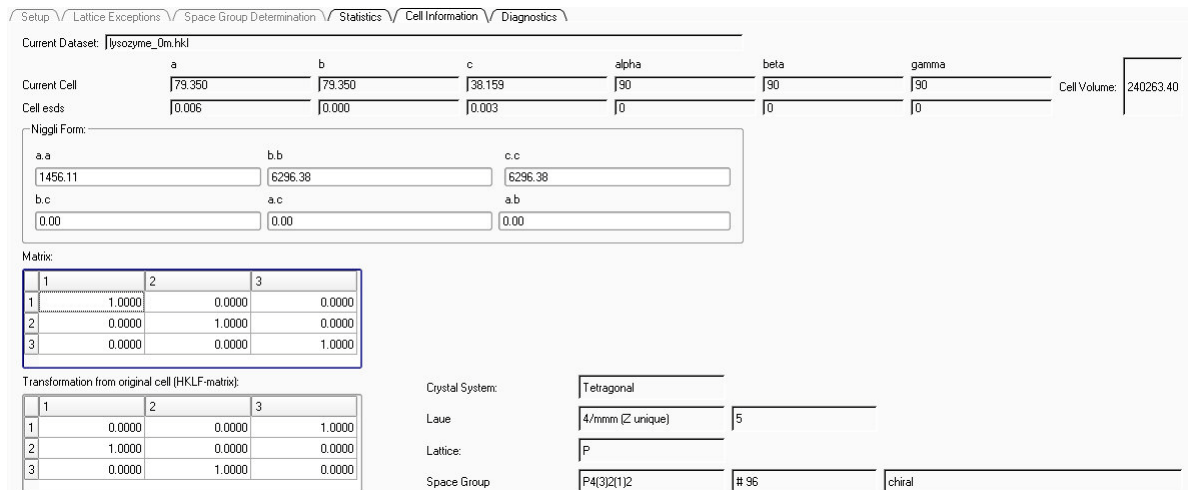


4. Click **Accept** to write the .ins file.

8.1.7 Examine Cell Information

Click the “Cell Information” tab to examine additional cell information.

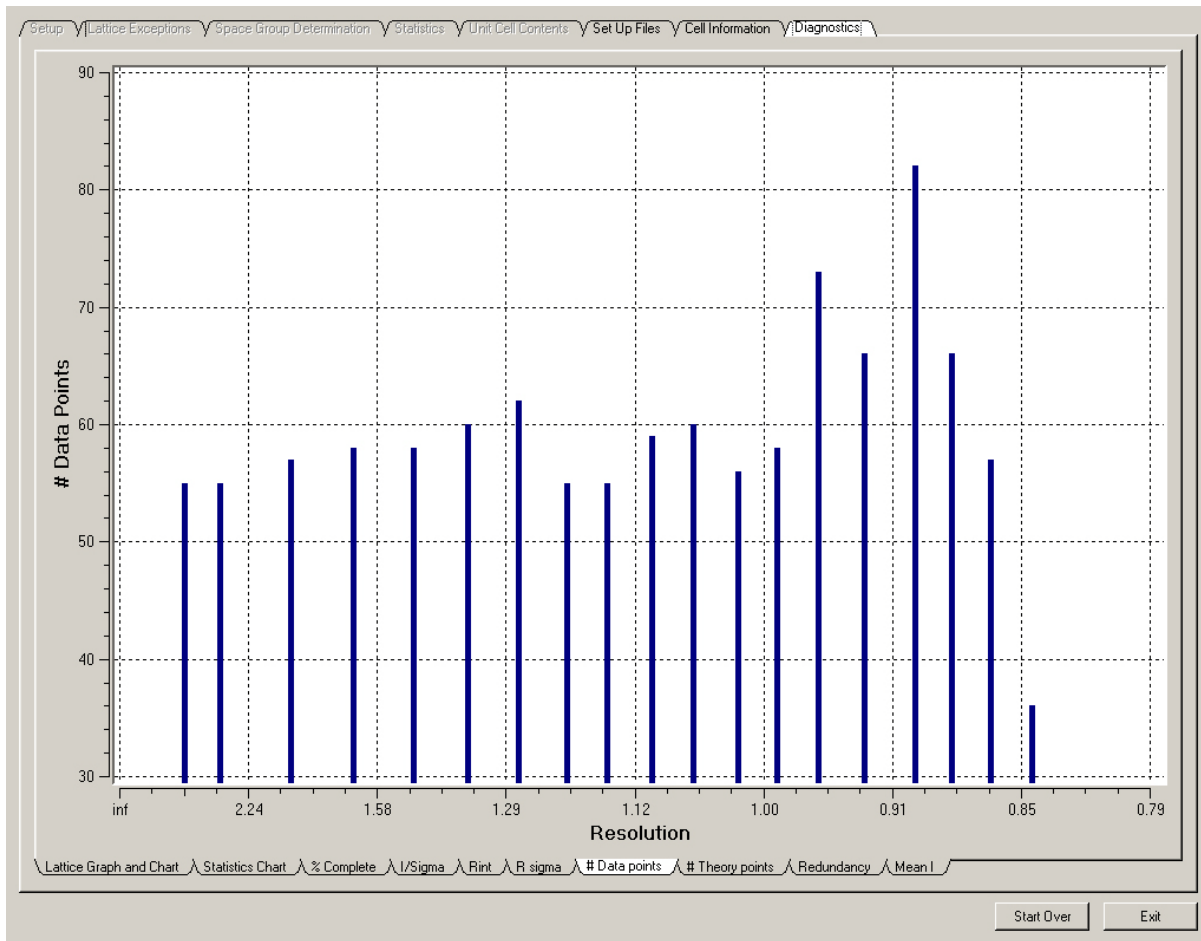
Figure 8.8 — “Cell Information” tab



8.1.8 Examine Diagnostics

Click the “Diagnostics” tab to examine additional diagnostic information.

Figure 8.9 — “Diagnostics” tab (“# Data Points” Section shown)



8.1.9 Exit the Determine Space Group Plug-in

Click the **Exit** button in the plug-in’s lower right-hand corner.

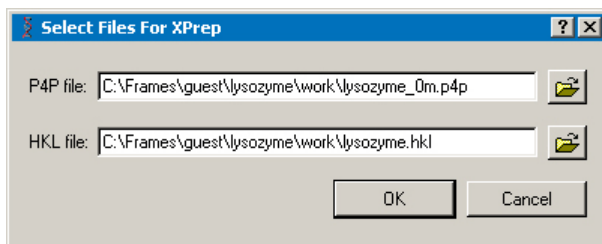
The plug-in closes.

8.2 Determining the Space Group with XPREP



1. Under the Task Bar's "Examine Data" category, click the **Analyze Data** icon .
The plug-in starts, and the "Select Files for XPRep" window opens.
2. Check that the two files in the "Select Files for XPRep" window are correct for your data set (Figure 8.10) and click **OK**.

Figure 8.10 — "Select Files for XPRep" window



NOTE: In this example, the integration process has created two files:
lysozyme_0m.p4p containing the final unit cell parameters from integration, and lysozyme.hkl containing the corrected intensities. Typically, these are the files to use for determining space groups, but you can browse to choose other files.

NOTE: In addition to space group determination, many other features of XPREP can be accessed from the general menu. Resolution cutoff, reciprocal space plots, simulated powder patterns, and a test for merohedral twinning are very useful tools.

3. Follow the program flow. Default options are automatically suggested and shown in square brackets. Make manual adjustments where necessary.

8.3 Creating Simulated Precession Images


The Synthesize Precession Images plug-in provides an undistorted view of layers of the reciprocal lattice. The plug-in generates simulated precession images by finding the appropriate pixels in a series of frames. You must specify the images to examine and the zones to calculate.

1. In the Task Bar's "Examine Data" category, click the **Synthesize Precession Images**



icon

The Synthesize Precession Images plug-in opens.

2. Select the runs for image synthesis by clicking the **Browse** button  beside the "Images from" field.

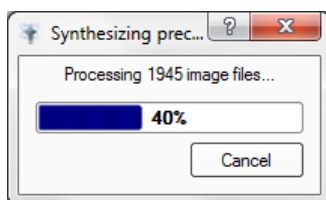
A "Select Runs" dialog opens.

3. Choose the sets of images that you want to use for the calculation by activating and deactivating the checkboxes. Click **OK**.
4. Check the default input values. Modify as needed (decimal values such as "0.5k1" are also allowed), and click **Calculate**.

Calculating more zones does not significantly increase the calculation time. Reducing the resolution may speed the calculations slightly. The thickness defines the range of pixels above and below the requested range. For example, if the 0kl zone is requested with a thickness of 0.1, then the simulation will use all pixels that have $-0.1 < h < 0.1$, and any value (including fractional values) for k and l.

A progress bar appears (Figure 8.11).

Figure 8.11 — Progress bar



After a short time (depending on the speed of the computer and the number of frames read), the simulated precession image will appear on the screen. The calculated frames are written to a "precession" subdirectory in the sample's folder.

5. Display other planes by clicking on the calculated images in the bottom right of the Task Display Area, or by browsing as usual with the View Images plug-in.

6. Use the simulated patterns to check space group symmetry (Figure 8.12) and to find signs of twinning or incommensurate modulation (Figure 8.13).

Figure 8.12 —Example 0kl plane

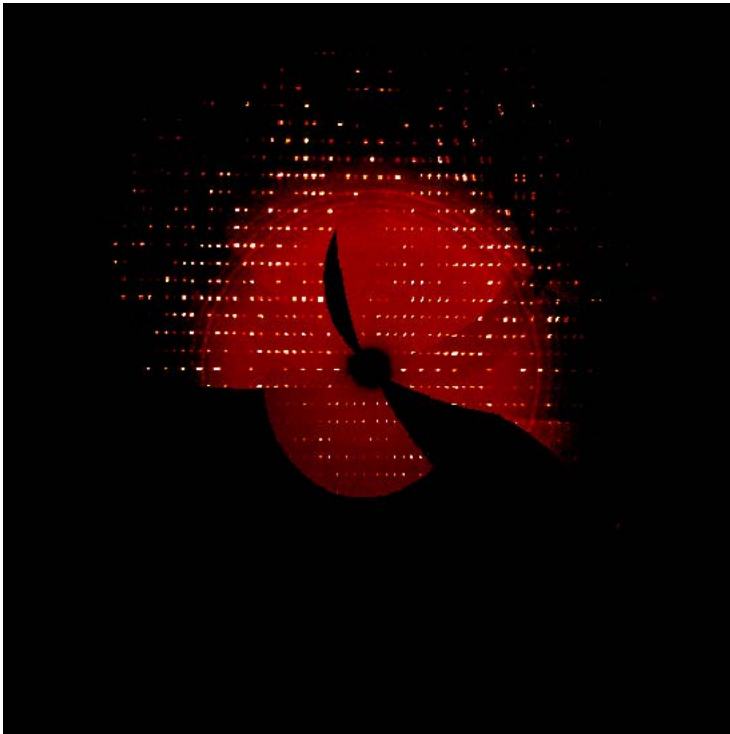
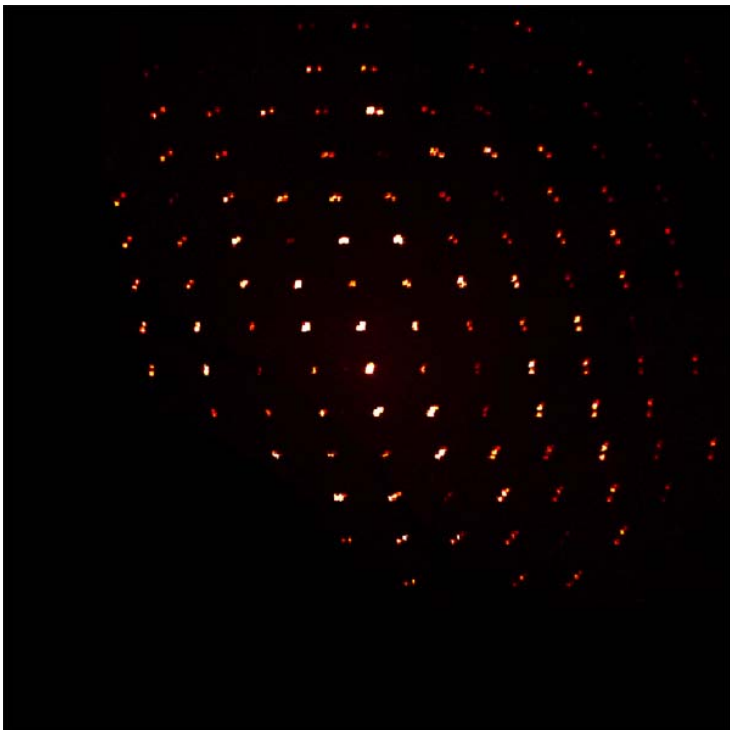


Figure 8.13 —A plane from a rotationally-twinned crystal, showing the two lattices



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9 Structure Solution and Phasing

The .hkl file (output by the Determine Space Group plug-in or, alternatively, XPREP) is all that is required to begin the structure solution and refinement process. The Determine Phases plug-in uses the SHELX suite (SHELXC, D and E) to provide an initial chain trace and phases for structure solution:

9.1 Perform SAD Phasing on the Lysozyme

9.1.1 Start the Determine Phases Plug-in

In the Task Bar's "Solve Structure" category, click the **Determine Phases** icon



The plug-in opens with the "Setup" tab active.

