Standard Operating Procedure – Bruker Quest Diffractometer with copper wavelength X-ray microsource

X-ray Crystallography Laboratory

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The following is a guide for collecting data and solving structures using the Purdue Bruker Quest single crystal diffractometer with a copper wavelength X-ray microsource. It is intended as a "walk-through" user guide geared especially towards novice users, but also tries to cover more advanced features of the software where they are important for the collection of normal "simple" small molecule structural data. A basic knowledge of the fundamentals of diffraction and crystallography is expected.

This manual is based on an instrument and software produced around the year 2017. Most of the manual's content also applies to newer as well as older instrument makes. For the novice user, changes between different generation instruments are mostly limited to slightly different program layouts and color schemes. The general procedures described in this manual still apply.

For a more in-depth description of the features of a CCD or CMOS diffractometer, the reader should refer to the manuals and technique guides on specific topics by the manufacturer of the type of instrumentation you are using. The gold standard for a more in depth guide towards the use of Shelxtl for the refinement of single crystal structures is Peter Müller's book "Crystal Structure Refinement". Every crystallography lab should have at least one copy.

Among the many programs commonly used for crystal structure solution and refinement we recommend the Bruker ***Shelxtl*** package (including XPREP, XS, XM and XT), George Sheldrick's refinement program ***Shelxl2018***, the graphical interface ***Shelxle*** by Hübschle, Dittrich and Sheldrick, and ***Platon*** by Anthony Spek.

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Instrument Overview

The Bruker Quest instrument with a copper microsource consists of:

- The diffractometer including the X-ray enclosure, PhotonIII detector, mircosource "IμS$_{Cu}$" X-ray tube, multilayer optics for monochromatization and collimator, video microscope, kappa (4-axis) goniometer, electronic controls, power supply and other miscellaneous pieces of equipment essential for the operation of the instrument.
- An Oxford Cryosystems 800 variable temperature unit (pump, controllers and liquid nitrogen tanks located outside the enclosure) and an Oxford Cryosystems AD51 dry air unit.
- The instrument computer: This computer runs the Measurement Server, the Bruker Instrument Service (BIS), Diffrac.Maintenance and Apex3.

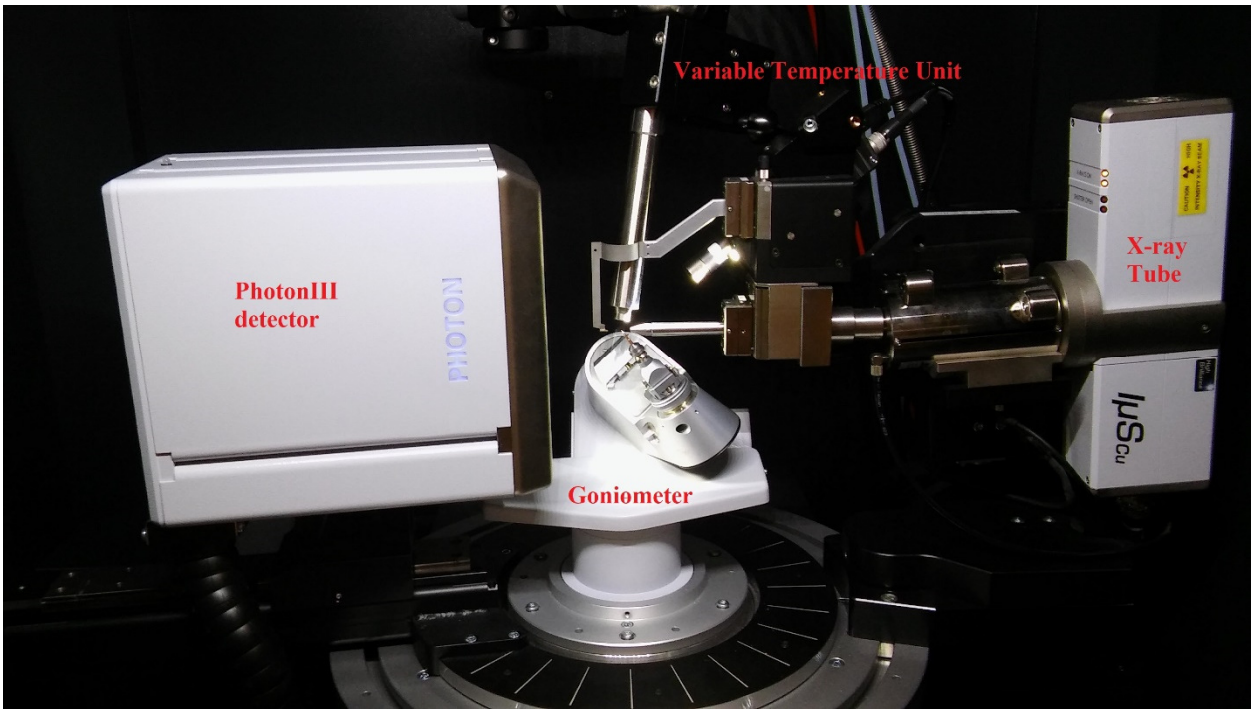


Figure 1, Inside of the Quest X-ray enclosure with microsource X-ray tube, PhotonIII Detector, Variable Temp Unit and Goniometer. Between X-ray tube and detector are the Optics (Multilayer Optics for monochromatization, Collimator), the Video Microscope (in back, partially hidden), and the Beam Stop. On the Goniometer is mounted the Goniometer Head holding the crystal specimen.

Having a promising sample of crystals, it is best to start in the following order:

Checking the Status and Thermostat

All but very high melting samples (inorganics, ceramics, etc) should be measured at lower temperature to avoid extensive thermal motion of the atom cores, to obtain higher angle diffraction data and to minimize radiation damage to the sample.

The Oxford Cryosystems variable temperature unit can be operated as a stand-alone unit, operated through its own controllers. More often, it is controlled via Apex3 or BIS. The sample is embedded in a stream of cold nitrogen gas supplied by a low pressure liquid nitrogen dewar. To avoid buildup of ice, the sample is insulated from ambient humidity by an outer layer of warm dry air supplied by an Oxford Cryosystems AD51 dry air unit (located outside the lab in a facilities closet).

The Oxford Cryosystems variable temperature unit is able to achieve temperatures between 375 K (+102.5°C) down to ca. 85 K (-188.5°C). The recommended temperature for "low temperature" data collections is 100 K in winter, and 150 K in summer (due to higher humidity levels that can cause icing around the sample mount).

- Make sure, the tank is properly connected and full enough for the planned experiment.

- The Oxford Cryostream N2 tank (silver dewar behind the diffractometer) is automatically refilled from the larger low pressure liquid nitrogen tank (between the two diffractometers). Check that the Liquid Level Controller (the smaller of the two controllers, Fig. 2) is set to <AUTOFILL> for automatic refills. The fill level displayed on the controller should be between 20 and 80%. When it is below 20% (despite the controller set to AUTOFILL) the low pressure N2 tank is empty and needs to be refilled.

Figure 2, The external controller (bottom) and the circulation pump (center), and the liquid level controller (top) of the Oxford Cryosystems variable temperature unit.

- **Check that no data collection is running** (e.g., look at the BIS interface, check for "Frames left" or "Time left"). If a running data collection can be stopped, abort the data collection (see below), then make changes to the VT unit.
- Check that the dry air unit providing the shield gas flow is switched on.
- Tap the touchscreen of the Cryostream Controller (larger of the two controllers) to check the status of the VT unit. ***If it is running*** ("Cryostream running" beside the ℹ symbol on the screen), check if the temperature is appropriate for your experiment. If you need a different temperature, or if the system is on standby ("Cryostream ready" beside the ℹ symbol on the screen), use the <Run Experiment> interface of Apex3 to set the desired temperature (see below, Starting the System).
- ***If the unit is switched off*** it needs to be started. Press the main single button on the front of the controller. Follow the on screen prompts to switch on the unit. Use the <Run Experiment> interface of Apex3 to set the desired temperature (see below, Starting the System).

Starting the System

- Start BIS (Bruker Instrumentation System) if it is not already running. This will also start the measurement server if it is not active already.

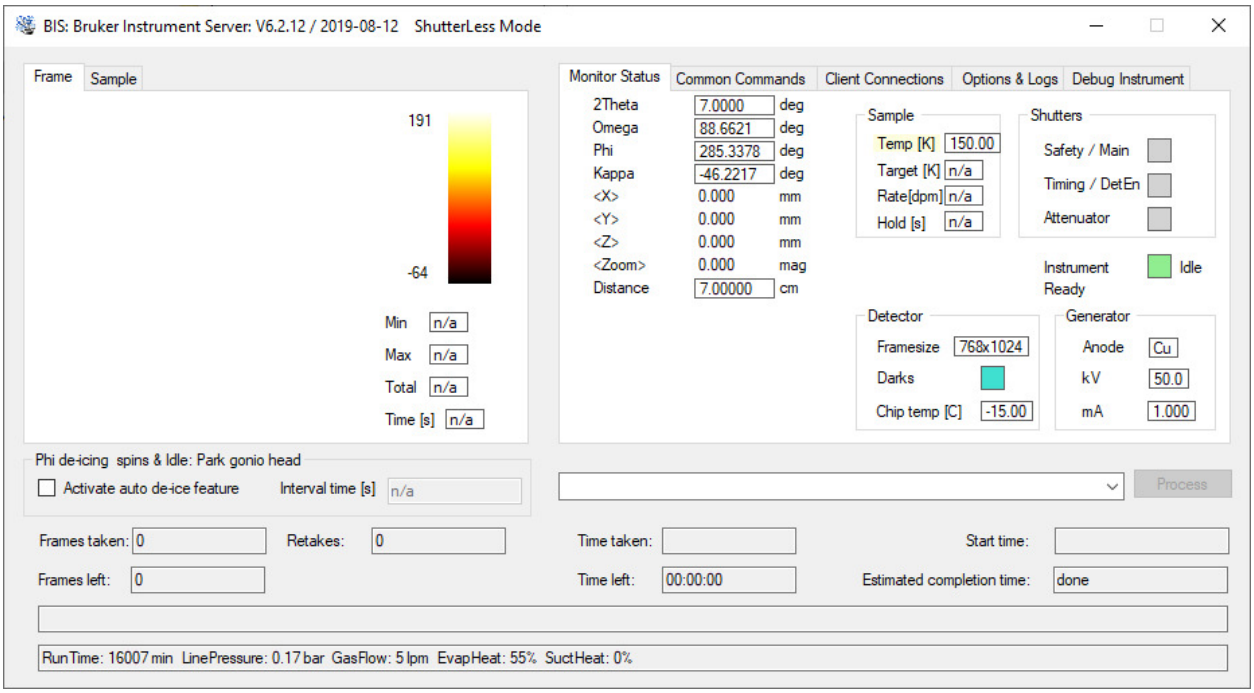


Figure 3, The BIS window

- Check if a data collection is still running (e.g., look at the BIS interface, check for "Frames left" or "Time left"). If a data collection is active but can be aborted, then stop this data collection first. Navigate to the active copy of Apex3 and click the STOP symbol in the Apex3 interface.
- Minimize any sessions of Apex3 that might be open.
  - Start a new session of Apex3. No login is required.
  - Connect to BIS (<Instrument>, then <Connect>).
- Via the <Sample> dropdown menu, either <Open …> an existing sample (continuation of old data collection) or
- Start a <New …> Sample
  - Fill in the project name. Avoid using special characters (including dashes and periods) or overly long project names.

- Leave the Group as “Users” and accept the automatically created project path (D:\frames\guest*ProjectName*)

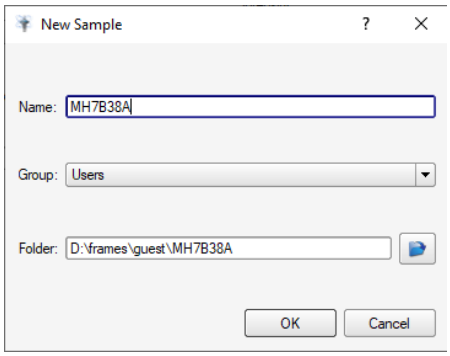


Figure 4, New Sample pop-up window

- If necessary, ramp the temperature:
  - Under <Collect> click on <Run Experiment>
  - In the <Operation> column of the table, click on the top row and select <Thermostat> Enter the target temperature (in K). Set the rate to 360 (K/hr). Click the <Execute> button. You can mount and center a crystal while the temperature is ramping. For unit cell measurements and data collections, wait for the temperature to have settled at the target value.

Unloading the Previous Sample

- Before using the video for crystal centering, make sure **only one copy of Apex3 is open** (trying to open the crystal centering tab from a second copy of Apex3 can crash the video feed).
- Under <Set Up>, go to <Center Crystal>
  - The video feed will automatically open showing you a life picture of the mounted crystal. ***Troubleshooting****: If the connection to the video feed fails, do the following: Close all other open copies of Apex3. Then close the “Center Crystal” subwindow. Disconnect from the Server (Click the <Instrument> dropdown menue, click <Disconnect>). Click <Center Crystal> under <Set Up> again. If this also fails, close Apex3 entirely. Open the Task Manager. Find and close “FrameGrabber.exe” (under “Details”, “End Task”). Restart Apex3 and try again. Repeat if necessary.*

- Once the live video feed is running, click <Center> in the lower right of the interface. The angles will drive to a position convenient for mounting a magnetic snap-on mount.
- Press the <door open> button on the front face of the diffractometer enclosure (to the right of the front door, below the light button). Doors need to be closed to drive any mechanical parts other than the phi (spindle) axis of the goniometer.
- Carefully dismount the magnetic snap-on mount from the previous experiment. Tilt it backwards until the magnet comes loose, then take it off.

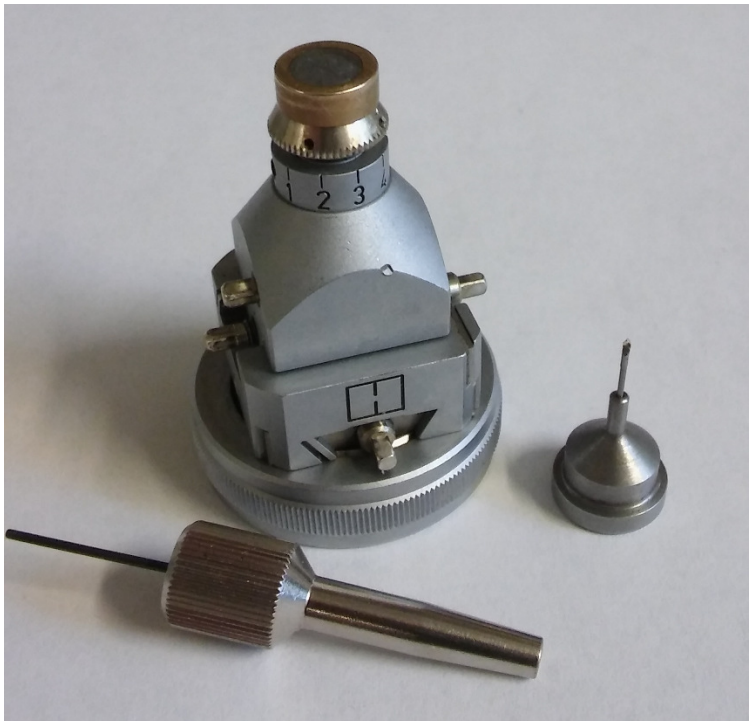


Figure 5, Goniometer head with magnetic snap on mount and adjustment screw

Selecting and Mounting of Sample

- Crystals can be mounted on glass rods, inside glass capillaries, on Nylon loops, or using mesh mounts. The Purdue X-ray lab uses Mitegen micromesh mounts for most samples and data collections.
- Two types of micromesh mounts for small (<0.1 mm) and medium and large (0.1 – 0.5 mm) crystals are stocked in the lab (Fig 6). Crystals collected at low temperature are mounted on the micromesh with the help of a trace of mineral oil, Fomblin (fluorinated mineral oil) or polybutene oil (very viscous oil to protect extremely oxygen sensitive samples) and flash

frozen in the cold stream. Crystals collected at room or elevated temperature might need to be fixed to the mesh with a trace of glue to avoid crystal movement during measurement.

