APEX2
User Manual

Version 1.22

USER MANUAL

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Version 1.22

This manual covers the APEX2 software package. To order additional copies of this publication, request the part number shown at the bottom of the page.

Notice

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1. Introduction

1.1 APEX II Systems for Chemical Crystallography

Bruker AXS Kappa APEX II and SMART APEX II systems are the newest members in the Bruker Nonius product line of instrumentation for single crystal X-ray diffraction. The systems provide the tools for complete small molecule structure determination. The hardware and software are completely redesigned. The software features a new start to finish graphical user interface (GUI). The hardware features a new CCD detector based upon four port readout of a 4K chip and a choice of two goniometers. APEX II systems are enclosed in a fail-safe X-ray enclosure system.

The Kappa APEX II system features the Kappa 4-axis goniometer. Two computers are used for experiments. One computer, the server, controls the goniometer. The other computer, the client, controls all other aspects of the experiment, from data collection through report generation.

The SMART APEX II system is an enhanced version of the SMART APEX fixed chi system. A single computer controls the data collection, and solution and refinement of the structure.

From a software and operational viewpoint, the APEX II systems use the GUI of the APEX2 software suite to control all operations, from crystal screening to report generation, for a typical crystallography study. This is a complete departure from the command driven, functionally separate modules of SMART, SAINTPLUS and SHELXTL. Enhanced versions of the proven and widely accepted programs used by these modules (e.g., SAINT, SADABS, XPREP, XS, XM, XL, etc.) underlie the GUI and provide powerful tools.

From a hardware viewpoint, APEX II systems share common hardware components with other

Bruker products. Other members of this new generation of instruments include the D8 ADVANCE and D8 DISCOVER, and the D8 GADDS systems for general diffraction. Documentation on some of these common hardware and software components is available in the user's manuals for the D8 family of instruments.

1.2   User Manual Features

This user manual and associated YLID test data are intended to provide you with a step-by-step guide to data collection and processing using the APEX2 software program.

The test data supplied was collected on an **X8 (or Kappa, per Ludger)** diffractometer with an APEX II camera, and graphite monochromated molybdenum radiation from a sealed tube generator. The high quality data (resolution=0.75 Å) allows easy refinement of the hydrogen atom positions and determination of the absolute structure of the sample.

NOTE: Before using this manual, check that your system is in proper working order (e.g., the optics and goniometer are aligned) and that the APEX2 suite is properly installed.

1.3   X-ray Safety

⚠ **WARNING**

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

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

2. Hardware Overview

The two hardware platforms for the APEX II systems are the Kappa APEX II, the four-axis advanced research instrument, and the SMART APEX II, the three-axis laboratory instrument. Software functionality is essentially the same for both platforms.

2.1 System Components

The system (Figure 2.1 and Figure 2.2) consists of the following basic components.

- APEX II™ CCD detector
- 4-axis Kappa or 3-axis goniometer
- K780 X-ray generator
- Radiation safety enclosure with interlocks and warning lights
- D8 controller
- Refrigerated recirculator for the detector
- Computer(s) (two for the Kappa APEX II and one for the SMART APEX II)
- Video microscope
- Accessories (temperature devices)

APEX **II**
Detector
Power
Supply

**Optional
LT Controller**

Generator

Electronics
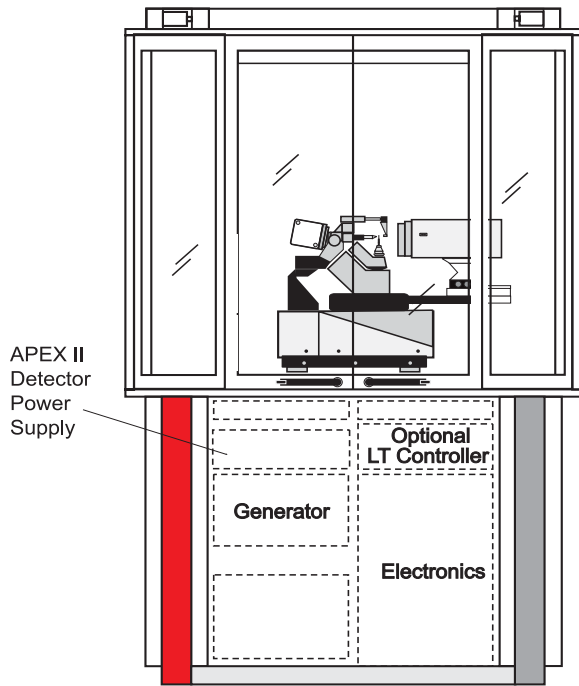
Figure 2.1 - Kappa APEX II system

APEX **II**
Detector
Power
Supply

**Optional
LT Controller**

Generator

Electronics
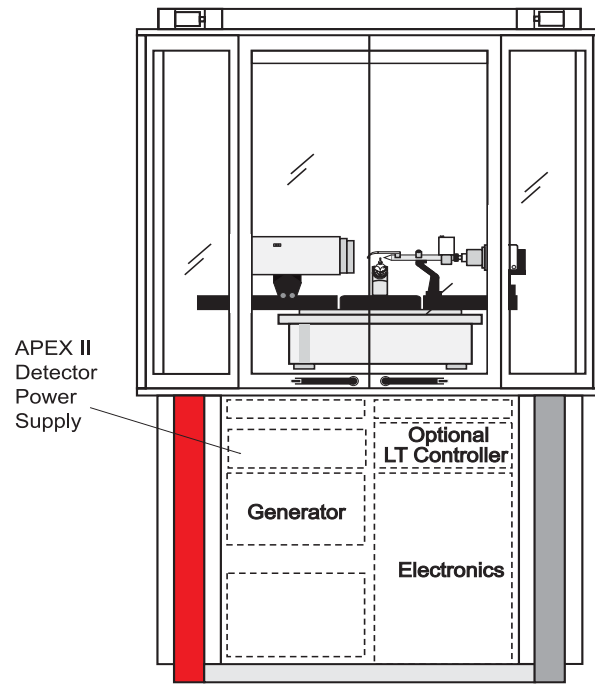
Figure 2.2 - SMART APEX II system

2.1.1 APEX II Detector

The APEX II detector is specific to this system. Status lamps on the top of the detector housing indicate when the detector is on (green) and off (red).

On Kappa APEX II systems, the detector is mounted on a motorized 2θ track. The camera distance is computer controlled (a typical distance for the camera is 40 or 50 mm).

On SMART APEX II systems, the detector is mounted on a 2θ dovetail track. The track has a scale that is calibrated in mm to indicate the distance from the crystal to the phosphor window (a typical distance for the camera is 40 or 50 mm).

2.1.2 Goniometer

The goniometer module and APEX II detector comprise the unique hardware of the system. This is the part of the instrument that actually performs the experiment.

Several components comprise the goniometer _module_ with APEX II detector.

- Goniometer (4-axis or 3-axis)

- APEX II detector

- X-ray source (including shielded X-ray tube, X-ray safety shutter, and graphite crystal monochromator)

- K780 X-ray generator

- Timing shutter and incident beam collimator (with beam stop)

- Video camera

Kappa APEX II Goniometer

The Kappa goniometer uses a horizontally oriented Kappa goniometer with 2-theta, omega, kappa and phi drives and a motorized DX track for setting the detector distance. It includes mounting points for the video camera and for optional attachments such as the optional low temperature attachment.
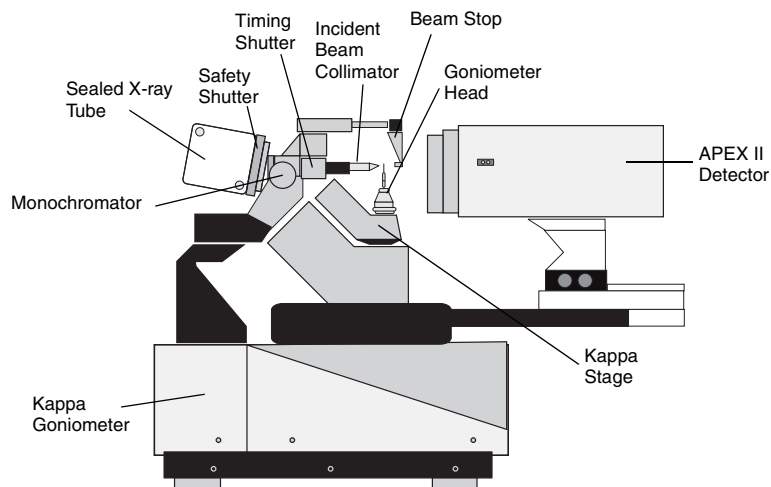
Figure 2.3 - Kappa 4-axis goniometer components

With the kappa angle, the crystal can be oriented at chi from *-92 to 92 °?*. This leaves the top of the instrument open for easy access.

SMART APEX II Goniometer

The SMART APEX II system uses a horizontally oriented D8 goniometer base with 2-theta, omega and phi drives, dovetail tracks for the X-ray source and detector, and an additional mounting track for accessories such as the video camera and optional low-temperature attachment.

The 3-axis system incorporates a fixed chi stage with chi angle of approximately 54.74° and a phi drive with 360° rotation, which is so compact that it **swings into** the incident beam collimator, allowing free rotation in omega. All four axes (2θ, ω, ϕ, and χ) intersect.
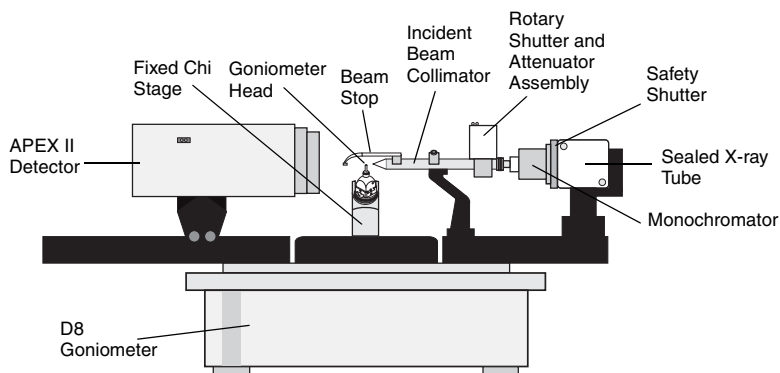
Figure 2.4 - SMART goniometer components

X-ray Source

Three components comprise the X-ray source: a shielded X-ray tube, an X-ray safety shutter, and a graphite crystal monochromator.

The sealed tube X-ray source, with a molybdenum (Mo) target, produces the X-ray beam used by the system.

The X-ray safety shutter is built into the X-ray tube shield. The shutter opens upon initiation of a set of exposures and closes upon the end of collection. Status lamps on the shutter housing indicate when the shutter is open (red) and closed (green). The shutter is also interfaced to the controller and to the safety interlocks.

A tunable graphite crystal monochromator selects only the K_α line (λ=0.71073 Å) emitted from the Mo X-ray source and passes it down the collimator system.

**Timing Shutter and Collimator**

On SMART APEX II systems, the monochromatic X-ray beam then passes through the labyrinth, the timing shutter, and the incident beam collimator before striking the specimen. On Kappa APEX II systems, the monochromatic X-rays pass through a small labyrinth, the timing shutter, a secondary labyrinth and the incident beam collimator before striking the sample.

- The labyrinth is a device that ensures that the collimator and shutter are tightly connected to prevent X-ray leakage.

- The timing shutter is a device which precisely controls the exposure time for each frame during data collection. Its status lamps indicate when the shutter is open (ON) and closed (OFF). For SMART APEX II systems, this assembly also houses an automatic attenuator. Kappa APEX II systems do not have an attenuator.

- The incident collimator is equipped with pinholes at both front (near crystal) and rear (near source). These pinholes help to define the size and shape of the incident X-ray beam that strikes the specimen. (Collimators are available in a variety of sizes, depending on your application.)

- The beam stop catches the remainder of the direct beam after it has passed the specimen. The beam stop has been aligned to minimize scattered X-rays and to prevent the direct beam from hitting the detector. The entire collimator assembly is supported by a collimator support assembly, which has been precisely aligned to guarantee that the X-ray beam passes through the center of the goniometer.

2.1.3 Video Camera/ microscope?--**heading?**

The video camera, an essential part of the system, allows you to visualize the crystal to optically align it in the X-ray beam and to measure the crystal dimensions and index crystal faces. The camera is interfaced to the computer and is operated through the VIDEO program. The video program includes several computer-generated reticules and scales to make centering and measuring the crystal easy.

2.1.4 K780 X-ray Generator

The K780 X-ray generator is a high-frequency, solid-state X-ray generator, which provides a stable source of power for operations up to 60 kilovolts (kV) and 50 milliamps (mA). For the APEX II system, power settings should never exceed the maximum power rating of the X-ray tube.

Typical maximum power settings for the APEX II system with a fine focus tube are 50 kV, 40 mA. For both types of tubes, the kV setting should not exceed 50 kV and the power (kV x mA) should not exceed the rating given on the tube cap.

This generator is interfaced to the controller and the power settings can be adjusted within the APEX2 software. This is usually not necessary though as the software automatically increases the power to the user defined values at the beginning of an experiment and lowers them when the instrument is inactive.

2.1.5 Radiation Safety Enclosure with Interlocks and Warning Lights

A common component of all systems in the D8 family is the radiation safety enclosure. This new design is fully leaded (leaded metal sides and panels, leaded windows) to protect you from stray radiation. The enclosure also includes warning lamps (a government requirement) that alert you when X-rays are being generated. And, as a special feature, the enclosure incorporates interlocks (for both hardware and software)—an automatic system-interruption device that senses when the doors and panels are open and prevents use of the shutter and data collection until you close the doors.

2.1.6 D8 Controller

The D8 controller is an electronic module enclosed in the rack behind the font panel of the instrument. It contains all of the electronics and firmware for controlling the generator, opening the X-ray shutters, and monitoring other instrument functions, such as safety interlocks, generator status, and detector statuses. For SMART APEX II systems, the goniometer is controlled by the D8. For Kappa APEX II systems, there is an additional module, the Kappa controller, for positioning the Kappa goniometer angles and driving the detector distance.

2.1.7 Refrigerated Recirculator for the Detector

The refrigerated recirculator uses an ethylene glycol/water mixture to absorb the heat from dual Peltier devices which are used to cool the CCD chip to approximately -58°F (-50°C). This minimizes dark current.

2.1.8 Computers

The Kappa APEX II system uses two high speed computers. The server controls the instrument and is used for crystal centering and screening. The client collects the data, stores the raw frames, processes the data, and solves and refines the structure. The two computers are linked via a hub and communicate with each other via TCP/IP protocols.

The SMART APEX II system uses a single high speed computer for control of the experiment, storage of raw frame data, integration of the data, and solution and refinement of the structure.

Often the computer(s) is (are) attached to a network of similarly configured computers with access to local and/or network printers.

NOTE: Connection to the external network must be done with care. Consult with local security experts.

2.1.9 Accessories

Various devices can be mounted on the goniometer base. These include optional low- and high-temperature attachments. Both instruments can be used with diamond-anvil cells.

3. Software Overview

This section presents an outline of the system software, including a brief description of the software layout, as well as the graphical user interface (GUI).

APEX2 runs on two computers: the server and the client. For SMART APEX II systems, the server and the client execute on the same computer, but the functionality remains separate. The flowchart in Figure 3.1 shows the software layout. For both Kappa APEX II and SMART APEX II systems, the server and client communicate using TCP/IP protocol.
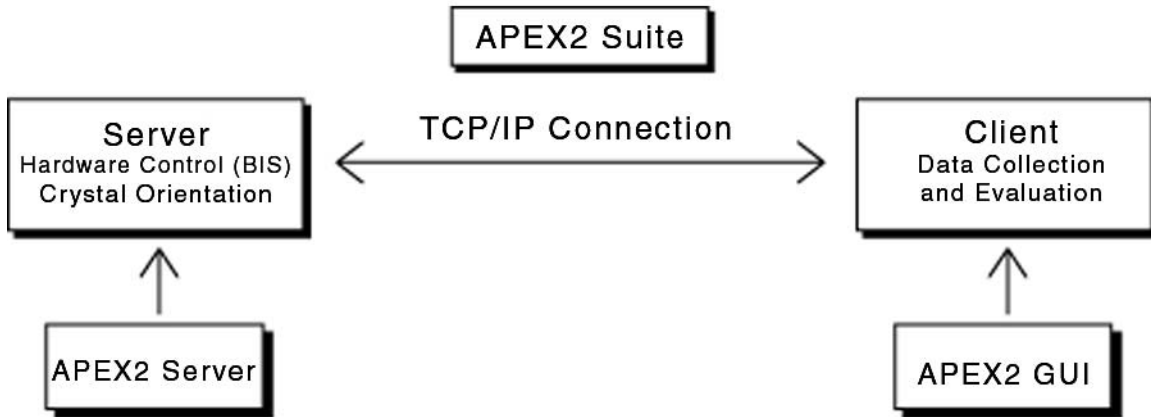
Figure 3.1 - APEX2 software diagram

3.1 The Server Computer

The server computer communicates with the hardware, allowing the user to control the instrument. The software to align the system, as well as to align and screen samples, runs on the server computer.

3.1.1 Bruker Instrument Service (BIS)

BIS provides the link between the hardware and software. Once a connection is established, BIS executes hardware commands sent by the APEX2 software. The instrument service can also be used as a service tool, displaying diagnostic messages during operation.

3.1.2 Bruker Control Program (BCP)

BCP is used to configure BIS, as well as to provide instrument control and alignment tools.
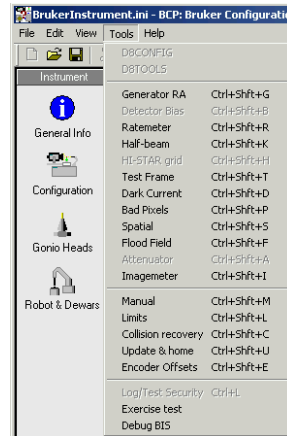

Figure 3.3 - BCP main window

Figure 3.2 - Image of start/stop in BIS

3.1.3 APEX2 Server

The APEX2 Server provides tools for aligning
and screening samples. There are two main
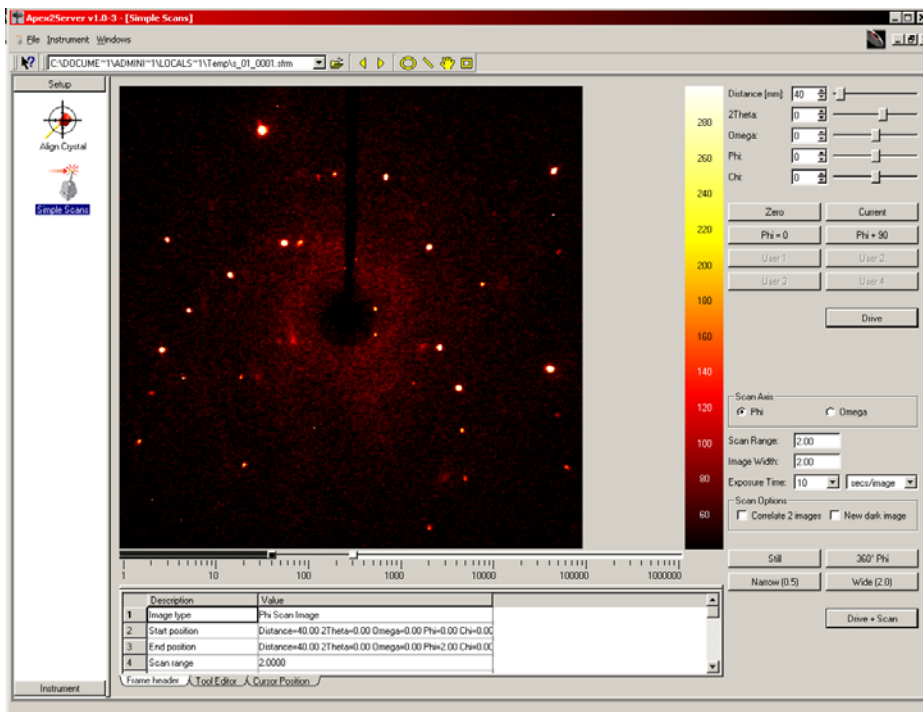items: Align Crystal and Simple Scans (see Fig-
ure 3.4).

Figure 3.4 - Simple Scans window

3.2 The Client Computer

The client can be any computer which is on the same network as the server. For SMART APEX II systems, it is usually the same computer as the server. The main portion of the APEX2 suite, the APEX2 client, runs on the client computer. The client is a GUI with multiple plug-ins or modules for different aspects of an experiment. The client includes a database which stores relevant data from each step in the experiment. Details of the functions available in the GUI will be explained in more detail later in the manual.

3.2.1 Database and Database Connection

As currently configured, the database is used internally by the APEX2 Suite and is not available for user customization or manipulation. It must be running before the APEX2 Suite is started and it should be stopped before the computer is shutdown (see Section 4.3.2).

The purpose of the database is for storage of data generated by the Bruker APEX2 software.

3.2.2 APEX2 GUI

The APEX2 GUI has one main window (see Figure 3.5). This window is divided into four sections:

- Window Tool Bar
- Tool Icon Bar
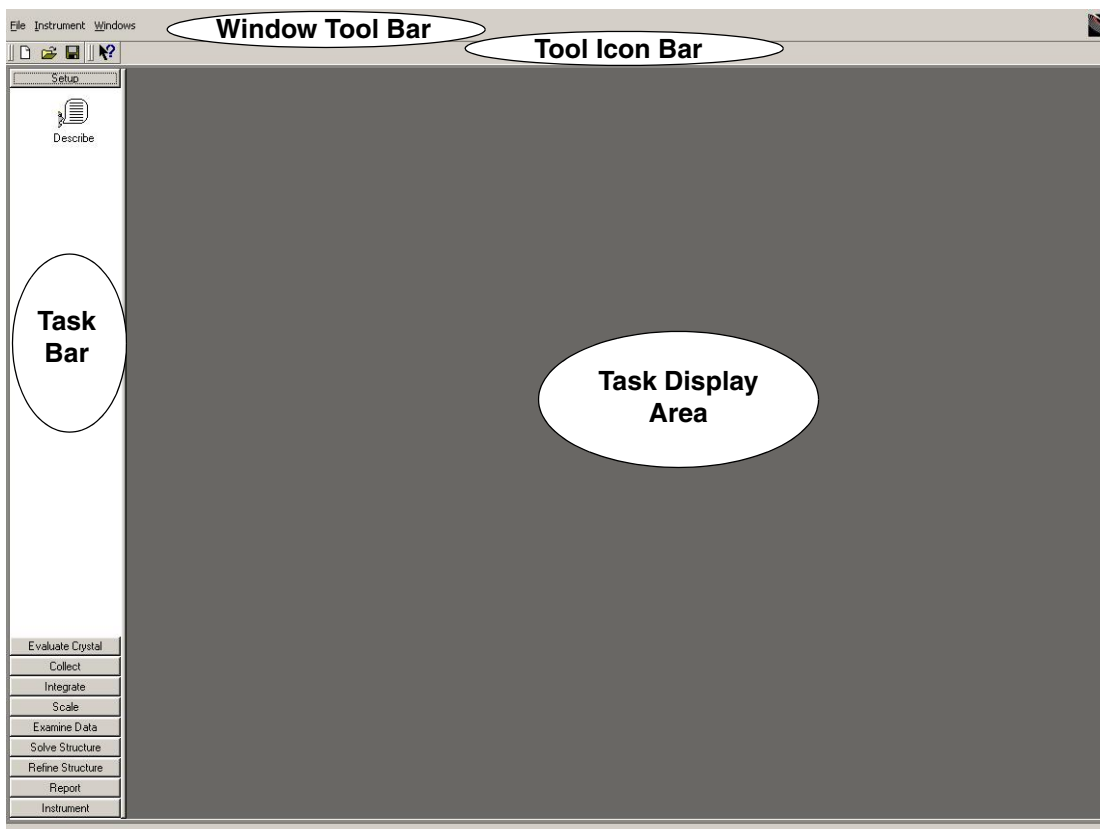- Task Bar
- Task Display Area

Figure 3.5 - APEX2 GUI

Window Tool Bar

The tool bar provides pull-down menus for a variety of file operations, image tools, and help files.