9.1.2 Set Up the Phasing Operation

1. Input the experimental phasing method.
2. Browse for the data files.
For SAD, the native and HA files can be different.
3. The unit cell is read in from a .p4p file or from the database.
4. Assign the correct space group.
5. Click **Run**.

Data statistics and methods are displayed.

Figure 9.1 — Resolution

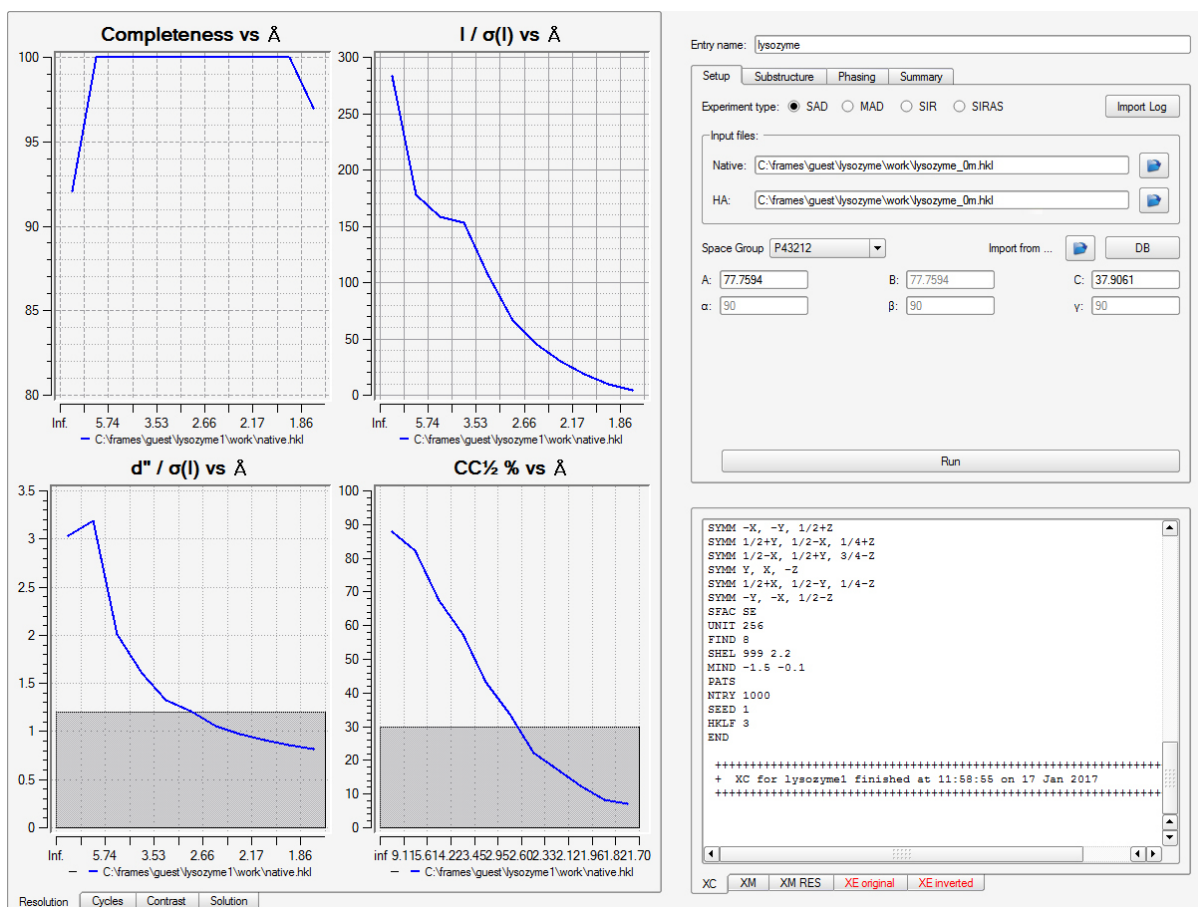


Table 9.1 — Resolution statistics descriptions

Completeness vs Å	For phasing experiments, the completeness should be above 95% for all resolution bins.
$I / \sigma(I)$ vs Å	This plot shows the signal-to-noise ratio for the measured diffraction intensities versus resolution with different data sets in different colors. As diffracted intensities are less at higher resolution than at low resolution, they become more difficult to measure, so the signal-to-noise ratio ($\langle I / \sigma(I) \rangle$) plot for the measurements should generally decrease with increasing resolution.
$d'' / \sigma(I)$ vs Å	This plot shows the signal-to-noise ratio for the anomalous differences. As the anomalous differences are measured as small differences between large numbers (the differences in the intensities between Friedel pairs) the signal-to-noise ratio for the anomalous differences is usually not very high. This quantity also decreases with increasing resolution. Anomalous data contain useful information as long as the d'' calculated by SHELXC is higher than 1.2. If data have been collected at several wavelengths, the signal-to-noise ratio should be higher for the wavelengths with larger anomalous signal (e.g., PEAK > HREM > INFL > LREM for a typical Se-Met experiment).
$CC\frac{1}{2}$ % vs Å	To a first approximation, the anomalous differences measured at different wavelengths should be related by the same constant factor for all reflections coming from the same crystal. Therefore, for ideal data, the correlation coefficient between anomalous differences measured at a wavelength i and at another wavelength j should be 1.0 or 100. In the real world, this is of course usually not the case. Nevertheless, the value of the correlation coefficient for data grouped in resolution bins is a very useful indicator of the accuracy of the measurements. Generally, the correlation coefficient goes down for increasing resolution. A correlation coefficient of less than 30% is a good criterion for truncating the data at the respective resolution. Beware that the correlation coefficient can be artificially high, if both data sets are not statistically independent or suffer from the same systematic errors. For SAD data, the correlation coefficient cannot be calculated between anomalous differences collected at different wavelengths. Instead, it is calculated by comparing different measurements of the same anomalous differences—note that this can only be done with unmerged data.

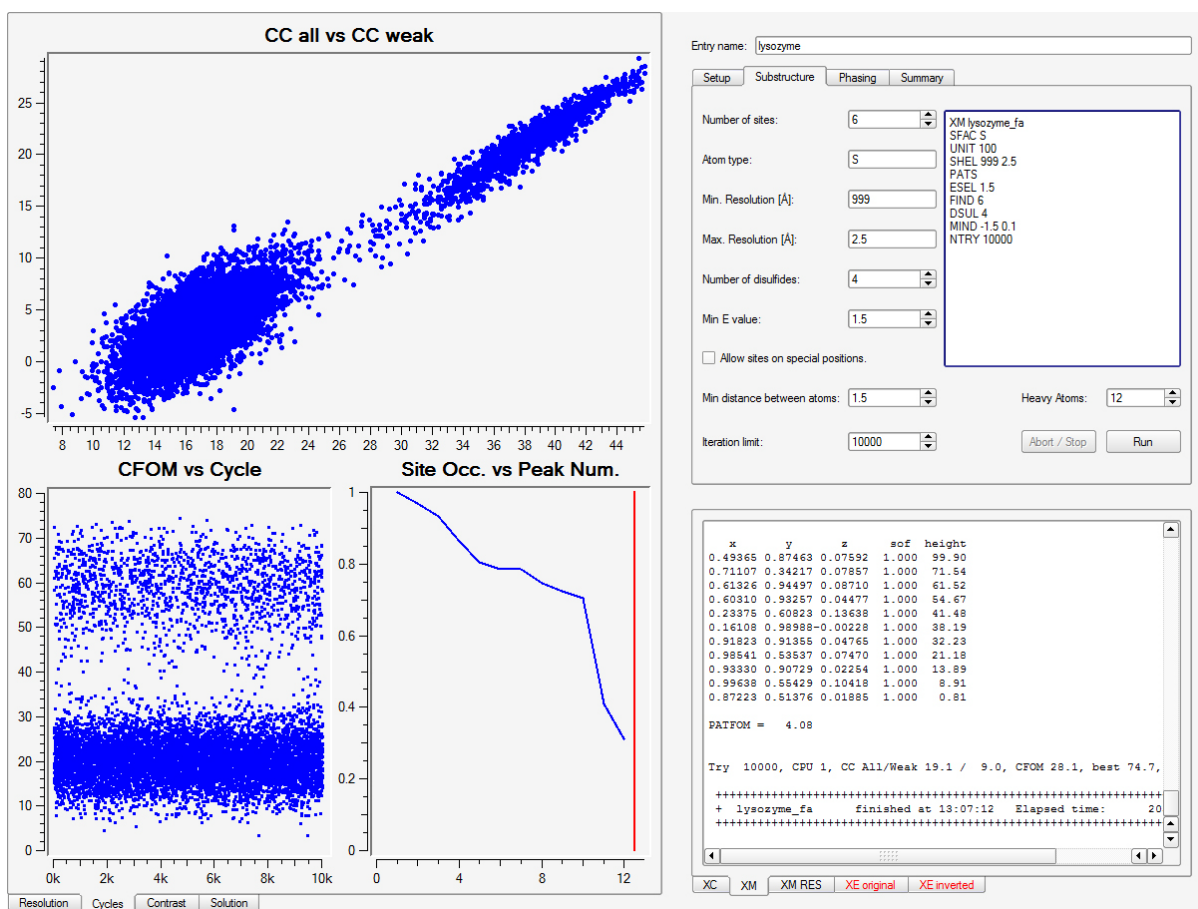
9.1.3 Find the Substructure

1. Input the number of sites based on the sequence or known metal sites.
2. Select the resolution limit.
Try lowering in steps of 0.5 Å to find a solution.
3. Select disulfides if known to be present in the structure; these should be input as a single site.
4. Set the iteration limit.
1000 trials is a good start.
5. Click **Run**.

If there are two distinct groups of solutions, that is a good sign that you have a correct substructure.

Look for a separation between the real peaks and noise for phasing.

Figure 9.2 — Cycles



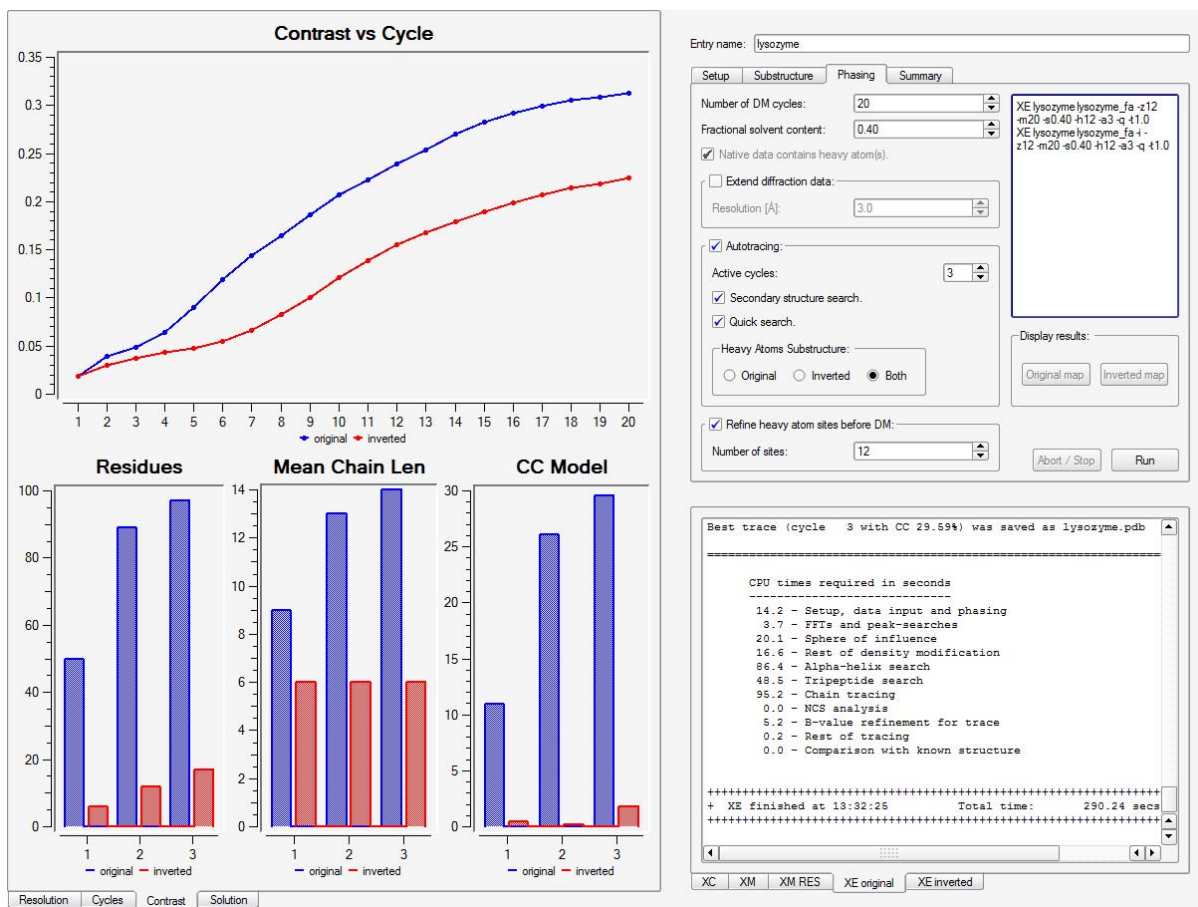
9.1.4 Perform Phasing

1. Input the number of direct methods cycles.
20 works well.
2. Select the solvent content.
3. For autotracing, 3-6 cycles is a good start.
4. Select the method of model tracing and which enantiomorph to use.
5. You can choose to refine the heavy atom sites.
6. Click **Run**.