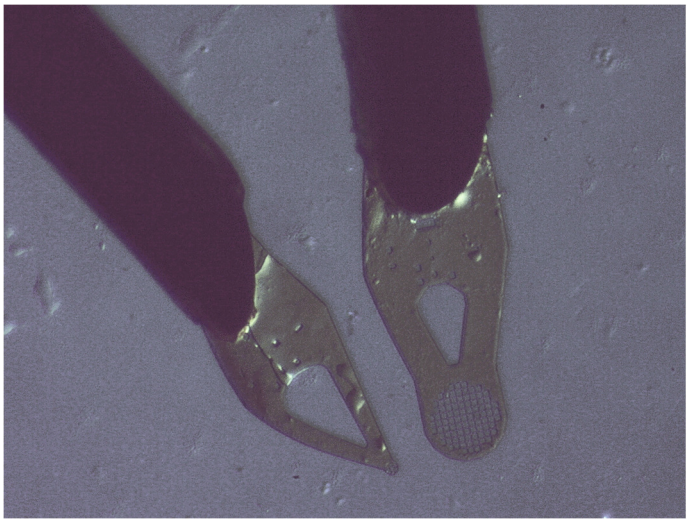


Figure 6, Tips of Mitegen Micromesh mounts as seen through a microscope (Left: small tipped mount with 15 μm openings for crystals 0.1 mm and smaller. Right: large mesh mount with 25 μm openings. Mesh is 0.4 mm across).

- For typical crystal selection and mounting, place a drop of oil on a glass slide under a microscope. Place several representative crystals in the oil. Inspect the crystals and select a suitable candidate.
- Crystals on the Quest instrument with **copper microsource** should ideally be not larger than 0.2-0.3 mm in any direction (the size of the X-ray beam is 0.1 mm). The minimum size depends on the diffraction intensity of the crystal. Larger crystals, especially if they are heavily absorbing, are usually more suitable for the Quest instrument with **molybdenum sealed tube X-ray source** (the size of its X-ray beam is 0.6 mm). Data collections are much faster using molybdenum radiation as long as the achievable diffraction is sufficiently intense. ***The higher intensity microsource and the intrinsically better diffraction with copper radiation are ideally suited for small, weakly diffracting crystals that are unsuitable for molybdenum radiation. Only those samples and organic samples for which absolute configuration is required should be run on the copper wavelength instrument.*** For highly absorbing samples limit the size of the crystal (make sure the minimum transmission will be above 10-20%).
- If no single specimen with suitable dimensions can be found, use a sharp razor blade to cut off a single piece from a larger crystal or cluster. Remove all smaller pieces and dirt from the crystal (the micromesh mounts can be used to brush off loose pieces from the crystal).

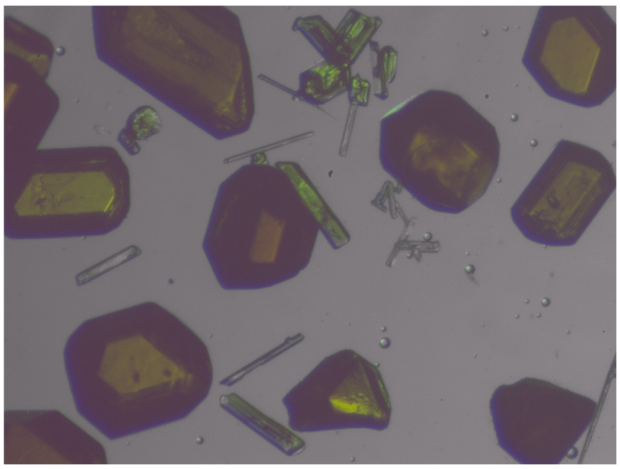


Figure 7, Crystals with well-developed faces on a microscope slide

- Scoop up a crystal with the micromesh mount and place it on the inside of the concave face of the mount in the center of the mesh. Remove as much excess oil as possible by tipping the back (convex) side of the mount onto a dry section of the microscope slide until only a minimum amount of oil remains on the mount and crystal. (If a crystal needs to be glued to the mount make sure that both crystal and mount are dry without any traces of oil. Dip the mesh carefully in a small amount of glue and pick up the crystal without touching the glass slide).

Crystal Mounting and Centering

- Press the <door open> button on the front face of the diffractometer enclosure (to the right of the front door, below the light button).
- Carefully mount the magnetic snap on mount onto the goniometer.
- Check the position of the mount by eye (is it aligned with the collimator and the beam stop?)
- In Apex3, click <Center Crystal> in the <Set Up> submenue. The crystal centering live video feed will automatically open (If the live video feed does not start, see "Unloading of Previous Sample", for troubleshooting). Is the crystal visible on the video screen? If you cannot see the crystal, its position is further off than usual and needs to be adjusted by eye on the goniometer head before the live video feed can be used.

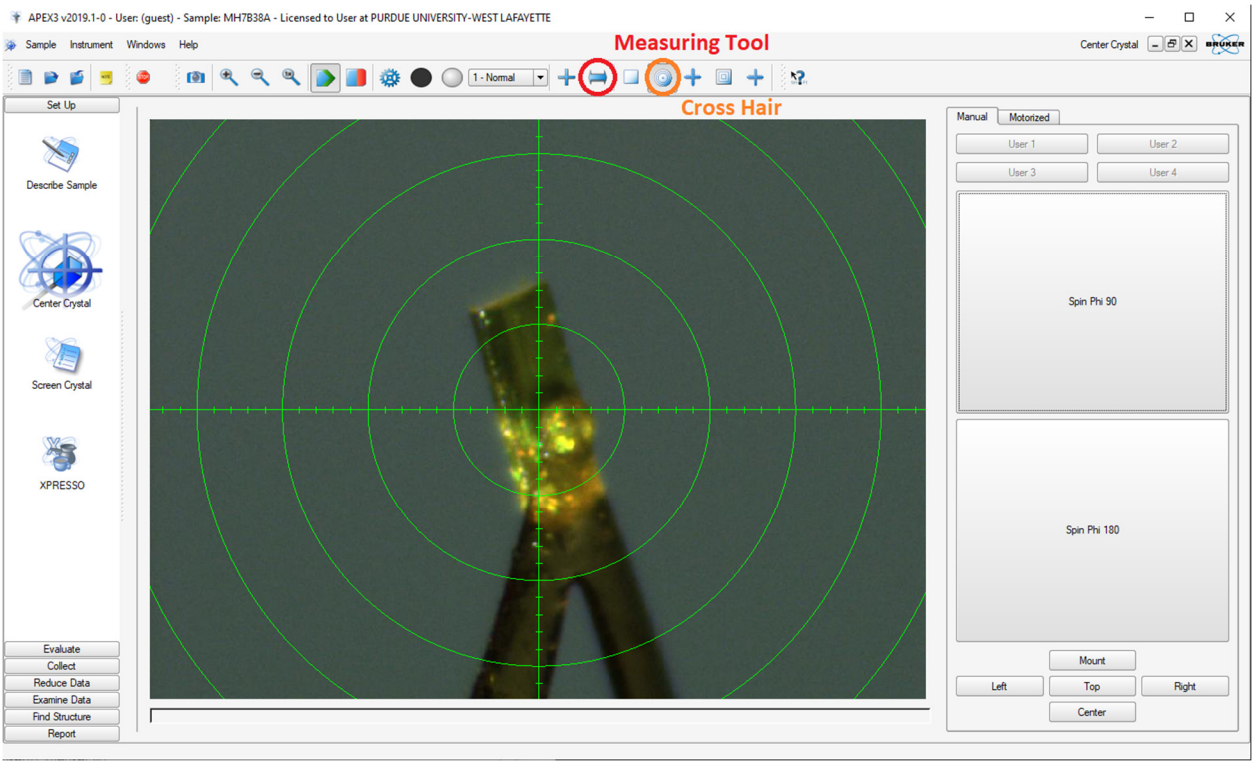


Figure 8, Crystal Centering Window.

- If the image is too dark to see the crystal well, you can place a piece of paper between the goniometer head and the detector.
- There are three adjustment screws (metal pins) on the goniometer head: for up-down, right-left, and forward-backward. Use the bold end of the adjustment screw driver to turn the pins to position the crystal in the center of the cross hair of the video feed.
- When the crystal is centered in the cross hair, click the large <Spin Phi 90> button (in the <Center Crystal> window of Apex3) to spin the crystal around the phi axis by 90°. Repeat the centering procedure using the adjustment screws as before. Repeat the process (<Spin Phi 90> followed by centering of the crystal in the cross hair of the video feed) until the crystal stays positioned in the center of the cross hair in all positions. If the cross hair seems to be slightly off try to center the crystal so that it "rotates in place".

  For oddly shaped crystals, try to center the mass center of the crystal. For long needles, try to align them along the axis of the mounting pin.
- Measure the dimensions of the crystal: Switch on the measuring tool (double sided arrow icon on top of <Center Crystal> window, Fig. 8). Click on one side of the crystal, hold the left mouse key and drag to the other side. The length of the green line dragged is given in the

bar below the video screen (“vector length”, value in µm). Write down the value and repeat for the other two directions using <Spin Phi 90>.

Crystal and Compound Description

- Under <Setup>, go to <Describe>
  - Fill in all values that apply. In the “compound” line, it is best to give the full name of the person that provided the sample. Crystal dimensions need to be entered in mm (video screen values are in µm!).

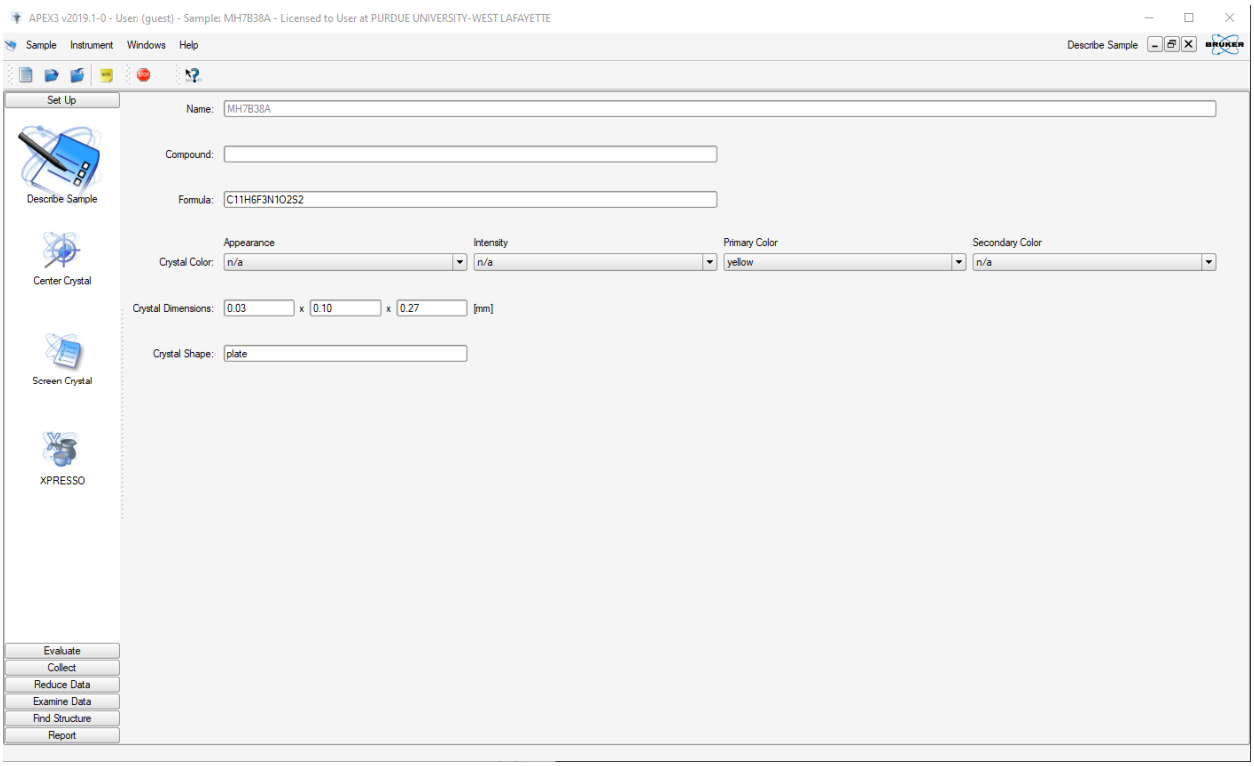


Figure 9, Crystal and compound description

Unit Cell Determination

- Under <Evaluate>, click on <Determine Unit Cell>.
  - Two procedures are available, <Automatic Mode> or <Manual Mode>.