Option	Description
[Symbol]	Use this menu to select the following: Restore, Move, Size, Minimize, Maximize, and Close.
File	Use this menu to select the following: Login, Logout, New, Open, Save, Close, Import (Spatial), Export (P4P File) and Exit.
Instrument	Use this menu to select the following: Connection, Status, Toggle Shutter and Abort.
Windows	Use this menu to select the following: Cascade and Tile.
RLATT (available when you select Reciprocal Lattice Viewer)	Use this menu to select the following: Rotate, Edit, Orientation, Unit Cell Tool, Measure Distance, Measure Angle and Visualization.
View (available when you select Data Collection Strategy)	Use this menu to select the following: Detailed Strategy.

Table 3.1 – Window tool bar options

Tool Icon Bar

The tool icon bar provides shortcuts to the options available through the window tool bar.

| Icon	Description
	Create a new file.
	Open a file.
	Save a file.
	"What's this?" -- context-sensitive help.
	Open an image.
	Select previous image. This icon is visible only when an image is open.
	Select next image. This icon is visible only when an image is open.
	Draw a circle. This icon is visible only when an image is open.

Table 3.2 – Tool icon bar options

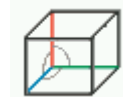| Icon	Description
	Draw a plotting line. This icon is visible only when an image is open.
	Change the part of the image displayed while zoomed in. This icon is visible only when an image is open.
	Select a region of the image. This icon is visible only when an image is open.

Table 3.2 – Tool icon bar options

Task Bar

The task bar provides menus for all of the options in the APEX2 Suite: crystal evaluation and indexing (Evaluate Crystal), data collection (Collect), data processing (Integrate and Scale), and instrument setup (Instrument).

Setup	
	Describe - Specify crystal size, color, shape, etc.
	Center -

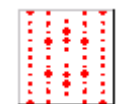| Evaluate Crystal	
	Determine Unit Cell - Determine unit cell and Bravais lattice type.
	Reciprocal Lattice Viewer - 3D visualization of lattice projected in reciprocal space.

Table 3.3 – Task bar options

Collect

	Data Collection Strategy - Simulated data collection and determination strategy.
	Experiment - Sequence editor for data collection experiments.
	Oriented Scans - Measure different images with the crystal aligned along the axes.

Integrate

	Integrate Images - Integration of different data.

Scale

	Scale - Scale intensities and perform absorption correction.

Examine Data

Table 3.3 – Task bar options

	XPREP (Space Group Determination) - Run XPREP.
	Precession Images - Create synthesized precession images based on measured frames.

Solve Structure

	Structure solution.

Refine Structure

| | |

Report

	Run XCIF to generate a report.

Instrument

Table 3.3 – Task bar options

Task Display Area

The task display area is the main area for tasks, user input and selected output. This area displays images, the reflections used in indexing, and the observed and predicted diffraction patterns. It also displays the runs for data collection and solution and refinement. (For version 1.22, space group determination, SaintChart output, XShell refinement, and Xcif report generation do not use the Task Display Area; they open in a new window). All other plug-ins open in the Task Display Area of the GUI.
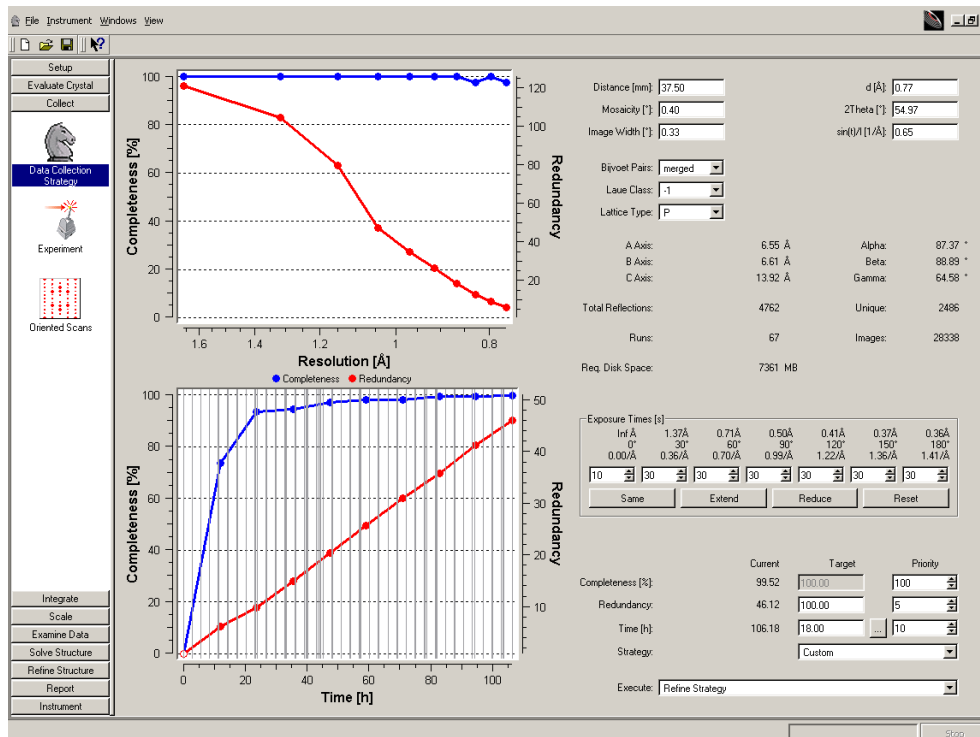

Figure 3.6 - The Task Display area showing COSMO

4. Program Start-Up and Shutdown

As mentioned previously, the APEX2 Suite is composed of several programs. All of the programs are started in a similar fashion. For ease of use there is usually a desktop icon for the folder containing these programs and desktop icons directly to these programs. However the Click on Start > Programs > Bruker … path is always available. This more complicated method is what will be used in this discussion.

4.1 Server Computer Start-Up

Two programs must be running: Bruker Instrument Service and APEX2 Server.

NOTE: For Kappa APEX II systems, the programs will be on the server computer in the goniometer cabinet. For SMART APEX II systems, there is typically only one computer.

4.1.1 Starting Bruker Instrument Service (BIS)

1. Click on Start > Programs > Bruker AXS Programs > Bruker Instrument Service.

After a brief initialization period, a window will appear (see Figure 4.1). On Kappa APEX II sys-

tems, the goniometer will move to reference positions.

Figure 4.1 - The BIS window

If a small pop-up window appears that says "This second instance of BIS is exiting" (see Figure 4.2), BIS was already running. Click on ok to clear this informational message.

Figure 4.2 - BIS warning message

NOTE: With a SMART APEX II or a Kappa APEX II, the kappa goniometer will home and the kappa server will activate when BIS is started. This may take a minute or two. The server is a service tool and should not be used to control the instrument.

4.1.2 Starting the APEX2 Server

1. Click on Start > Programs > Bruker Nonius Programs > APEX2 Server.
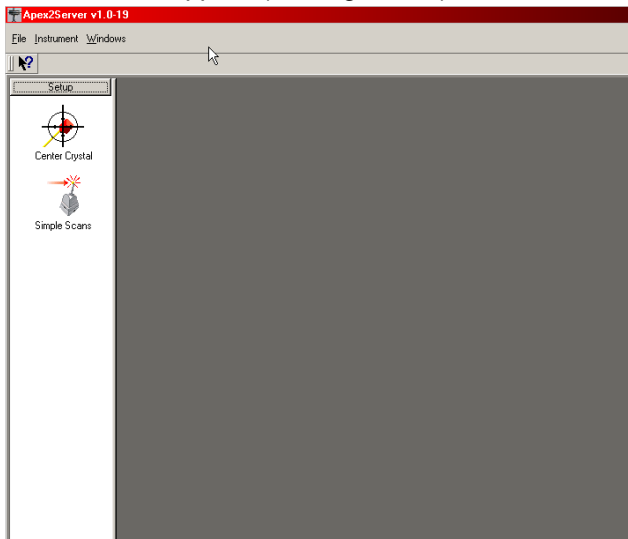
A window will appear (see Figure 4.3).

Figure 4.3 - Initial APEX2 Server window

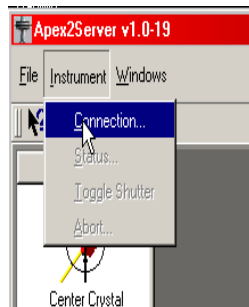2. In the top left corner, click on the pull-down menu Instrument (see Figure 4.4).

Figure 4.4 - Connecting to the instrument

3. Click on Connection and a new window will appear (see Figure 4.5). The name of your server should already be filled in.
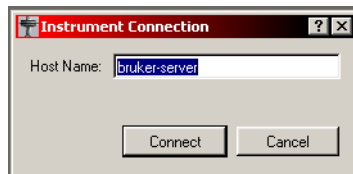
4. Click on connect.

Figure 4.5 - Connection window

NOTE: If the name is wrong then the instrument is not properly configured and you should consult the system manager. (It is possible to configure the instrument to automatically connect and then this window will not appear). **tell how to do this.**

4.2 Client Computer Start-Up

On the client computer, two programs are also required: the database and APEX2. It is best to start the database before starting APEX2.

NOTE: For the SMART APEX II, there is typically only one computer for the client and server software.

4.2.1 Starting the Database

1a. For Windows systems, click on Start > Programs > Bruker AXS Programs > Start Database or click on the Start Database icon on the desktop.

1b. For Linux systems, open a terminal window and type "bnrun startdb" or click on the Start Database icon.

A window should appear that says the database system is ready.

Figure 4.6 - The database is ready

You can minimize this window. If the database has not previously been closed properly (e.g.,

after a power failure), a window will appear (see Figure 4.7) that states that another postmaster is running. If this happens, stop the database and then start it again.

Figure 4.7 - Database failure message

4.2.2 Starting APEX2

1a. For Windows systems, on the client computer click on Start > Programs > Bruker Nonius Programs > APEX2 or click on the APEX2 icon on the desktop.

1b. For Linux systems, open a terminal window and type bnrun apex2 or click on the APEX2 icon.

2.   A window will appear to login to the data-base by entering a user and password (see Figure 4.8).

2.1 If the system manager has set up the system to automatically enter the user name and password, step 2 is skipped.
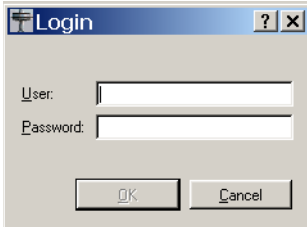

Figure 4.8 - Login request

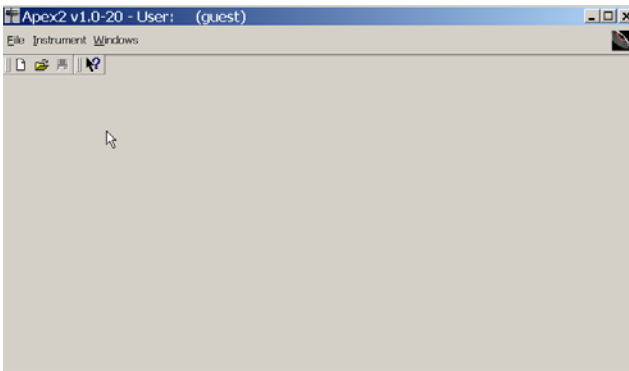3.   An empty start-up window will appear (see Figure 4.9).

Figure 4.9 - APEX2 start-up window
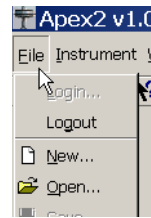
4.   Click on File.

Figure 4.10 - clickfile2.bmp

5.   Use the options in this menu to create a new project or to open an existing project.

6.   If the window in Figure 4.11 appears then APEX2 thinks the database is already in use. Answer "Yes" to close the window.
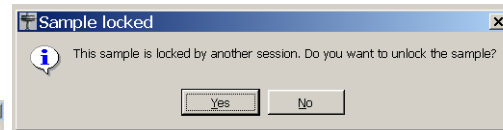

Figure 4.11 - Sample Locked menu

4.3   Client Computer Shutdown

NOTE: The order of stopping these programs is important. If you attempt to close the database before APEX2 is stopped, the database will remain open until APEX2 is stopped.

4.3.1 Stopping APEX2

1. For Windows or Linux systems, click on the X in the upper right corner of the window or click on File > Exit in the upper left. It is not necessary to disconnect from the instrument.

4.3.2 Stopping the Database

1a. For Windows systems, click on Start > Programs > Bruker Nonius Programs > Stop Database or click on the Stop Database icon.

1b. For Linux systems, in a terminal window enter bnrun stopdb or click on the Stop Database icon.

A window will appear and quickly disappear, and the Start Database window will close.

Figure 4.12 - Stop database screen

NOTE: Occasionally the windows won't disappear and the Start Database window will display a "smart shutdown request" (see Figure 4.12). This message means that the database is waiting to close until applications that it might write to are closed. Exit APEX2 to solve this problem. If the message still appears, check for other processes that may still be running (e.g., COSMO).

4.4   Server Computer Shutdown

Stop APEX2 Server before BIS. It is ok if the order is inverted. Generally, BIS is never stopped.

4.4.1 Stopping the APEX2 Server

1. Click on the X in the upper right corner of the window or click on File > Exit in the upper left. It is not necessary to disconnect from the instrument.

4.4.2 Stopping BIS

It is almost never necessary to stop and exit BIS. If necessary, click on the stop BIS button on the bottom of the BIS window and then click on the exit button at the bottom of the window.

5. Crystal Orientation

We are now ready to begin data collection with the instrument using the YLID test crystal. We assume that your system manager has set up the system properly and that all system default parameters have been set appropriately.

The data collection process is divided into five steps:

See Section 5:

1. Centering/aligning on the diffractometer (from the APEX2 Server—the center module)

2. Crystal quality check (from the APEX2 Server—the simple scans module)

See Section 6:

3. Cell determination (from APEX2—the cell determination module)

4. Data collection setup (from APEX2—the strategy module)

5. Data collection (from APEX2—the experiment module)

The first steps, mounting, aligning and screening a crystal, are performed on the server computer.

5.1   Mount the Goniometer Head on the Instrument

⚠ **CAUTION**

Use extreme care when handling the goniometer head to prevent damage to your sample on the end of the small glass fiber.

1.   Open the enclosure doors. Push either of the rectangular green Open Door buttons on the side posts. This will release the door locks for approximately five seconds. During this time, pull out on one or both of the handles to physically open the doors.

2.   In the APEX2 Server GUI, under Setup click on Center Crystal.

The centering buttons will appear and the video window will open.
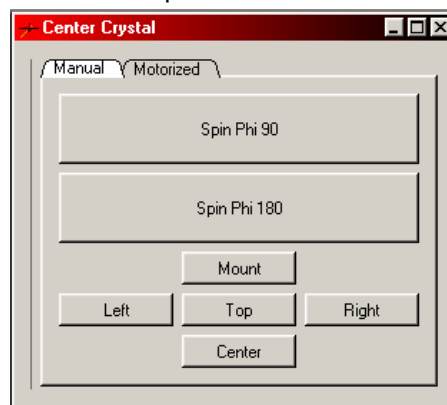
Figure 5.1 -

The bottom five buttons will drive the goniometer to various predefined positions that are designed to simplify crystal centering. The top two buttons will drive phi by either 90 or 180 degrees.

3.   Click on Mount to mount an empty goniometer head and determine the position.

NOTE: On Kappa APEX II systems, in the mount position, the key or pin on the mounting base will be toward the back of the instrument. On SMART APEX II systems, at $\phi=0°$, the key on the mounting base of the goniometer head will be at the 12:00 position.

⚠ **CAUTION**

Use extreme care when handling the goniometer head to prevent damage to the sample on the end of the small glass fiber.

4.   Carefully remove from its case the goniometer head containing the YLID test crystal.

5.   Place the goniometer head onto its base on the phi drive, aligning the head's key slot with the key (pin) in the base.

6.   Snugly screw the head's collar to the base so the head does not move. Do not overtighten it.

5.2   Center and Align the Sample

To obtain accurate unit cell dimensions and to collect good quality data, align the center of the sample with the center of the X-ray beam and maintain the alignment for the entire experiment. Your video camera should be aligned so the crosshairs of the video camera coincide with the center of the goniometer and the center of the X-ray beam (see manual M86-Exx024 for instructions on aligning the microscope to the center of the instrument). Although, if the microscope is not centered, you can still align the sample—the key here is that the crystal stay in the same place in the microscope in all orientations.

NOTE: On SMART APEX II systems, adjust the height while in the mount position.

NOTE: Use the thin end on the goniometer wrench to unlock the adjustment slides at the beginning of the centering process and to lock them at the end—locking needs only a very slight touch. The other end of the wrench is used to make adjustments.

NOTE: If the image of the crystal is difficult to see, illuminate the sample with a high-intensity lamp and/or temporarily place a light-colored piece of paper on the front of the detector.

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, drive to one of the centering positions, loosen the screw that locks the goniometer head, rotate the crystal to a suitable orientation and then tighten the screw again.

1. Click on the Center button—the crystal and goniometer head will be positioned perpendicular to the microscope. To center the sample, make adjustments in the height and with the translation screw that faces the front of the diffractometer.
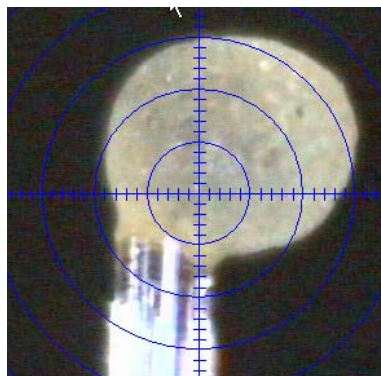

Figure 5.2 - Crystal initially mounted

2. Use the z-axis (translation) screw and the x- or y-axis (translation) adjustment screw that is perpendicular to the microscope axis to move the crystal so it is centered in the microscope (see Figure 5.8 and Figure 5.9).

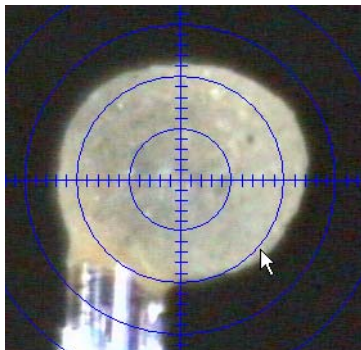   2.1 (For the SMART APEX II) Make adjustments in the x and y translations, but not to the height (z).

Figure 5.3 - Crystal centered

3. Adjust the height with the z-axis screw.

    3.1 (SMART APEX II) Click on the Right button. Adjust the height and the translation screw that is facing up.

4. Adjust the translation with the x- or y-axis screw, whichever is pointed toward you.

5. Adjust the height again with the z-axis screw.

6. Adjust the crystal position using the x- or y-axis screw.

7. Click on Drive Phi 180 and adjust **(adjust what? x/y)** again.
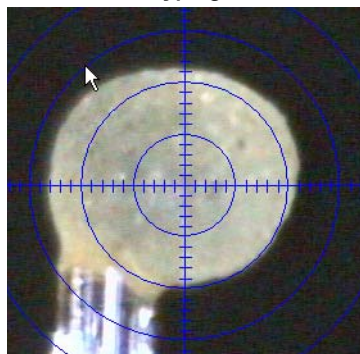
Figure 5.4 - Drive Phi 180

8. Check the other translation. **(how? which one?)**

Repeat steps 3 and 4 as necessary to keep the crystal in-line with the microscope.

9. Click on Drive Phi 90.
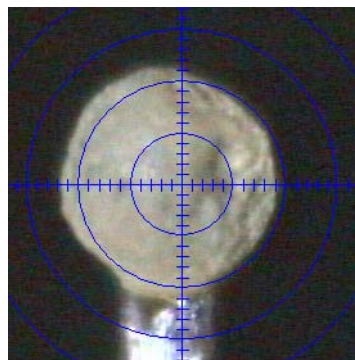
Figure 5.5 - Drive Phi 90

~~Repeat steps 6 and 7 as many times as necessary to keep the crystal in the same place in the microscope.~~

10. Adjust the crystal position using the goniometer screws. **Why adjusting now--what are we adjusting?**

11. Click on Drive Phi 180 and adjust again. **(adjust what? x/y)**

Repeat steps 3–11 as necessary until x is centered. Then adjust the height using the following steps.

1. Click the Left button and note the height. The goniometer drives to place the fiber horizontal and to the left.

2. Click the Right button and check that the crystal height stays in the same place in the microscope.

   2.a If the height is in the same place, you are done.

2.b If the height is not in the same place, adjust the height and repeat steps 1 and 2.
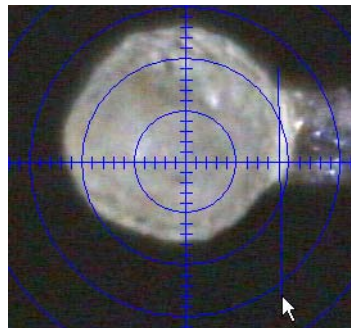
Figure 5.6 - Check the crystal height

3. Click the Top button.
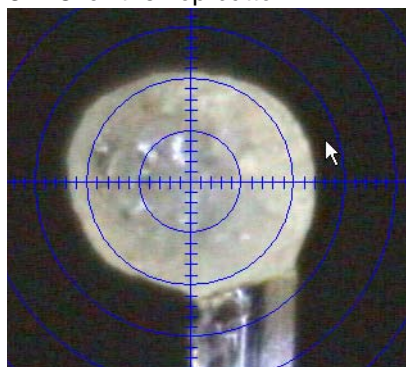
Figure 5.7 - The crystal is centered

The crystal is now centered on the instrument. All of the next steps (except removing the crystal from the diffractometer) are performed with APEX2 on the client computer.
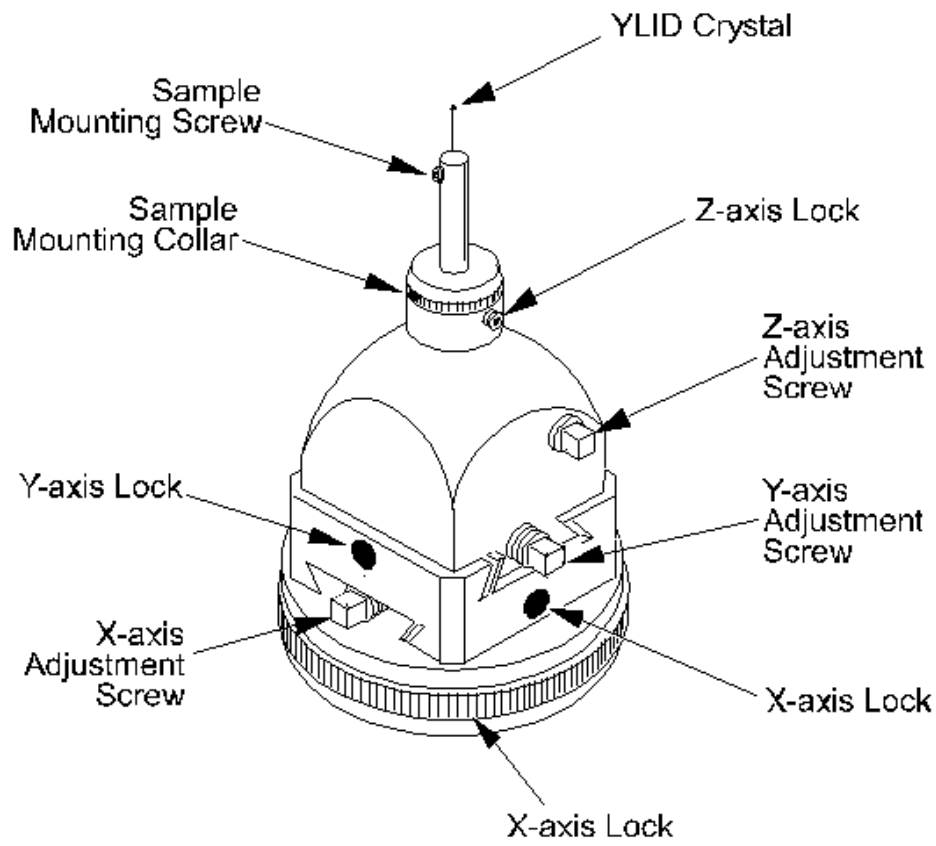
Figure 5.8 - Huber goniometer head in detail

Sample
Mounting Screw

YLID Crysal

Sample
Mounting Collar

Z-axis Lock

Z-axis
Adjustment
Screw

Y-axis Lock

Y-axis
Adjustment
Screw

X-axis
Adjustment
Screw

X-axis Lock
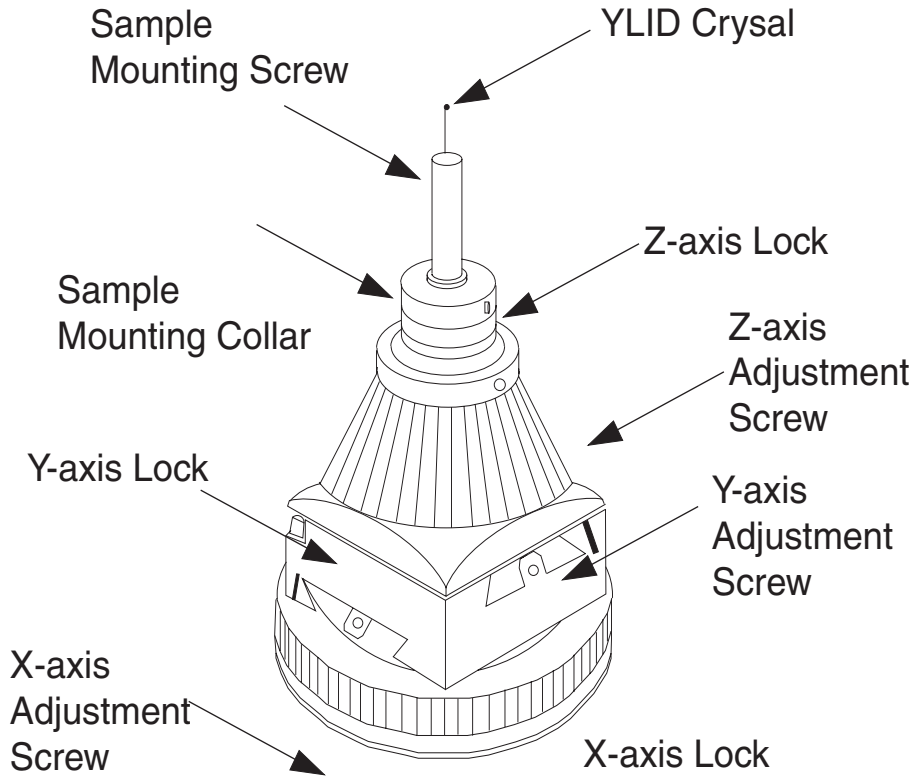
Figure 5.9 - Standard goniometer head in detail

6. Data Collection

The data collection process is carried out on the client computer using APEX2. Once data collection is started, exit APEX2. Data collection will continue.