If there is a clear difference in the contrast between enantiomorphs, that is usually a good sign.

The residues' fit, mean chain lengths, and the model's correlation coefficient are displayed.

Figure 9.3 — Contrast

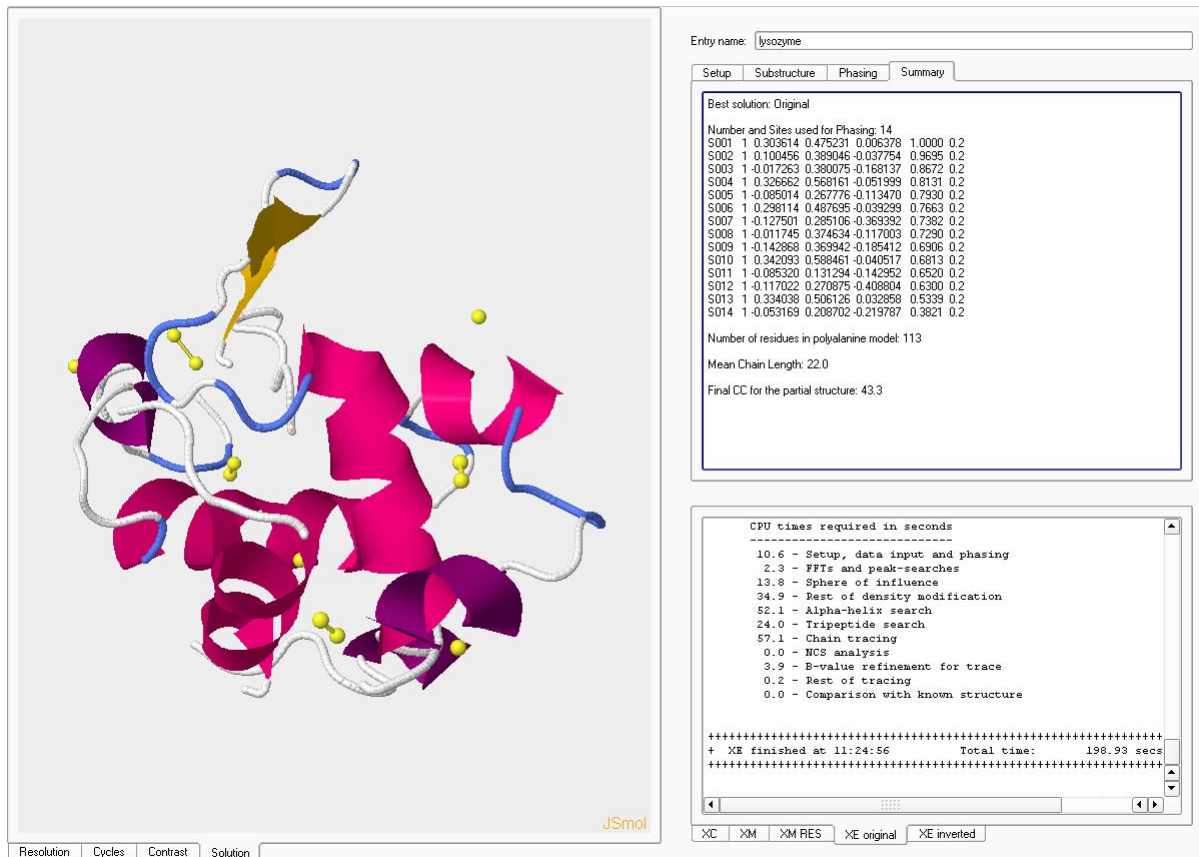


9.1.5 View the Summary

The summary highlights the results from SHELXE:

- Sites for substructure
- Number of residues traced
- Mean chain length
- Final CC for the model
- JSmol display for the trace

Figure 9.4 — Summary



The screenshot displays the Proteum3 software interface. On the left, a 3D molecular model of lysozyme is shown in a ribbon representation, with a yellow surface highlighting a specific region. The model is rendered in shades of pink, purple, and blue. On the right, a summary window is open, showing the following information:

Entry name: lysozyme

Setup Substructure Phasing **Summary**

Best solution: Original

Number and Sites used for Phasing: 14

S001	1	0.303614	0.475231	0.006378	1.0000	0.2
S002	1	0.100456	0.369046	-0.037754	0.9695	0.2
S003	1	-0.017263	0.380075	-0.168137	0.8672	0.2
S004	1	0.326662	0.568161	-0.051999	0.8131	0.2
S005	1	-0.085014	0.267776	-0.113470	0.7930	0.2
S006	1	0.298114	0.487695	-0.039299	0.7663	0.2
S007	1	-0.127501	0.285106	-0.365392	0.7362	0.2
S008	1	-0.011745	0.374534	-0.117003	0.7280	0.2
S009	1	-0.142868	0.369942	-0.185412	0.6906	0.2
S010	1	0.342093	0.588461	-0.040517	0.6813	0.2
S011	1	-0.085320	0.131294	-0.142952	0.6520	0.2
S012	1	-0.117022	0.270875	-0.408804	0.6300	0.2
S013	1	0.334038	0.506126	0.032858	0.5339	0.2
S014	1	-0.053169	0.208702	-0.219787	0.3821	0.2

Number of residues in polysalanine model: 113

Mean Chain Length: 22.0

Final CC for the partial structure: 43.3

CPU times required in seconds

```

-----
10.6 - Setup, data input and phasing
 2.3 - FFTs and peak-searches
13.8 - Sphere of influence
34.9 - Rest of density modification
52.1 - Alpha-helix search
24.0 - Tripeptide search
57.1 - Chain tracing
 0.0 - MCS analysis
 3.9 - B-value refinement for trace
 0.2 - Rest of tracing
 0.0 - Comparison with known structure

```

+ XE finished at 11:24:56 Total time: 198.93 secs

Resolution Cycles Contrast Solution

JSmol

XC XM XM RES XE original XE inverted

10 Using the Generate Report Plug-in

10.1 Starting the Plug-in



In the Task Bar's "Report" category, click the **Generate Report** icon .

The Generate Report plug-in opens, retrieves your project's data, and populates the report based on the default template.

Figure 10.1 —Generate Report plug-in: initial view

lysozyme report

Compound: lysozyme
Source: hen egg white
Sequence: 129 AA, 14313 MW
KVFGRCLEAA AMKRHGLDNY RGYSLGNVVC AAKFESFNPT QATNRENTDGS TDYGLIQINS
RWCNDGRTP GSRNLGNIPC SALLSSDITA SVNCAKGIKVS DGNCHNAWVA WEMRCKGTDV
QAWIRGCRLE

Crystal characteristics
A colorless rectangular crystal with approximate dimensions of 0.300 x 0.300 x 0.300 mm was used for data collection.
Unit cell constants: a=77.76, b=77.76, c=37.91 Å, α=90, β=90, γ=90°
Lattice type: Primitive
Laue group: 4/mmm
Space group: P4(3)2(1)2
Diffraction limit: 1.8 Å
Matthews Coefficient:

Number (mol)	Overall Probability	Vm	Solvent content (%)
1	0.998	2.0	39
2	0.002	1.0	0

Data Collection:
The sample temperature was 100 K. The PROTEUM3[®] software suite was used for data collection and reduction. The total data consists of 1 scan containing 150.0 degrees of data (table 1). The data collection time was 00.25 hr:min.

Table 1: Data collection strategy

Axis	dx/mm	2θ/°	ω/°	φ/°	χ/°	Width (")	Sweep (")	Frames	Time (s)
Phi	49.877	0.00	372.00	-150.00	23.00	0.50	150.00	300	5.00

The frames were integrated and scaled using SAINT[®] and SADABS. The relevant scaling statistics are listed in table 2.

Table 2: Data statistics

Resolution	% Complete	Redundancy	Mean I/σ	R(int)
20.63 - 7.62	86.6	5.73	49.95	0.0251
7.62 - 5.08	98.5	7.89	45.55	0.0319
5.08 - 4.00	100.0	8.37	48.72	0.0333
4.00 - 3.47	100.0	8.57	46.91	0.0372
3.47 - 3.14	100.0	8.61	40.17	0.0425
3.14 - 2.91	100.0	8.62	32.78	0.0480
2.91 - 2.73	100.0	8.65	26.52	0.0591
2.73 - 2.59	100.0	8.58	23.80	0.0629
2.59 - 2.47	100.0	8.66	21.01	0.0753
2.47 - 2.37	100.0	8.62	19.17	0.0844
2.37 - 2.29	100.0	8.76	16.99	0.0895
2.29 - 2.21	100.0	8.44	16.10	0.0995
2.21 - 2.15	100.0	8.44	13.52	0.1235

Template File: C:\bn\src\report\proteum2.html

Template Sections: No sections available.

Save Report...

Open Report in Default Application

10.2 Choosing a Template File

To change the formatting for your report, you can choose a template on which your report will be based. Template files are contained in the directory “C:\bn\src\report”.

In the plug-in’s right-hand section, choose the “proteum2.zip” template if it is not already chosen.

10.3 Creating an HTML-Formatted Report for Online or Print Distribution

To format your report as HTML, simply choose “C:\bn\src\report\apex2.zip” in the **Template File:** drop-down menu.

Bruker’s report format is an HTML file, along with necessary Java files to display the structure using the free structure viewer JSmol. The report may be viewed on any computer with Java and a web browser (Mozilla Firefox recommended). The HTML file may also be opened in Microsoft Word or other word-processing programs to produce printed manuscripts.

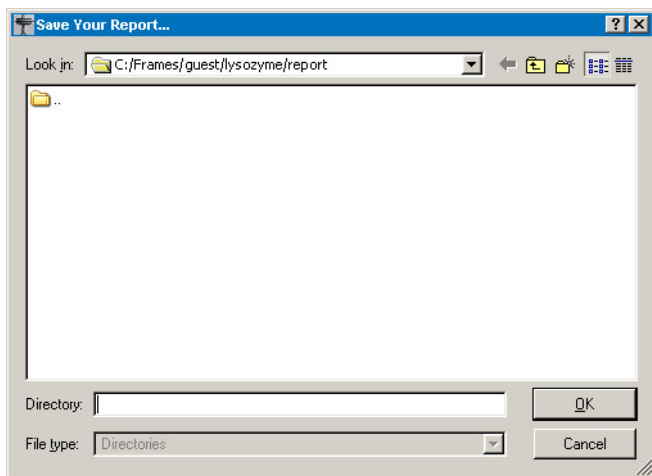
The report is shown in the “Report” tab. Additional tabs are available for specific details. It is possible to select specific bond lengths, bond angles, and torsion angles from the corresponding tabs by activating the checkboxes.

Before saving the report, add any additional information (e.g., information from your .cif file, your run lists, your contact information) to the report (for details, see Section 10.3).

1. In the lower right-hand corner, click the **Save Report...** button.

The “Save Your Report...” dialog opens.

Figure 10.2 —“Save Your Report...” dialog



2. Choose the desired directory for your report (there is no file name to type in; the final report will be named “report.html”, located in the chosen folder with the other required files).