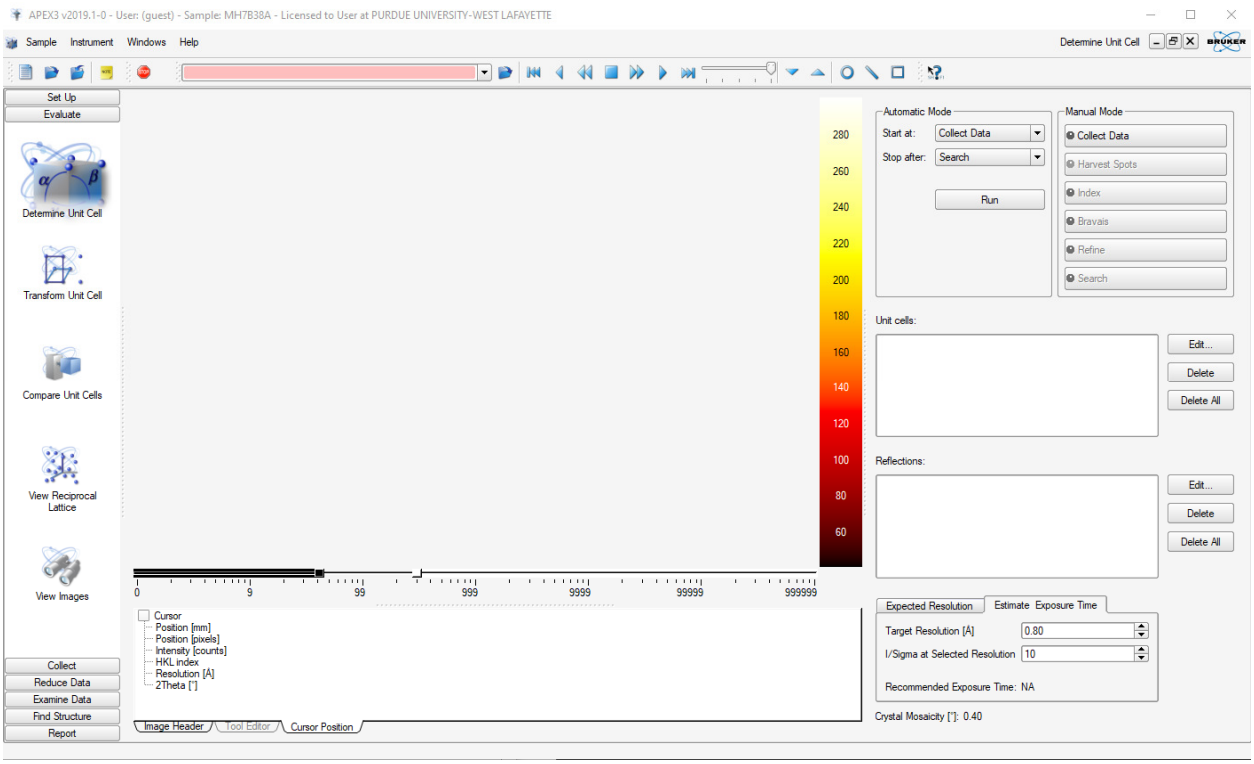


Figure 10, Starting Window of the Unit Cell determination

We encourage to use the manual procedure. To do so click <Collect Data>, this will open the Unit Cell Data Collection Window.

- Choose an exposure time (default is 10 seconds). Change the <Scan Option> to <Sensitive>, then click <Collect>.
- 3 × 20 frames will be collected which will be displayed in the frame window. The first sweep will be at low angle, with the beam stop visible at the center (Figure 11). The second and third run (Figure 12) will be at high angle. Make sure that your specimen diffracts to sufficiently high angle. Use the resolution arc tool (Figure 12) to check the resolution limit. Only samples that show at least some diffraction above ca. 0.9 Å (with ten second exposures) are likely to meet the IUCr requirements for publication.
- The high sensitivity of the Photon III detector leads to capturing of natural decay events (γ-rays from radioactive decay), which show up in the diffraction images as very bright

but very sharp signals, often only one pixel in size. They do not affect the final data quality and are corrected for during data integration via a high energy event discrimination (HEED) algorithm, but might be harvested during unit cell determination and reduce the fraction of harvested spots that fit a selected unit cell. These are not diffraction spots and can be ignored.

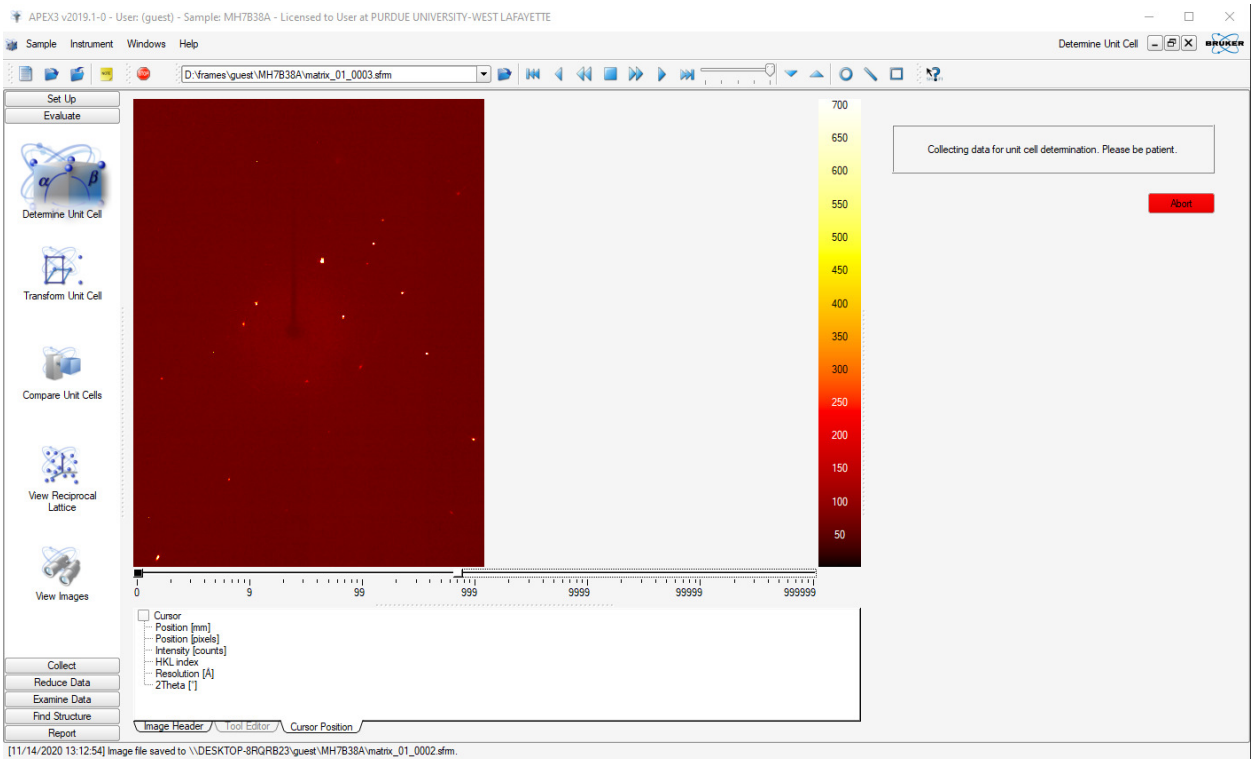


Figure 11, Unit Cell window with the first (low angle) sweep running.

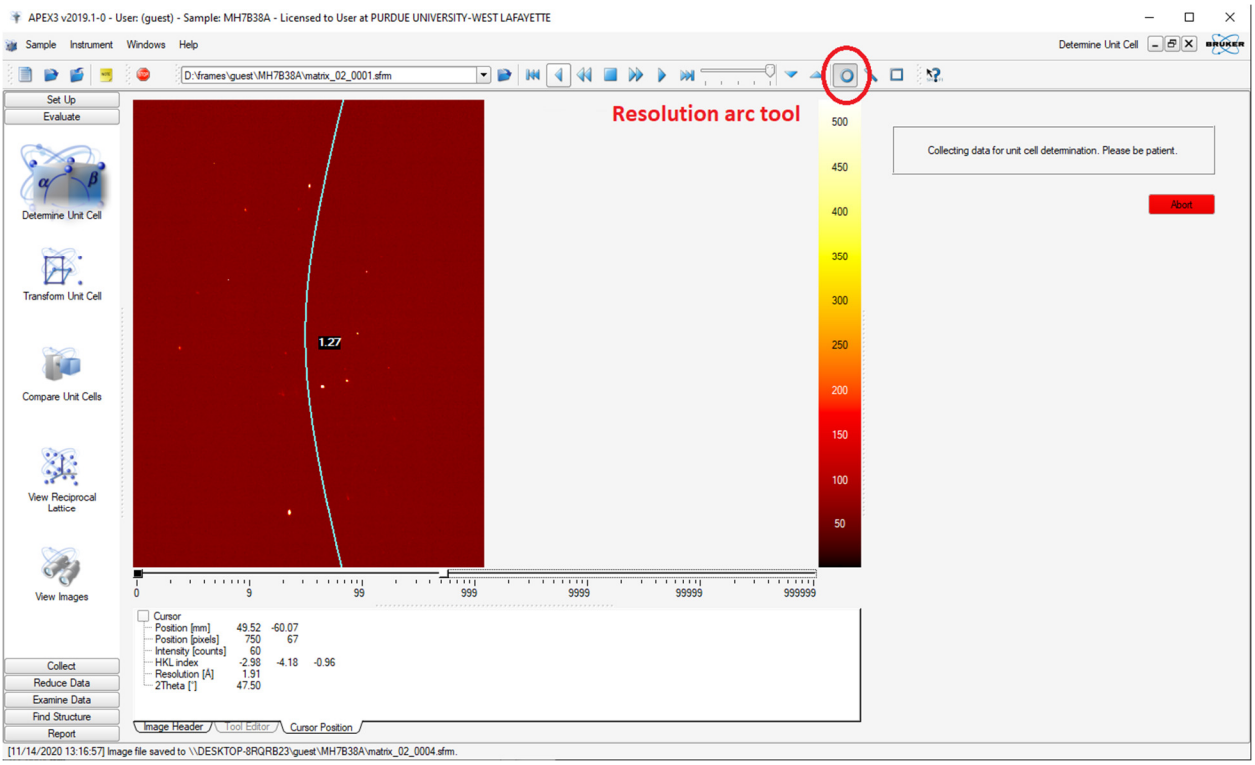


Figure 12, Unit Cell window with one of the high angle sweeps running and resolution arc tool.

- When all 60 frames are collected, the program will automatically proceed to <Harvest Spots>, select an I/sigma(I) cutoff value for the diffraction spots to be used ((circled in green in the diffraction image). To obtain a reasonable suggestion for the exposure time use the default value of 10.0. Click on <Harvest>.

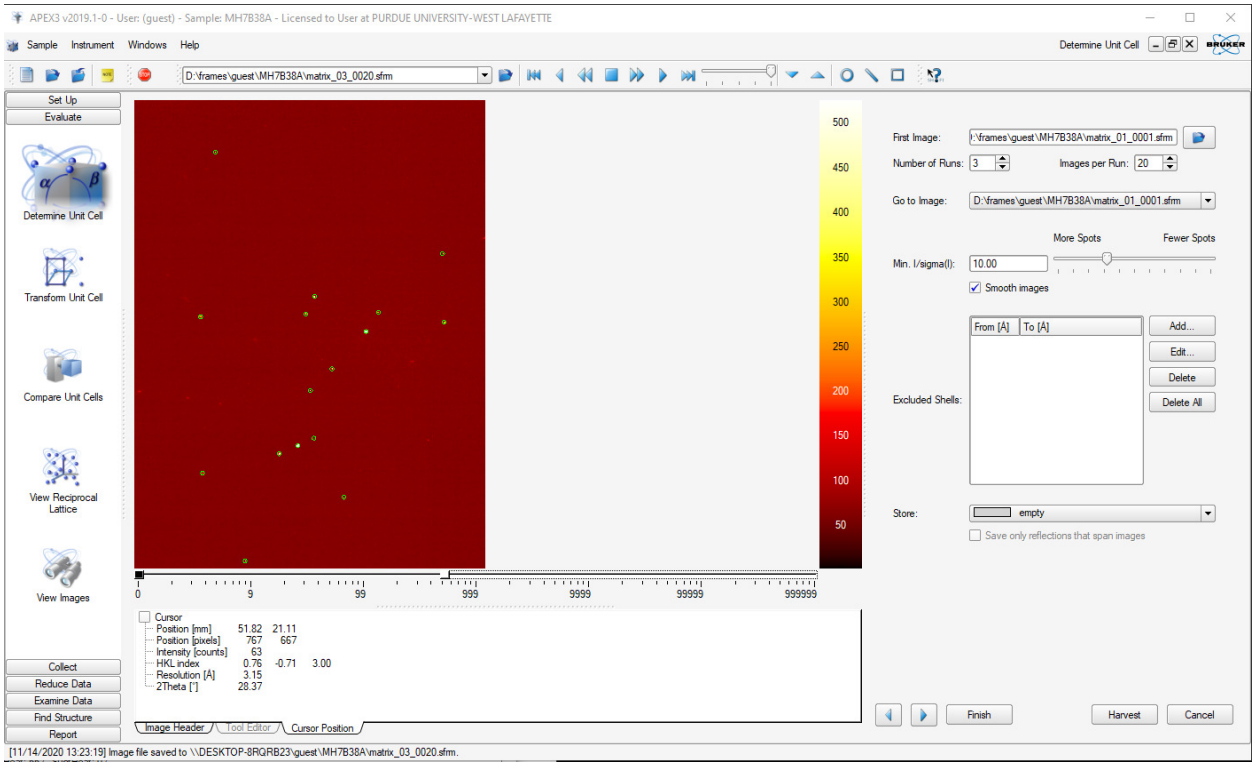


Figure 13, Unit Cell Harvesting Window

- Proceed to <Index>, use all default values and click on <Index> at the bottom of the window.

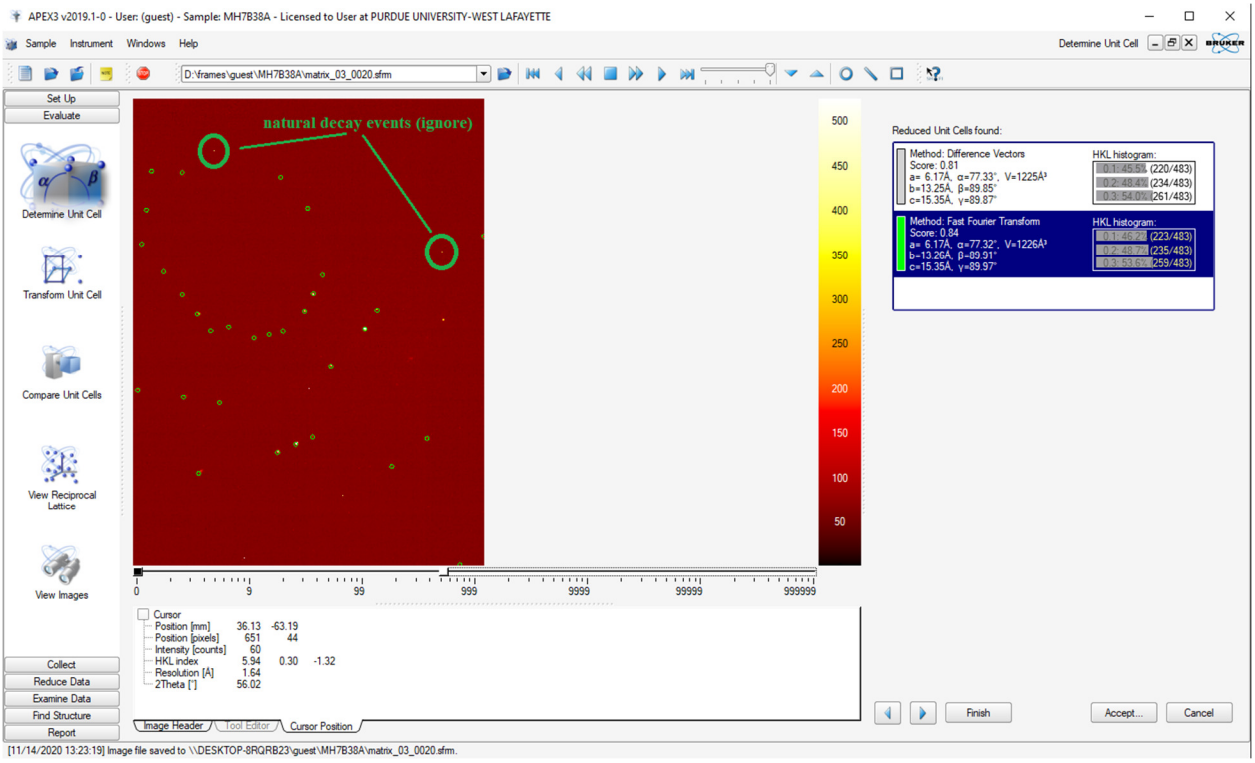


Figure 14, Unit Cell Indexing Result Window

Check the unit cells obtained. The positions of the diffraction spots (ignoring natural decay events) should agree with the predicted positions (circled in diffraction images). If there are excess spots not assigned to the cell a non-merohedral twin might be present, or the crystal is not single. **Try to find a better, more obviously single crystal.** If this is the best crystal that can be found, investigate if it can be processed as a twin. Export the data as a p4p file using <Sample>, then <Export> and use the program Cell Now to determine the unit cell and relationships between domains (see the Purdue Twinning Manual for details).