6.1 Start a New Project and Describe the Sample

1. Left-click on File > New.

2. In the window that appears enter the sample name.
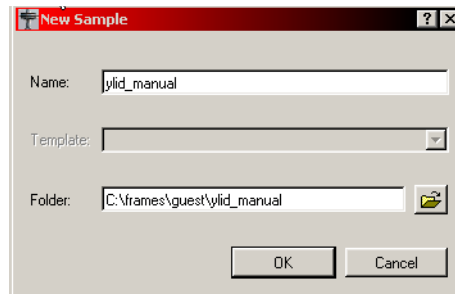
Figure 6.1 - The New Sample window

3. Click OK.

4. The task bar will appear with the Setup section open. Left-click on Describe.

5. Enter the requested information into the
    Describe window.

Figure 6.2 - Describe window

6. Close this module. The data will automati-
    cally save to the database.

6.2   Determine the Unit Cell

6.2.1 Collect Images

1. In the task bar, left-click on Collect and then on Experiment

Since there was no connection to the instrument when APEX2 was started, the program recognizes that it needs a connection in order to collect images. It will ask to connect (see Figure 6.3).
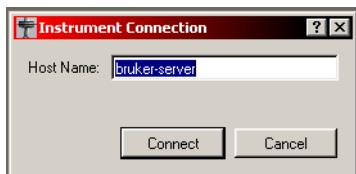
Figure 6.3 - Instrument Connection window

2. Click on Connect.

3. Click on Append Matrix Strategy at the bottom left of the window.

Figure 6.4 - Append matrix runs

1. Adjust the scan time and scan width if desired. The default values are usually good. The default time of 10 seconds is acceptable for most samples, but shorter times will not adversely affect most experiments.

2. Click on Execute/Resume. The view will shift to the Monitor Experiment view (see Figure 6.5). The program will collect a series of three runs with twelve frames per run. This typically takes less than ten minutes. The images will stop changing when the experiment is done.

NOTE: Adjust the time (upper right) to match the scattering ability of the crystal (e.g., shorter times for strong diffractors and longer exposure times for weak diffractors). If the exposure times are five seconds or less, click on the check mark by Correlate Frames to turn off this feature. Frame correlation measures each frame twice at typically 1/2 the time and then combines the two together. This is usually not necessary with shorter exposure times.

NOTE: The format for frame names is shown in Figure 6.5. Every frame is assigned a name. For this figure the name is ylid_manual_01_005.sfrm. This means that the

frame is for the project ylid_manual and that this
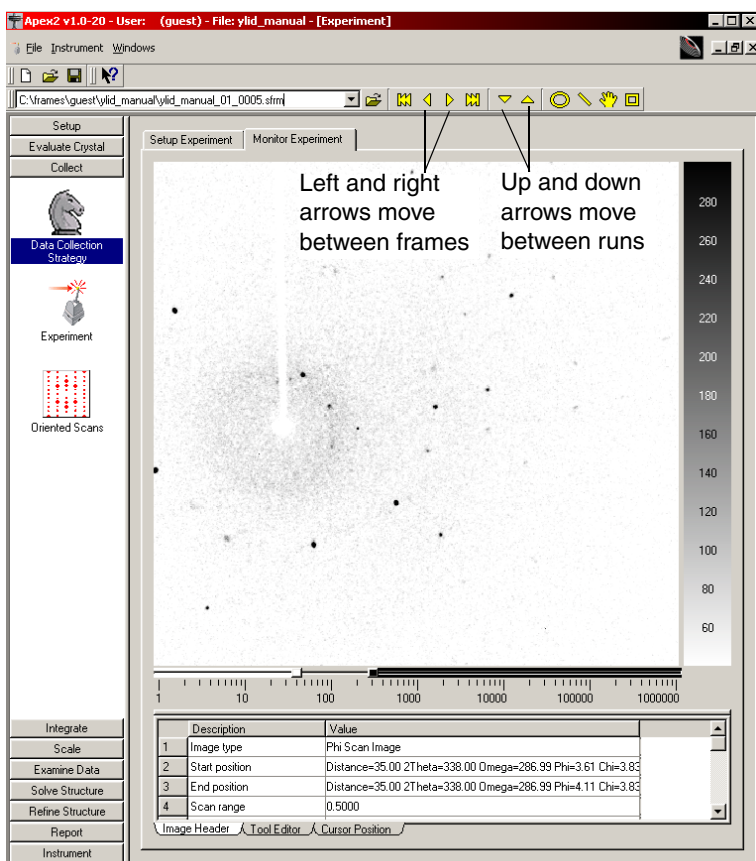is the fifth image of the first run.

Figure 6.5 - Monitor Experiment view

To change the color of the image display (e.g., Black on White), right-click in the intensity bar to the right of the image display (see Figure 6.6).
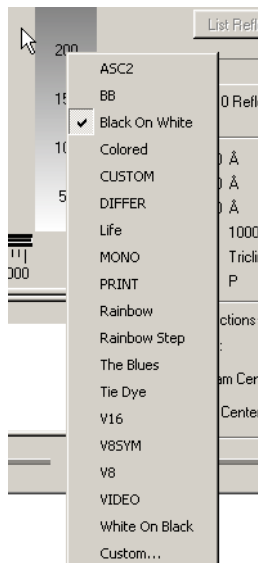

Figure 6.6 - Color tool

After the first run is completed, there **usually** is sufficient information to start the indexing step.

If state "usually" then need to give information about what to do if there isn't enough information.

NOTE: APEX2 assigns to every collected frame a name. **Per Ludger, basename.run#.frame#.sfrm**

6.2.2 Harvest the Reflections

1. Left-click on Evaluate Crystal > Determine Unit cell.
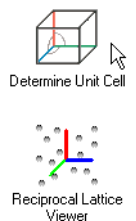
Figure 6.7 - The Determine Unit Cell (Indexing) icon

This will open the image viewer, but with a tool bar to the right for indexing (see Figure 6.8). The plug-in initializes with the first run (e.g., matrix_01).
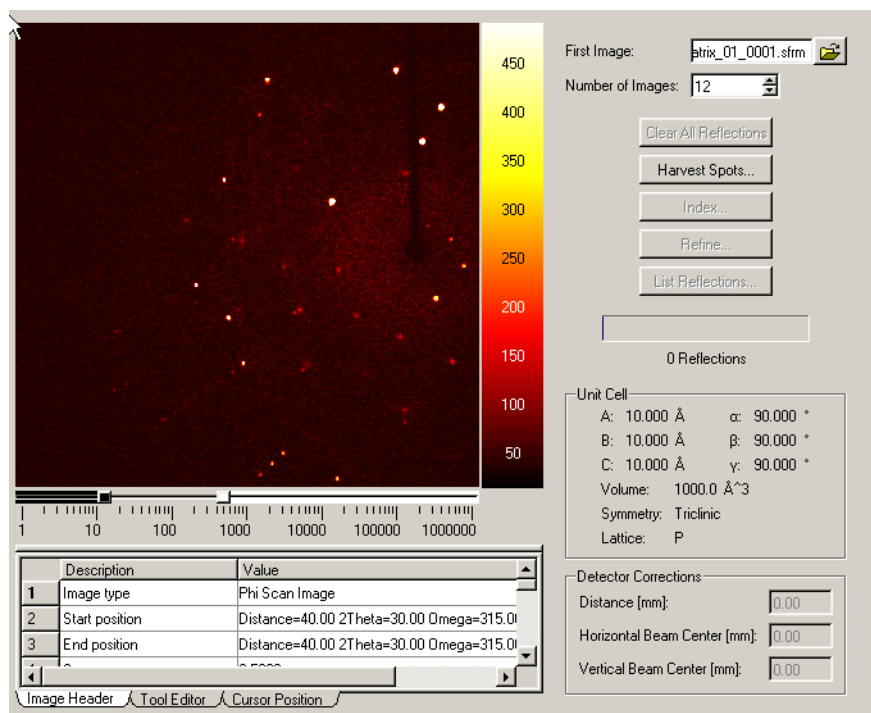

Figure 6.8 - Image viewer with indexing tool

2. The name of the first image is already entered. Click on Harvest Spots.

NOTE: All other options are gray at this point because there are no reflections available.

A blue progress bar will appear as the software determines the best background level to use for harvesting. Then a window with two sliders will appear.
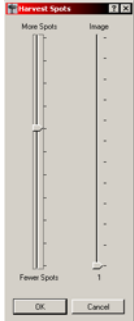
Figure 6.9 - Indexing sliders

The right slider selects which image is displayed. The left slider increases or decreases the I/s(I), the cut off criteria for accepting reflections. Generally, the defaults are fine.

3. Change the run number in the "First Image" box to matrix_02_0001 and click on Harvest Spots. The run number is 02. The image or frame number is 0001.

At this point you should have 100 to 300 reflections harvested.

4. Change the run number in the "First Image" box to matrix_03_0001 and click on Harvest Spots.

NOTE: If run three is not completed, a window will pop-up that says "Do you want to continue with the images that could be read?" If this happens, skip step 3 and go to Section 6.2.3. Then return to Section 6.2.2 and harvest the spots before refining.

6.2.3 Index the Reflections
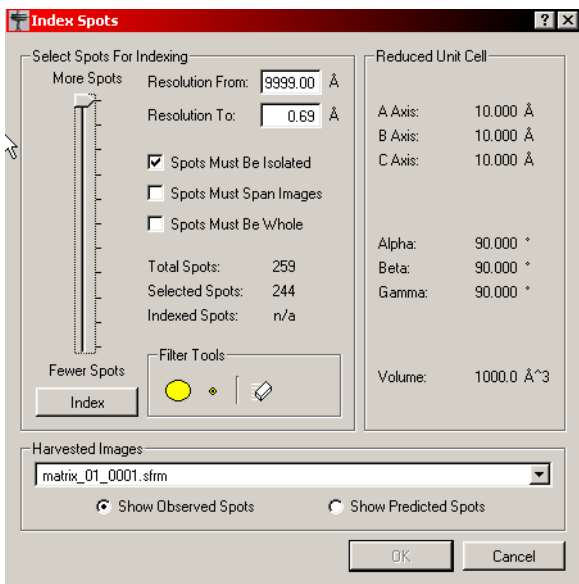
1. Click on Index.

A window will open.

Figure 6.10 - Indexing tool

The defaults are usually OK. Use the slider to omit reflections with lower I/sigma from the calculations. If indexing is difficult, try reducing the number of reflections used.

If indexing is difficult, use the RLATT tool. This tool is described in **Appendix ?**.

2. Click on Index.

After approximately 30 seconds the index window will display a possible cell and the OK button will no longer be gray. The values shown in Figure 6.11 are reasonable for the ylid.
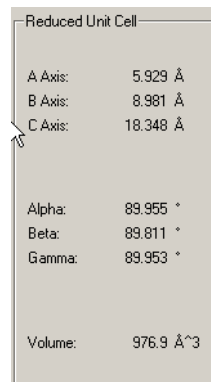
Figure 6.11 - The unit cell

The spot statistics are also acceptable with 98% (i.e., (238/244)x100) of the selected spots indexed.
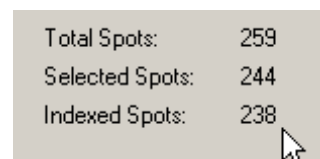
Figure 6.12 - Focus on the spot results

There are often a few reflections that are not indexed. The reciprocal lattice viewer can be used to look at the spots used in the indexing, but first refine this cell.

3. Click on OK to accept the indexing results.

6.2.4 Refine the Unit Cell

There is not a correct order for the following steps. Use this as a guideline with the main goal of creating a stable converged refinement.

1. Click Refine in the Indexing Tools menu. The refinement window will open.
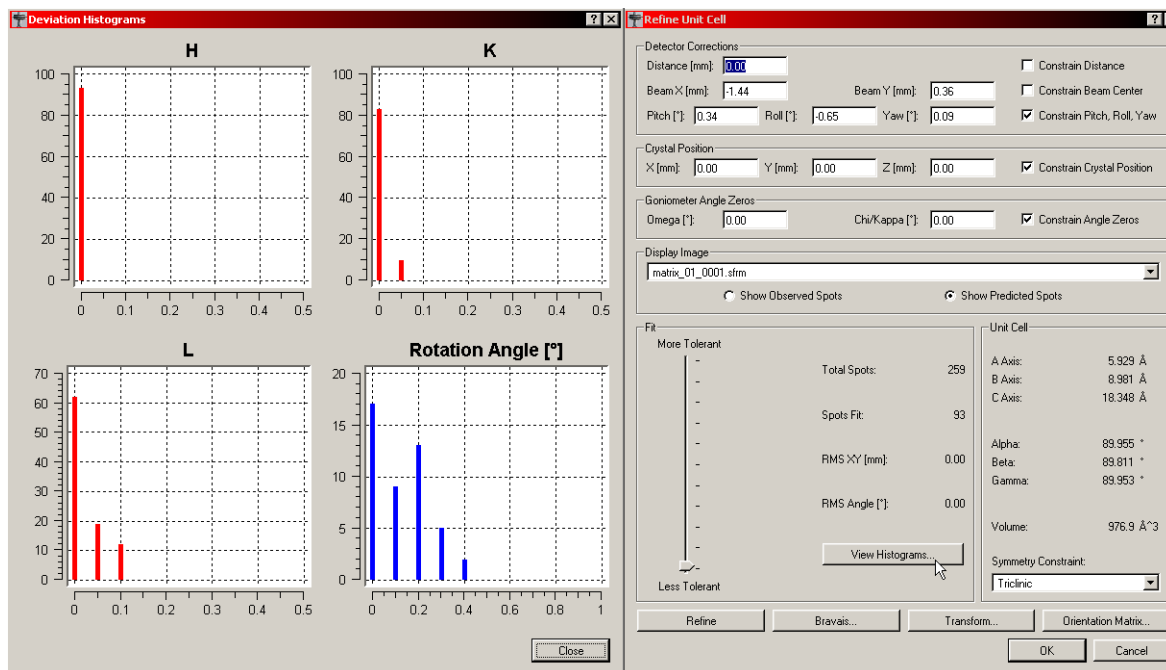

Figure 6.13 - The Refine menu with histograms displayed

The YLID test crystal should have an orthorhombic primitive cell with approximate cell dimensions of a=5.95Å, b=9.03Å, c=18.38Å, and $\alpha=\beta=\gamma=90°$.

2. Click View Histograms. The histograms show how observed data compares to the data calculated using the current unit cell. The HKL values should be close to integers and the rotation angle differences should not be significantly larger than the step size used to collect the matrix frames.

3. Click Refine several times.

4. Check the Constrain Distance and Constrain Beam Center boxes.

NOTE: Check the constraints to fix the parameters listed. Uncheck the constraints to allow the parameters to refine.

5. Uncheck Constrain Pitch, Roll, and Yaw.

6. Click Refine several times.

7. Uncheck Constrain Distance and Constrain Beam Center, and check Constrain Pitch, Roll, and Yaw.

8. Click Refine several times.

NOTE: In most cases, the angle zeroes are close to zero and should not be refined. The crystal should now be aligned, so refinement of the crystal center is not necessary.

6.2.5 Determine the Bravais Lattice

1. After refining, click Bravais Lattice and look for other unit cell choices (i.e., look at fit values).

	Fit	A Axis [Å]	B Axis [Å]	C Axis [Å]	Alpha [°]	Beta [°]	Gamma [°]	Volume [Å^3]
Cubic F	379.54	21.36	21.36	21.37	129.93	60.85	147.60	3970.79
Cubic I	436.63	19.37	10.82	20.51	68.44	31.37	80.30	1985.39
Cubic P	491.52	5.96	9.04	18.42	89.94	89.97	89.90	992.70
Hexagonal P	215.27	5.96	9.04	18.42	89.94	90.03	90.10	992.70
Rhombohedral R	236.36	5.96	10.82	56.31	94.32	96.06	123.32	2978.09
Tetragonal I	174.92	5.96	9.04	38.40	76.42	81.08	89.90	1985.39
Tetragonal P	154.90	5.96	9.04	18.42	89.94	89.97	89.90	992.70
Orthorhombic F	102.67	5.96	19.03	37.32	87.11	99.16	108.15	3970.79
Orthorhombic I	81.26	5.96	9.04	38.40	103.58	98.92	89.90	1985.39
Orthorhombic C	90.74	5.96	19.03	18.42	90.04	90.03	108.15	1985.39
Orthorhombic P	0.59	5.96	9.04	18.42	89.94	89.97	89.90	992.70
Monoclinic C	90.74	19.03	5.96	18.42	89.97	90.04	71.85	1985.39
Monoclinic P	0.52	9.04	5.96	18.42	90.03	90.06	89.90	992.70
Triclinic P	0.00	5.96	9.04	18.42	89.94	89.97	89.90	992.70

Figure 6.14 - Bravais lattice display

Note that even though monoclinic has a slightly better fit, the software makes the correct choice of the higher symmetry cell.

2. Click on the appropriate Bravais Lattice, in this case orthorhombic.

3. Press OK to accept the suggested lattice settings.

4. Refine again.

5. Refine for several more cycles, changing the constraints one or two at a time.

Now you have a unit cell ready for determining a data collection strategy.

6.3   Determine the Data Collection Strategy

APEX2 includes a powerful algorithm, COSMO, for determining an efficient strategy that fully utilizes the flexibility of your instrument.
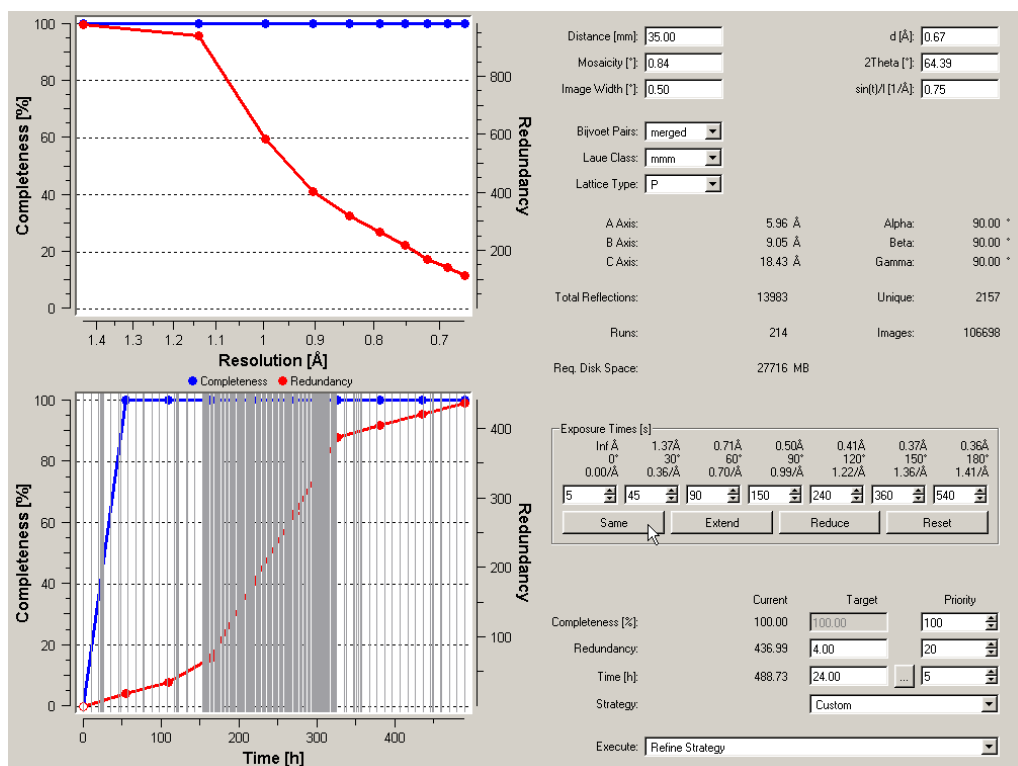
1. Left-click on Collect > Data Collection Strategy.

Figure 6.15 - The strategy display

NOTE: COSMO will use information from cell determination to set defaults. You can modify the suggested values. **How?**

2. Check the inputs for defining the data collection.

    2.1 Set the data collection distance. For SMART APEX II systems this should be set to the actual detector distance. For Kappa APEX II systems there is a variable (DX) and the distance will default to the shortest reasonable distance. Generally for the APEX II detector, the distance should be about the same as the longest cell dimension. Typically distances ranging from 35 to 45 are reasonable.

    2.2 Set the exposure time and press return. For normal crystals on an APEX II, five seconds is a reasonable time.

    2.3 Click Same to set all of the times to be the same.

NOTE: If the "Same" feature is not chosen, **the times for shells can be set** to collect high angle data more slowly than inner shell data.

    2.4 Set the desired redundancy (**0.50** is a reasonable value).

2.5 Check the other values (Laue class, Lattice, etc.).

2.6 Each time a value is changed, COSMO recalculates the statistics for the runs. The results are displayed in the column labeled Current.

2.7 Below the Target and Priority columns is a pull-down menu with several different strategies. Choose the one that best meets the needs of the experiment (**for the example, "Best in 2 hours"**).--cut this text, we don't need to give a step by step analysis of the YLID.**

At this point, if all of the runs available were collected it would take 183.98 hours and get a redundancy of 452.86. Clearly this is not desirable.