NOTE: A “report” folder is automatically created in the work directory once the “Save Your Report...” window opens.

3. Click **OK**.

The report and additional files are saved to the specified directory (which takes a few seconds; please do not move the folder before all files are present).

11 Shutdown

11.1 Shut Down PROTEUM3

Choose **Sample > Exit**.

PROTEUM3 closes.


11.2 Shut Down BIS

Click the  button in BIS' upper right-hand corner.

BIS closes.

11.3 Stop the Measurement Server

On the computer controlling the instrument (i.e., the single computer or the Server in a dual-computer

configuration), right-click the Measurement Server icon  in the Windows Task Bar. Choose **Exit**.

The Measurement Server stops.

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12 Appendices

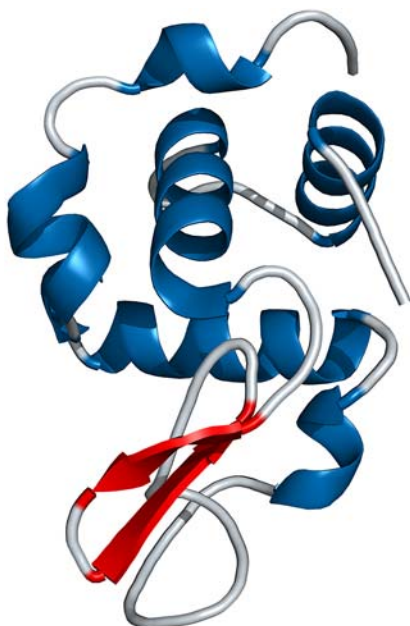
12.1 The Lysozyme Crystal

Because it is readily available, inexpensive, and easy to crystallize, lysozyme (isolated from hen egg white) is commonly used in protein crystallography as a test sample. Lysozyme crystals exhibit very strong and consistent diffraction properties, which make them ideal as standards. The crystals flash-freeze reproducibly and are resistant enough to radiation damage to allow data collection on capillary-mounted samples at room temperature.

Table 12.1 — Lysozyme information

Parameter	Value
Crystal system	Tetragonal
Space group	$P4_32_12$
Unit cell lengths (mean value of the 479 tetragonal structures in the PDB)	a = 78.50° b = 78.50° c = 38.02°
Unit cell angles	$\alpha = 90^\circ$ $\beta = 90^\circ$ $\gamma = 90^\circ$
Sequence	KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDYGILQINSRWWCN DGRTPGSRNLCNIPCSALLSSDITASVNC AKKIVSDGNGMNAWVAWRNRCKGTDVQAWIRGCR L

Figure 12.1 — Lysozyme (ribbon diagram)



12.2 The View Reciprocal Lattice Plug-in

The View Reciprocal Lattice plug-in is a very powerful tool for viewing the harvested reflections in a reciprocal lattice.

12.2.1 Start the View Reciprocal Lattice Plug-in



In the Task Bar's "Screen" section, click the **View Reciprocal Lattice** icon .

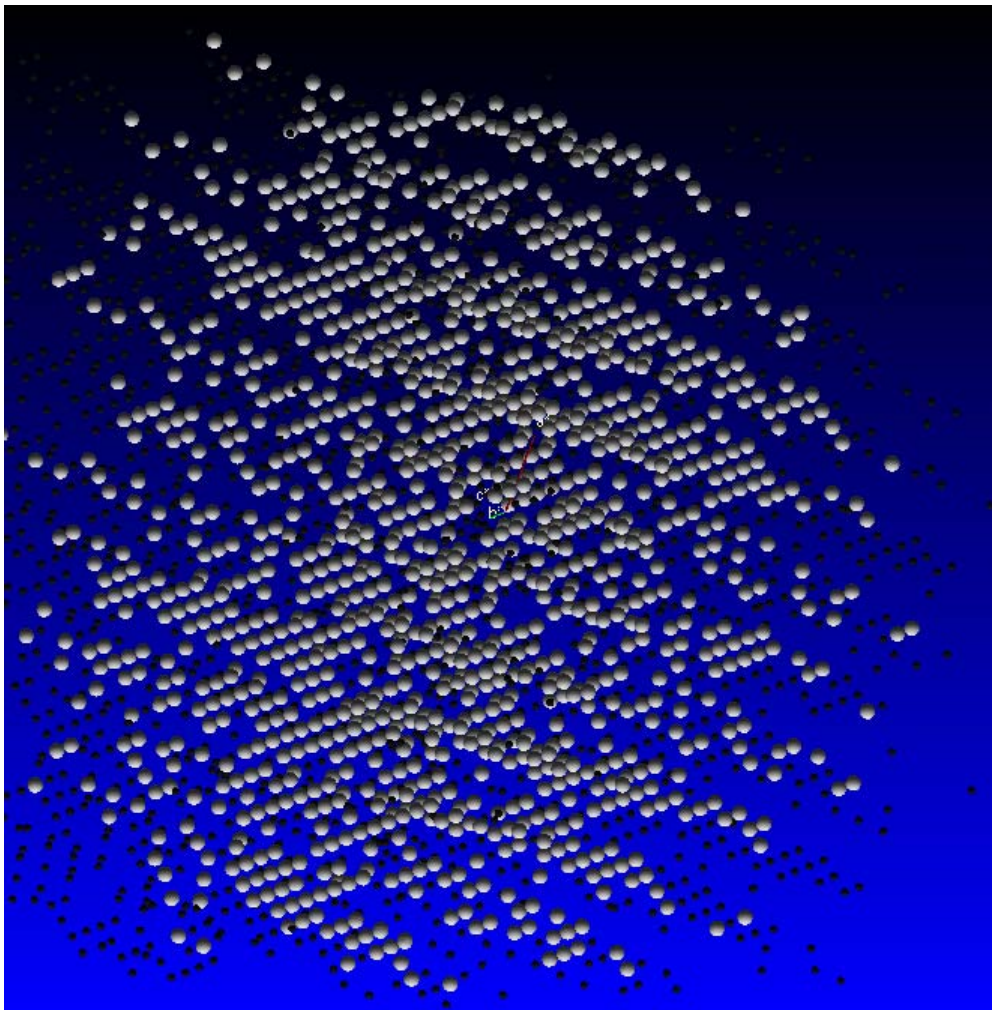
The Reciprocal Lattice Viewer plug-in opens.

The plug-in automatically reads the reflections from the current project and displays them as a reciprocal space plot.

12.2.2 Views and Tools

Figure 12.2 is a typical unoriented view after opening the plug-in. Harvested reflections are shown based on their color slot (gray in this case). Black dots are weaker reflections that were filtered by the "Intensity Filter" slider at the bottom of the screen.

Figure 12.2—Unoriented view



Other tools can be accessed by right-clicking on the reflection viewer to give the “Quick Tools” menu (Figure 12.3), and by choosing **RLATT** in the Menu Bar (Figure 12.4).

Figure 12.3 —Quick Tools menu

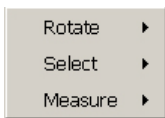
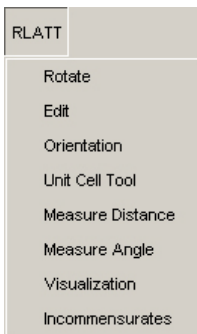


Figure 12.4 —Full menu tools



Additional context-sensitive information is available by clicking the “What’s This” help arrow in the Menu Bar.