- If one of the two unit cells looks reasonable, select it and click <Accept>. This will open the Refine Window. Use all defaults and click on <Refine> to run a least squares optimization of the unit cell against the diffraction spot position. Select more or less reflections using the sliding bar and repeat, then click <Accept>.

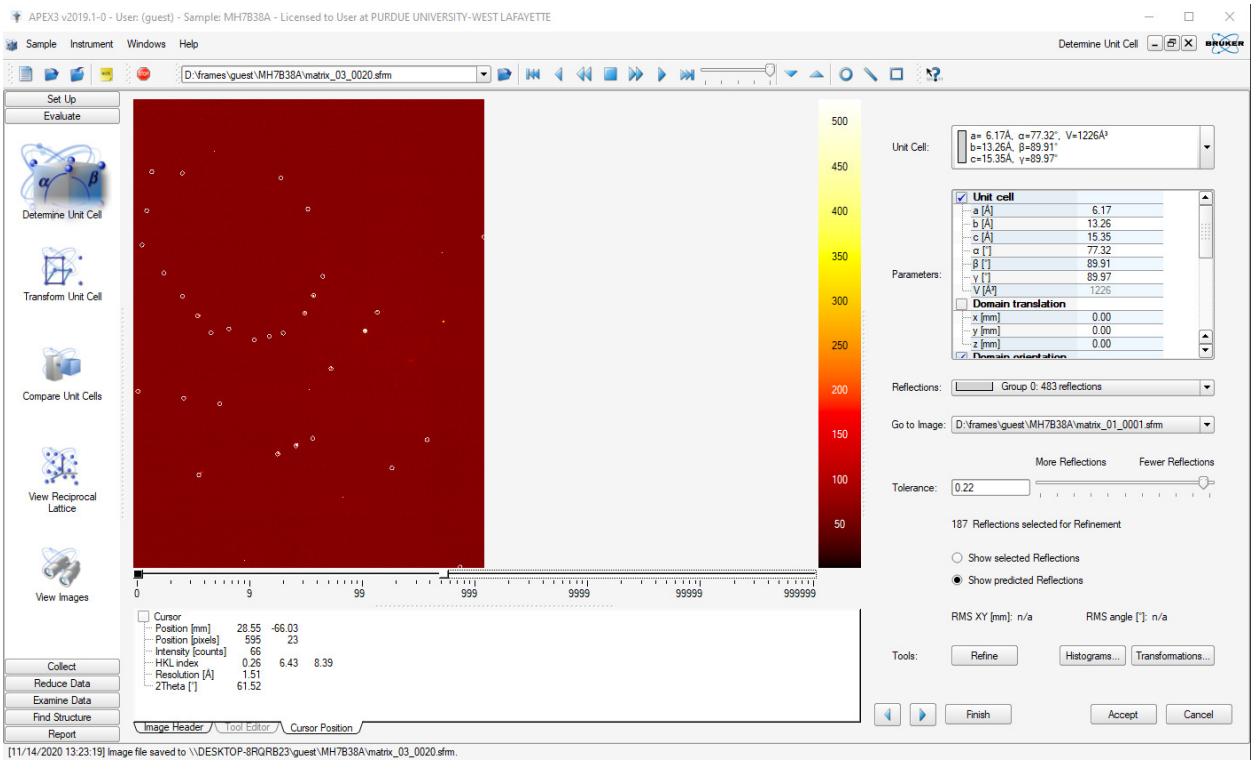


Figure 15, Unit Cell Refinement Window

- Proceed to <Bravais>. A list of possible Bravais lattices will be displayed with the software's choice highlighted in green.

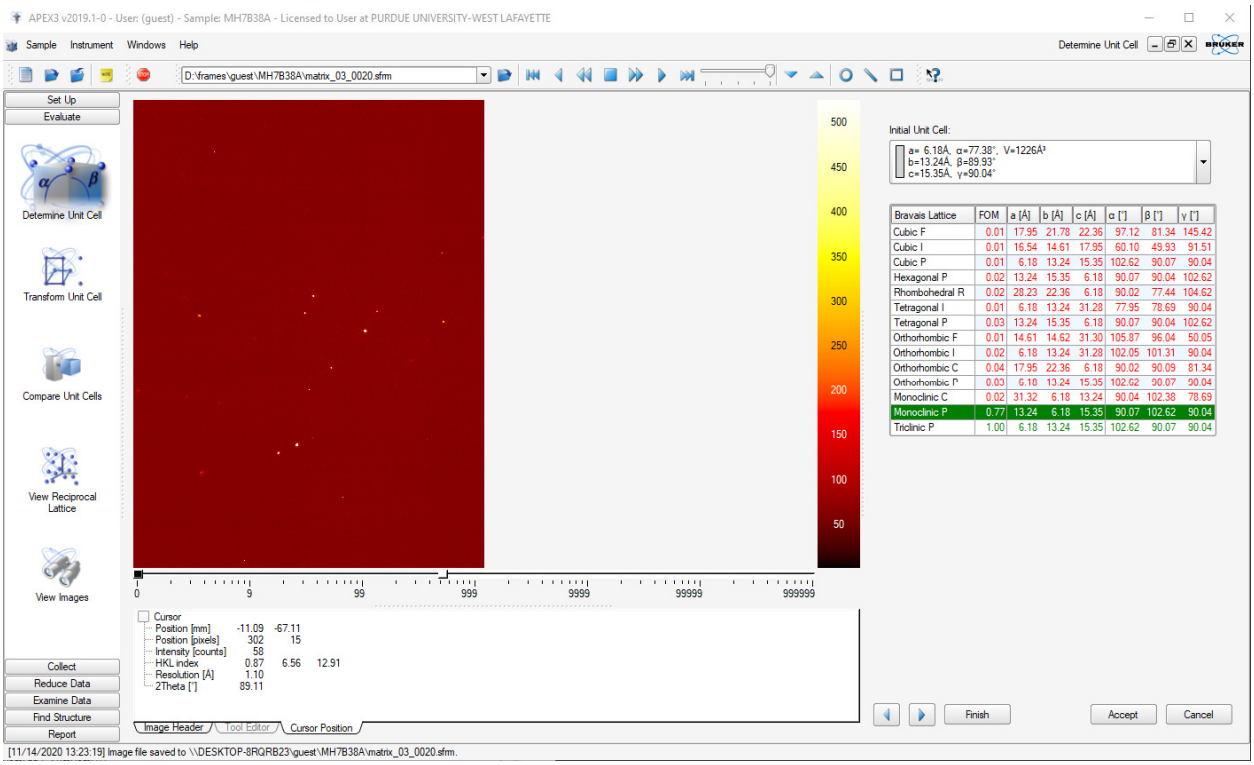


Figure 16, Bravais Result Window

The correct choice should have a Figure of Merit (FOM) value significantly higher than the others. If several solutions have similarly high FOM values, the one with the highest symmetry is likely to be correct. If you are not sure (e.g. when a high symmetry solution has a high FOM value, but significantly lower than a lower symmetry solution) select the lower symmetry solution to avoid collecting incomplete data.

- Make your choice of Bravais lattice, then click <Accept>. This will open the second Refine Window.
- Use all defaults and click on <Refine> to run a least squares optimization of the unit cell against the diffraction spot positions (constrained to the chosen Bravais lattice). Select more or less reflections as desired and repeat, then click <Accept>.
- Proceed to <Refine>, click <Refine>, then <Accept>.
- Please note the resolution prediction at the bottom of the window
- This finishes the Unit Cell Determination.

Data Collection

- Click < Calculate Strategy> under <Collect>.
  - Under Step 1., change the resolution to d = 0.82 Å or smaller. The worst acceptable value, by IUCr standard, is 0.83 Å. The best resolution that can be set on this instrument is 0.78 Å. Collecting to 0.78 rather than 0.82 significantly increases the data collection time and is only recommended for samples that do indeed diffract all the way to the detector edge. Click <Apply> if you made any changes.

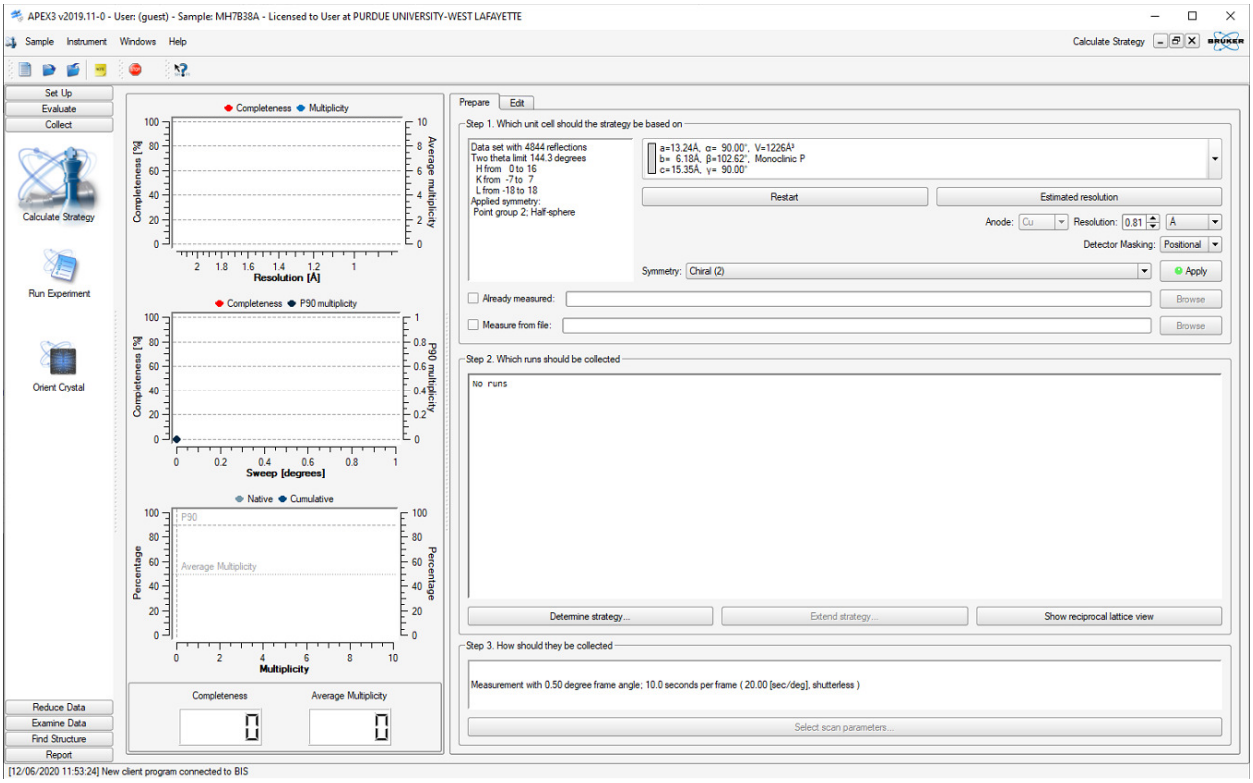


Figure 17, Strategy Starting Window

- Under Step 2., click <Determine Strategy>. In the pop-up window, it is usually safe to use the default values. Click <OK>,

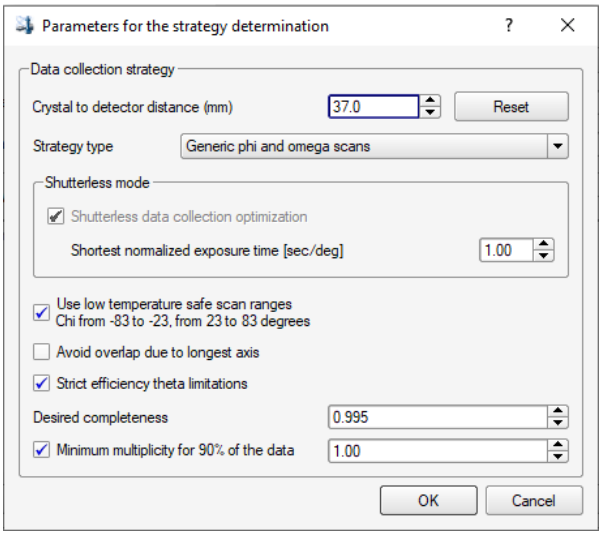


Figure 18, Determine Strategy popup window

- Under Step 3., click <Select Scan Parameters>. A popup window will emerge. Click the two <reset> buttons for "Frame angle" and "Frame time" to obtain (usually reasonable) suggested values (Fig 19).
- If the crystal is weakly diffracting, change the <Scan Options> to <Sensitive>. Otherwise leave at <Dynamic>.