3. Click on Refine Strategy.

4. A list of options will appear. Again click Refine Strategy.
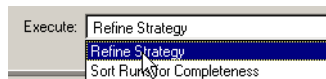
Figure 6.16 - UserManual017.bmp

NOTE: The objective in refine strategy (COSMO) is to get good completion (98% or better) with high redundancy in a reasonable amount of time. When COSMO is first started it will tell you the completion, redundancy, and time for all of the available runs. It is almost

never necessary to let COSMO run to comple-
tion. Typically it should be stopped when com-
pletion is greater than 99% and the time is close
to what is desired.
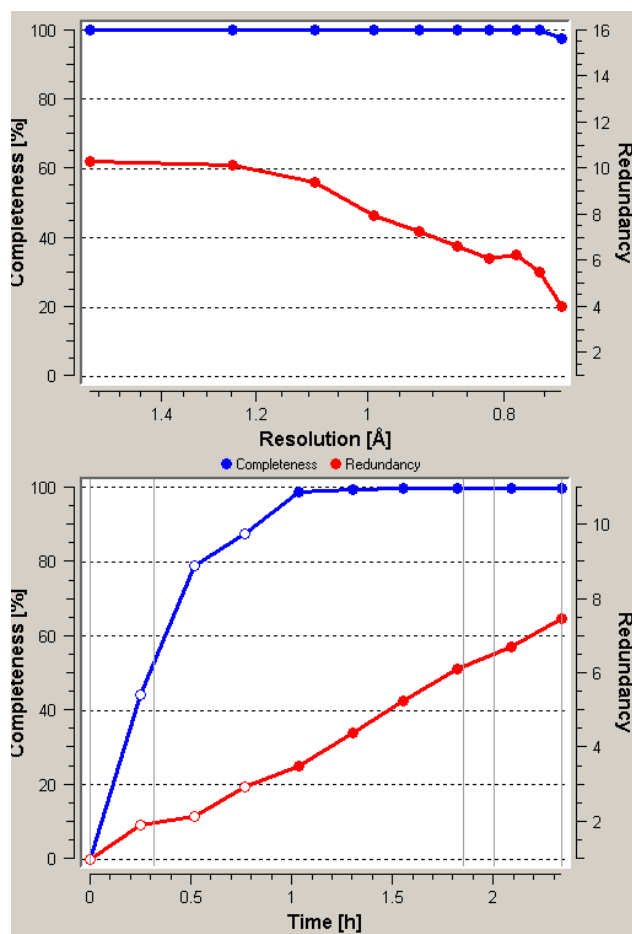
Figure 6.17 - Completeness and redundancy chart

In this example, as shown in Figure 6.18, the completion is 99.76% and the time is approximately 2.33 hours.

NOTE: Time estimates are approximate. They depend on the number of rescans, general instrument overhead, backlash compensation, etc. If estimated times are consistently longer or shorter, modify the COSMO hardwire profile.

5. Click Stop when the completeness nears 100% and the time and redundancy approach the desired values. It is not necessary to wait until the refinement reaches 100%.

Figure 6.18 - Strategy status and priority control

6. Click Refine Strategy.

7. A list of options will appear. Click on sort runs for completion.

Figure 6.19 - Choose "Sort Runs for Completeness"
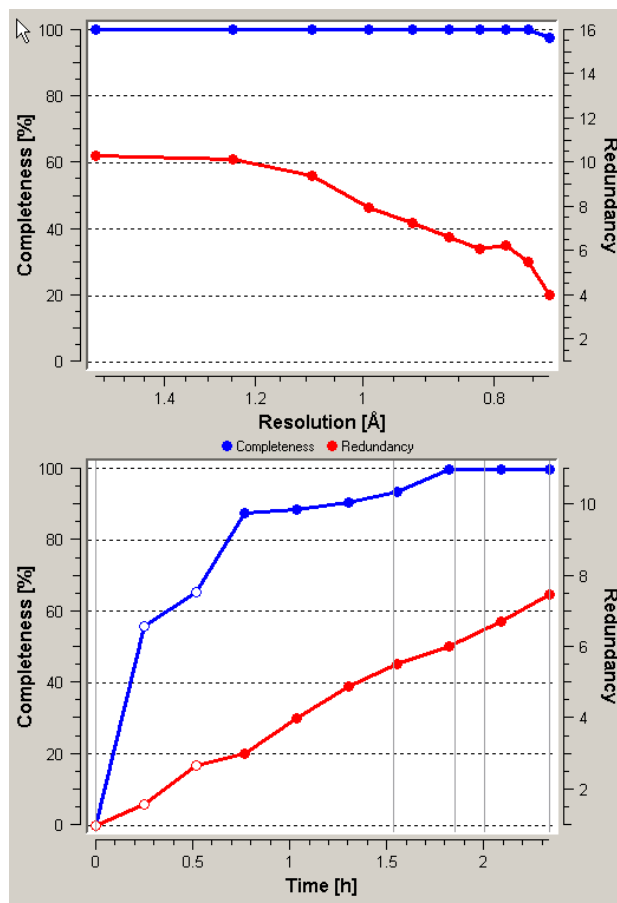
Figure 6.20 - UserManual022.bmp

8.   Go to View > Detailed Strategy.

The next window shows the runs to be collected.

		Scan Type	CCD	2Theta	Omega	Phi	Chi	Axis	Width	#Images	Sweep	Time
	1	Strategy	35.00	-22.00	+286.99	-358.39	+3.83	Phi		739	369.50	7.5
	2	Strategy	35.00	-32.00	-24.79	+27.75	-99.47	Omega	permanent	153	76.50	7.5
	3	Strategy	35.00	-7.00	-4.01	-26.26	-55.59	Omega	as-is	74	37.00	7.5
	4	Strategy	35.00	+28.00	+48.51	-14.91	-34.09	Phi	partial	162	81.00	7.5
	5	Strategy	35.00	-32.00	+317.27	+63.87	+93.86	Phi	disabled	127	63.50	7.5
	6	Strategy	35.00	-32.00	-170.70	+23.43	+98.83	Omega	+0.50	96	48.00	7.5

Figure 6.21 -

NOTE: If for some reason it is necessary to start over, change the distance slightly (by 0.02 for example) and press Return. COSMO will reload all of the possible runs.

You are now ready to collect data.

6.4   Data Collection/Run Experiment

1.   Click Collect > Experiment.

2.   Go back into the experiment window and delete the three matrix runs if they are still there.
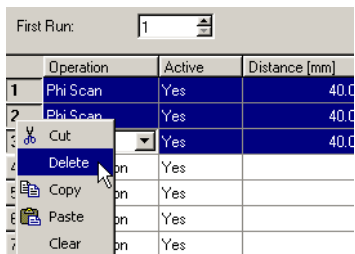
Figure 6.22 - UserManual014.bmp

3.   Click Append Strategy.

4. The program changes the name to the
    name of the current project (in this example,
    ylid_manual).
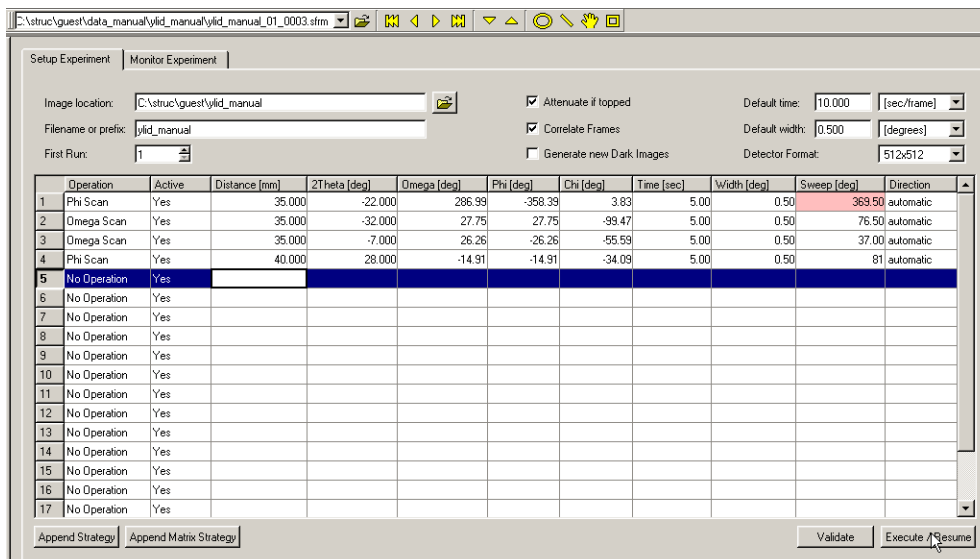
Figure 6.23 - Experiment view with strategy appended

NOTE: At the top of the experiment window are controls for data collection. Usually the default values are correct. For data collection times of less than five seconds, correlation can usually be turned off. If new dark frames are required, APEX2 will automatically collect them. Checking "Generate New Darks" will force the collection of darks before every run. In Figure 6.23, the time and width are explicitly set for each run, so changing the default width and time will have no affect. If the explicit time or width for a run is deleted so the box is empty, the default values will appear and be used.

5. Click Execute/Resume. The focus will shift to Monitor Experiment and images will start to appear. This may take a minute or two if new darks are being collected. Images will start to appear.

NOTE: When resuming after a power failure, APEX2 will automatically skip images that were previously collected with matching angles and generator settings. Otherwise it will ask if you want to overwrite the images.

NOTE: After data collection is started, the experiment window can be closed and APEX2 can be stopped. The server computer must be left on. If communications are lost between the client and the server, frame data is stored on the server.
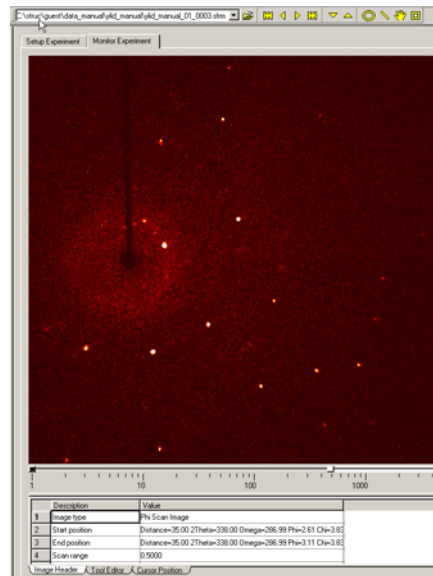
Figure 6.24 - Monitor experiment view

7. Data Integration and Scaling

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

7.1   Integration

1.   Click on Integrate in the Task Bar.

2.   Click on the Integrate Images icon. The fol-
     lowing window will open.
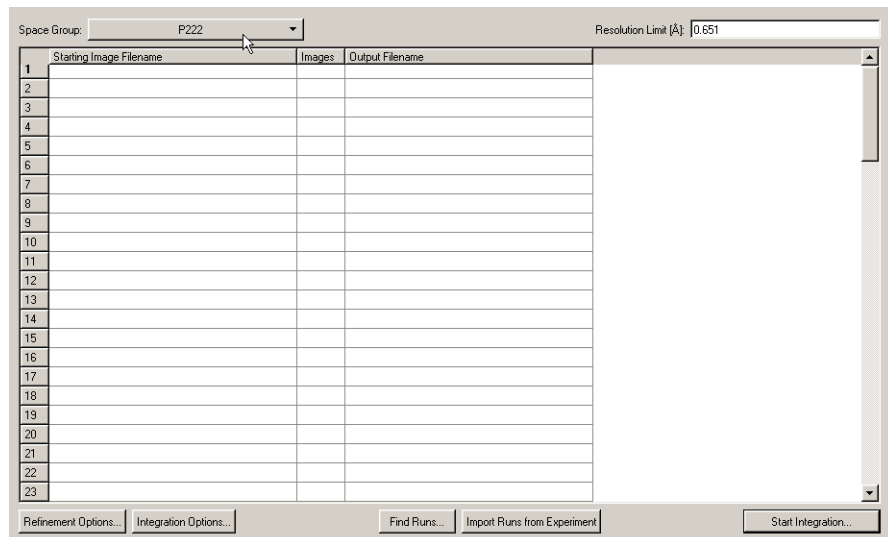
Figure 7.1 - Initial integration window

3.   Check the default values.

There are two items of interest on the top of the
window. The space group tool and the resolu-
tion limit value.

Figure 7.2 - The space group tool

The space group tool allows the user to set the symmetry for integration. Typically this value is correct when the integration window opens.
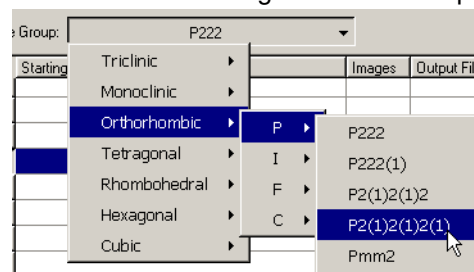
Figure 7.3 - Choosing P2(1)2(1)2(1) with the space group tool

The resolution window on the upper right sets the upper resolution for integration.

Figure 7.4 - The resolution tool

The APEX2 Suite will suggest a resolution cut-off. In this case the suggested value changed to 0.75.

In the center of the bottom are two buttons for defining the data collection runs to be integrated.

Figure 7.5 - The Find and Import Runs buttons

The import runs button determines the runs to be integrated from the experiment that has just been submitted. Using this button allows the user to start integration while the data is still being collected.

NOTE: SAINT will integrate all of the data currently measured and then will wait for the next image, integrate that, and wait for the next, etc.

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

1. Click on Find Runs.

The following window will open, usually it will
have the correct directory and basename as the
defaults. If these are not correct, then use the
browse button to find the runs for the data col-
lection of interest.
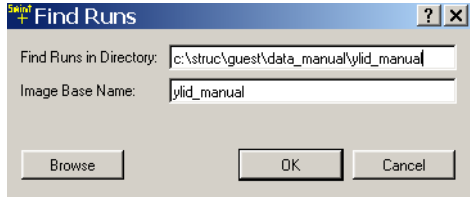
Figure 7.6 - Find Runs tool view

Both the Import Runs from Experiment and the
Find Runs buttons will generate a list of runs as
shown in Figure 7.7.

Figure 7.7 - Completed runs list

Figure 7.7 shows a run list entry being changed.
The original run is shown first and then the run
with the starting image number modified. In the
last strip, the number of frames to process has
been changed.

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

Figure 7.8 - Example of manually editing a run list

2. Check the refinement defaults.

The two buttons on the bottom far left are used to change the default options for the refinement of the data.

The default values are generally very good. There is seldom any need to change those in the refine window.
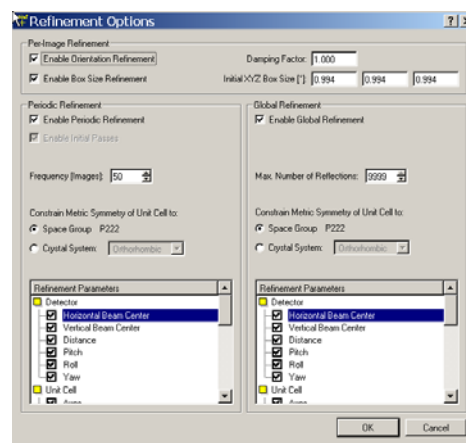
Figure 7.9 - Refine Window

3. Change the default options for the integration in the "Integration Options" window.

The integration window has a few values that are changed more frequently.
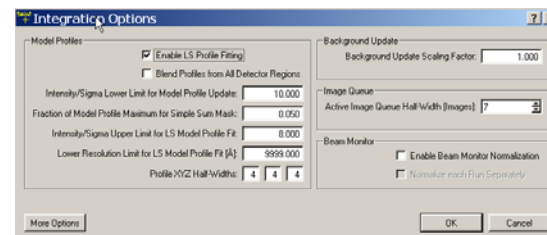
Figure 7.10 - Integration Options

The default values in the Model Profiles window are usually good.
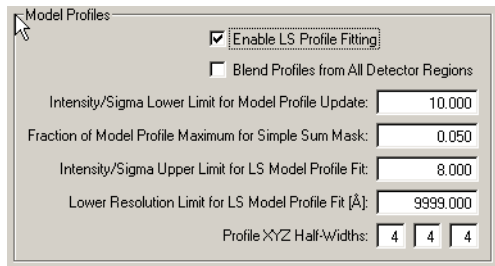
Figure 7.11 - Model Profiles

For weakly diffracting crystals it is sometimes helpful to change some options in the Model Profiles window. Reducing the Intensity/Sigma ratios from 10 and 9 to, for example, 5 and 4 or 3 gives the profile fitting algorithm more reflections to model. Clicking on Blend Profiles from All Detector Regions to increase the profile blending can also help with weakly diffracting crystals.

4. Click on More Options. This results in an expanded window (see Figure) and gives access to several other useful features, in particular Active Mask (see Figure 7.13), Algorithm (see Figure 7.14), and Image Queue (see Figure 7.15).
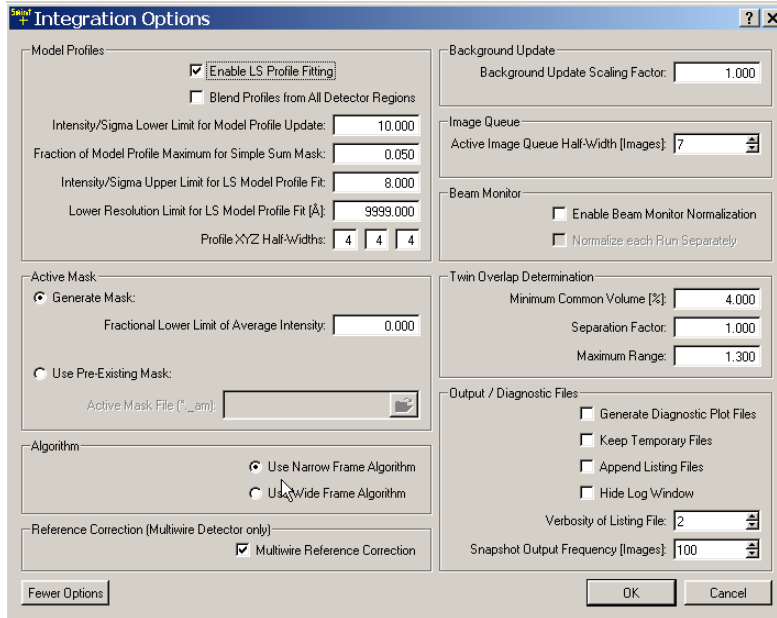
Figure 7.12 - The Integration Options window with "More Options" selected

7.1.1 Active Mask
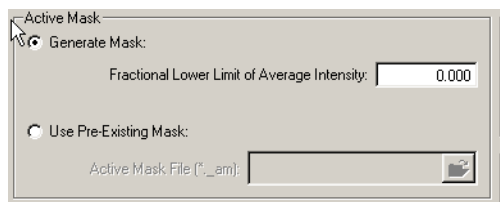
Figure 7.13 - The Active Mask tool

The Active Mask is used to mask out areas that are covered by the beam stop, low temp nozzle or other obstructions. Typically a value of 0.7 is good here. SAINT will determine an active mask for each run.

NOTE: If this option is set, the user should examine the active mask images that are written into the work directory. These images can be opened with the image viewer. They should be all red except for a black area showing the shape of the obstruction, typically the beam stop.

7.1.2 Algorithm

Figure 7.14 - The narrow/wide algorithm tool

The algorithm buttons are used to change from narrow to wide frame integration. Frames collected with narrow scans width (less than one) should be integrated as narrow frames. Those greater than one should be integrated as wide frames.

7.1.3 Image Queue
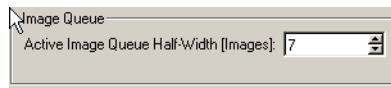
Figure 7.15 - The Image Queue tool

The Image Queue sets the number of frames being used to determine profiles. For crystals with high mosaic spread (big wide reflections) increasing the queue size can improve integration.

5.   Click on the button on the far right of the bottom row, Start Integration. This will start the integration and will open the SaintChart window for monitoring the integration.

6.   Examine the output. Double-click on any of the output entries for one additional tool—the ls button at the far right of the top. This will open the SAINT listing file for that integration run (see Figure 7.16).
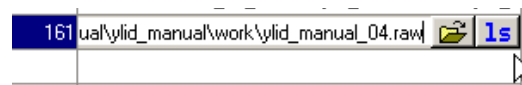
Figure 7.16 - Using the runs list ls tool

7.2   Monitor the Integration Run with SaintChart

SaintChart is a powerful tool for monitoring the progress of the calculation and for graphically presenting the results of the integration process.
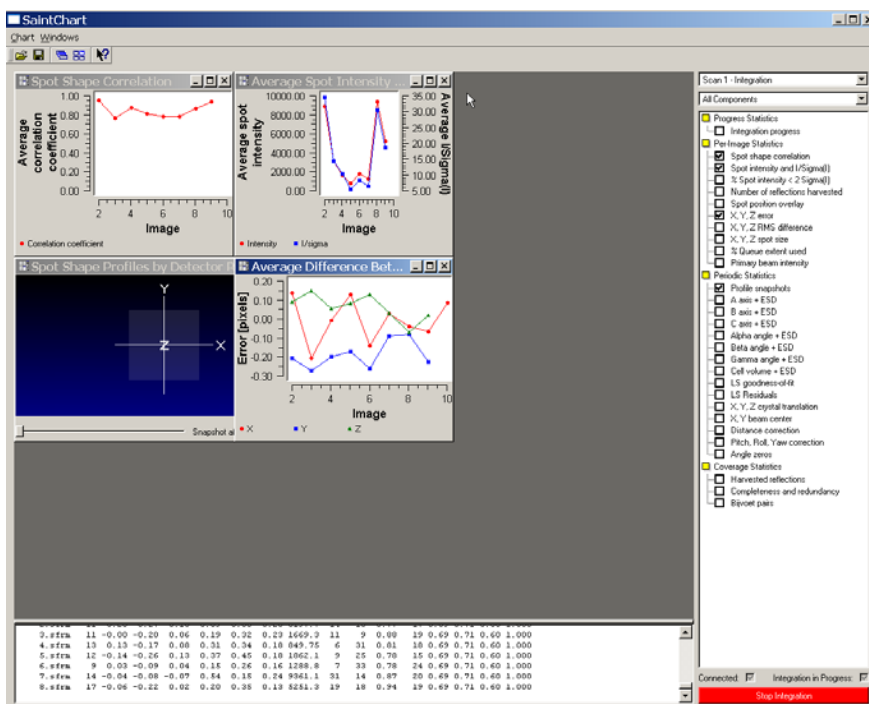
Figure 7.17 - SaintChart view

As shown in Figure 7.17, when the SaintChart window opens the graphs are small, and under Linux the text area is large.

1. Change the view.

    1.1 Change the text area by clicking and dragging on the line between the text and graphical areas.

1.2 Expand the windows to fill the available graphical area by clicking on the four-square icon in the upper right.
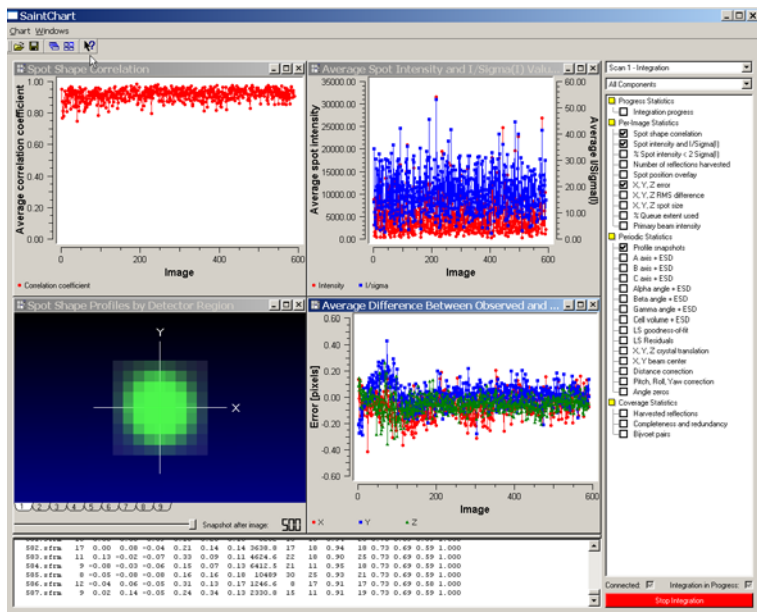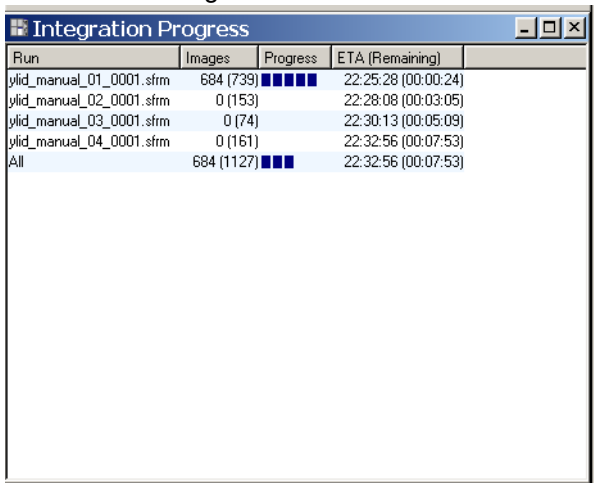
Figure 7.18 - SaintChart view with graphical area expanded

To the left of the graphical area are a series of check boxes that select the displays.