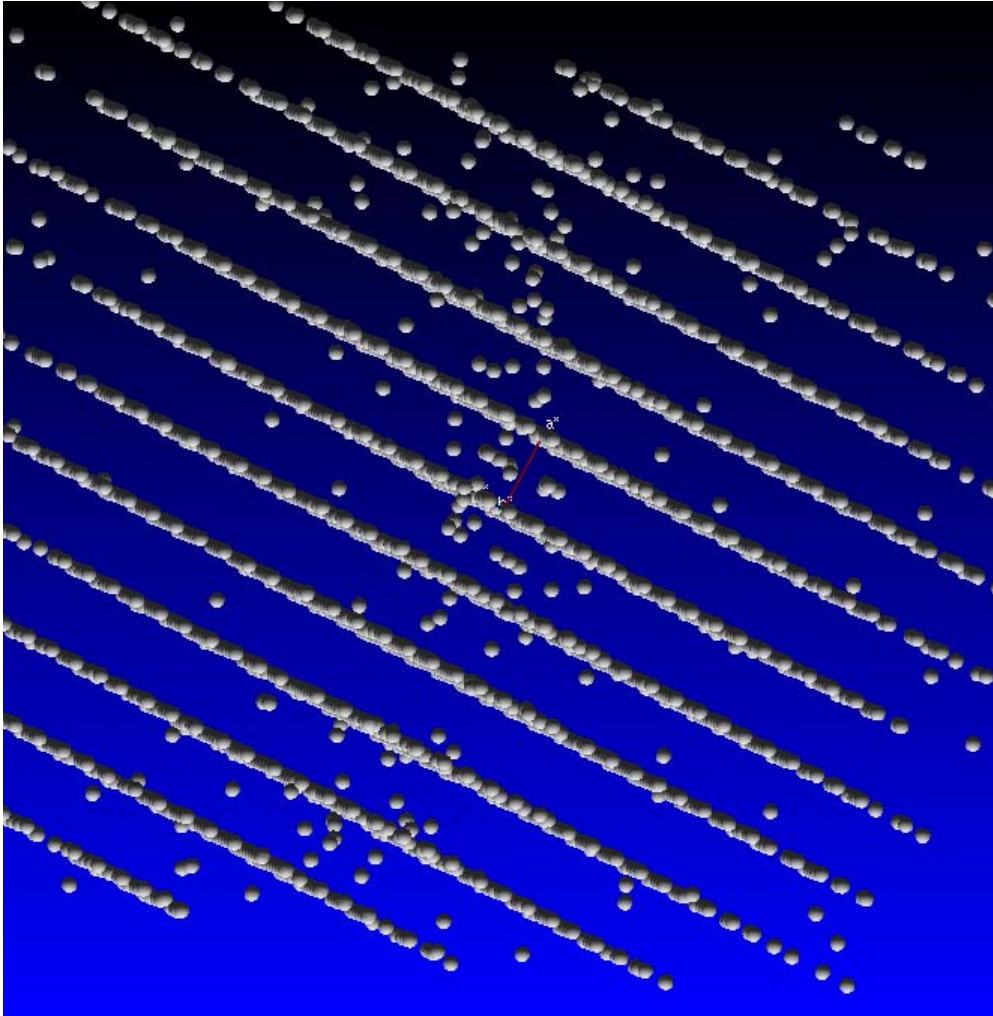
Figure 12.5 —“What’s This?” help icon



12.2.2.1 Orienting Views

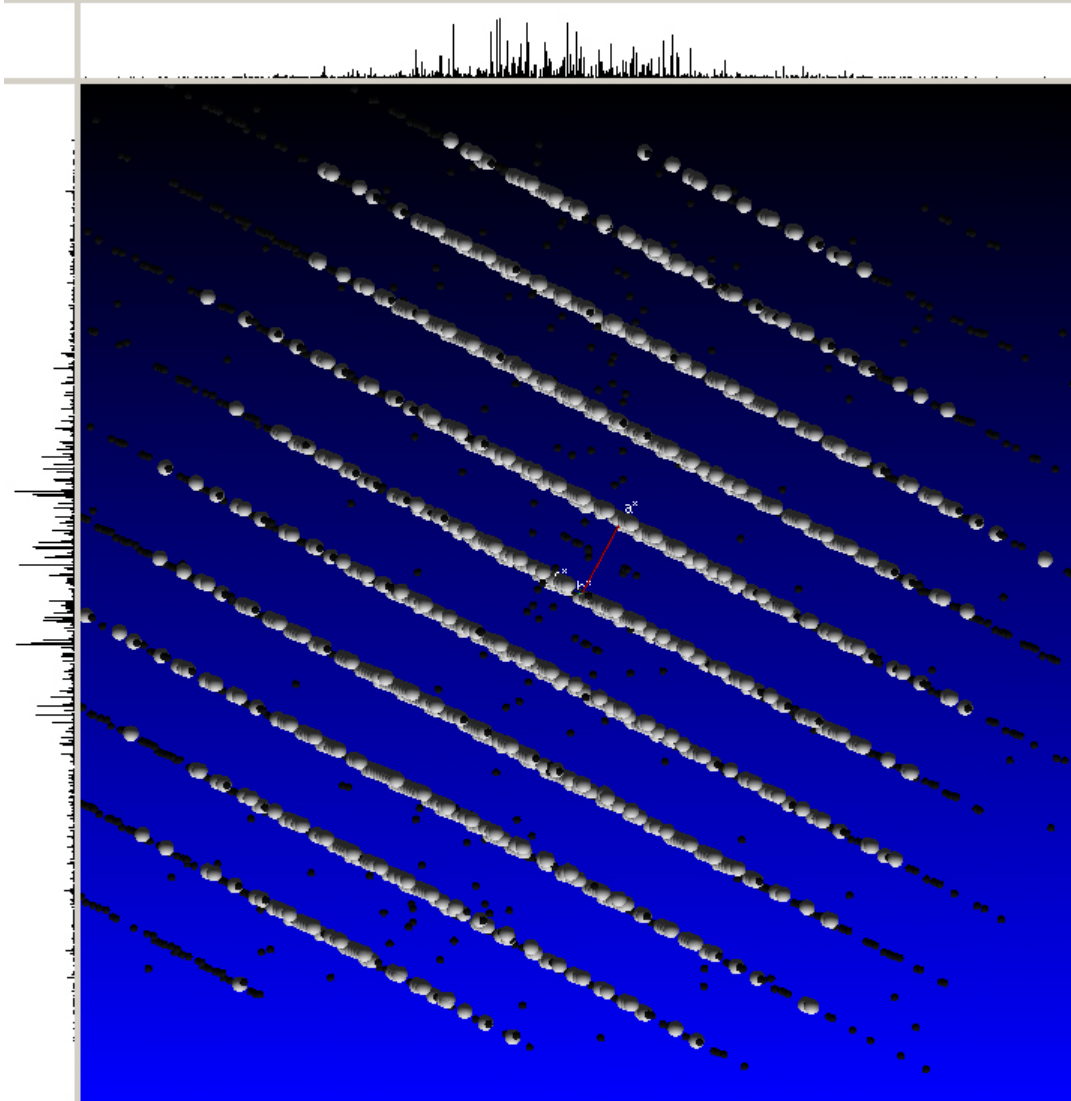
Left-click and drag the mouse to rotate the lattice display. It is possible to easily see rows and non-fitting peaks (see Figure 12.6).

Figure 12.6 —Display looking down layers of reflections



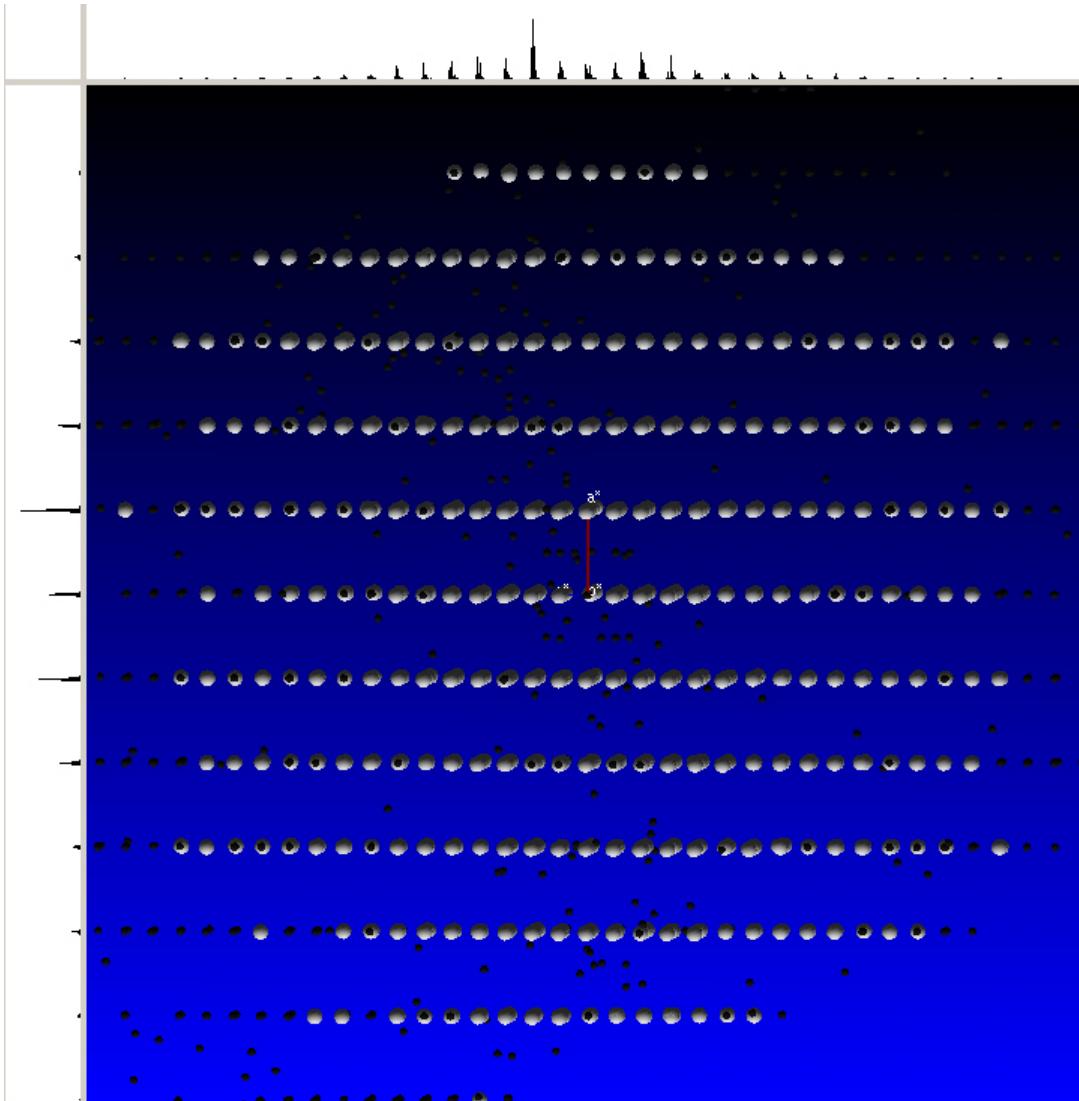
By moving the intensity slider at the bottom of the display, it is easy to deselect weak reflections. In Figure 12.7, the reflections between the layer lines all turn black when the intensity filter is moved to the left.

Figure 12.7 —Display looking down layers of reflections. Weaker reflections, i.e., those with smaller $I/\sigma(I)$, are black because the "Intensity Filter" slider has been moved to the left.



With additional rotations, the layers of reflections can be further oriented so that stacks of reflections become visible. Sometimes it is quite useful to right-click on the background and choose Z-rotations from the rotation options. The 2D profiles on the top and to the left are a valuable aid in this process. With practice, the 2D profiles can be organized into clusters. As shown, the 2D profiles are counting intensity. The counting mode can be changed to spot count or turned off completely using the Visualization menu (**RLATT > Visualization**) or by pressing the [s] key to cycle through the counting modes.

Figure 12.8 —An oriented lattice view. The 2D profiles are to the left and to the top.



The distance between layers can be measured by clicking on one of the clusters of lines in the 2D view and then dragging to the next cluster. If you drag over three clusters, then the distance as measured would need to be multiplied by three.

Figure 12.9 —Horizontal measured distance



The Orientation menu allows easy orientation if the cell is indexed (Figure 12.10). Press the [F1], [F2], or [F3] key (these can be pressed at any time to orient the view) or click the appropriate button.

Figure 12.10 —Orientation menu

Pre-Defined Orientations

1 0 0 = along a* (F1)

0 1 0 = along b* (F2)

0 0 1 = along c* (F3)

Reset View (F4)

User-Defined Orientations

A (F5) Define Display

B (F6) Define Display

C (F7) Define Display

D (F8) Define Display

HKL Layers

HKL Layer Mode = DISABLED

*KL * Layer -20 0 +20

H*L * Thickness ±0.0 ±0.5 ±1.0

HK*

Runs Displayed

Scan 1

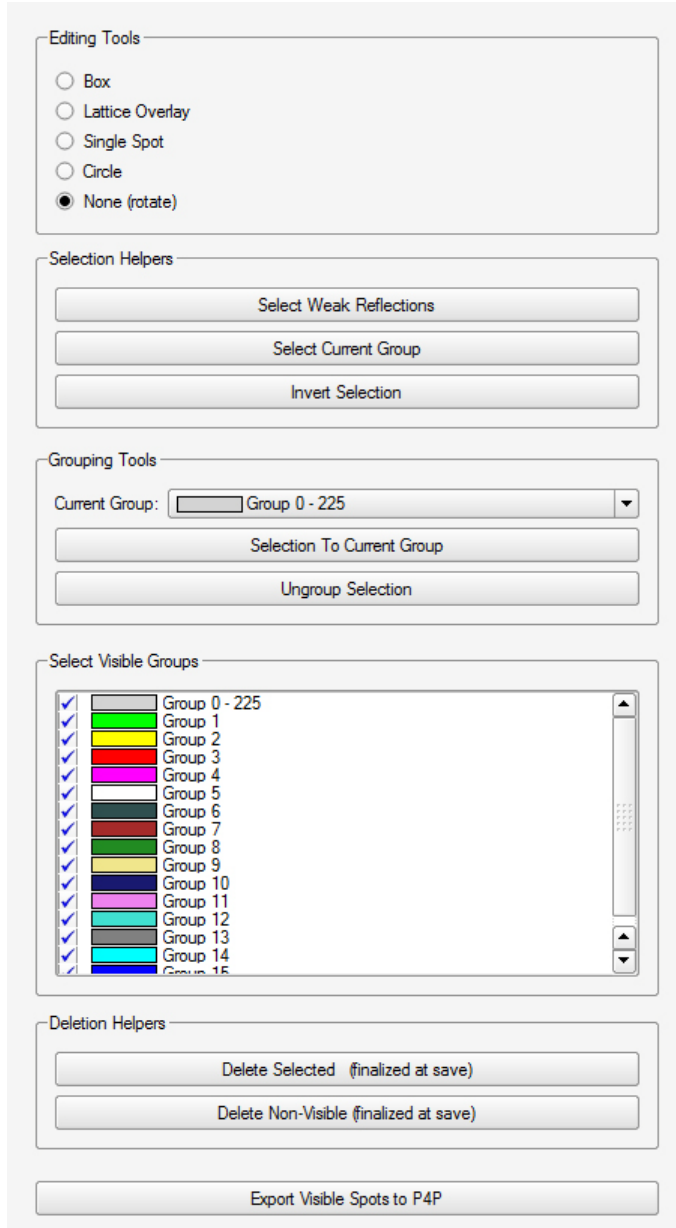
Scan 2

Scan 3

12.2.2.2 Editing Options

The Edit menu is already shown in the right work area, when the plug-in is opened (Figure 12.11). You can also show it by selecting **RLATT > Edit**. It contains useful editing tools for selecting, sorting and deleting reflections.