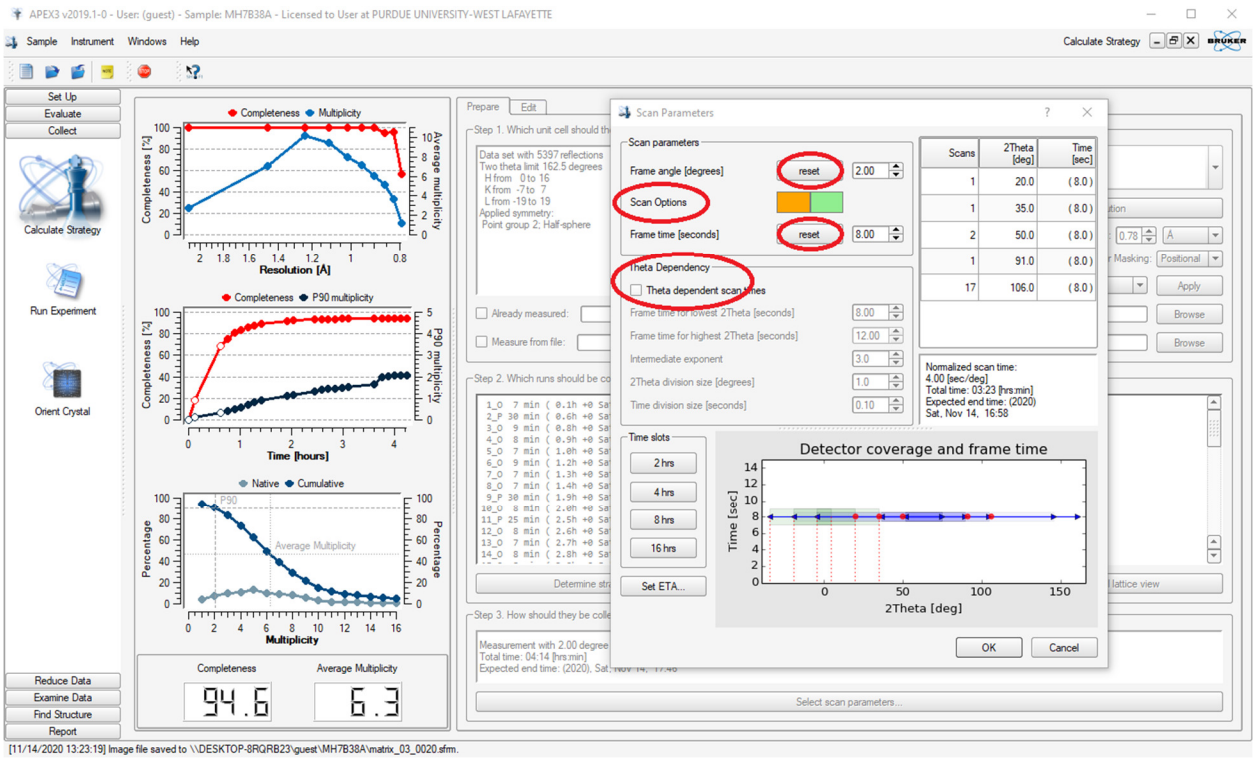


Figure 19, Strategy Window with Scan Parameters sub-window

- The frame angle value can usually be accepted it is.
- Check the box for <Theta dependent scan times>. Use the suggested value for the <Frame time for highest 2Theta>. Use a substantially lower exposure time for <Frame time for lowest 2Theta>. The value to use depends on how quickly the data intensity drops off with angle. If high angle data are intense, then using four times the low angle exposure time is a good guess. If high angle data are very weak but low angle data are intense, then ten times or more can be used for the high angle fraction.
- If you feel the suggested values are completely out of whack, make your own choice!!
- Adjust the <intermediate exponent> as required.
- Click <OK> to proceed.

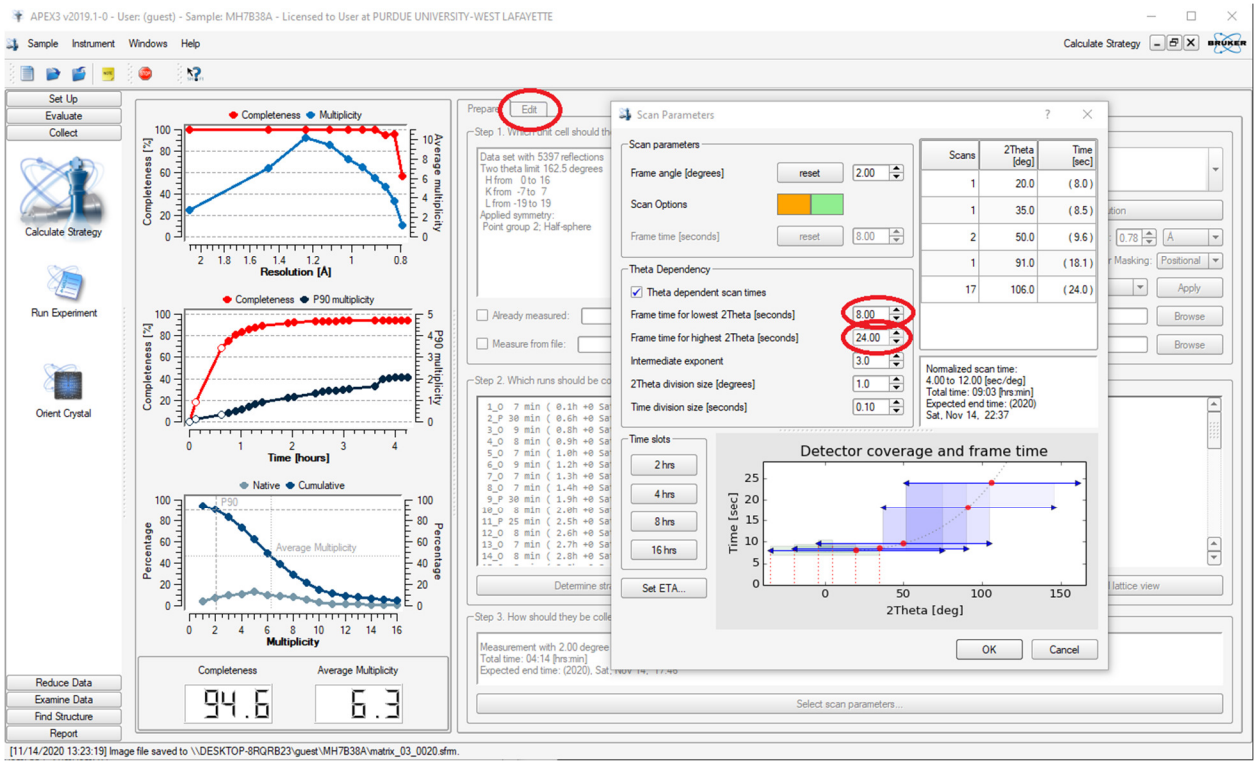
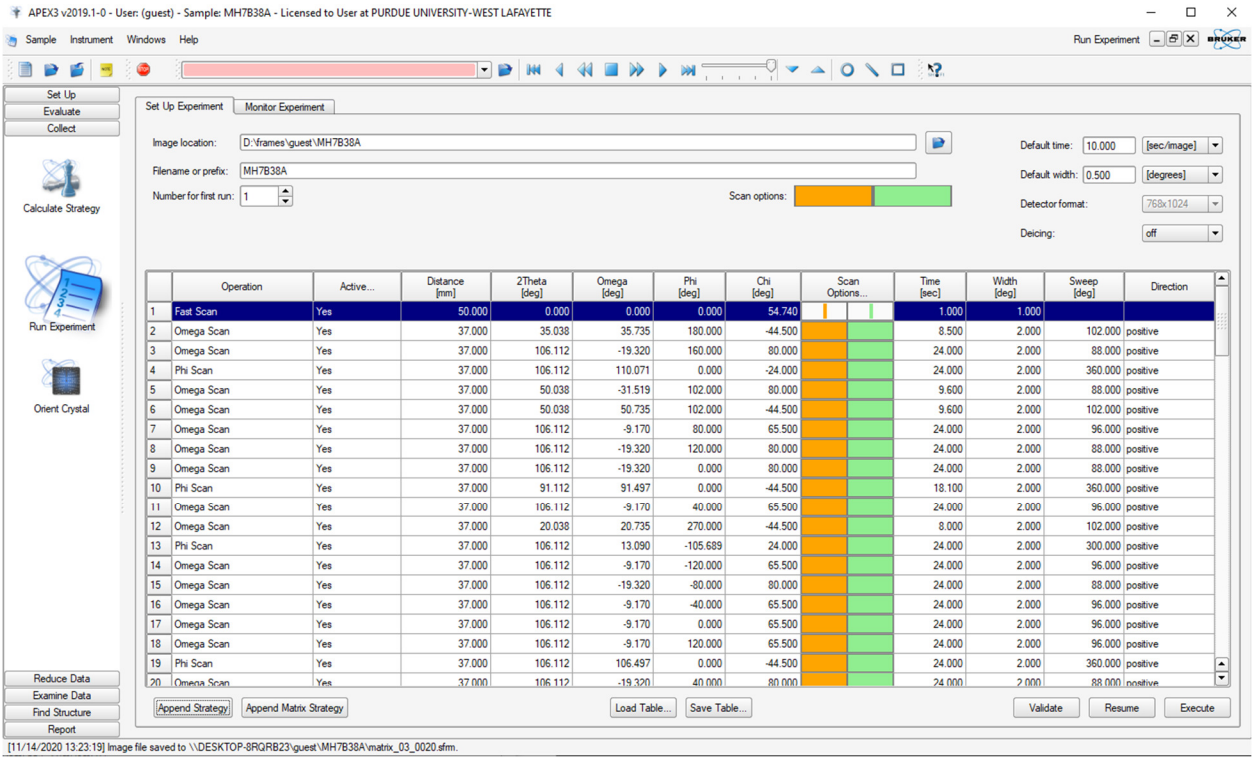


Figure 20, Strategy Window with Scan Parameters sub-window

- After assigning low and high angle exposure times, you can re-sort the frames for optimal data collection time. Click the <Edit> button, then click the <Sort> button.
- Optional: If you expect the structure to be centrosymmetric (most structures are), but would like the option to collect complete non-centrosymmetric data without needing to "starting over", you can re-sort the frames so that complete centrosymmetric data are collected first, followed by frames that fill in Friedel pairs. Change <Symmetry> to the centrosymmetric case and click <Apply>, but do not click <Determine Strategy> again. Click the <Edit> button, then click the <Sort> button.

- Under <Collect> click on <Run Experiment>
  - Click <Append Strategy> to add the runs from the strategy calculation.
  - Apex3 does automatically add two <Fast Scans>, one with 1 second exposure per degree, and one with one second exposure and use of an attenuator (a thin metal foil automatically moved into the beam path during the measurement to further diminish extremely intense reflections). Fast Scans are usually not needed when using the copper wavelength instrument (the PhotonIII detector has a sufficiently large dynamic range to avoid large numbers of saturated diffraction spots). If you think the Fast Scans are not needed, highlight the line of the Fast Scan with a left mouse click. Then right mouse click the line number and select <Delete> from the options.
  - If you chose to use the Fast Scan, edit the exposure time for the fast scan. Use ten times less exposure time / degree for the Fast Scan than for the other low angle runs.



	Operation	Active...	Distance [mm]	2Theta [deg]	Omega [deg]	Phi [deg]	Chi [deg]	Scan Options...	Time [sec]	Width [deg]	Sweep [deg]	Direction
1	Fast Scan	Yes	50.000	0.000	0.000	0.000	54.740		1.000	1.000		
2	Omega Scan	Yes	37.000	35.038	35.735	180.000	-44.500		8.500	2.000	102.000	positive
3	Omega Scan	Yes	37.000	106.112	-19.320	160.000	80.000		24.000	2.000	88.000	positive
4	Phi Scan	Yes	37.000	106.112	110.071	0.000	-24.000		24.000	2.000	360.000	positive
5	Omega Scan	Yes	37.000	50.038	-31.519	102.000	80.000		9.600	2.000	88.000	positive
6	Omega Scan	Yes	37.000	50.038	50.735	102.000	-44.500		9.600	2.000	102.000	positive
7	Omega Scan	Yes	37.000	106.112	-9.170	80.000	65.500		24.000	2.000	96.000	positive
8	Omega Scan	Yes	37.000	106.112	-19.320	120.000	80.000		24.000	2.000	88.000	positive
9	Omega Scan	Yes	37.000	106.112	-19.320	0.000	80.000		24.000	2.000	88.000	positive
10	Phi Scan	Yes	37.000	91.112	91.497	0.000	-44.500		18.100	2.000	360.000	positive
11	Omega Scan	Yes	37.000	106.112	-9.170	40.000	65.500		24.000	2.000	96.000	positive
12	Omega Scan	Yes	37.000	20.038	20.735	270.000	-44.500		8.000	2.000	102.000	positive
13	Phi Scan	Yes	37.000	106.112	13.090	-105.689	24.000		24.000	2.000	300.000	positive
14	Omega Scan	Yes	37.000	106.112	-9.170	-120.000	65.500		24.000	2.000	96.000	positive
15	Omega Scan	Yes	37.000	106.112	-19.320	-80.000	80.000		24.000	2.000	88.000	positive
16	Omega Scan	Yes	37.000	106.112	-9.170	-40.000	65.500		24.000	2.000	96.000	positive
17	Omega Scan	Yes	37.000	106.112	-9.170	0.000	65.500		24.000	2.000	96.000	positive
18	Omega Scan	Yes	37.000	106.112	-9.170	120.000	65.500		24.000	2.000	96.000	positive
19	Phi Scan	Yes	37.000	106.112	106.497	0.000	-44.500		24.000	2.000	360.000	positive
20	Omega Scan	Yes	37.000	106.112	-19.320	40.000	80.000		24.000	2.000	88.000	positive

Figure 21, Experiment setup window with one Fast Scan and a typical strategy as determined by <Data Collection Strategy>

- Click <Execute> to start the data collection.
- The instrument will now collect your dataset. Open <Show Status> from the Instrument drop down menue. This will open the <Instrument Status> window where you can check variables and completion time. You can also check many of these items via the BIS interface.

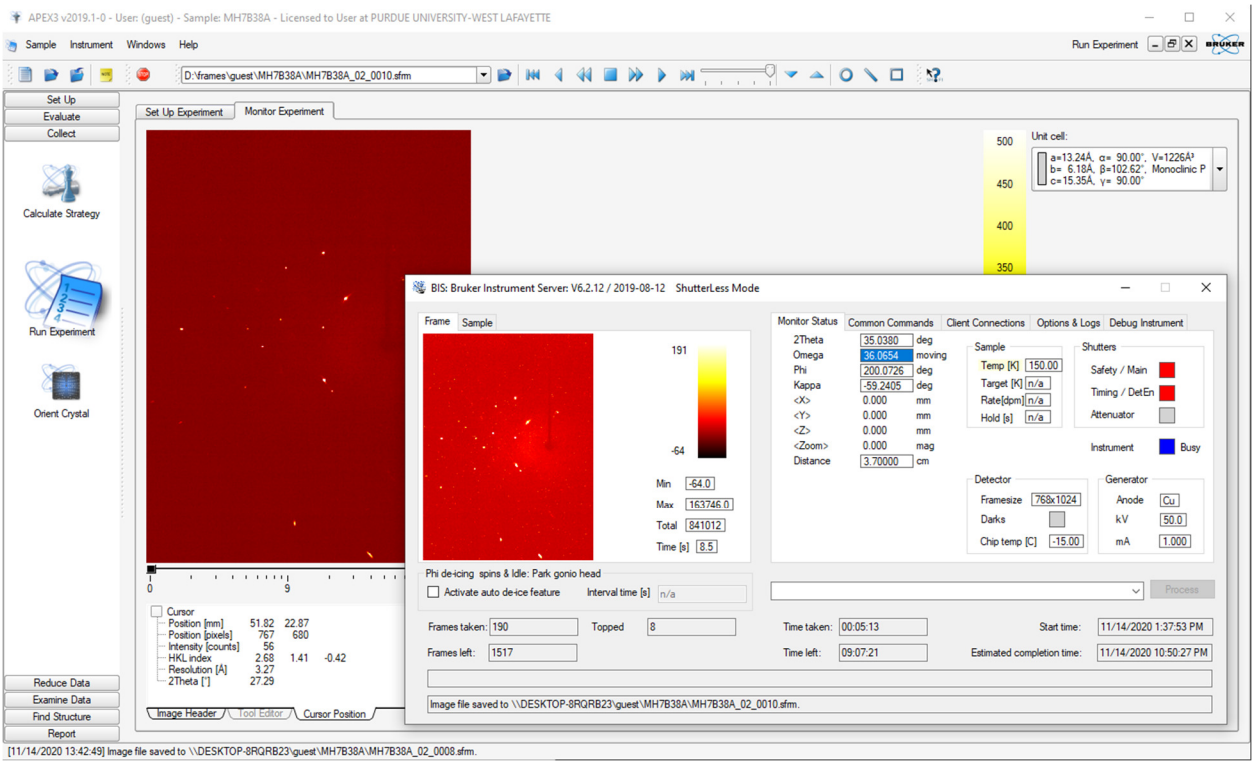


Figure 22, Data Collection Window with active data collection and BIS Window in foreground

- Users are encouraged to check the progress of their data collection and to test-solve and refine the data while the data collection is still running to check that the data quality is good enough to proceed, that the structure is indeed of interest, that the unit cell is correct, and if data might be already complete. It is highly advisable to not stop a data collection until you are sure the data are complete enough for an efficient absorption correction and to pass checkcif (i.e. data should be integrated, scaled, solved, refined, the refinement quality checked, the completeness should be checked in the cif file, and preliminarily checked using checkcif prior to stopping the data collection!!). Once a crystal is taken off the instrument a data collection cannot be resumed!