1.   Add other displays.

     1.1 Click on integration progress to add the new display seen in Figure 7.19. When the integration is complete, the blue progress bars will be full and the remaining time will be zero.

Figure 7.19 - Integration progress display

One of the most powerful windows for monitor-
ing the progress of the integration is Spot Posi-
tion Overlay (in Per-Image Statistics). Pull up on
the Window Tool Bar for Integrated Spots (the
cursor arrow is pointing to this tool bar in Figure
7.20) to display the entire window (see Figure
7.21).
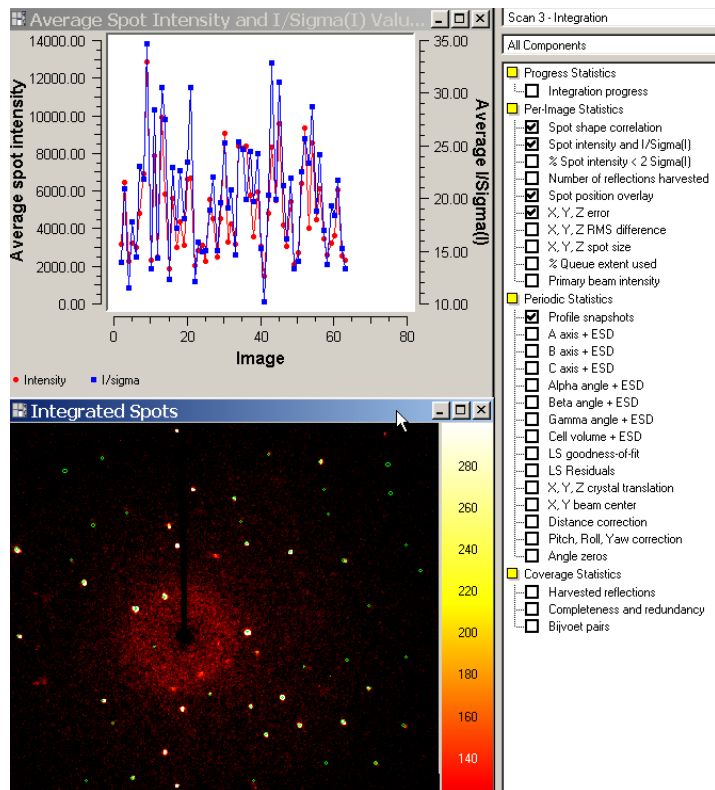
Figure 7.20 - Spot Position Overlay

In this full view, the Integrated Spots window looks much like the image viewer used previously, but there are no tools. At the bottom of the window is an entry box that can be used to change the display frequency of the Integrated Spots images. The default is shown every fifth image.
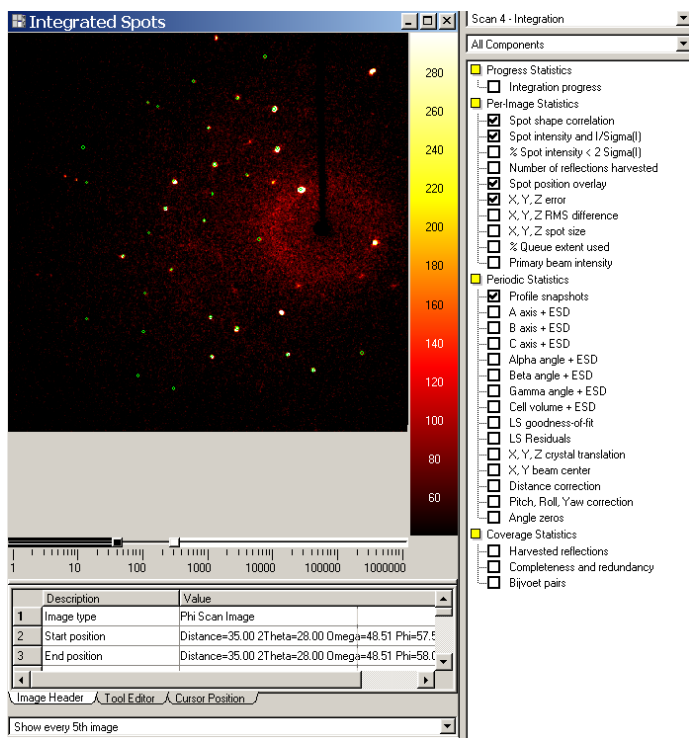
Figure 7.21 - Full view of the Spot Position Overlay

1. Examine SAINT text output.

At the bottom of the SaintChart window is a scrolling text window that displays the text output from Saint. (This window can be expanded by dragging the horizontal bar above the text as indicated by the arrow in Figure 7.22).

2. Examine the final results.

At the end of the integration, examine this text critically. In particular, look at the Overall Rsym, the coverage statistics and the unconstrained unit cell refinement.

For a typical small molecule structure, the overall Rsym is usually less than 8%. Higher values may indicate problems with absorption, twinning or poor crystal quality.

7.2.1 Overall Rsym and Coverage Statistics


```
Spot Shape Correlation                     _ □ X    Integrated Spc
  1.00
coefficient

  0.80


     25.000

   Overall      #  Pairs   Uniq   Merg %<2s        <I> <#Sig>    <Bg>  Rsym   dI/I dI/
     0.000   12956   1442   1451  12948 17.3  4707.540  27.07    2.89 0.038  0.000  0.
     1.000

   Centric      #  Pairs   Uniq   Merg %<2s        <I> <#Sig>    <Bg>  Rsym   dI/I dI/
     0.000    2413    447    454   2407 21.5  7064.442  39.39    3.08 0.033  0.000  0.
     1.000

Coverage Statistics for ylid_manual_0m.raw
                                                          .......Shell......
   Angstrms  #Obs Theory %Compl Redund   Rsym Pairs %Pairs Rshell #Sigma %<2s
to  1.615    178    178 100.00  11.78  0.032   178 100.00  0.032  82.97   4.7
to  1.282    327    328  99.70  11.38  0.033   327  99.70  0.038  31.34   2.6
to  1.120    473    475  99.58  10.95  0.034   473  99.58  0.039  21.81   7.6
to  1.018    614    616  99.68  10.71  0.034   614  99.68  0.045  13.70  11.0
to  0.945    760    763  99.61  10.29  0.035   759  99.48  0.050   9.88  13.5
to  0.889    897    903  99.34  10.03  0.035   896  99.22  0.063   7.32  18.9
to  0.845   1033   1036  99.71   9.77  0.036  1031  99.52  0.076   4.84  28.1
to  0.808   1183   1189  99.50   9.44  0.037  1181  99.33  0.083   4.45  30.9
to  0.777   1317   1320  99.77   9.18  0.037  1313  99.47  0.098   3.34  40.3
to  0.750   1451   1452  99.93   8.93  0.038  1442  99.31  0.116   2.65  50.8

Averaged "local" cell least squares refinement ============== 05/06/04 22:32:31
Local refinement results for component 1
Number of local refinements averaged:    21
```

Figure 7.22 - Final Saint Integration Statistics

Add text.

7.2.2 Unconstrained Unit Cell Refinement

```
Unconstrained global unit cell refinement ============== 05/06/04 22:32:31

Performing final unit cell least squares on file d:\structures\data_for_manual\ylid
Input file contains      5552 reflections

Global Refinement -- single-component data...
Maximum allowed reflections =       9999
One reflection will be stored in memory per     1 reflection(s) read

Orientation least squares, component  1 (5552 input reflections)...
Wavelength, relative uncertainty:  0.7107300,  0.0000089

Reflection Summary:
'RLV.Excl' are reflections excluded after cycle 1 because RLV error exceeded 0.0250
 Component      Input  RLV.Excl     Used  WorstRes   BestRes   Min.2Th   Max.2Th
         1       5552         0     5552    8.1065    0.7884     5.025    53.580

Orientation ('UB') matrix:
  -0.0584509   0.0333484  -0.0483062
   0.0005328   0.1049404   0.0175150
   0.1573586   0.0121009  -0.0180036

         A         B         C     Alpha      Beta     Gamma        Vol
    5.9572    9.0273   18.3668    90.088    89.999    90.033     987.72
    0.0001    0.0002    0.0004     0.001     0.001     0.001       0.05
Corrected for goodness of fit:
    0.0005    0.0008    0.0019     0.005     0.005     0.005       0.22
```

Figure 7.23 - Unconstrained unit cell refinement

Check the angles in the unconstrained unit cell refinement to get a quick confirmation that the initial lattice determination was correct. In the unconstrained refinement, angles that are required to be 90 or 120 are allowed to refine. If they differ considerably from the expected value then the data should be checked carefully.

7.3   Scale
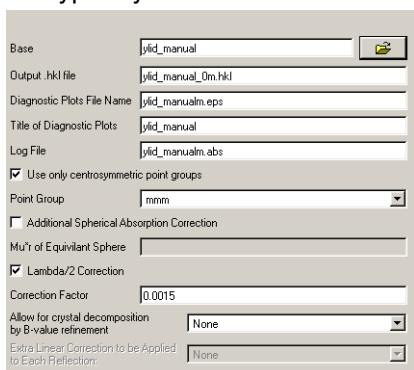
The scaling process uses the SADABS program to put all of the measured data on the same scale. This process involves five steps:

1.   Setup input files.

2.   Parameter refinement.

3.   Error model refinement.

4.   Display diagnostics.

5.   Exit.

7.3.1   Setup Input Files

1.   Click on Scale > Scale in the task bar. Figure 7.24 shows a portion of the initial Scale work area. The defaults and file names are typically correct.

Figure 7.24 - FIG7.28a

2.   Click Next and check that the defaults are correct. Generally the Absorber Type is all that changes.

7.3.2 Parameter Refinement

1. Click on Refine. Figure 7.25 shows a typical
    refinement result. The R-values are reduced
    and the Mean weight increases. Typical
    final values are 3–4% for the R-values and
    0.95–0.98 for the Mean Weight.
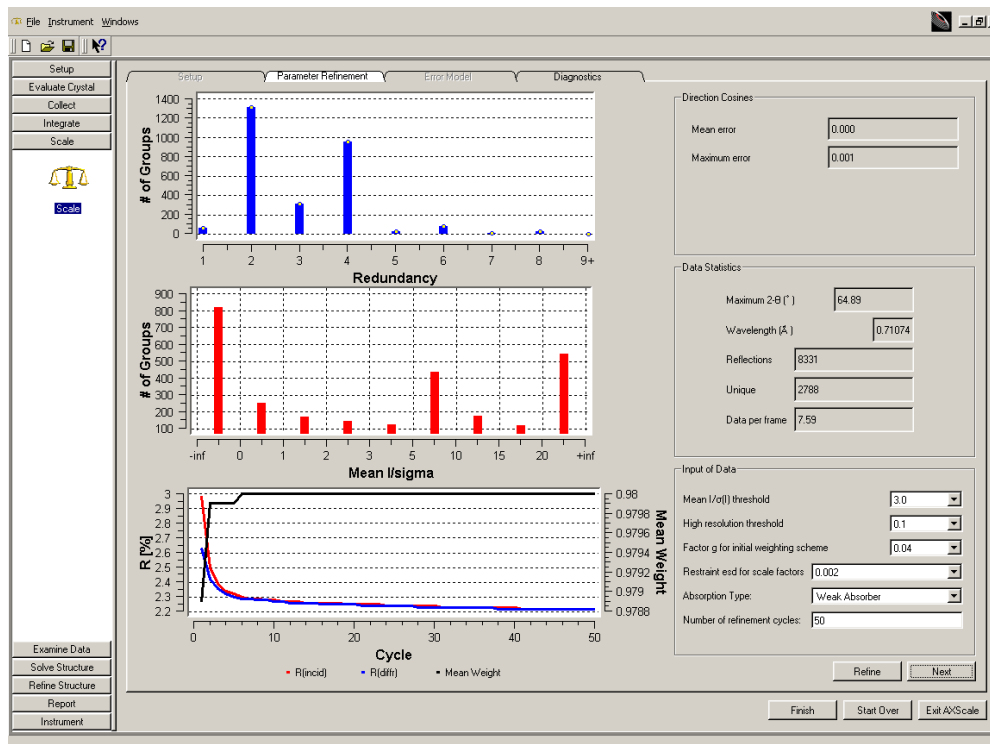
Figure 7.25 - Xscale window after refinement

7.3.3 Error Model Refinement

Figure 7.26 shows typical Error Model results.
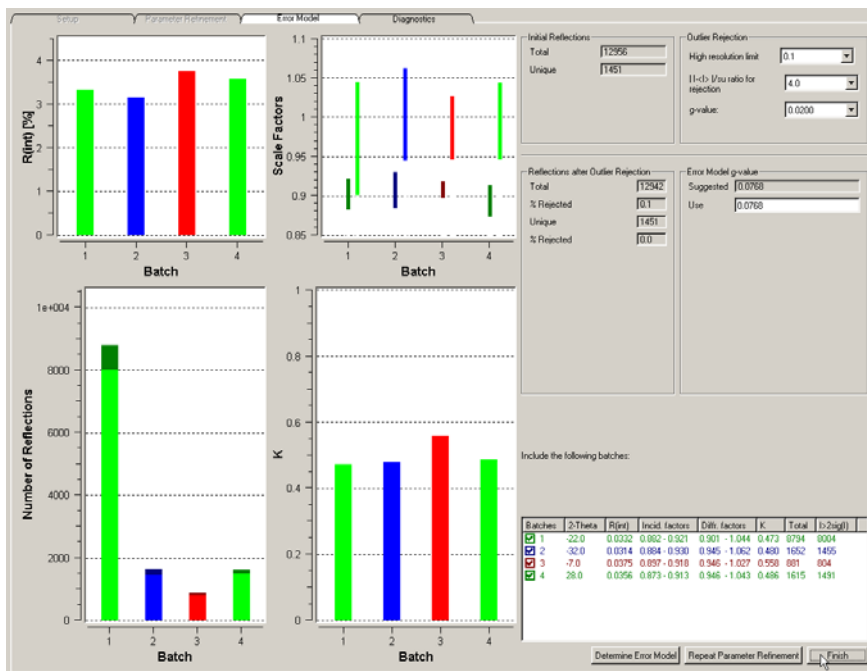
Figure 7.26 - Error model results

NOTE: If the R-values are significantly higher than this step, repeat the entire scaling calculation. Click the check box by the "bad" run and then click Repeat Parameter Refinement.

7.3.4 Display Diagnostics

1. Click Finish to produce a series of diagnos-
    tic plots. Some of these are recaps of the
    plots that have already been displayed.

2. Click on the "folder" tab at the bottom of the
    screen to select the individual plots as
    shown in Figure 7.27. **Why? To do what?**
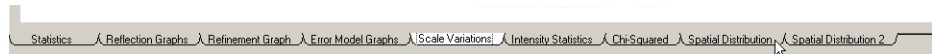
Figure 7.27 - Tabs for selecting diagnostic plots

The Scale Variations plot shows the overall vari-
ation in Scale and R(int) for the individual
frames. The Scale plot should be flat (for highly
absorbing, irregularly shaped crystals it will
probably be sinusoidal) and the R(int) plot
should not show large variations (more than
2%).
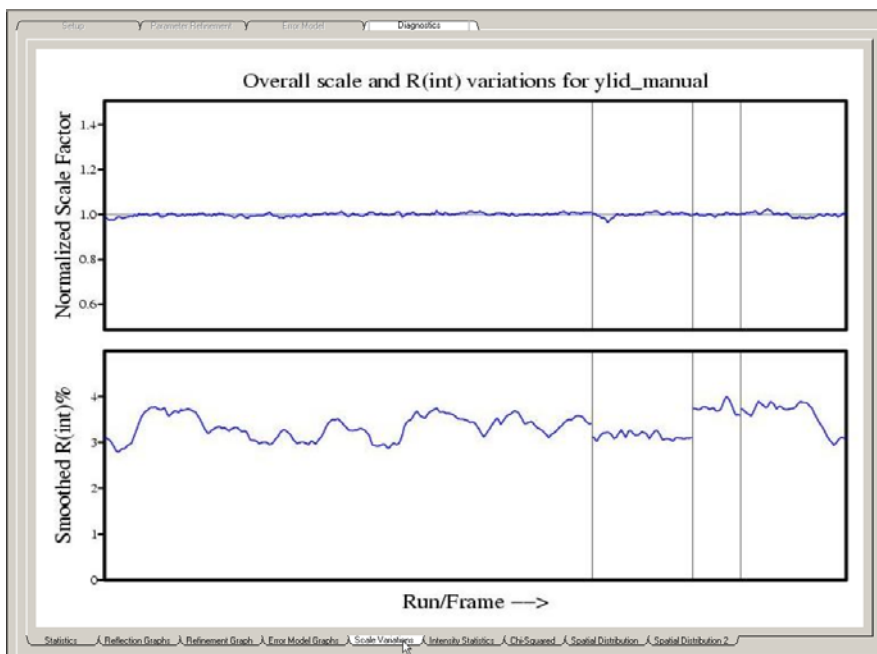
Figure 7.28 - Overall scale and R(int) variations

The Intensity Statistics plots look at R(int) and |E2-1| as a function of resolution. Typical plots of R(int) versus Resolution increase to the right as seen in Figure 7.29. An |E2-1| plot should have a constant resolution. |E2-1| is a strong indicator of centric and acentric space groups. The two horizontal lines indicate the expected values for centric (top) and acentric (bottom) space groups. This plot clearly suggests an acentric space group.

The plots Chi-Squared values for the data as a function of resolution and intensity should be mostly flat. Values below one are not unusual for weaker high resolution data. The plots shown in Figure 7.30 are typical.

Figure 7.30 - Chi-squared
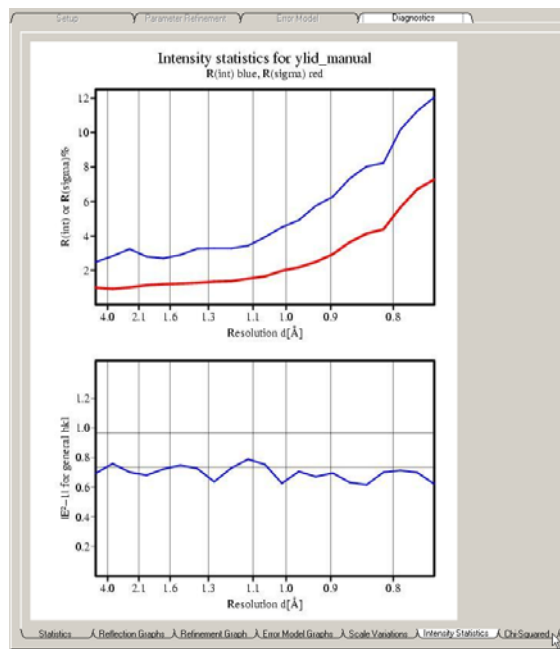
Figure 7.29 - Intensity Statistics
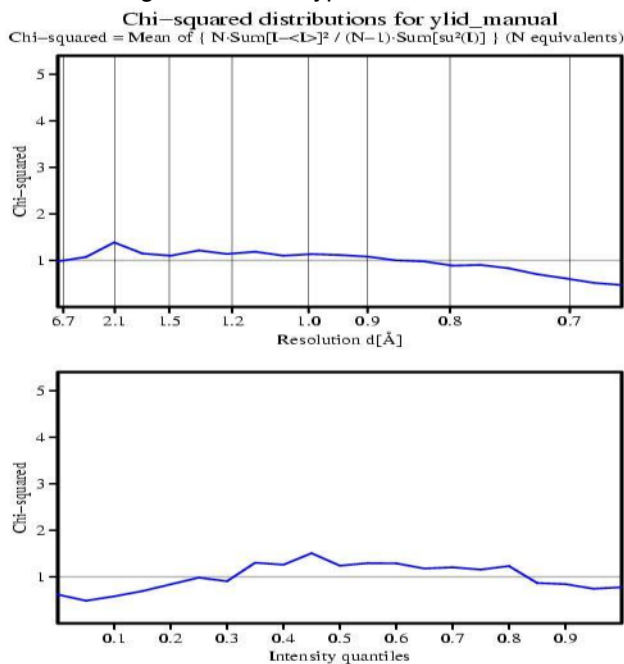
The special distribution plots are generated for each data collection run. They indicate, using red and blue dots, points of average intensity for a particular reflection after scaling. The actual intensity measured at that spot on the detector vary by more than three standard deviations. Figure 7.31 show two spatial distribution plots side by side. This is the way they are typically output by Scale.
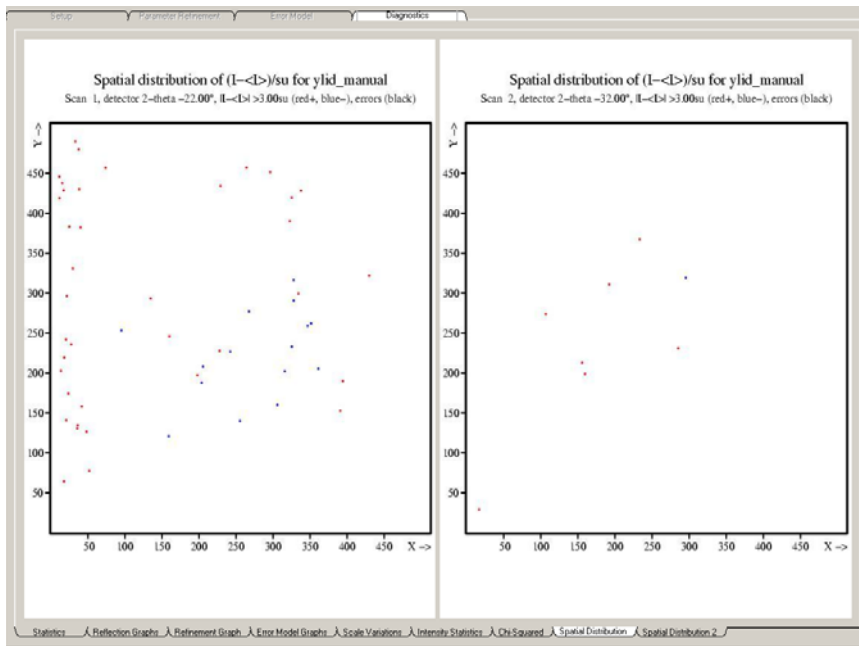
Figure 7.31 - Spatial distribution

Figure 7.32 shows a single plot so that the dots are easier to see. Note that the points of disagreement are spread fairly evenly over the entire detector face.
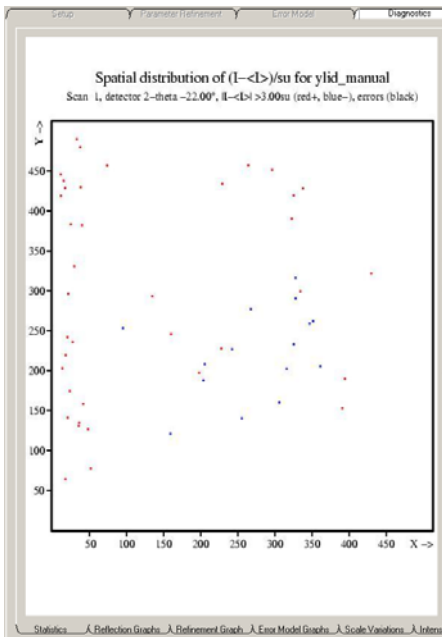
Figure 7.32 - A single Spatial Distribution

If the spots are clustered in an area or if there are significantly more spots of one color than there are of another, then the data should be examined critically. Figure 7.33 is from another data set where the active mask was not used during integration and the reflections collected in that area are consistently smaller than

expected. This kind of problem may affect the final results.
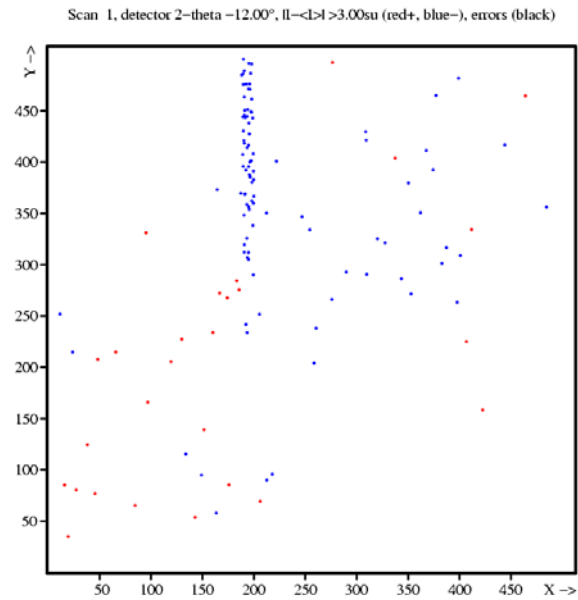
Figure 7.33 - A Spatial Distribution plot showing a problem area

7.3.5 Exit

1. Click on Exit XScale to close the scaling GUI.

Figure 7.34 - Exit XScale

NOTE: Scale is one of the few GUIs where it is necessary to exit using a special exit button. This avoids an error message that SADABS has exited improperly.