Figure 12.11 —RLATT edit menu



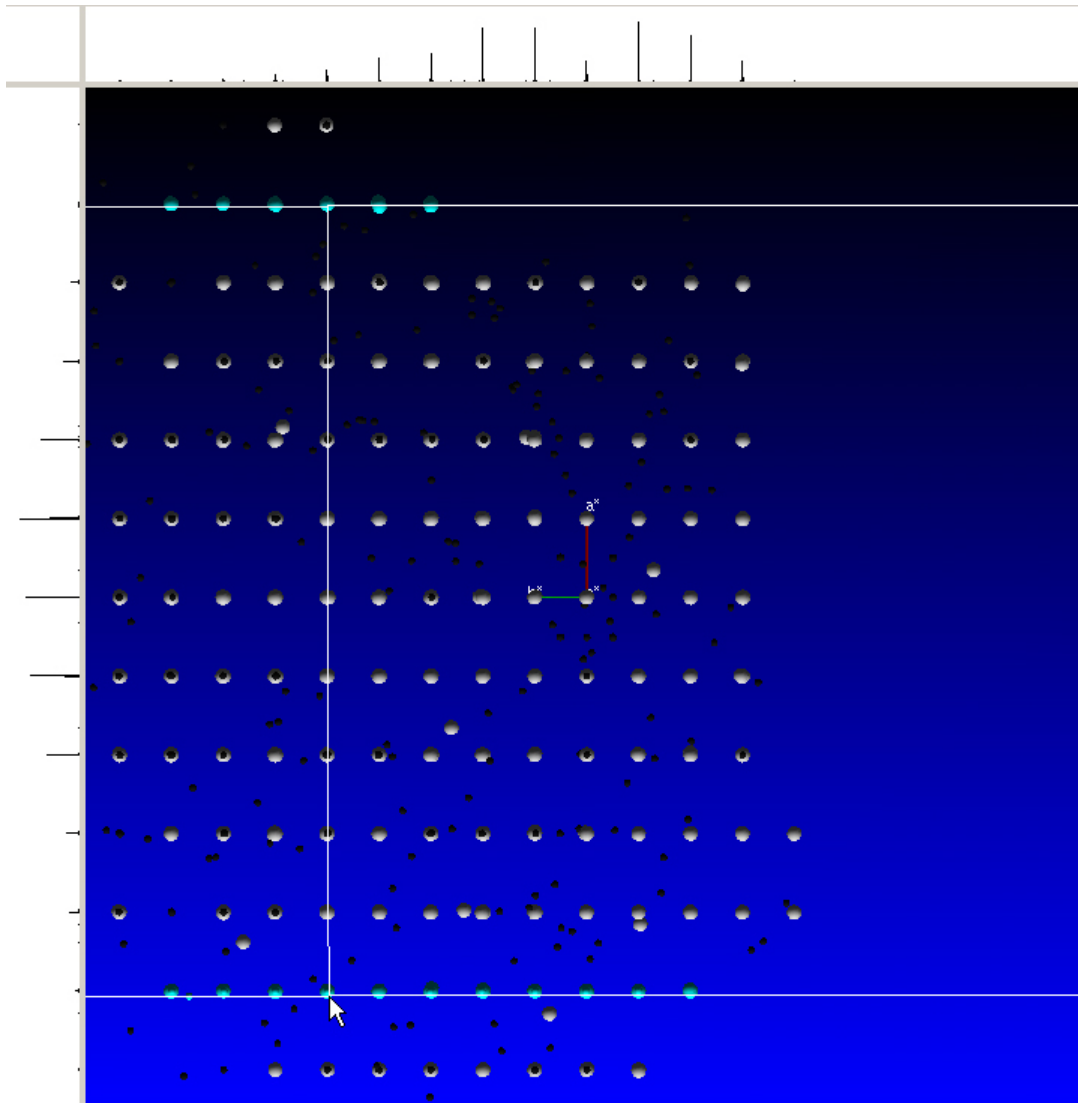
There are four different reflection selection tools available, which work in the reflection view area using the mouse control. You can either select reflections by drawing a Box, a Circle, or by clicking on individual reflections (Single Spot).

The most useful selecting tool is Lattice Overlay. When activated, you move the mouse pointer to the origin of the selection (normally a trusted reflection), left-click and hold the mouse button and finally drag the mouse pointer to another location. Normally you want to select reflection rows, so the perpendicular lines between the selected points should line up with the reflections. Once you release the mouse button, all reflections ending up on the perpendicular lines are selected (Figure 12.12).

You can hold the [Alt] key down while selecting your starting point. This will lock to the centroid of the reflection on which the mouse pointer was placed.

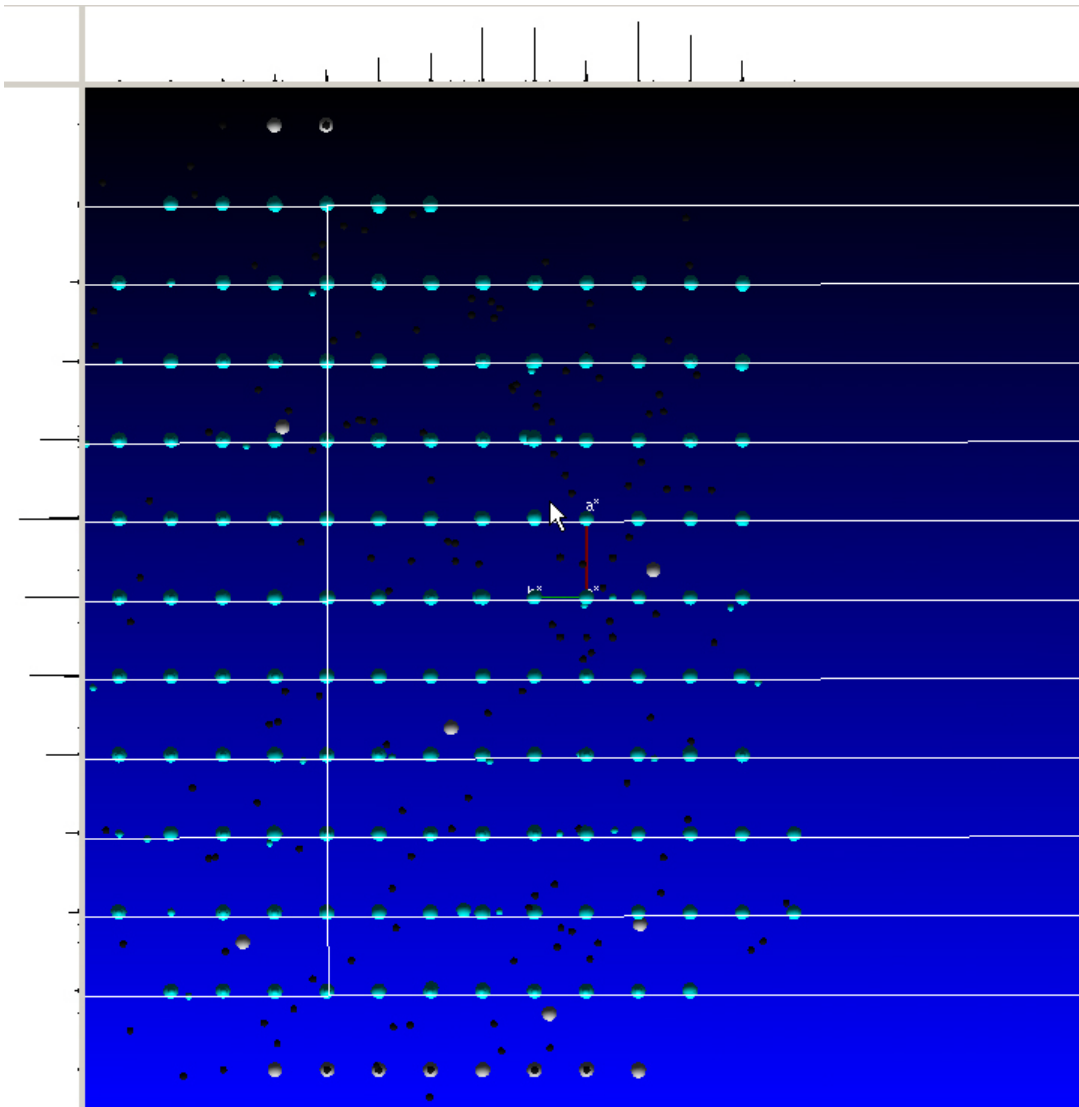
If you want to start over, click on another spot and repeat the process.

Figure 12.12 —Two lattice lines selected



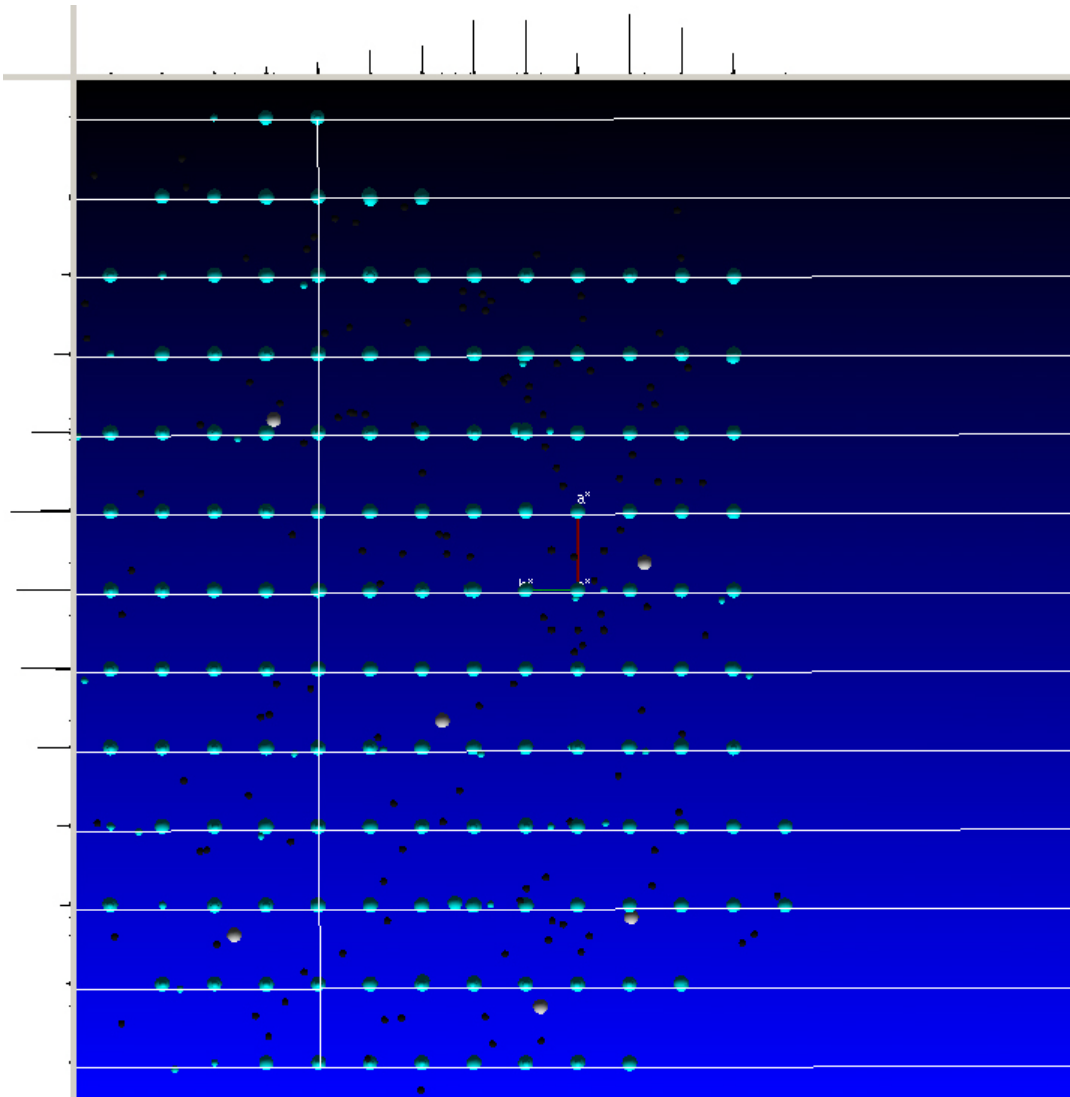
You can press the [+] key to add lines between the two you have marked. Every time you press the [+] key, one additional line appears. The [-] key removes one line.

Figure 12.13 —View with most of the lattice lines selected (lines to the top and bottom are not selected).



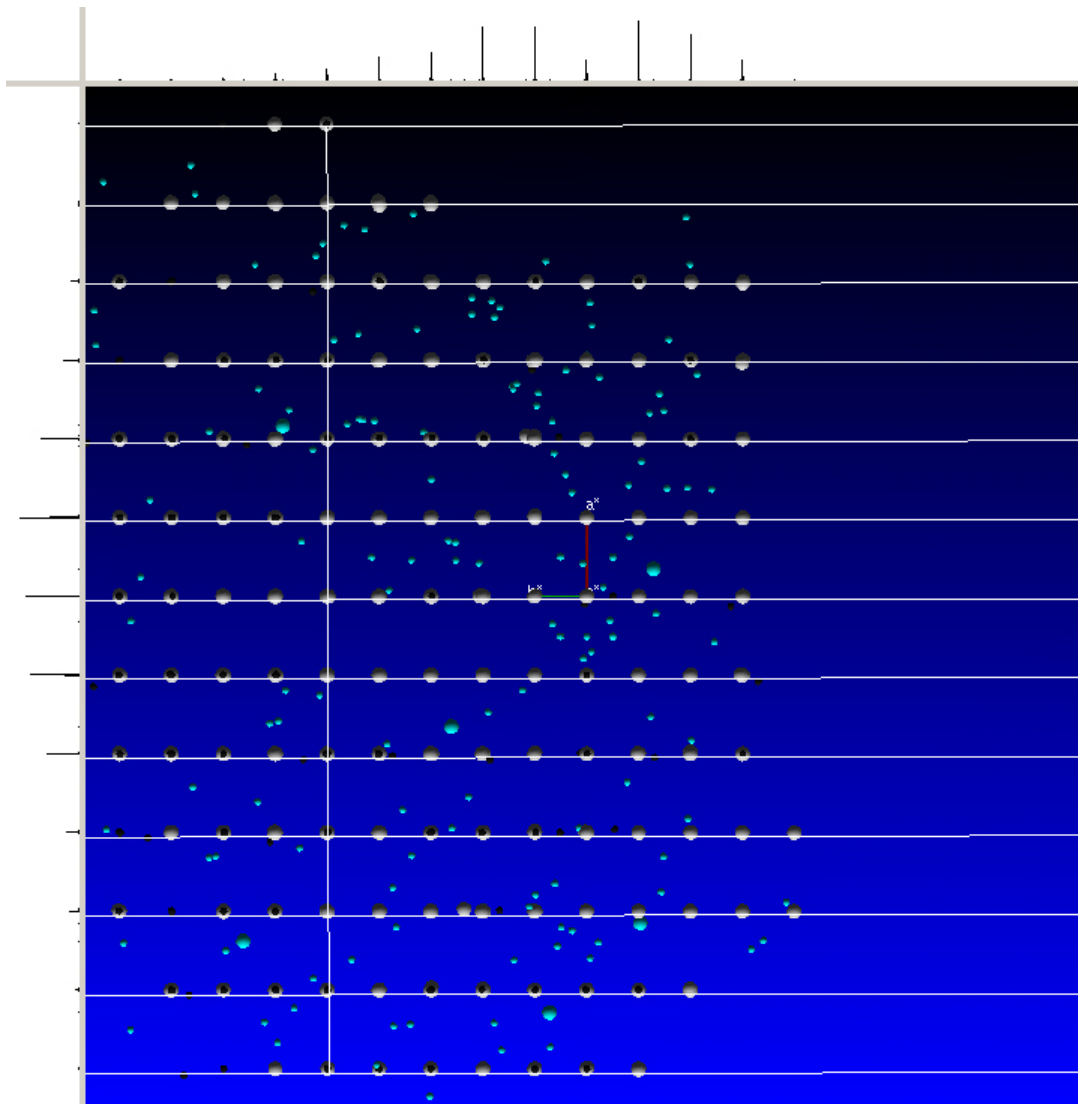
The [Page Up] key adds lines to the outside of the previously-selected line with the same line spacing. [Page Down] removes lines from the outside (Figure 12.14).