Data Integration

When the data collection is complete (or when enough data are collected for an initial structure solution and refinement attempt) open, <Reduce Data>, then <Integrate Images>

- Change the resolution limit to a value of 0.83 Å or lower.
- Click on <Find Runs>, check the runs you would like to integrate (leave out the Matrix runs).

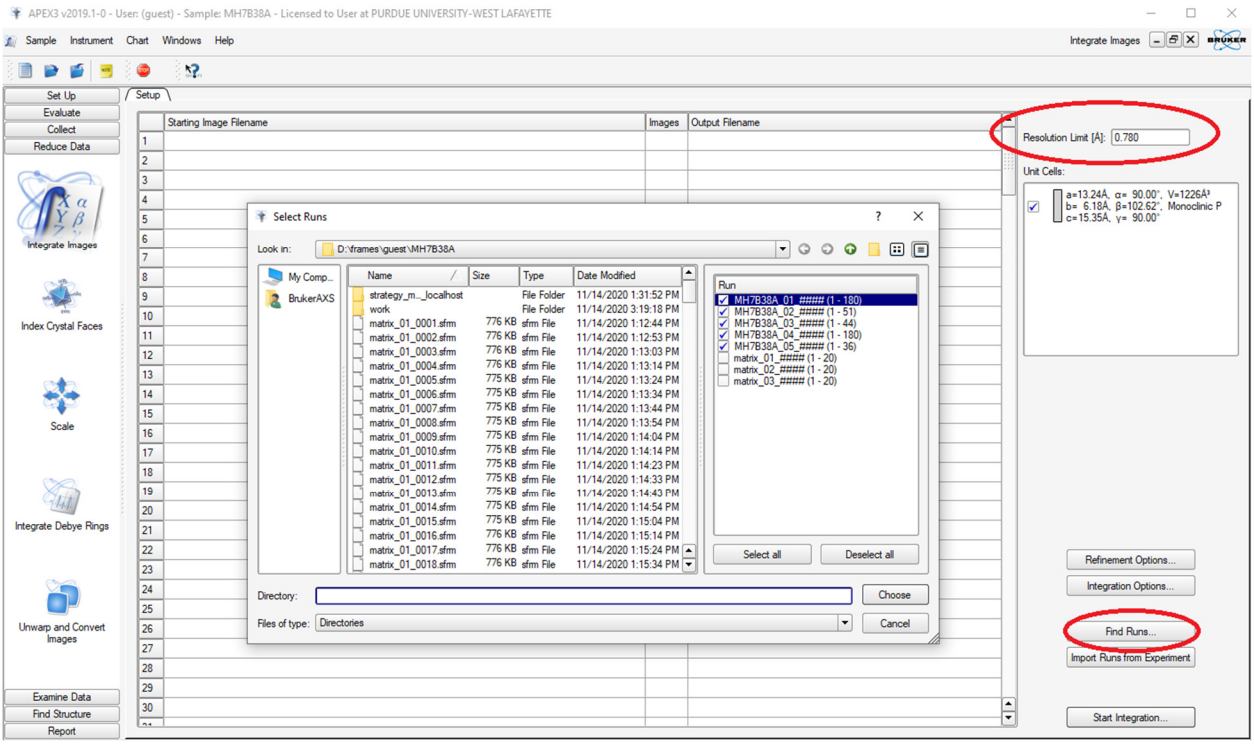


Figure 23, Integration Start Window with <Select Runs> Window open

- If your structure features at least one long unit cell axis (> 30 Å), increase the queue length. Click <Integration Options> and increase the <Image Queue> value (to e.g. 20 or 30, default is 7).
- Click on <Start Integration>. This will start the integration. Wait for it to finish, this might take several minutes.

  Troubleshooting: If the integration window does not open properly (graphics cut off), close the "integrate images" subwindow and reopen it by clicking <Integrate Images> in the <Reduce Data> menue again.

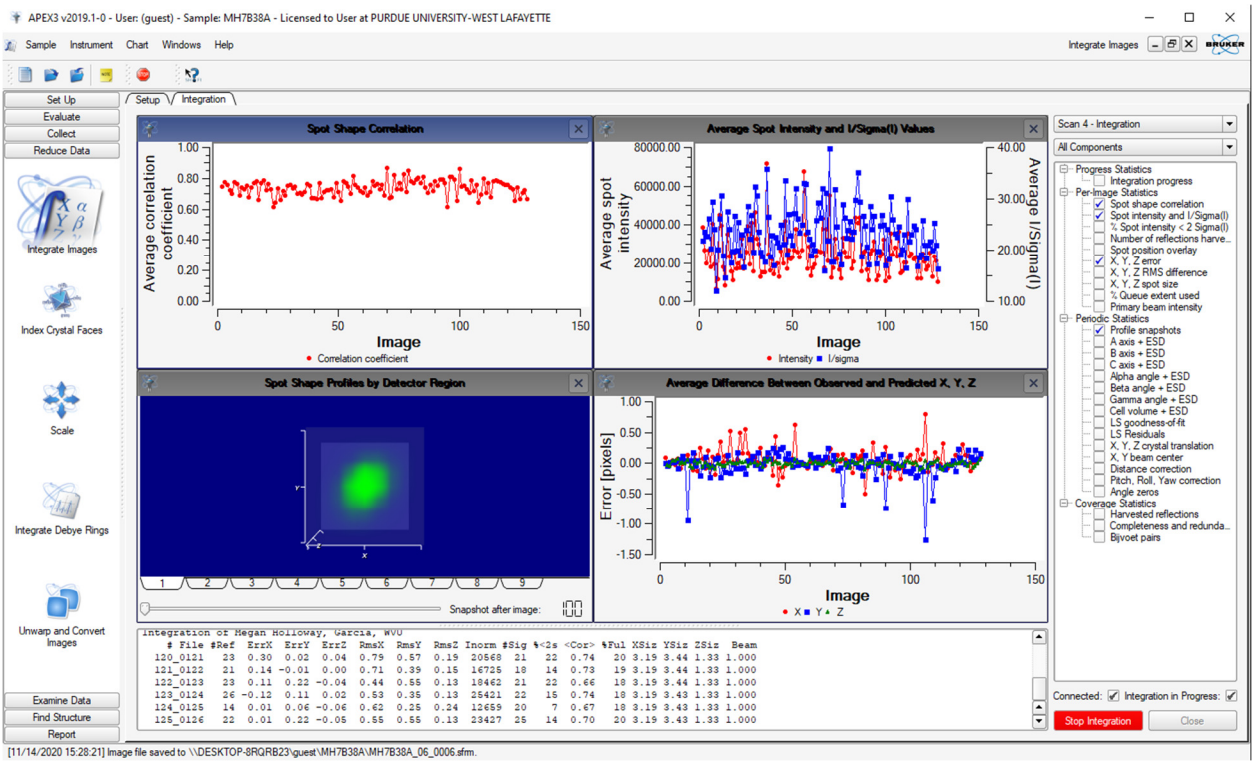


Figure 24, Integration Window with active Integration running

- Spot Shape Correlation should show values around or above 80% and be mostly even. Values of 40% or lower are statistically mostly meaningless. Pixel Errors should be on average between ±0.2. Average I/sigma(I) intensities should be at least around 3 (good datasets have values larger than 20). Shape profiles should be round to slightly ellipsoid. Double spots, large pixel errors and erratic uneven lines are possible signs for twinning. If you suspect a crystal to be split or twinned, consult the Purdue Twinning Manual.
- **IMPORTANT** (especially for data collected using a microsource X-ray tube): "Recycle" the p4p file and repeat the entire integration before proceeding to the final data work-up steps. Click <Sample>. From the dropdown menu, select <Import>, then <P4P/SPIN file>. Browse for the p4p file ending in _0m.p4p (the result of the most recent integration). Import this file (defaults are fine) and repeat the integration.

Scaling and Absorption Correction

• Under <Reduce Data> Click on <Scale>

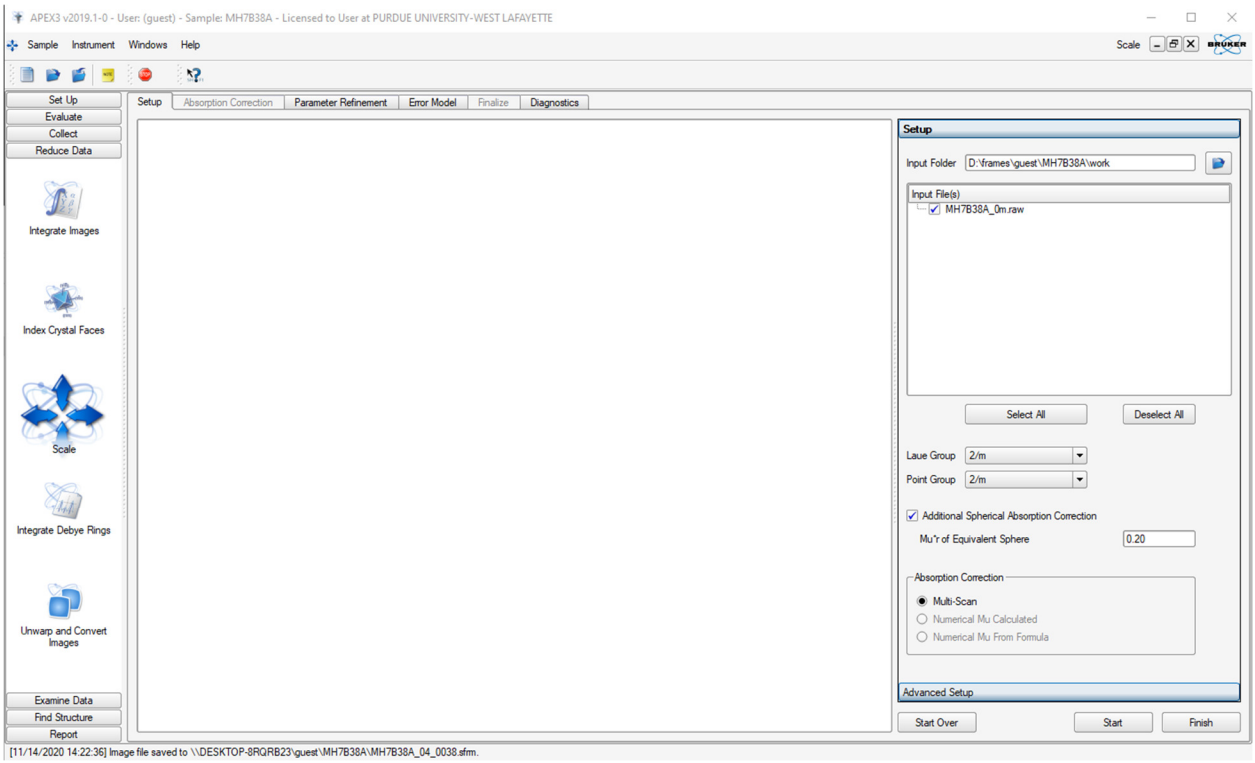


Figure 25, Absorption Correction and Error Model (<Scale>) Starting Window

- Unless only one raw file exists, the merged batch raw file from the integration is loaded automatically.

  If you did initially integrate a single run you will have to manually load the merged raw file. Click the blue browse symbol, click on the first raw file (ending in _0m.raw), then click <OK>. If you did not integrate all runs at once you need to load individual raw files. Click the blue browse symbol, click on any raw file you want to load, then click <OK>. Uncheck raw files you would like to not use.
- Check the Laue Group and Point Group.
- Keep Multi-scan as the absorption correction method. It is sufficient for > 99% of all samples. For heavy absorbers, adjust the number for <Mu*r> (r is the average radius of the crystal in mm, mu is the absorption coefficient in mm^{-1}; its value can be looked up in the cif after assignment of all atoms). If the value of mu is different than initially expected, repeat scaling and absorption correction with a more suitable Mu*r value prior to writing the final cif.
- Proceed by clicking <Start>.

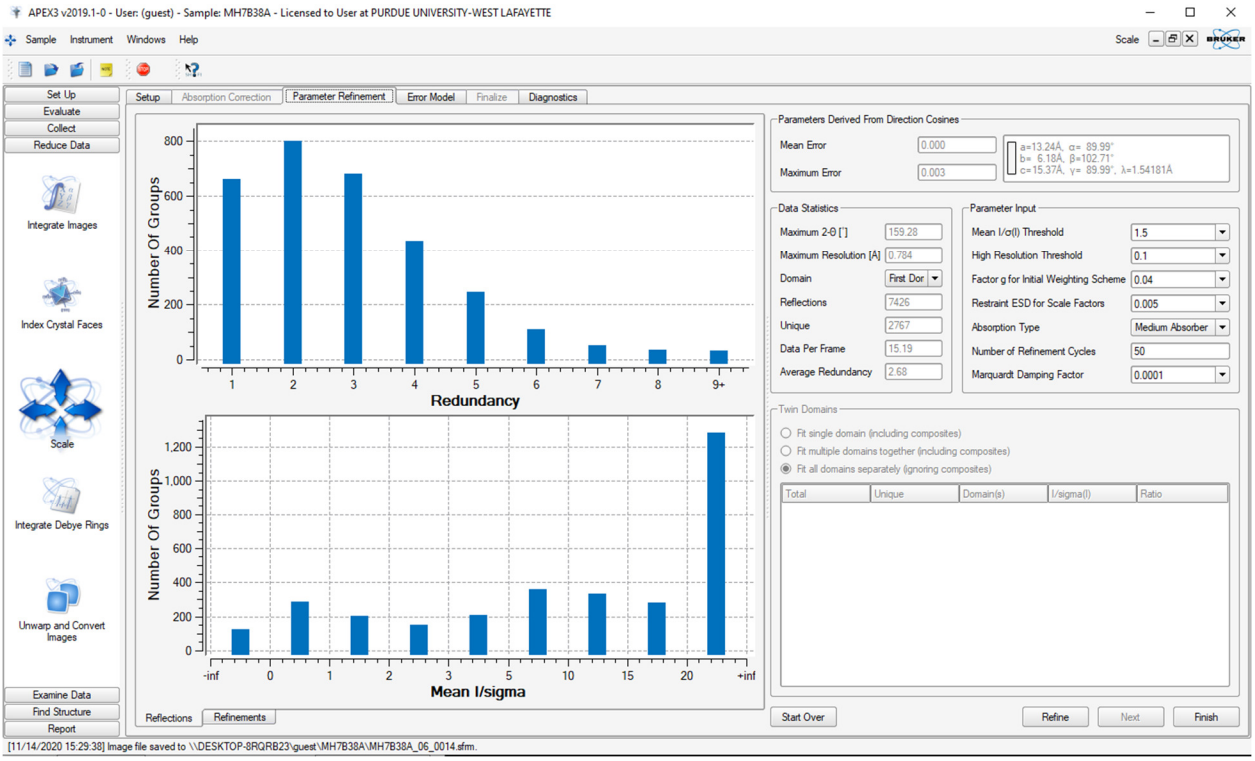


Figure 26, Absorption Correction window (second Scale Window) before refinement

- The parameter refinement window will open (Fig. 26). An average redundancy of around 5.0 is usually sufficient for weak to medium absorbing samples, but initial data can be processed with much less. For oddly shaped and heavily absorbing samples a higher redundancy is recommended for the final data.
- Click <Refine>, wait for the refinement to finish and inspect the results. The parameter refinement curve should show a smooth and steady decline (as in Fig. 27) and the final R value should be reasonably low. Large up and down fluctuations in R[%] combined with higher than expected R values could be a sign that scaling and absorption correction effects are highly correlated. This can especially be a problem for heavily absorbing crystals that are also aspherically shaped when a small beam size (microsource) is used. If the refinement results appear less than ideal, select a higher value for <Restraint ESD for Scale Factors> and repeat the parameter refinement (click <Refine> once more). Default is 0.005, the maximum allowed value is 0.02. Check if the refined data are indeed improved via a Shelxl refinement, otherwise use the defaults!