---

8. Examine Data

In this step, the space group for the sample is determined and optional simulated precession photographs are calculated to further evaluate the overall quality of the data. APEX2 provides two tools for this: XPREP for space group determination and Precession Images for looking at undistorted slices of reciprocal space. This is the final step before beginning the structure solution and refinement process.

8.1 XPREP

1. Click on XPREP (Space Group Determination) under Examine in the task bar.

2. Check that the two files in the pop-up window are correct (see Figure 8.1) and click OK.
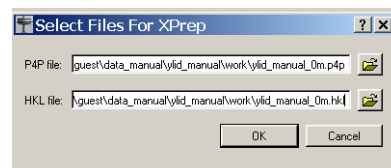
Figure 8.1 - Select files for XPREP input

NOTE: The scaling process has created two files: ylid_manual_0m.p4p containing the final unit cell parameters from integration and ylid_manual_0m.hkl containing the corrected intensities. Typically these are the files to use for

space group, but you can browse to choose other files.

8.2   Space Group Determination

1. XPREP evaluates the data and looks at the mean intensities and the mean int/sigma. Since these are large for all groups except P, XPREP suggests that the lattice is P (see Figure 8.2). Press Enter to accept.
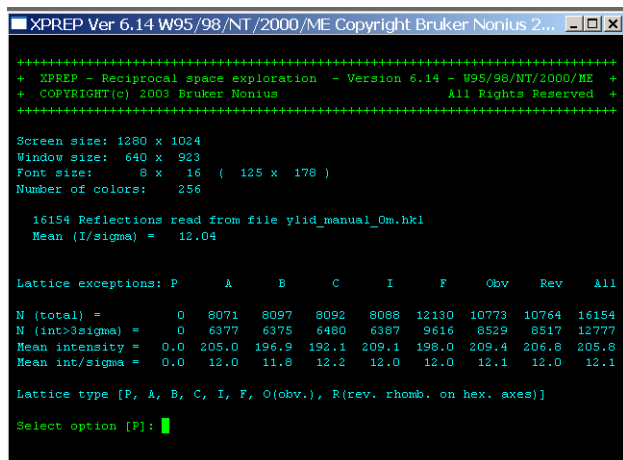
Figure 8.2 - Lattice statistics

2. XPREP determines the reduced cell based on the lattice entered above. Since the lattice was primitive and the magnitudes of the cell dimensions were proper (a<b<c), the original and reduced cells are the same (see Figure 8.3). Press Enter to search for a higher symmetry cell.
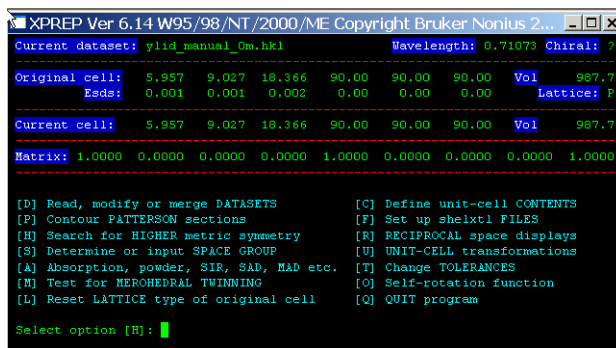
Figure 8.3 - Reduced cell

For the YLID, there is no higher symmetry cell found. The program has determined that the YLID crystal has an orthorhombic primitive lattice (see Figure 8.4). Press Enter to accept.
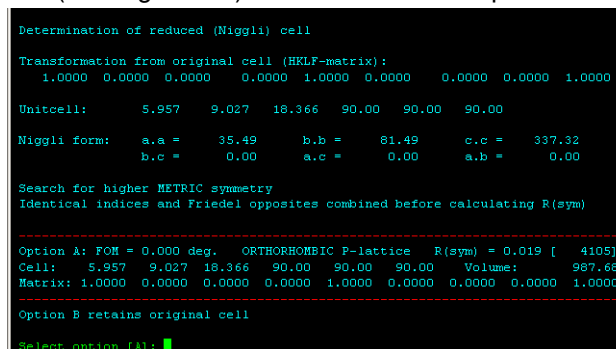
Figure 8.4 - Higher symmetry cells

3. The next logical step is to determine the space group. XPREP suggests this (see Figure 8.5). Press Enter to determine the space group.

Figure 8.5 - Determine the space group

4. If the space group is known or if the compound is known to be chiral, enter that information (see Figure 8.6). Generally, it is sufficient to press Enter to start the space group determination.

Figure 8.6 - Space group options

5. XPREP has chosen the crystal system [O] (see Figure 8.7). Press Enter to accept.

Figure 8.7 - Choose the crystal system

6. XPREP has chosen the crystal lattice [P] (see Figure 8.8). Press Enter to accept.

Figure 8.8 - Choose the lattice

7. XPREP evaluates the data and looks at the systematic absences for all possible glide planes and screw axes (see Figure 8.3). These are displayed across the middle of the figure. By examining the number of reflections with I>3 sigma(I), the mean intensities, and the mean int/sigma, which should all be very small for a systematic absence, XPREP derives a suggested space group, P2(1)2(1)2(1). Press Enter to accept.

8.3   Reflection Statistics.

Figure 8.9 - Systematic absences and a suggested space group

1. XPREP returns to the general menu seen previously (see Figure 8.5). This time D is chosen to evaluate the dataset. Press Enter to accept.

2. There are multiple choices for data manipulation (see Figure 8.10). "S" chooses a display of statistics.

Figure 8.10 - The data manipulation menu

3. The data can be merged in several ways. Choose the merge all equivalents including Friedel mates option [A] (see Figure 8.11). Press Enter to accept.

Figure 8.11 - The merge data menu

4. A table of statistics appears (see Figure 8.12). Examine the data presented. Is the completion near 100%? Is the redundancy good? Are Rint and Rsigma small and increasing smoothly from top to bottom? In particular look at the last two lines which compares all of the data with the high resolution data. The completion should be near 100% for both the high resolution shell and the complete data set. The redundancy and Rint should be similar for the two. The Mean Intensity and the Mean I/sigma(I) will usually be quite different. The Mean I/sigma(I) for the high resolution data should be greater than 3.0.

For this data set the difference between the Rint for the high resolution data and the complete data set is slightly larger than expected. Press Enter to continue.

Figure 8.12 - Intensity statistics

5. Since the difference between the Rint for the high resolution data and the complete data set is slightly larger than expected, it is reasonable to apply a high resolution cutoff using the H option in the data manipulation menu. (see Figure 8.13). Type H and press Enter.

Figure 8.13 - Requesting a high resolution cutoff

8.4   Applying High Resolution Cutoff

1. Enter a high resolution limit of 0.75 (see Figure 8.14) and press Enter to accept the low resolution cutoff of infinity.

Figure 8.14 - The resolution cutoff menu

2. Applying the cutoff removes approximately 3000 reflections from the data set (see Figure 8.15). The next step is to see if the cutoff has improved the statistics. Type S and press Enter.

Figure 8.15 - Requesting statistics after applying a high resolution cutoff

3. As before in step 3, enter [A] to merge all equivalents.

4. After the cutoff, all of the statistics look better (see Figure 8.16). Press Enter to continue.

Figure 8.16 - Statistics after high resolution cutoff

5. This response will return to the data manipulation menu (see Figure 8.10, Figure 8.13 or Figure 8.15). The default answer should be E for exit. Press Enter to accept this default.

8.5   Preparing an Output File

1. Exit the data manipulation menu to return to the general menu. This time chose C to define the unit cell contents.

2. A window will open displaying the current formula, Z, the density and the atomic volume (see Figure 8.17). In this example, the formula is incorrect and Z has been set to six to try to achieve a reasonable density and atomic volume. Since this formula is incorrect it must be modified now. The correct chemical formula for the YLID crystal is C11 H10 O2 S.

   2.1 At Select Option do not accept the default answer of E. Type in F to enter a new formula.

   2.2 In response to the question "Enter Formula," type the correct formula C11 H12 O2 S1 and press Enter.

   2.3 Check that the information is correct. Check that Z seems reasonable for the space group, that the density is as expected (1.1 to 1.4 for organic molecules, higher for inorganic compounds), and that the atomic volume is around 17 or 18. Significant variation from the expected values may indicate an incorrect molecular formula or missing counter ions or solvates. The values at the bottom of the window look fine for the YLID. Press Enter to accept.

Figure 8.17 - Defining and checking the unit cell contents

3. The next default action for XPREP is to write out the files necessary for the structure solution process (see Figure 8.18). Press Enter to accept.

Figure 8.18 - Requesting output files

4. The program asks for an output file name. Since this data has been cutoff at a resolution of 0.75A, it makes sense to change the output file name to ylid_res75 to reflect the cutoff (see Figure 8.19). Press Enter to accept.

Figure 8.19 - Changing the file name

5. After entering the file name (or hitting return to accept the default file name) an input file for the structure solution module is created and displayed on the screen. And the program asks "Do you wish to (over)write the intensity data file ylid_res75.hkl." Since the file name has been changed, this question must be answered with a y (see Figure 8.20).

Figure 8.20 - The input file for structure solution and a final question

6. Exit XPREP (see Figure 8.21). Press Enter to exit the program.

Figure 8.21 - Exit XPREP from the general menu

NOTE: There are many other features in XPREP that can be accessed from the general menu; reciprocal space plots, simulated powder patterns and a test for merohedral twinning are very useful tools.

8.6   Simulated Precession Images

1. Click on Precession Images under Examine
    in the task bar. Precession Images provide
    an undistorted view of layers of the recipro-
    cal lattice. APEX2 generates simulated pre-
    cession images by finding the appropriate
    pixels in a series of ".sfrm" images. You
    must specify the images to examine and the
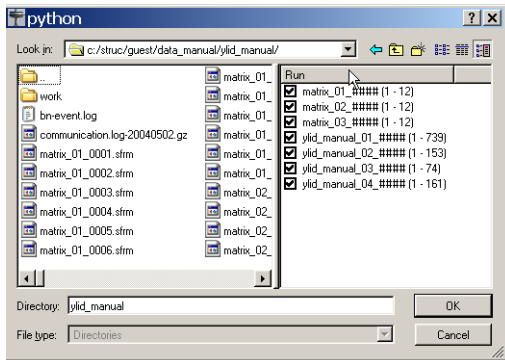   zones to calculate.

Figure 8.22 - The simulated precession work area

2. To open the file selection window, left-click
    on the folder beside the file name. Choose
    the sets of images that you want to use for
    the calculation by clicking on the check
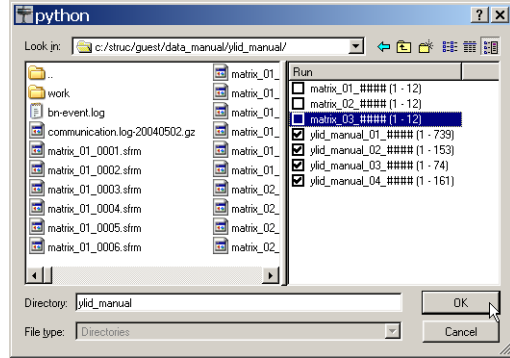   boxes (see Figure 8.23).

Figure 8.23 - File Selection window with matrix runs
deselected

3. Check the default input value. Modify as
    needed and press Calculate.

Calculating more zones does not significantly
increase the calculation time. In the example,
1kl, h1l and hk1 have been added to the
defaults of 0kl, h0l and hk0. Fractional values
(e.g., 0.5kl) are allowed. Reducing the resolu-
tion 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 is looking for all pixels that
have -0.1<h<0.1, and any value, including frac-
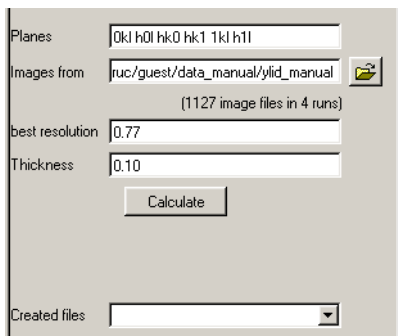tional values, for k and l.

Figure 8.24 - Detail of the input section

4. A progress bar will appear and after approximately 5–10 minutes (depending on the number of "sfrm" files read) an image will appear on the screen. Display other planes by clicking on the calculated images to the bottom right of the work area or by browsing as usual with the view plugin.
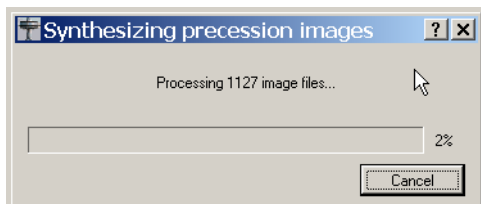

Figure 8.25 - The progress bar

NOTE: This is a relatively slow calculation since every sfrm file is examined to determine if it has pixels that would contribute to a requested zone. For example, if the 0kl zone is requested with a thickness of 0.1 then the simulation is looking

for all pixels to have -0.1<h<0.1 and any value, including fractional values, for k and l.

5. User the simulated patterns to check space group symmetry (see Figure 8.25, Figure 8.26 and Figure 8.27) and to find signs of twinning (see Figure 8.28).

I am having trouble generating acceptable images for 8.26 to 8.29, but they will get done eventually.

Include SIMPREC5.bmp here

Figure 8.26 - The 0kl plane for the test YLID

Include SIMPREC6.bmp here

Figure 8.27 - The h0l plane for the test YLID

Include SIMPREC7.bmp here

Figure 8.28 - The hk0 plane for the test YLID

Include SIMPREC7.bmp here

Figure 8.29 - A plane from a rotationally twinned crystal showing the two lattices

Curt will add images of a good and bad example.

9. Structure Solution and Refinement

9.1 Overview

You are now ready to solve and refine the crystal structure for the YLID test crystal. The various steps in solving and refining the structure are carried out within the APEX2 GUI. These tools will prepare input files for the solution and refinement programs of the SHELXTL package. The integration process (SAINT) has produced two important files—YLID1M.p4p, containing the final unit cell parameters and other important information on how the experiment was carried out, and YLID1M.raw, containing the actual intensity data.

These files are all that is required to begin the structure solution and refinement process. The various steps in solving and refining the structure are carried out using the programs of the **SHELXTL package**.

9.2   Solve the Structure

The process of obtaining an initial model of the compound is started by clicking in the Task Bar on Solve Structure and then on Structure Solution.

1.   This will open the Structure Solution GUI. Check that there is a reasonable formula in the formula window.

1.1 If you entered a correct formula in the display GUI, then all is ready to start a normal direct method run.

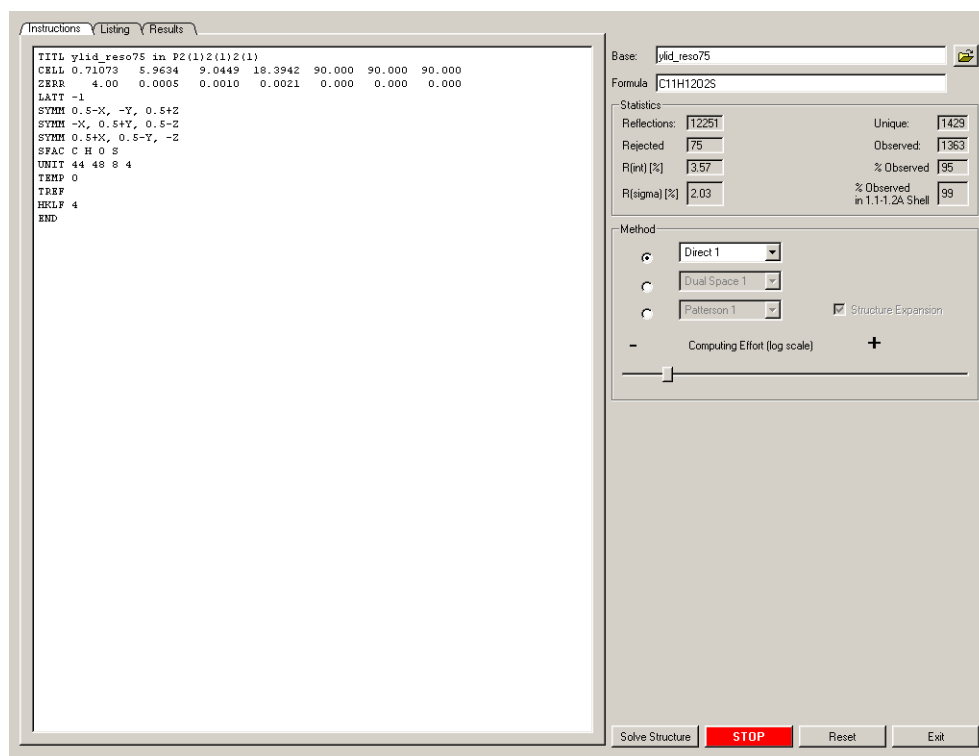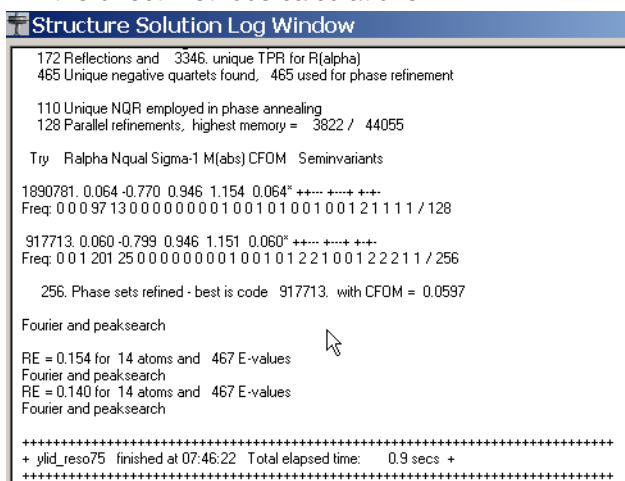1.2 If the formula is incorrect, change it in the formula window and press Return. The input file will update.

Figure 9.1 - Default Solve Structure view

2. Click on Solve Structure to start the calcula-
    tions and open an output window. This win-
    dow can be used to monitor the progress of
    the direct methods calculations.

Figure 9.2 - Structure Solution output

3. Click on the arrow to the right of Direct 1 in
    the Method box (see Figure 9.3). This gives
    three choices for structure solution using
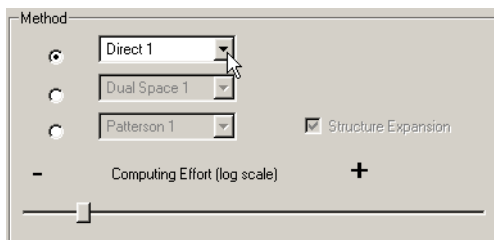   direct methods.

Figure 9.3 - Method box

In most cases the default values will give a good
initial model, but there are several other options
available for more difficult problems.

1. Chose the solution method by clicking the
    appropriate radio button on the left in the
    Method box.

2. Highlight and click on Direct 1 to set up a
    default run with a simple TREF instruction.

3. Highlight and click on Direct 2 to set up an
    extended run with TREF 10000. By adding
    10000 to the TREF command, more
    attempts are made to determine a solution.
    This will take more time, but if you see a
    good solution in the output window it is pos-
    sible to stop at that point by clicking on the
    STOP button. Good solutions typically have
    a CFOM of 0.06 or less.

4. Highlight and click on Direct 3 to set up an
    extreme run with two instructions, ESEL 1.0
    and TREF 100000. By adding ESEL 1.0,
    more reflections are used in the solution
    process. This is particularly useful for acen-
    tric triclinic structures and for pseudo sym-
    metric structures. Increasing the number of
    tries in the TREF command to 100000 runs
    until ended by clicking on the STOP button.
    There is only one predefined option for the
    dual space calculations and also only one
    for the heavy atom Patterson methods.

At the end of the direct methods runs, the output
display will look like the figure. Good figures of

merit are near 1.0 for Sigma-1 and M(abs) and less than 0.06 for Ralpha and the CFOM. Structures can be solved with figures of merit that deviate from these numbers, but they may require more effort.

If all looks reasonable, click OK in the output display and Exit in the solutions plug-in. The next step is structure refinement using XSHELL.

9.3   XSHELL

9.3.1 Refine the Structure

The structure solution step produced statistics that indicate a solution had been found. The real proof is in the initial model that is produced. XSHELL provides the tools to view the model and determine if it makes chemical sense.

1.   Start XShell, the molecular viewing and refinement plug-in, by clicking on Refine Structure in the Task Bar and then on the Structure Refinement icon.

2.   This will open a window to select files for XShell. In usual operations, the file name will be preselected. If a different file is desired, select Browse.
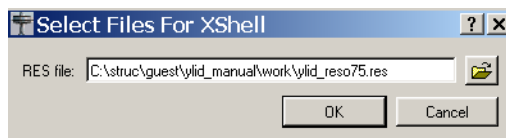

Figure 9.4 - The Select Files tool

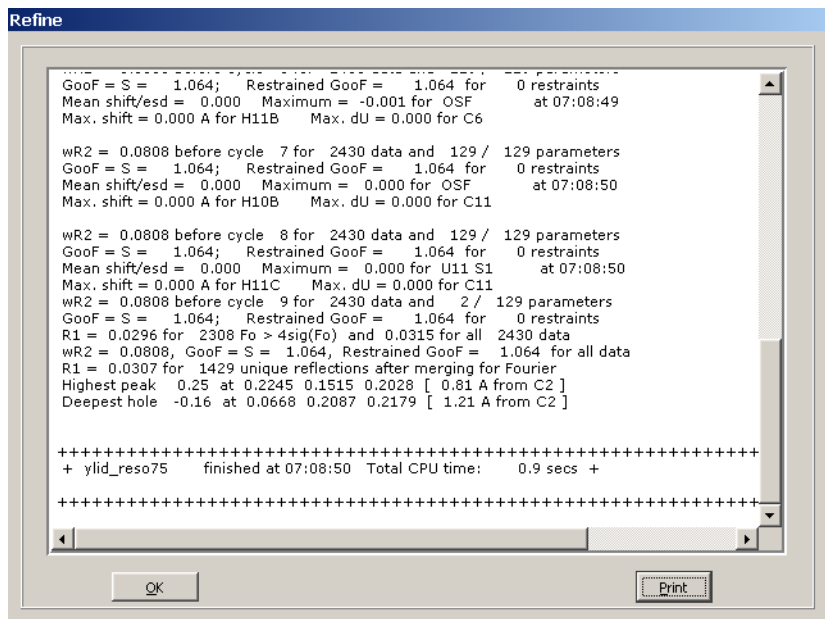3. Click on OK to open a molecular display
    window.