Figure 12.14 —A view with all the layers selected



Click **Invert Selection** under “Selection Helpers”. Now, all the non-fitting spots that did not touch the layer lines are selected.

Figure 12.15 —Spots selected between layers



You can click **Delete Selected (finalized at save)** to mark the selected reflections to be deleted. First, they turn black and could be in principle selected again. They are finally removed from the reflection slot(s) if the **Save** button is clicked or the plug-in is closed.

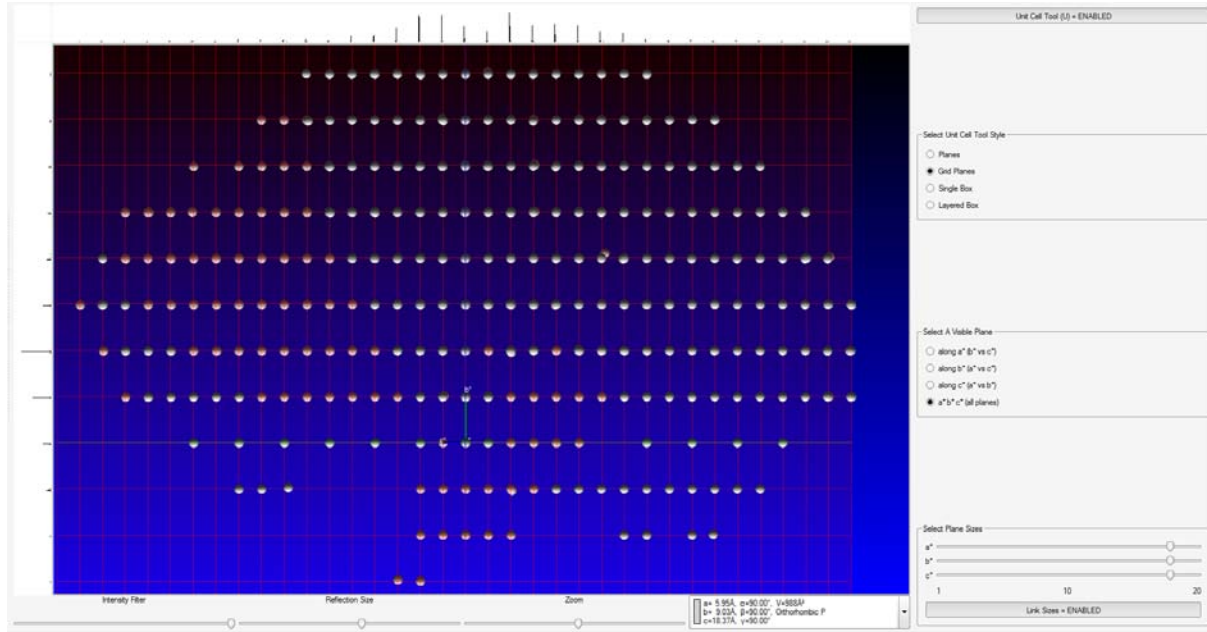
Alternatively, these selected reflections can be added to a group by choosing a color with the “Current Group” combo box and clicking **Add to Current Group**.

You can hide the newly-assigned group by deactivating its checkbox in the “Select Visible Groups” section.

12.2.2.3 The Unit Cell Tool

Enable the Unit Cell tool either by using the [u] key or by choosing **RLATT > Unit Cell Tool** and clicking the **Unit Cell Tool** button at the top, to put a unit cell lattice in the reflection viewer. This allows you to see if reflections are actually falling on the corners of the box (see Figure 12.16). **Select Plane Size** determines the boundaries of the planes and the number of unit cells displayed.

Figure 12.16 —Unit Cell tool enabled

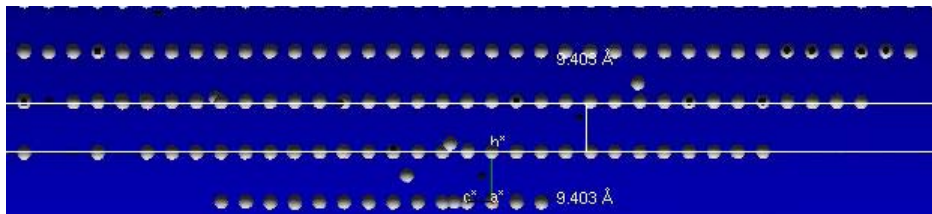


12.2.3 Measuring Distances and Angles

Right-click on the background, and choose **Measure** to get tools for measuring distances and angles (or select the Options from the **RLATT** menu).

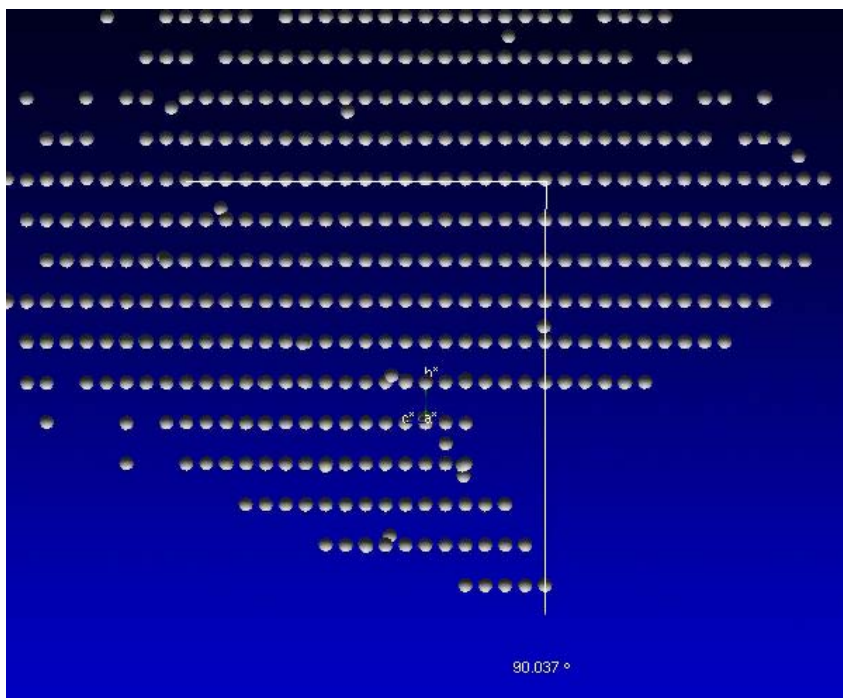
The Measure Distance tool gives two lines, much like the lines in the Lattice Selection tool, that can be oriented and dragged to get lattice spacings (Figure 12.17).

Figure 12.17 —Measure distance tool



With the Measure Angle tool, left-click, hold, and drag a line on a lattice layer ending on the spot that will become the vertex of the angle. When the mouse is released, that point becomes the pivot point for a new line. Moving the mouse with no buttons depressed gives angle measurements (Figure 12.18). As described, the angle measurements will be done in 2D mode (i.e., the angle between the two lines displayed). To measure an angle in 3D mode, hold the [Alt] key while selecting spots as above. The tool will then lock on to spot centroids and the angle will be calculated using spot coordinates. This eliminates errors that might result from measuring using the 2D projection of the current orientation.

Figure 12.18 —Measure angle tool



12.2.4 Writing a .p4p File

At the bottom of the **Edit** menu, there is a button for exporting all visible reflections to a .p4p file. The .p4p file is a text file of crystal, instrument, and reflection information. If the cell and crystal orientation is known, that information is included also (otherwise, dummy values are included).

The sequence of cleaning up a set of reflections using the Reciprocal Lattice Viewer plug-in and then exporting the results for input to CELL_NOW is a useful tool for dealing with hard-to-index crystals.

12.3 SaintChart Output Reference

12.3.1 Run Selector (Top Right Combo Box)

This drop-down list contains entries for each run that is integrated, plus the initial passes that are made before each run. When the integration has finished, an additional "Integration Finished" entry is added.

Selecting an entry from this list will update the charts and graphs on the left-hand side to the corresponding run. If "Integration Finished" is selected, the global statistics for the whole data set will be displayed instead.

Entries can be selected while the integration is still in progress (it will continue running in the background).

12.3.2 Twin Component Selector

This list contains an entry for each domain of a twinned crystal and an additional entry for the combination of all domains. Selecting an entry from this list will update the charts and graphs on the left side to the corresponding domain(s).

Only the "All Components" entry will be available if data from a non-twinned crystal is integrated.

12.3.3 Chart Selector

This list, along the right-hand side of the screen, contains a checkbox for each chart or graph that is available.

A chart/graph window appears in the "Charting Area" on the left-hand side when one of the checkboxes is activated. Likewise, the window disappears when the checkbox is deactivated. Alternatively, a window can be closed by clicking the "X" symbol in the upper right-hand corner of the chart/graph window.

The various charts and graphs are grouped in the following categories:

- **Progress Statistics:** Statistics on the progress that has been made integrating the data and the estimated remaining time for each run and for the integration at large to finish.
- **Per-Image Statistics:** Charts/graphs in this group are updated with each image that is integrated.
- **Periodic Statistics:** Charts/graphs in this group are updated in periodic intervals when a local least squares refinement of the crystal and instrument parameters is performed.
- **Coverage Statistics:** Charts/graphs in this group contain statistics about the data set after the integration process has finished.

12.3.4 Default SaintChart Items

12.3.4.1 Spot Shape Correlation

The Spot Shape Correlation graph is the best indicator for a successful integration. It plots the degree of correlation between the measured 3D reflection profiles and the model 3D profiles computed from the strong spots.

- For well-diffracting crystals with properly-chosen exposure times, the spot shape correlation should be in the range of 0.7 or better.
- For weak diffractors, the spot shape correlation will be lower but should be at least 0.4 if the exposure time is chosen correctly.
- Correlation factors below 0.4 indicate a serious problem with the indexing, the experimental setup, or both. However, a very low spot correlation factor may occur for twins with a significant number of overlapping reflections—this is normal.

You can investigate any unusual features in the spot shape correlation by checking the images corresponding to the region of concern using the Movie Tool. To start the movie, right-click in the Image Display Area and choose **Movie**.

12.3.4.2 Average Spot Intensity and I/Sigma(I) Values

Spot intensity and I/sigma plots the average intensity and average I/sigma (intensity divided by standard deviation) for the integrated reflections on a given image.

12.3.4.3 Spot Shape Profiles by Detector Region

Spot shape profiles displays the model 3D reflection profiles in pixels for the nine regions of the detector. Holding the left mouse button down while moving the mouse enables the box to be rotated.

In general, each 3D profile should be centered in the box but the spot shapes may change from region to region. Multiple or deformed 3D profiles indicate that your choice of initial box size or indexing may be incorrect. Split spot shapes may indicate diffraction from split crystals or twins.