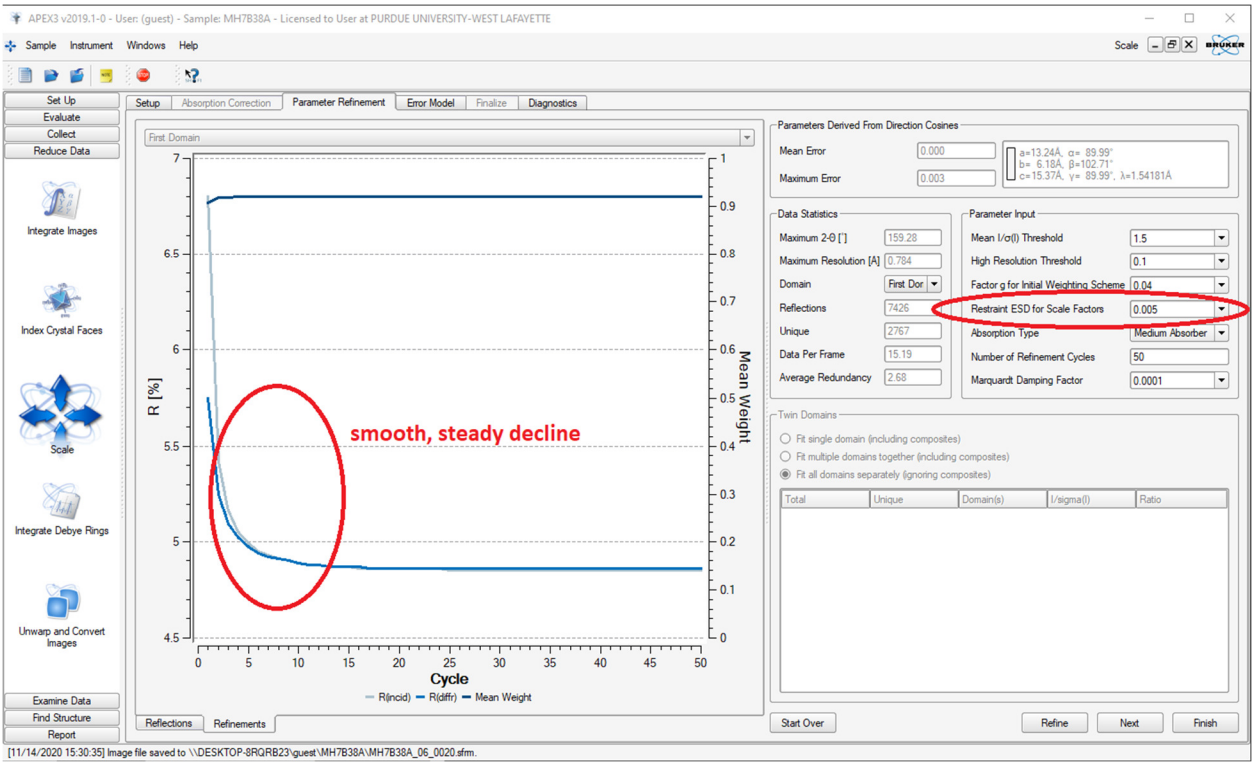


Figure 27, Absorption Correction window (second Scale Window) after refinement

- Once satisfied, click <Next>. The Error Model page will open.
- If you used a Fast Scan, check the R value of the Fast Scan. If it is substantially larger than the R values of the other scans, check the "Fast Scan" checkbox and click <Repeat Parameter Refinement> (the checkbox might be automatically selected; uncheck it if you would like to use all data from the Fast Scan).
- Check the number of rejected reflections. If this number is extremely high, in conjunction with higher than expected data R values, then there is a possibility that the selected Laue group is too high in symmetry ("metric pseudosymmetry"). Revise your unit cell assignment and reintegrate the data.
- The number of rejected reflections can be reduced (assuming that the Laue group is correct!) by increasing the value for "<|I-<I>|/su ratio for rejection>" (default is 4.0). If you do so, click <Error Model> to update the outlier rejection.

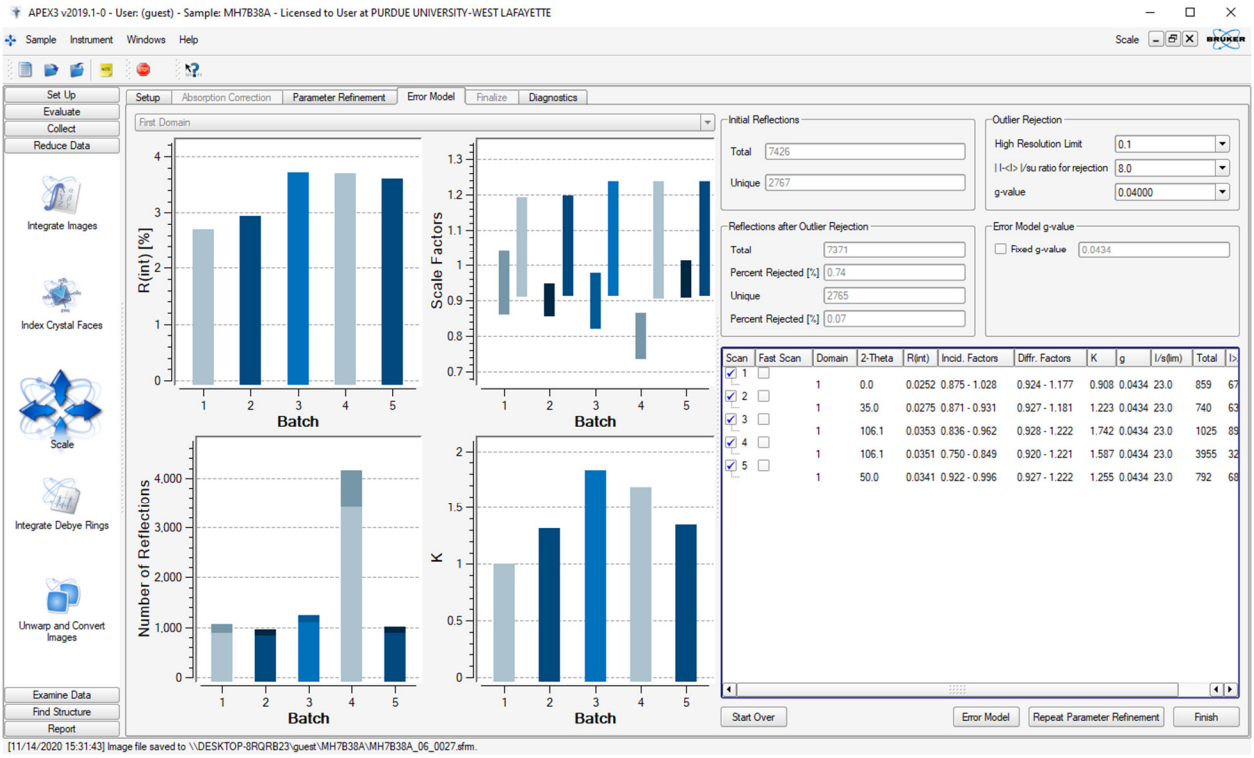


Figure 28, Error Model Window (third Scale Window)

- Click <Finish>, then <Exit> to finalize scaling and absorption correction.
- You have now finished the data collection and absorption correction. To allow other users to use the instrument you should proceed with analysis on a different computer at this point.

Transferring the Data

• Move the data via e-mail, USB flash drive or online to the data workup computer or your personal computer.

• For the refinement, you will need the *.hkl and the *.p4p files (located in the work folder of your project)

e.g. 04mz02a_0m.hkl

04mz02a_0m.p4p

• For publication purposes you will also need copies of the *.abs and the *._ls files:

e.g. 04mz02a.abs or 04mz02a_0m.abs (A copy of what you did in SCALE, SADABS, or TWINABS, contains the ratio of Tmin/Tmax).

04mz02am_0m._ls (A copy of the last lines of the integration procedure, contains parameters of unit cell refinement (2THETA min, 2THETA max and the number of reflections used), crystal colour and shape, crystal dimensions).

• If you used Cell Now to obtain a unit cell, you will also need a copy of the *._cn file

Setting up Data for XPREP

• On your computer save the files from above into a new file folder. Making a backup of your files is strongly recommended! (with older operating systems you need to uncheck the "read only" flag, by highlighting your files plus right mouse click, go into properties and uncheck.)

• Open the SHELXTL program. Select <PROJECT> and <New>. Find the appropriate file and open it. Give it a project name e.g. 04mz02a_0m, then <open>.

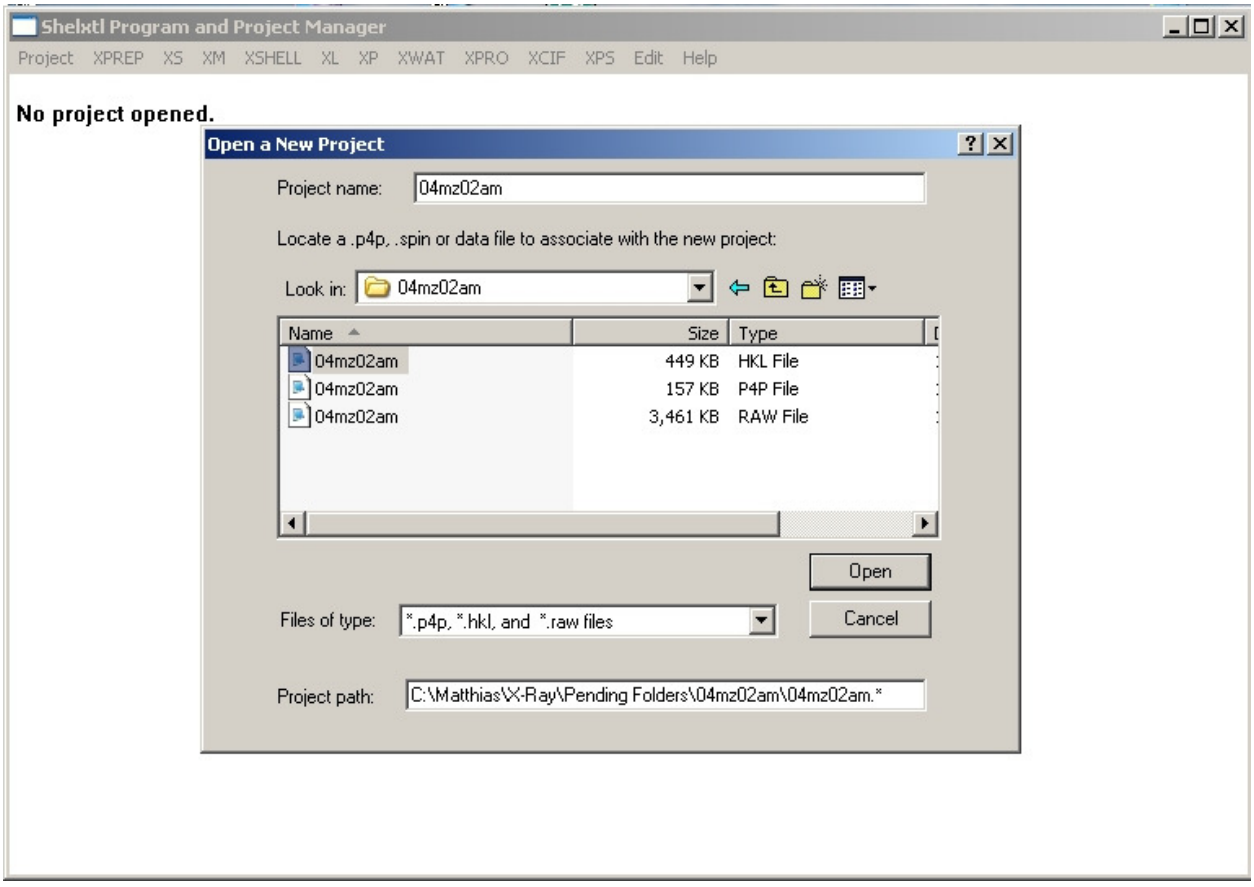


Figure 29, Shelxtl Program and Project Manager Window with New Project Window open

XPREP

• Select <XPREP> on the toolbar.

In the next steps, the computer will make suggestions that can usually be accepted (i.e. for high quality data).