Refine

```
GooF = S =    1.064;    Restrained GooF =     1.064  for      0 restraints
Mean shift/esd =  0.000    Maximum =  -0.001 for  OSF          at 07:08:49
Max. shift = 0.000 A for H11B     Max. dU = 0.000 for C6

wR2 =  0.0808 before cycle   7 for   2430 data and  129 /  129 parameters
GooF = S =    1.064;    Restrained GooF =     1.064  for      0 restraints
Mean shift/esd =  0.000    Maximum =  0.000 for  OSF          at 07:08:50
Max. shift = 0.000 A for H10B     Max. dU = 0.000 for C11

wR2 =  0.0808 before cycle   8 for   2430 data and  129 /  129 parameters
GooF = S =    1.064;    Restrained GooF =     1.064  for      0 restraints
Mean shift/esd =  0.000    Maximum =  0.000 for  U11 S1       at 07:08:50
Max. shift = 0.000 A for H11C     Max. dU = 0.000 for C11
wR2 =  0.0808 before cycle   9 for   2430 data and    2 /  129 parameters
GooF = S =    1.064;    Restrained GooF =     1.064  for      0 restraints
R1 = 0.0296 for   2308 Fo > 4sig(Fo)  and  0.0315 for all   2430 data
wR2 = 0.0808, GooF = S =  1.064, Restrained GooF =   1.064  for all data
R1 = 0.0307 for   1429 unique reflections after merging for Fourier
Highest peak    0.25  at 0.2245  0.1515  0.2028 [ 0.81 A from C2 ]
Deepest hole   -0.16  at 0.0668  0.2087  0.2179 [ 1.21 A from C2 ]


++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
 + ylid_reso75     finished at 07:08:50  Total CPU time:     0.9 secs  +

++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
```

[OK]   [Print]

Figure 9.5 - Initial view of the YLID molecule

4. Right-click in the background to open a pop-up window with options to change the image.

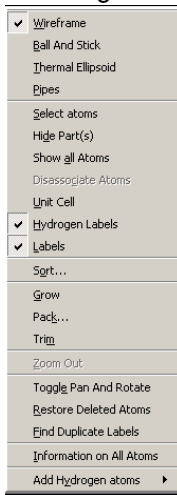| |
|---|
| ✔ <u>W</u>ireframe |
| <u>B</u>all And Stick |
| <u>T</u>hermal Ellipsoid |
| Pi<u>p</u>es |
| <u>S</u>elect atoms |
| Hi<u>d</u>e Part(s) |
| Show <u>a</u>ll Atoms |
| Disasso<u>c</u>iate Atoms |
| <u>U</u>nit Cell |
| ✔ <u>H</u>ydrogen Labels |
| ✔ <u>L</u>abels |
| S<u>o</u>rt... |
| <u>G</u>row |
| Pac<u>k</u>... |
| Tri<u>m</u> |
| Zoom Out |
| Togg<u>l</u>e Pan And Rotate |
| <u>R</u>estore Deleted Atoms |
| <u>F</u>ind Duplicate Labels |
| <u>I</u>nformation on All Atoms |
| Add H<u>y</u>drogen atoms ▸ |

Figure 9.6 - XSHELL menu

The Information On All Atoms option shows a list of the peaks and their heights. The drop in height between Q13 (the last real peak) and Q14 (the first noise peak) is typical of a correct solution.

INFORMATION ON ALL ATOMS

	LABEL	PEAK HEIGHT	TYPE	X	Y	Z	HYBRID	CHARGE	UEQ	
1	S1	0.0000	S	0.8105	0.1820	0.2401	sp3	0	0.05	
2	Q1	161.0300	Q	0.6925	0.10	0.1253	sp3	0	0.05	
3	Q2	158.7400	Q	0.3333	0.3057	0.1756	sp3	0	0.05	
4	Q3	155.9800	Q	0.4332	0.1961	0.1488	sp3	0	0.05	
5	Q4	141.7900	Q	0.5055	0.3	0.685	sp3	0	0.05	
6	Q5	141.6800	Q	0.8344	0.898	0.1307	sp3	0	0.05	
7	Q6	140.8200	Q	0.6372	0.1262	0.1726	sp3	0	0.05	
8	Q7	140.6700	Q	0.3537	0.1161	0.834	sp3	0	0.05	
9	Q8	127.0800	Q	0.1821	0.1416	0.360	sp3	0	0.05	
10	Q9	109.9200	Q	0.8324	0.3761	0.2281	sp3	0	0.05	
11	Q10	109.4100	Q	0.1499	0.486	0.244	sp3	0	0.05	
12	Q11	104.8200	Q	0.2908	0.615	0.380	sp3	0	0.05	
13	Q12	98.8900	Q	0.4789	0.856	0.93	sp3	0	0.05	
14	Q13	85.8200	Q	0.6561	0.1836	0.3232	sp3	0	0.05	
15	Q14	66.1600	Q	0.5674	0.1814	0.2395	sp3	0	0.05	
16	Q15	64.7300	Q	0.8062	0.4621	0.2409	sp3	0	0.05	
17	Q16	54.7300	Q	0.9493	0.1856	0.1563	sp3	0	0.05	
18	Q17	40.2200	Q	0.52	0.989	0.237	sp3	0	0.05	

OK   Print

Figure 9.7 - Atom information screen

At the bottom left of this window is a slide bar which is used for deselecting peaks. The arrow can be moved by left-clicking and dragging the arrow or by left-clicking on either side of the arrow. Left-clicking to the left of the arrow (i.e., where the cursor is pointed in Figure 9.8) will remove peaks.
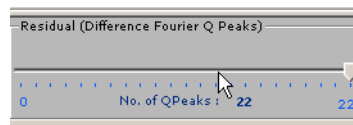
Figure 9.8 - The Q-peak slider

5. Slide the pointer down while watching the molecular display. Change the number of Q peaks to 15.

At 15 the molecule is much cleaner, but there are still peaks that do not make sense.
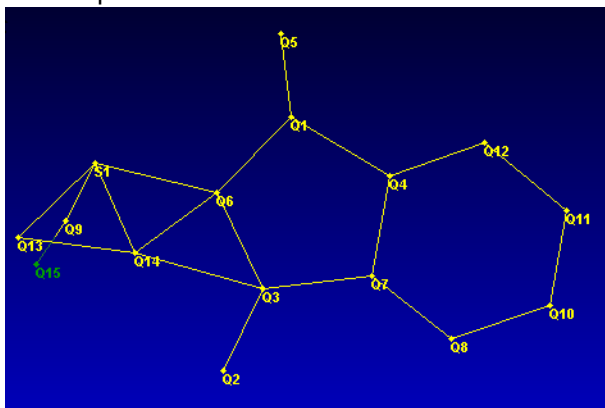
Figure 9.9 - Image after the number of Q peaks was changed to 15

Two more clicks left on the Q-peak slider gives a reasonable model.
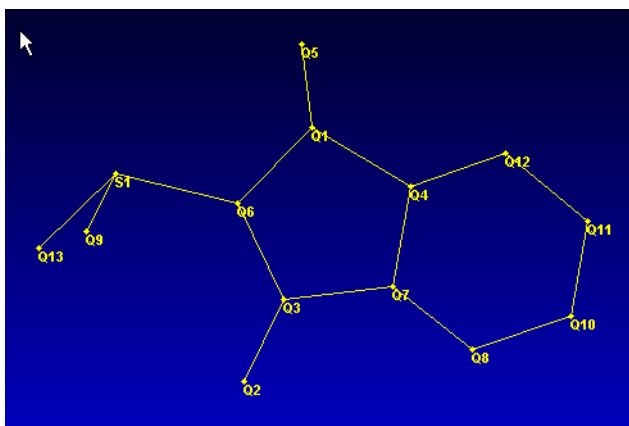
Figure 9.10 - Image with all noise peaks removed

6. Put the cursor over the bonds to check distance. The distance will be displayed at the bottom right. **The distance of 1.178 is typical for C=0 in aromatized ring systems.**

9.3.2 Label the Atoms

1. Label the atoms and set the atom types. Left-click on atoms to select them. Left-click on the peaks for the two oxygen atoms (Q2 and Q5 in this example).
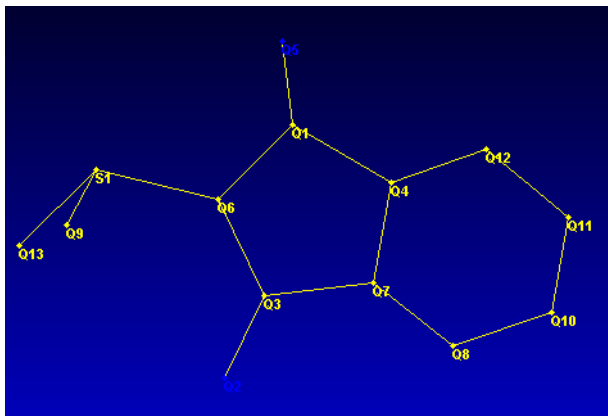
Figure 9.11 - View of model with the probable oxygen peaks selected

2. If it is difficult to see the color and labels, change the color scheme by left-clicking on Preferences and then right-clicking on Background Color. Choose colors and click Apply. Click Cancel to exit the background color mode. Note that the selected peaks are blue in Figure 9.13.
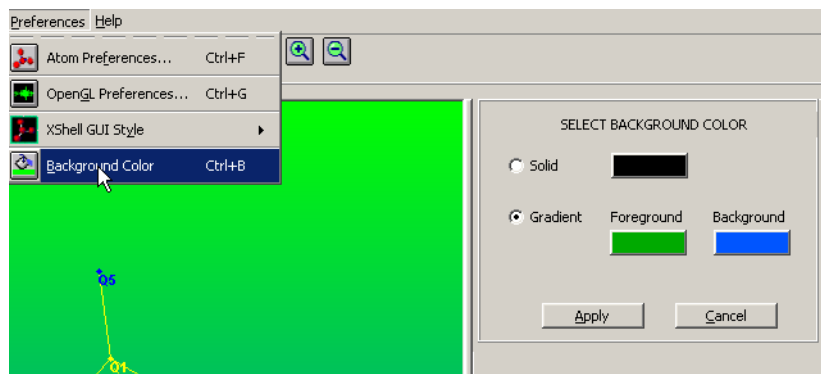
Figure 9.12 - Selecting the background color
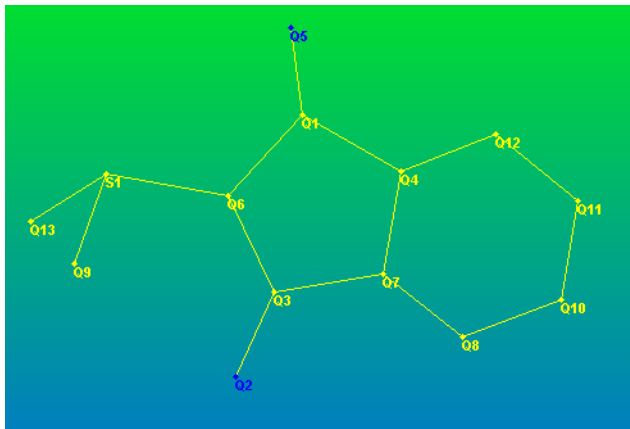
Figure 9.13 - New background color

3. Right-click to view a pop-up menu and select Labelling.
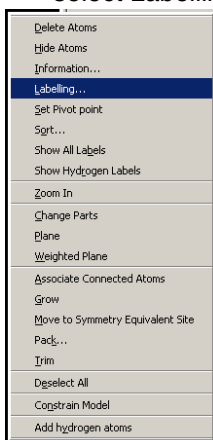
Figure 9.14 - Select Labelling

The Atom Labelling box will open.
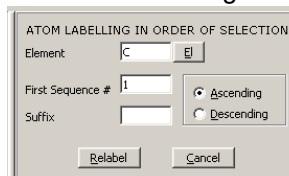
Figure 9.15 - Atom Labelling box

4. The two selected atoms need to be changed to oxygen atoms. Do this one of two ways:

•   Click on the Element field and type in the element symbol (case does not matter).

•   Click on the El button to the right of the Element field. This will open a periodic table. Left-click on the appropriate element symbol to select it (the periodic table will automatically close).
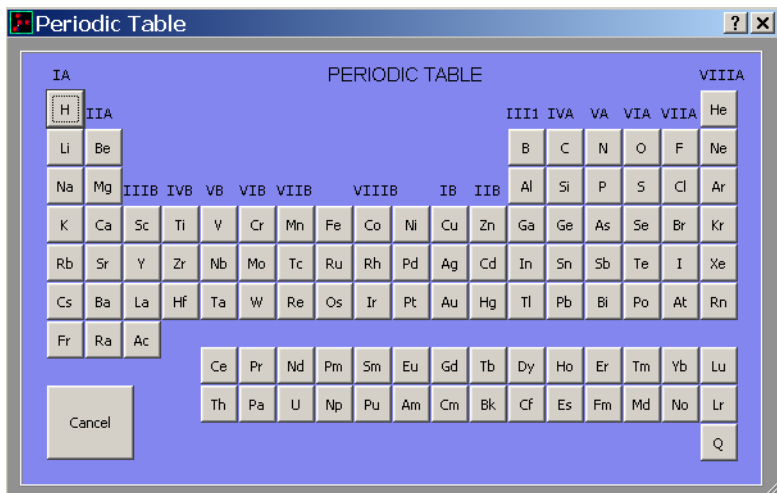
Figure 9.16 - Refine014

5.   Once the element type is set correctly, click
      on Relabel to number the peaks sequen-
      tially in the order they were selected starting
      with the number in the First Sequence #
      field.

6. Seeing the color of atoms can be difficult in the Wireframe view used so far. Right-click in the background with no atoms selected to get a slightly different action menu. In this menu, highlight and left-click on Pipes. This

makes the bonds thicker and makes it obvious that there is an incorrectly labeled atom. That will be corrected when the carbon atoms are labeled.
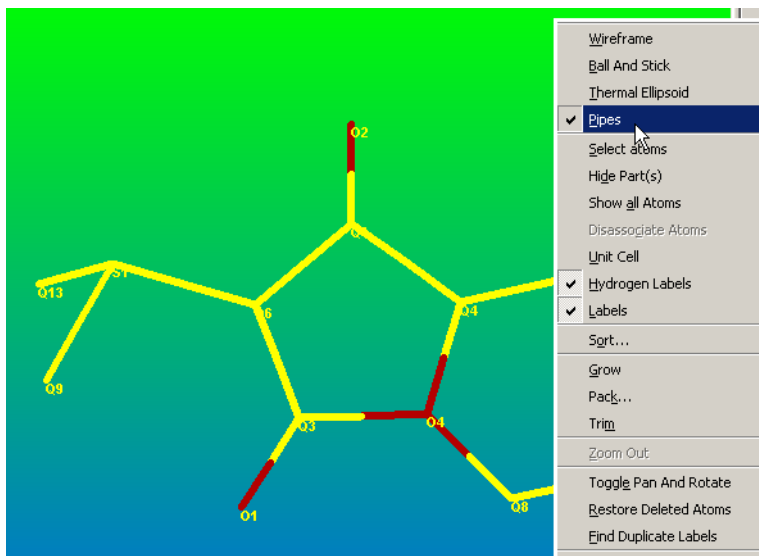
Figure 9.17 - Choose the pipes view

7. Left-click on the remaining peaks in the order that you want them labeled.

NOTE: If working on the YLID test data, look at Figure 9.18 and click on the peaks to give the same order as used here. Right-click and choose Labelling. Select Carbon and apply the labels.

---

8. In the labelling tool, change the el type to C (carbon). The starting atom number will change to 1—the first available number for carbon atoms.

9. Click Relabel.

10. Click Cancel to close the window.

9.3.3 Refine the Molecule

The labeled ylid molecule is now ready for refinement.
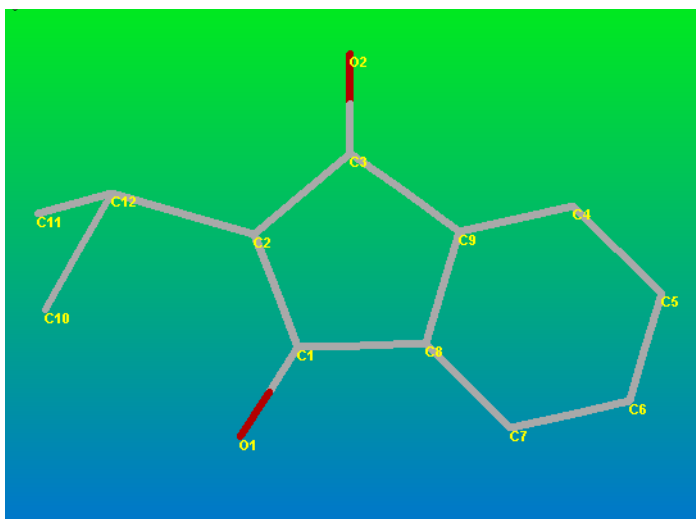
Figure 9.18 - Correctly labeled model

1. Left-click on the Refine icon in the tool bar (the cursor is pointing to it in Figure 9.19) or Type Control+R.

Figure 9.19 - The Refine icon

This will open the Refine Box. Click on Refine to launch the least-squares refinement program, XL using the default parameters as given.
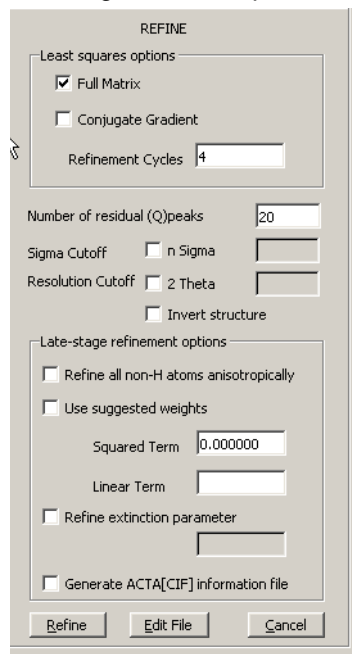
Figure 9.20 - The Refine menu

After a few seconds, a Refine window will open with output results for the calculation.
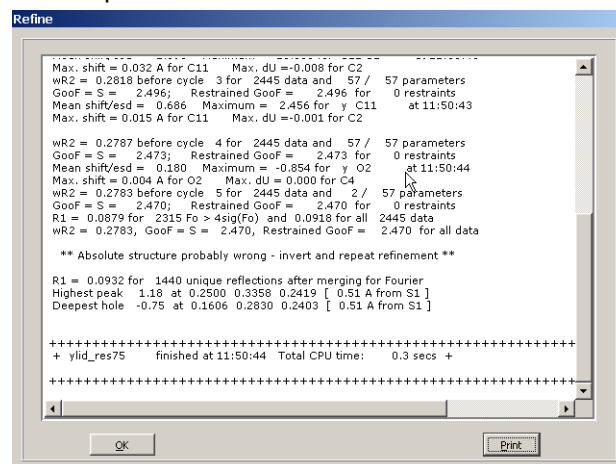
Figure 9.21 - Isotropic refinement output

In the output displayed above, note that the R1 value is 0.08. This is typical for a preliminary isotropic refinement for an organic molecule with data to a resolution of 0.75 and no hydrogen atoms included. The refinement program also indicates that the model needs to be inverted to get the correct absolute structure.

NOTE: This indication of the absolute configuration is usually reliable, but all assignments of absolute structure should be confirmed later in the refinement process by including TWIN and BASF cards in the instruction input.

2. Click on OK to return to XShell. The mole-
    cule display will refresh with the results of
    the least-squares calculations.
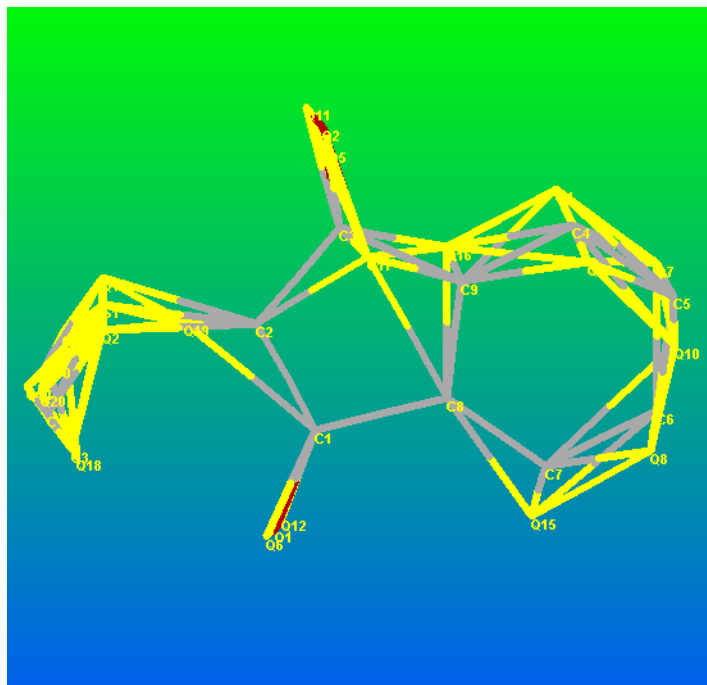
Figure 9.22 - The model after isotropic refinement
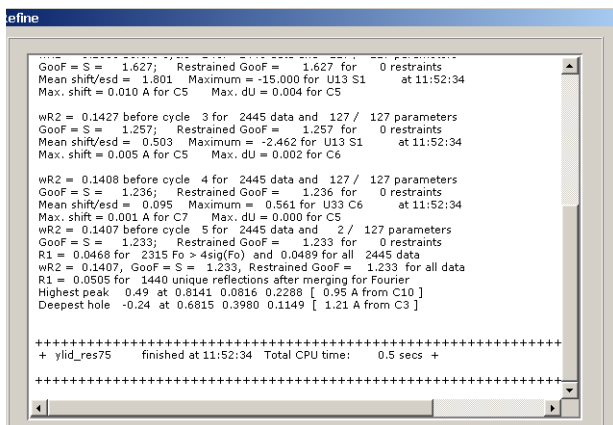
The peaks in the diagram represent difference
in the electron density between the refinement
model and the experimental electron density as
defined by the measured data. Many of these
difference peaks are near the sulfur and oxygen
atoms. Refinement of all atoms anisotropically
should correct the model.

In the refinement box click on Invert Structure
and Refine All Non-H Atoms Anisotropically as
in Figure 9.23.

Figure 9.23 - Inverting and choosing anisotropic refinement

3. Click on the Refine button to launch the
    least-squares refinement program. The out-
    put window will open and a summary of the
    results of individual cycles of refinement will
    appear.

Figure 9.24 - Refine023

The value for R1, 0.047, is typical for an initial
anisotropic refinement with no hydrogen atoms
included. Since the mean shift/esd is greater
than 0.1, the model is still changing. Since the
highest peak in the difference map has a height
of 0.49 and is 0.5A from C10, there are certainly
some hydrogen atoms to be included.

Examination of the difference peaks in the resulting difference electron density map shows that the top difference peaks all correspond to hydrogen atoms.
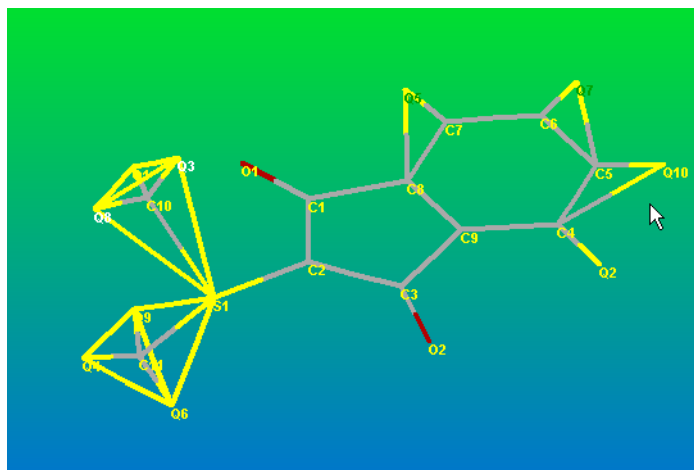
Figure 9.25 - Difference peaks correspond to expected hydrogen atom positions

9.3.4 Look at Atomic Displacement Parameters (Thermal Ellipsoids)

It is generally good practice to examine the displacement parameters at this point in structure determination.

1. Remove difference peaks with the slider tool.
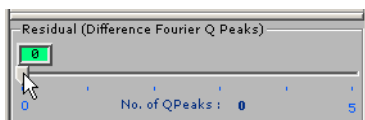
Figure 9.26 - Removing all Q-peaks with the slider bar

2. Right-click on the background and select Thermal Ellipsoids.
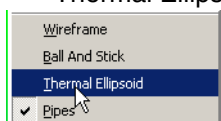
Figure 9.27 - Selecting Thermal Ellipsoids
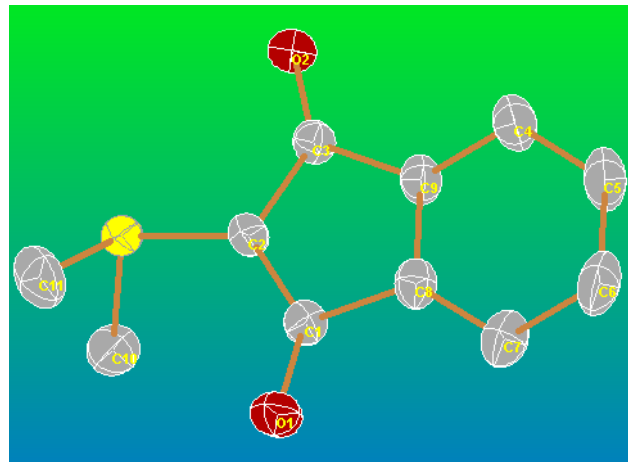
Figure 9.28 - Refine2015

Alternatively, a list of Ueq's (equivalent isotropic displacement parameters) can be generated by requesting information on all of the atoms. Examining these values for outliers is good practice.