12.3.4.4 Average Difference Between Observed and Predicted X, Y, Z

X, Y, Z error plots the average difference in pixels (X and Y) and frame number (Z) between the observed and predicted reflection positions on a given frame. These parameters are useful indicators of the plug-in's ability to track reflections.

Errors in X, Y, and Z should be small (less than 1 pixel) and should not vary during the integration of a run. Large variations indicate problems with slipping crystals, misalignment of the instrument, or other problems.

12.3.5 Non-Default SaintChart Items

Table 12.2 — Other SaintChart display outputs

Display	Description
Connected	This indicator shows whether the SAINT integration engine is running in the background.
Integration in Progress	This indicator shows whether the integration process is still in progress. This is useful, because even if no changes to the charts and graphs or to the log file can be seen, the integration engine may still be working.
Stop Integration	Clicking this button will stop the integration process.
SAINT Log File Area	This is the text output from the SAINT integration engine. It contains a textual representation of the same information as the graphical charts, plus some additional data that is not available in the charts and graphs.

12.4 Using CELL_NOW

CELL_NOW is an extremely powerful tool for determining unit cells for difficult crystals. It analyzes a list of reflections to find a cell and orientation matrix despite the presence of several twin domains or other problems.

In initial search mode, the program tries to find sets of reciprocal lattice planes that pass close to as many reflections as possible. The corresponding real space vectors are sorted on a figure of merit (1.0 being a perfect fit). After the vector list has been output, CELL_NOW attempts to suggest a suitable cell. This will not necessarily be the conventional cell, so it should be checked using XPREP.

Once a cell is found, it may be rotated to locate further twin domains iteratively using only the reflections that have not yet been indexed.

If CELL_NOW fails to suggest a sensible cell, for example something may be seriously wrong with the reflection list (e.g., a wrong detector distance) or a cell axis is longer than the given search range.

12.4.1 Running CELL_NOW on a List of Reflections

1. Open a Command Prompt window in your “\work” directory by choosing **Sample > Run Command...**

The exported .p4p file should be available in this directory.

2. For Windows or Linux, enter the command `cell_now -t`.

The terminal prints out an explanation of the program, which contains more detailed information about its working principles than is found in this Section’s introduction.

3. CELL_NOW requests the name of the input file and suggests a name for the output file. Enter the name of the input file including the file extension (for the output file, press [Enter] to accept the default).

4. Press [Enter] to start a general search.

Initial search (<Enter>) or specified cell search (S):

5. If the default superlattice threshold is suitable (which it normally is), press [Enter].

Superlattice threshold: an axis will be rejected if less than this percentage of reflections has indices not equal to 2n or 3n resp. [10]:

6. If the default minimum and maximum distances are acceptable, press [Enter] again.

The default range is 5 to 45 Å, which may not fit your crystal. In order to get an impression about the unit cell dimensions, you can use the **Measure Distance** tool in the “View Reciprocal Lattice” plug-in.

NOTE: Usually CELL_NOW is used to search generally for a cell, but if the cell is known, choose the specified cell option and enter the cell dimensions. CELL_NOW will then search for a matching cell.

After some period of time, CELL_NOW presents a list of possible unit cells. This will not necessarily be the conventional cell, so it should be checked using XPREP (even possible without an .hkl file) taking the lattice type found with CELL_NOW into account. If necessary, this conventional cell may then be used in “specified cell” search mode to find the orientation matrix—or the transformation matrix from XPREP can be used to reorient a cell (R option).

The following cells would appear to be plausible, but should be checked using XPREP because they are not necessarily the conventional cells.

```
FOM, % with i      n 0 .2, a..gamma, volume and lattice type for potential
unit-cells:
      1      1 .000      85.3      12.692      11.016      14.648      89.70      90.95      90.00
2047.7 P
```

```
-----
Cell for domain 1:  12.692  11.016  14.648  89.70  90.95  90.00
Figure of merit: 0.619  %(0.1): 68.3  %(0.2): 85.3  %(0.3): 91.0
```

CELL_NOW has identified a domain that fits 85.3% of the data with a tolerance of 0.2 (deviation from integer HKL indices).

7. Enter the maximum deviation from integer index cutoff (the default of 0.25 is somewhat high; normally use 0.1 or 0.15). Press [Enter].

```
New cell from list (number), reorientate (R), accept (A) or quit (Q) [A]: A
```

8. Enter A or just press [Enter] to accept this cell. The program asks for a name for a .p4p file. Enter the name of the .p4p file for this solution (in this example, domain1.p4p).

```
.p4p or .spin file to write domain to:domain1.p4p
```

```
RLATT color-coding employed in file: domain1.p4p
```

```
White: indexed for first domain
```

```
Red: not yet indexed
```

```
      877 reflections within 0.250 of an integer index assigned to domain 1,
      877 of them exclusively;  122 reflections not yet assigned to a domain
```

9. 122 reflections, approximately 1/10 of the data, have not yet been indexed (assigned to a domain). This is more than might be expected for random noise peaks. A search for an additional domain makes sense. The domain 1 cell will be rotated to locate further twin domains iteratively using only the reflections that have not yet been indexed. Enter S to search for another orientation of the newly chosen cell.

```
Re-refine initial cell (R), search for next domain (S), quit (Q) or choose new
cell from list (enter number) [S]:S
```

```
Cell for domain 2:  12.692  11.016  14.648  89.70  90.95  90.00
```

```
Figure of merit: 0.643  %(0.1): 69.7  %(0.2): 97.5  %(0.3): 98.4
```

```
Orientation matrix:  0.00598966  0.00402327  0.06807401
                   -0.03367313 -0.08180340  0.00480114
                   0.07099302 -0.03914713 -0.00221035
```

```
Rotated from first domain by 179.7 degrees about
```

```
reciprocal axis 1.000  0.001 -0.032 and real axis 1.000  0.002 -0.010
```

```
Twin law to convert hkl from first to      0.999  0.004  -0.019
this domain (SHELXL TWIN matrix):          0.003 -1.000  -0.004
                                           -0.064  0.006  -0.999
```

CELL_NOW has identified an additional domain that fits 97.5% of the remaining data with a tolerance of 0.2. The relationship between this domain and the first domain and the SHELXL TWIN matrix are reported. This example is for a rotational twin about the a* axis.

10. Enter the maximum deviation from integer index cutoff for the second domain.
11. Enter the name of the .p4p file for this solution (in this example "domain2.p4p").

```
.p4p or .spin file to write domain to:domain2.p4p
```

```
RLATT color-coding employed in file: domain2.p4p
White: indexed for first domain
Green: current domain (but not in a previous domain)
Red: not yet indexed
```

```
691 reflections within 0.250 of an integer index assigned to domain 2,
119 of them exclusively;      3 reflections not yet assigned to a domain
```

```
Re-refine initial cell (R), search for next domain (S), quit (Q) or choose
new cell from list (enter number) [Q]
```

12. Check the number of indexed reflections, and how many reflections belong exclusively to the second orientation.

If the program does not find a suitable twin rotation, it will find a solution that indexes the first domain again. The rotation angle is then close to 0° , and almost no reflections belong exclusively to the second domain.

13. Enter <q> to exit CELL_NOW, only 3 reflections are not indexed (and therefore it makes no sense to search for further twin orientations).

```
C:\struc\guest\twin>
```

NOTE: At this point CELL_NOW has indexed a two-component twin. The orientation matrices for the two components are in the "domain2.p4p" file.

12.5 Configuring the Suite

The Bruker Suite includes the ability to customize many features of the program. Most customizations should be left to the site administrator, but some introduction is appropriate.

The following is a collection of examples of possible changes to the configuration files that control the look and feel of the GUI.

12.5.1 The bn-config Configuration File

The appearance and behavior of this Suite of programs can be modified by a configuration file. The programs read a series of configuration files in following order. As described below, a specification in a later file overwrites a setting in an earlier file.

For Windows: these files are (in order):

1. bn-config.py in the system drive folder (C:\)
2. bn-config.py in the "USERPROFILE" folder
3. bn-config in the current folder

For Linux/UNIX: these files are (in order):

1. /usr/local/lib/bn-config.py
2. .bn-config in your home directory (Please note the initial "." character, which renders this file invisible for the normal `ls` command).
3. bn-config in your current directory.

If none of these files are present (e.g., when the program has just been installed), all parameters use built-in defaults. To change a single configuration parameter, create or edit one of the three files and put in a value for the parameter to be customized. You should:

- put a parameter in (1) if it should be the system default for all users;
- put it in (2) if it is your own default, and other people might want to use other values; and
- put it in (3) if it is only required for a single project.

The configuration files are read as Python programs, and therefore syntax is very important. The best way to use it is to only use variable assignments or function calls. If required, you can comment out lines from your configuration file by preceding them with a # character.

NOTE: Leading space is significant. Start all assignments in the first column.
Variable names are case sensitive.

NOTE: PROTEUM3 needs to be restarted before the changes are applied. Some parameters are stored within the project, therefore only new projects will have the changed defaults.

12.5.2 Configuration File Examples

12.5.2.1 Comments

A few notes about Python comments:

Three quotation marks in a row on a line (""") start a comment section. The next three quotation marks (""") end the section.

The “pound” or “number sign” character (#) starts a simple comment. This can be anywhere on a line.

12.5.2.2 Default Color Scheme for Image Display

The default color scheme for the image display is given in:

```
colorramp= 'Colored_SCD.ICM'
```

This is the typical BAXS orange display.

The most frequently used other color choices are:

```
Black_On_White_SCD.ICM
```

```
White_On_Black_SCD.ICM
```

```
The_Blues_ANY.ICM
```

Other color ramps are in:

- Windows: C:\bn\src\gui\intensitycolormaps
- Linux: /usr/local/bn/src/gui/intensitycolormaps

12.5.2.3 User Positions

Up to four user positions can be activated in the Center Crystal and Screen Crystal plug-ins.

The detector distance is specified in millimeters. The goniometer angles are specified in degrees. To activate a user defined goniometer position, its userpos<#> variable and at least one of the positional variables must be uncommented. For instruments with a manual detector track, the detector distance variable (dx) should normally not be uncommented because of the risk of setting the distance to a different value than the actual distance. The chi angle setting must be uncommented for instruments with a Kappa goniostat.

An example user position:

```
userpos1 = "User Position 1"  
userpos1_dx = 40.0  
userpos1_2theta = 23.0  
userpos1_omega = 23.0  
userpos1_phi = 90.0  
userpos1_chi = 54.74
```

12.5.2.4 Automatically Connect to BIS

Automatically connect to the instrument when needed:

```
autoconnect = True
```

NOTE: This is a protected variable that is controlled by the System Administrator.

12.5.2.5 Frames in a Network Shared Folder

IP address or DNS name of the file server used for storing images, followed by the name of a network shared folder on that server. Add additional path segments as comma-separated and quoted strings:

```
fileserver=('x8-client', 'frames')
```

To find out the DNS, enter the following at a command prompt:

- Windows: `echo %computername%`
- Linux: `hostname`

12.5.2.6 Supported Filename Protocols

Currently-supported filename protocols are `smb` for files that are accessed over a Microsoft Windows network, and `file` for local files:

```
universalfnprotocols=('smb', 'file')
```

12.5.2.7 Scan Parameters for 2theta Dependency

These settings adjust the behavior of the “Calculate Strategy” plug-in’s “Scan Parameters” window (Section 6.2.4):

There are a few parameters you can tune:

```
strategy_exponent = 3.000
strategy_frametimehigh = 20
strategy_frametimelow = 5
strategy_slottimes = [2.0, 4.0, 8.0, 16.0]
strategy_thetadependent = False
strategy_timedivision = 0.1
strategy_twothetadivision = 1.0
```

Systems that typically always measure with theta-dependent times should set `strategy_thetadependent` to `True`. This has a direct effect on the sorted sequence. Otherwise the runs are sorted for a flat time schedule. Of course, you can also switch the option on, click **OK**, switch to the “Edit” tab, and sort the data. You can do that anyway when you make significant changes in the setup.

Systems that take dark images can set `strategy_timedivision` to an integer value; the classic value would be 5. This will somewhat restrict the possibilities of the time slot fitting.

12.5.3 Functionality Groups in the Configuration File

Sometimes a complete group of options need to be changed to get a specific functionality. For these cases, there are function calls that change a number of variables at once.

Due to the “default-like” nature of these function calls, they should probably be used only in the site-wide configuration files, and even there only at the beginning (but this is not enforced).

The available functions are:

- `default_protein()`

This switches from the default “small-molecule” parameters to more protein-like parameters. At this time, the following parameters are set:

```
chiralonly=1
autochiralpointgroup=1
resomode=1
resolution=2.5
```

- `default_noprotein()`

This sets the defaults back to small-molecule values.

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