3. Right-click on the background and click on Information on All Atoms.
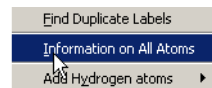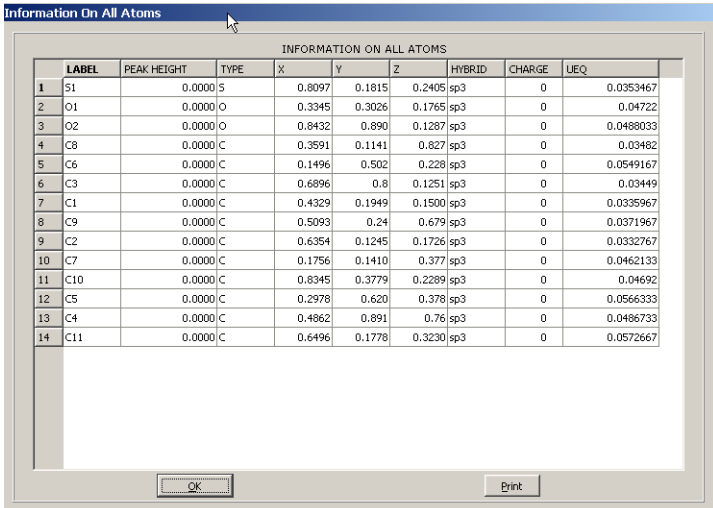
Figure 9.29 - refine 2005

This produces the table in Figure 9.30.

	LABEL	PEAK HEIGHT	TYPE	X	Y	Z	HYBRID	CHARGE	UEQ
1	S1	0.0000	S	0.8097	0.1815	0.2405	sp3	0	0.0353467
2	O1	0.0000	O	0.3345	0.3026	0.1765	sp3	0	0.04722
3	O2	0.0000	O	0.8432	0.890	0.1287	sp3	0	0.0488033
4	C8	0.0000	C	0.3591	0.1141	0.827	sp3	0	0.03482
5	C6	0.0000	C	0.1496	0.502	0.228	sp3	0	0.0549167
6	C3	0.0000	C	0.6896	0.8	0.1251	sp3	0	0.03449
7	C1	0.0000	C	0.4329	0.1949	0.1500	sp3	0	0.0335967
8	C9	0.0000	C	0.5093	0.24	0.679	sp3	0	0.0371967
9	C2	0.0000	C	0.6354	0.1245	0.1726	sp3	0	0.0332767
10	C7	0.0000	C	0.1756	0.1410	0.377	sp3	0	0.0462133
11	C10	0.0000	C	0.8345	0.3779	0.2289	sp3	0	0.04692
12	C5	0.0000	C	0.2978	0.620	0.378	sp3	0	0.0566333
13	C4	0.0000	C	0.4862	0.891	0.76	sp3	0	0.0486733
14	C11	0.0000	C	0.6496	0.1778	0.3230	sp3	0	0.0572667

Figure 9.30 - List of parameters with Ueq to the right

Examining the values here is difficult because the atoms are not in order.

9.3.5 Sort Atoms

1. Right-click on the background and click on "Sort…" to sort the atoms into a sensible order.
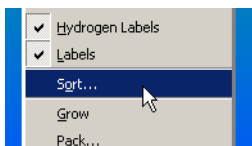
Figure 9.31 - Choose the Sort option

A box will appear to the right of the molecule display. Since S1, O1 and O2 are in a reasonable order, only the carbon atoms need to be ordered.
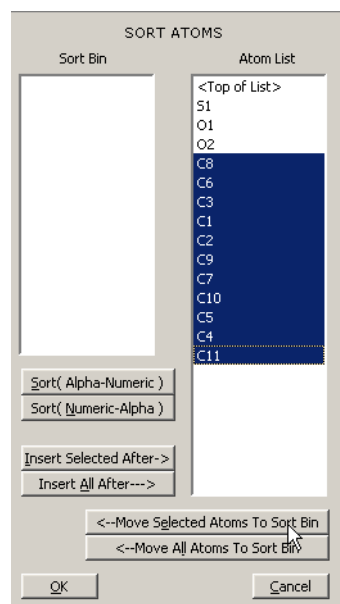
Figure 9.32 - refine2016

NOTE: Generally, it is easier to sort and number atoms before adding hydrogen atoms.

2. Highlight the atoms to sorted (i.e., left-click and drag over the atoms) and move them to the sort bin (i.e., left-click on Move Selected Atoms to Sort Bin).
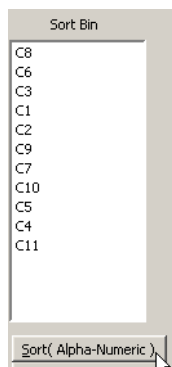
Figure 9.33 - refine2017

3. After the atoms are in the sort bin, click on Sort(Alpha-Numeric) to get them into a normal order.

4. Highlight O2 and click on Insert All After --- > as in Figure 9.34.
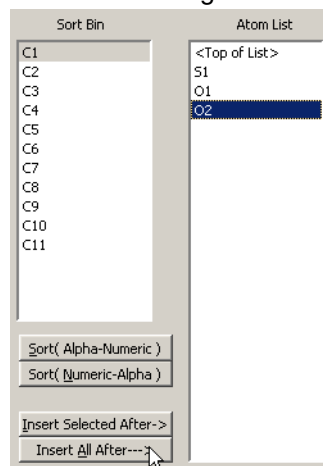
Figure 9.34 - refine 2019

5. Click on OK to accept the sorting.

Figure 9.35 - refine2011

The resulting list of atom information is easier to examine.

Figure 9.36 - Sorted atoms

The Ueq are reasonable if there are:

*   lower values for the sulfur atom and the five member ring

*   higher values for the terminal atoms—methyls and carbonyls

*   sensible numbers for the six member ring—lower for C8 and C9 higher for C5 and C6 and in between for C4 and C7

9.3.6 Add Hydrogen Atoms

At this point, refine the hydrogen atoms either by renaming them using the labelling tool as before and then including them in the refinement.

or

Include the hydrogen atoms in fixed idealized positions riding on the atom to which they are attached.

In this manual we will illustrate the later fixed idealized method.

NOTE: In some cases (e.g., hydrogen atoms on hetero atoms or on geometrically strained carbon atoms), it is better to refine the hydrogen atom positions. A general rule is to refine if there is some doubt about the position of the hydrogen atom and there is different electron density present.

---

1. Using the slider tool, remove all of the hydrogen atoms.
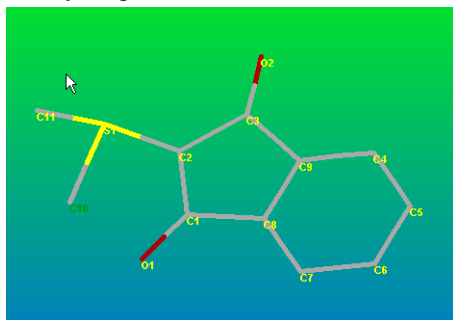
Figure 9.37 - Retread model with difference peaks removed

2. Right-click in the background and the click on Hybridize All.

Figure 9.38 - Refine025

The colors of the atom labels will change to indicate the atom hybridization.

NOTE: If the atom hybridizations are hard to see, right-click on the background and open the Information on All Atoms window.

Figure 9.39 - Refine028
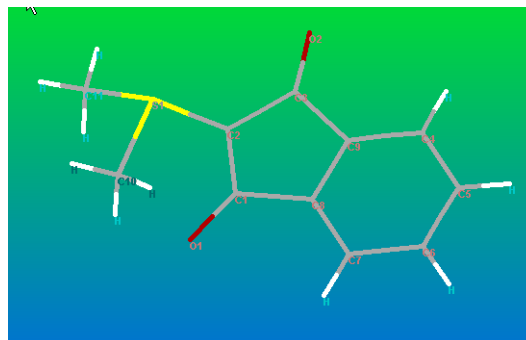
Figure 9.40 - Model with added Hydrogen atoms

3. The following warning will appear indicating that the hydrogen atoms have been provisionally added and that the final addition with naming will take place when the least-squares calculations are started. Click OK.
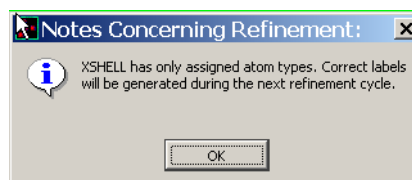
Figure 9.41 - Hydrogen atom warning

4. Click on the Refine button to launch the least-squares refinement program. The output window will open and a summary of the results of individual cycles of refinement will appear.

Figure 9.42 - Refinement results

The value for R1, 0.029, is typical for an anisotropic refinement with riding hydrogen atoms and a data set measured to 0.75Å. Since the mean shift/esd is less than 0.01, the model is not changing significantly. Since the highest peak and the deepest hole in the difference map are similar there are probably no missing atoms.

The resulting difference electron density map appears rather ugly. This is because the number of difference peaks to be displayed has been left at 20.
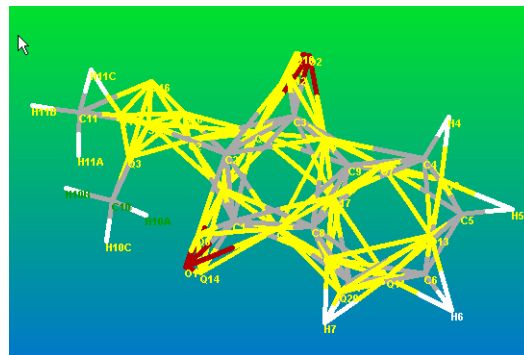
Figure 9.43 - Difference peaks in a well refined model

9.3.7 Final Refinement for Publication

1. For the final cycles of refinement reduce the number of peaks to be displayed to five and turn on Use Suggested Weights and Generate ACTA(CIF) Information File as shown in the next two figures.

NOTE: If the suggested weights vary significantly from 0.06 and 0.00, then change the values to 0.06 and 0.00.
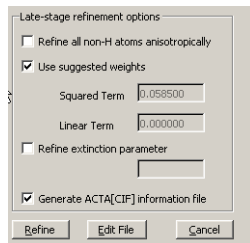

Figure 9.44 - Refine032

Figure 9.45 - Select weights and request ACTA output

2.   Click on Edit File, choose INS and click OK to open the instruction file for the XL. This allows you to add instructions that are not directly available in the GUI.
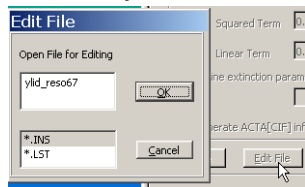

Figure 9.46 - Open the .ins file for editing

3.   Check that the temperature card is correct.

   3.1 If torsion angles are of interest, add a CONF (confirmation).

```
TEMP 23
conf
mpla 12 c1 c2 c3 c4 c5 c6 c7 c8 c9  s1  o1 o2 c10 c11
mpla 6 c4 c5 c6 c7 c8 c9 c1 c2 c3  o1 o2 s1 c10 c11
mpla 5 c8 c9 c1 c2 c3 c4 c5 c6 c7  o1 o2 s1 c10 c11
mpla 10 c1 c2 c3 c4 c5 c6 c7 c8 c9  s1  o1 o2 c10 c11
```

Figure 9.47 - Add instructions in the .ins file
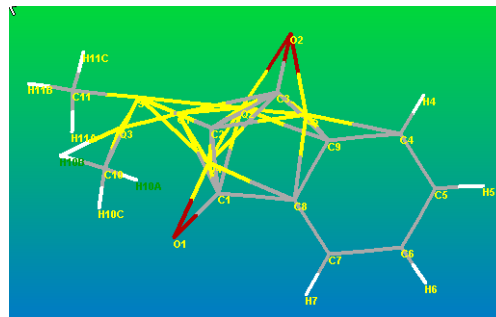
Figure 9.48 - Difference peaks

4.   Display the anisotropic atoms displacement parameters for the YLID.

5.   If planarity and angles between planes are of interest, add mpla cards.

9.3.8 Generate an Atomic Displacement (Thermal Ellipsoid) Plot

1. Remove difference peaks with the slider tool.

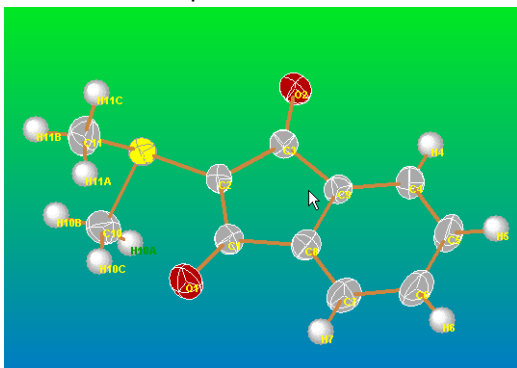2. Right-click on the background and select Thermal Ellipsoids.

Figure 9.49 - Thermal ellipsoids with large hydrogen atoms

3. If the hydrogen atoms in Figure 9.49 are large, change their size.

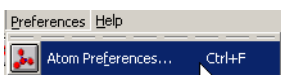   3.1 Click on preferences in the Window Tool Bar and choose Atom Preferences.

Figure 9.50 - Atom preferences tool

At the top of the box that is opened there are three values that can be changed to change the size of atoms and the bonds to them.
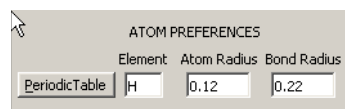
Figure 9.51 - Default atom preferences

4. Change the hydrogen radius to 0.08 and the bond radius to 0.15 to make the hydrogen atoms smaller.
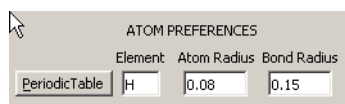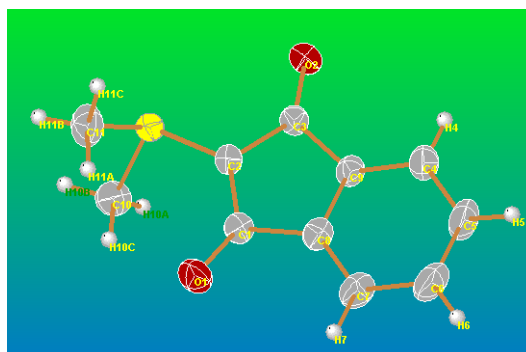

Figure 9.52 - Modified atom preferences

Figure 9.53 - Thermal ellipsoid plot with smaller hydrogen atoms

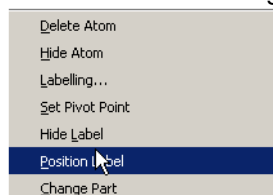5. Position atom labels by right-clicking on the atom and choosing Position Label.

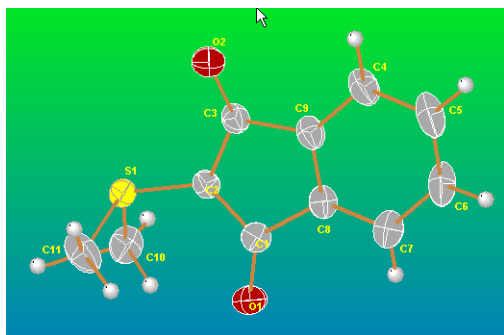Figure 9.54 - Position Label menu

Figure 9.55 - Positioned labels

6. Convert this image into a high quality jpeg for inclusion into reports by clicking on render in the Windows Tool Bar and choosing Black and White to High Quality JPEG file.
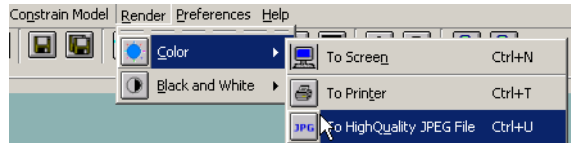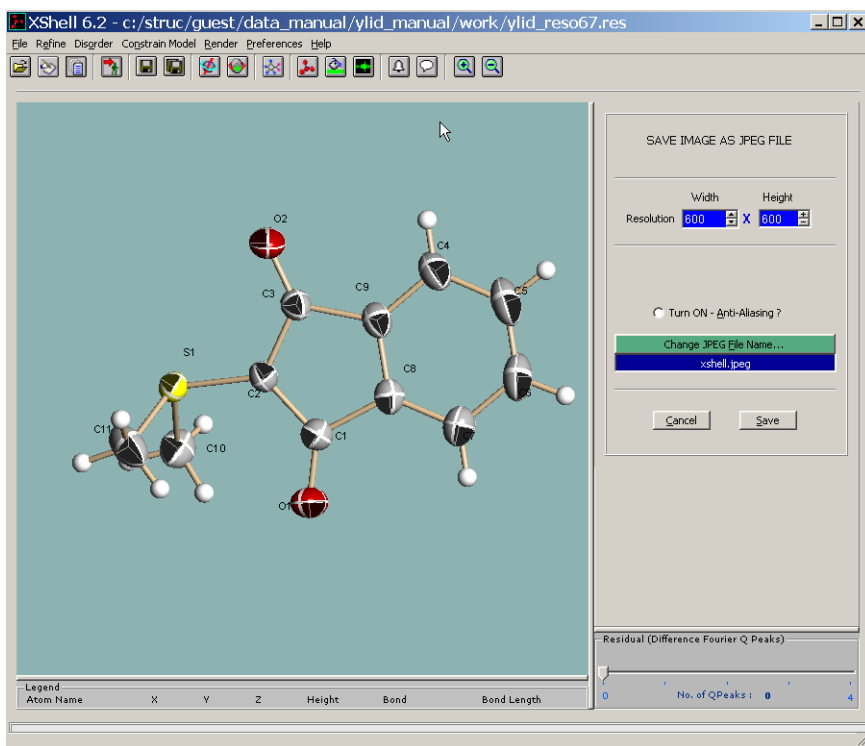

Figure 9.56 - Generate JPEG

NOTE: A message may appear that the font file needs to be defined "Please set your font directory for the first time for opengl rendering." If this happens, click on Preferences > OpenGL Preferences. Then click on the true type font For.OpenGL. In Windows browse to C:\bn\xshell\fonts\ or in Linux go to /usr/local/bin/xshell/fonts. Choose a font.

.

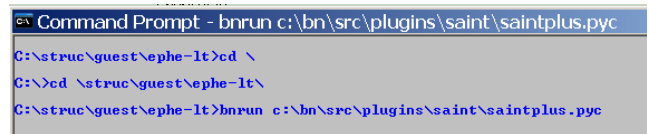Figure 9.57 - The final rendered thermal ellipsoid/atomic
displacement parameter plot

Appendix A. SAINTPLUS

Occasionally it is necessary to integrate images outside of the APEX2 GUI. Usually this is necessary in order to import a matrix (p4p) file. SaintPlus executed from a command window is designed for this purpose.

1. Open a Command Prompt window.

2. Change directories to the folder of interest.

3. For Windows, enter the command
bnrun c:\bn\src\plugins\saint\saintplus.pyc

(see Figure A.1). For Linux, enter the command bnrun /usr/local/bn/src/plugins/saint/saintplus.pyc.

Figure A.1 - Starting SAINTPLUS

After the command is executed, the SaintPlus
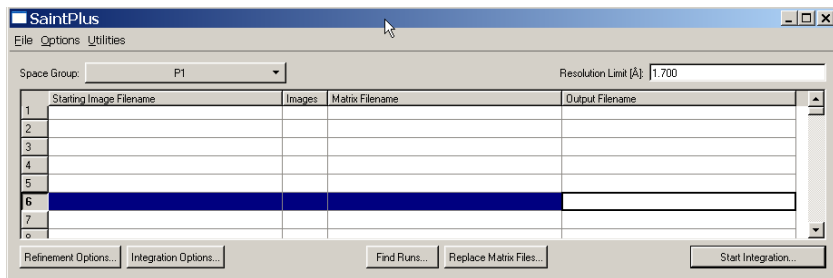window will open (see Figure A.2).

Figure A.2 - Initial integration window

This window is like the Saint window discussed
previously, but with the following differences:

• Defaults are not determined by previous
  knowledge of the sample.

• There is a button to replace the matrix files.

4. Click Find Runs.

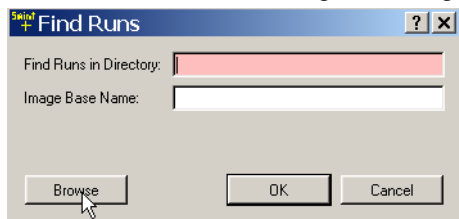5. Browse to find the images to integrate.

Figure A.3 - Find Runs tool view

6. Click on Replace Matrix Files and browse to
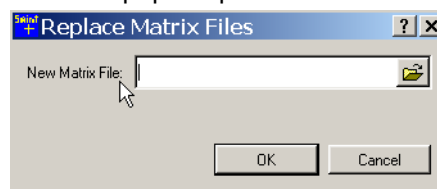   find a p4p or spin file to use.

Figure A.4 - Find Matrix tool view

7. This defines the information on the runs, but
   the defaults for space group and resolution
   have not been updated. Click on space
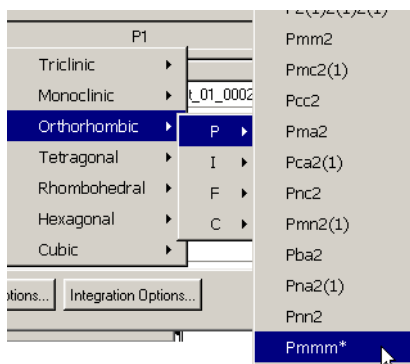   group and select the correct space group, in
   this case Pmmm.

Figure A.5 - Space group tool

8. Double-click on the Resolution Limit and enter an appropriate value.

NOTE: If the starting values for SAINT need to be changed frequently, change the default by modifying the SAINT.ini file. Consult the SAINT manual 269-0175xx for further information **on Windows or Linux**.

---

9. Use the Refinement Options and Integration Options buttons to set up the Saint run. In

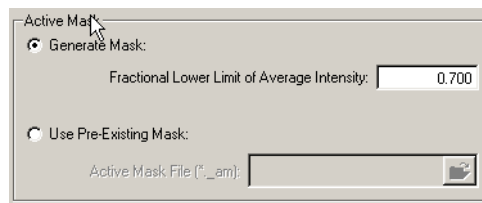this case Generate Active Mask has been turned on and set to 0.7.

Figure A.6 - Active Mask tool

10. Click on Start Integration to start the calculations and open the SaintChart window for monitoring the results.

Appendix B. Crystal Screening

crystal selection and mounting

Appendix C. RLATT

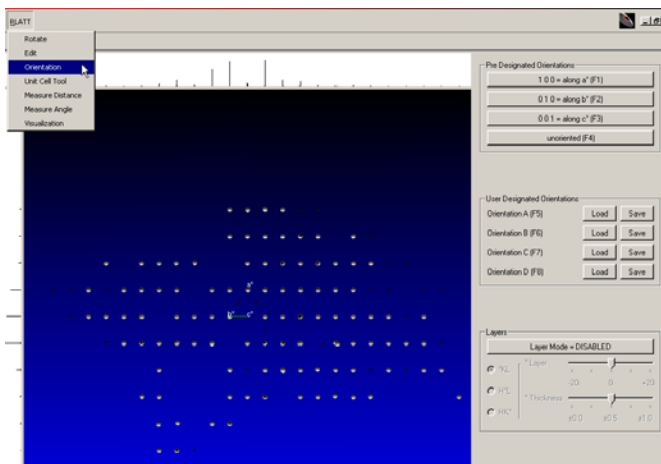1. Use the Reciprocal Lattice tool to look at the reflections.

Figure C.1 - UserManual013.bmp

Index

DPS

HKL

Kaercher Strategy

ProScale

SADABS

SAINT+

SMART

Sparks indexing

XM

XPREP

Curt--I'll add to this list of keywords. If there are any you specifically want added, write them in.