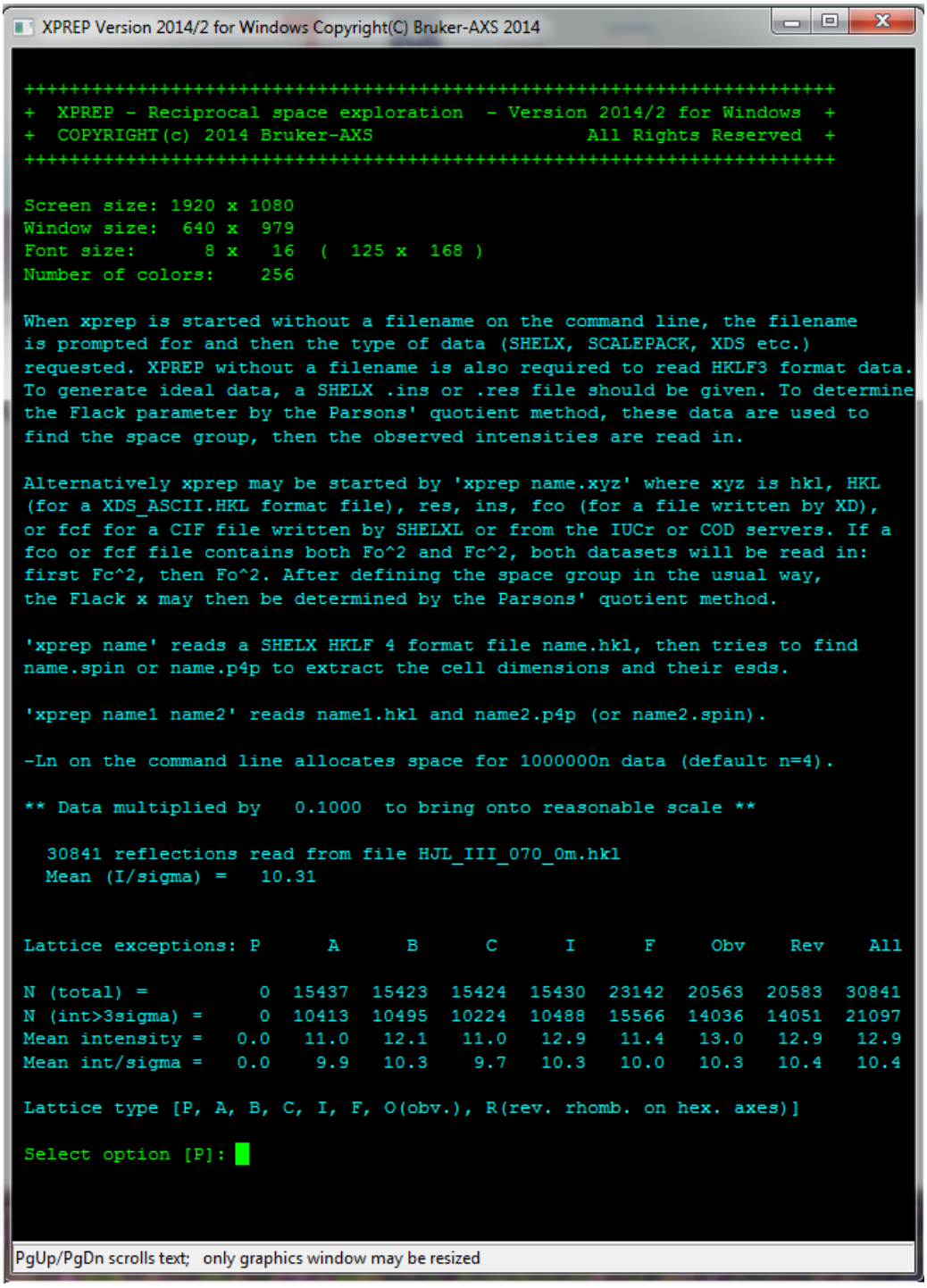


Figure 30, XPREP Window, initial lattice centering selection

- Select the suggested lattice type.
- Choose [H] to search for higher metric symmetry.
- Choose offered choice [A] for the Laue group (e.g. monoclinic).

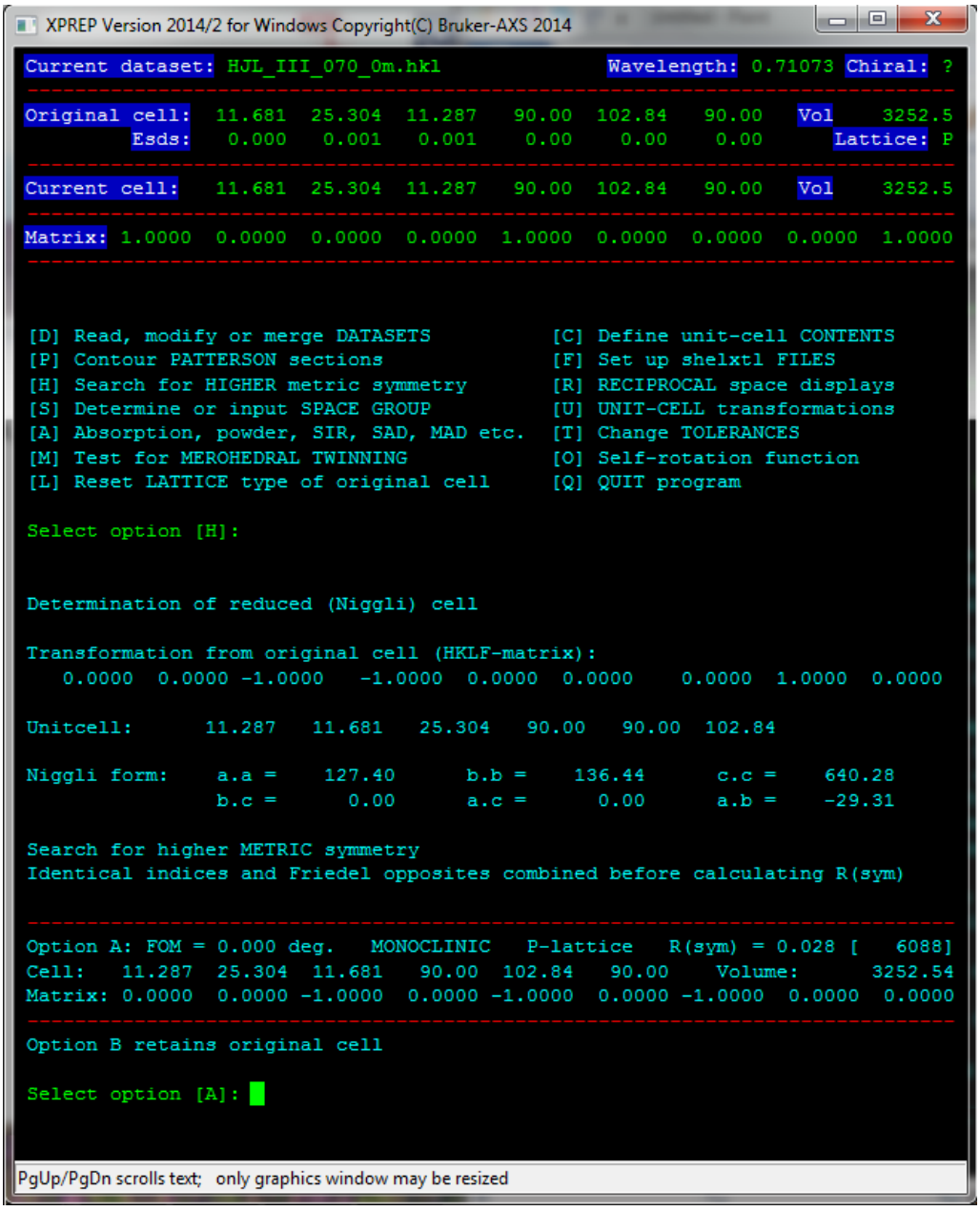


Figure 31, XPREP Window, initial lattice type selection

- Select [S] to determine or input space group.
- Select [S] again to determine space group.
- Select the suggested Laue group (e.g. [M] for monoclinic)

- Select the suggested lattice centering (e.g. [P] for primitive, [C] for C-centered, etc)

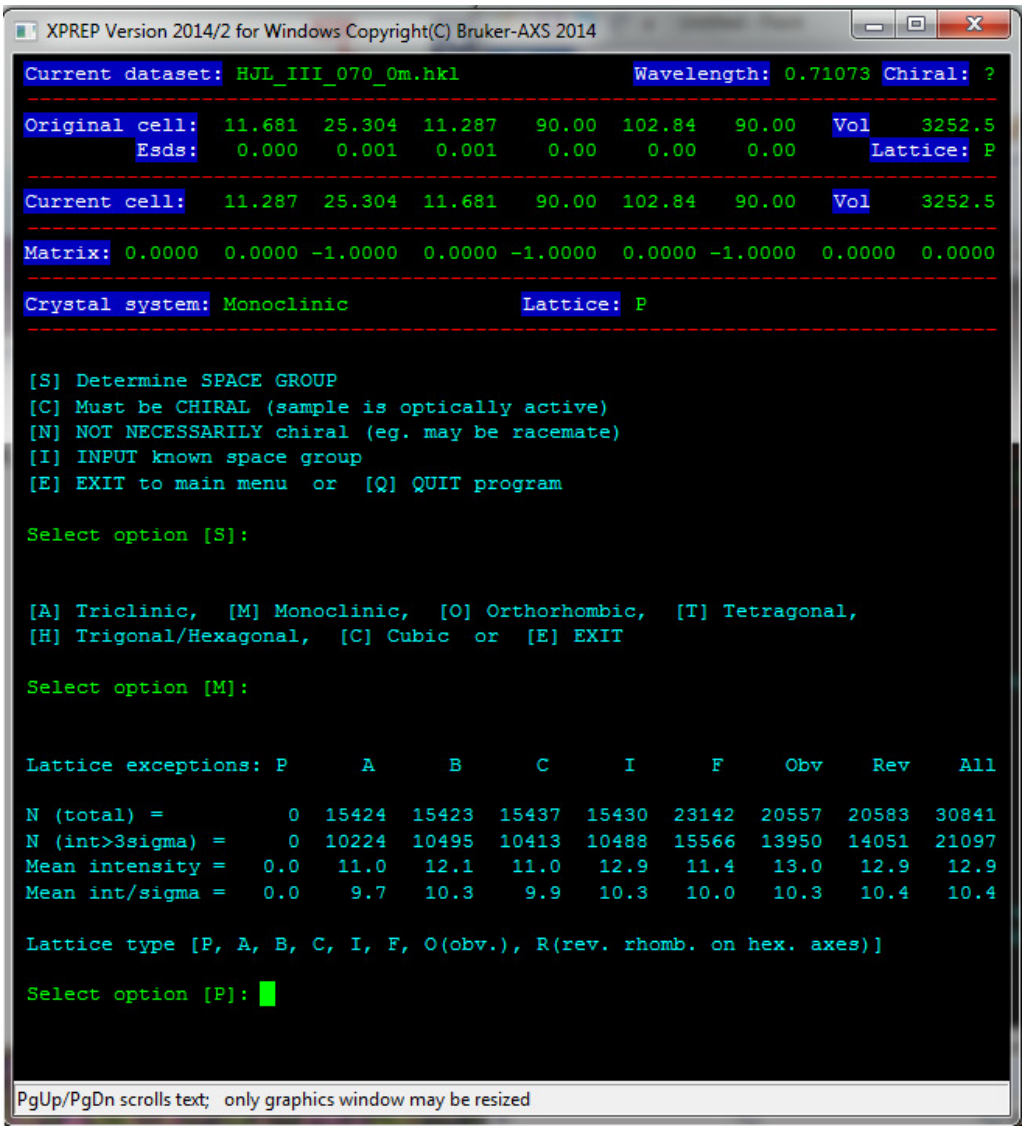


Figure 32, XPREP Window, new lattice centering selection

- Select the suggested option for the space group e.g. P2(1)/c or C222(1) (If several solutions are offered, take the one with the lowest CFOM value. If that does not work out later on, try the next best in the list).

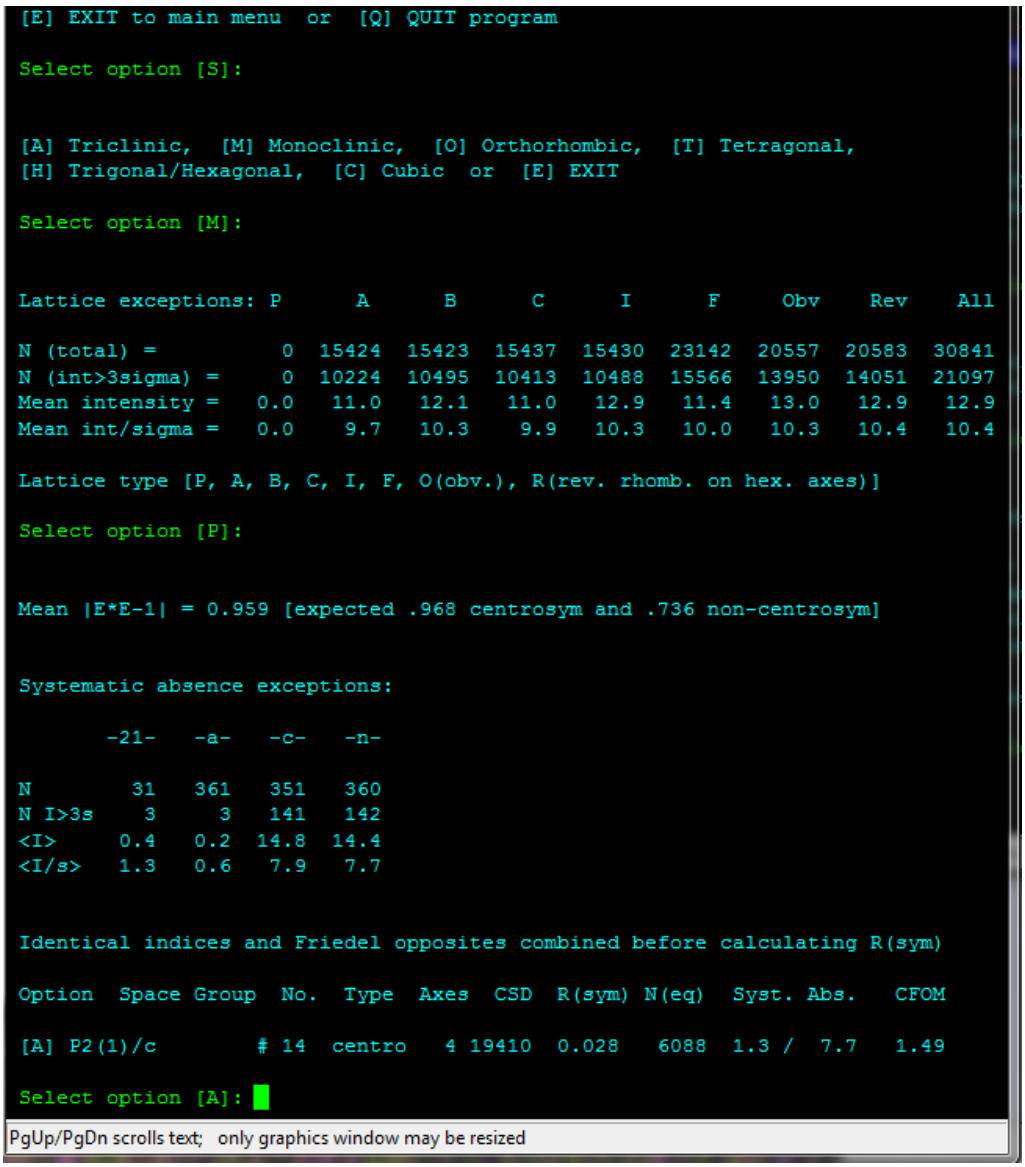


Figure 33, XPREP Window, space group selection

- Select [D] to read, modify or merge datasets
- Select [S] to display the intensity statistics
- Select [A] to merge all equivalent reflections (including Friedel opposites). This does not actually merge the reflections (which is not recommended to do), but only displays the data and statistics as if they would have been merged.

The intensity statistics will be displayed.

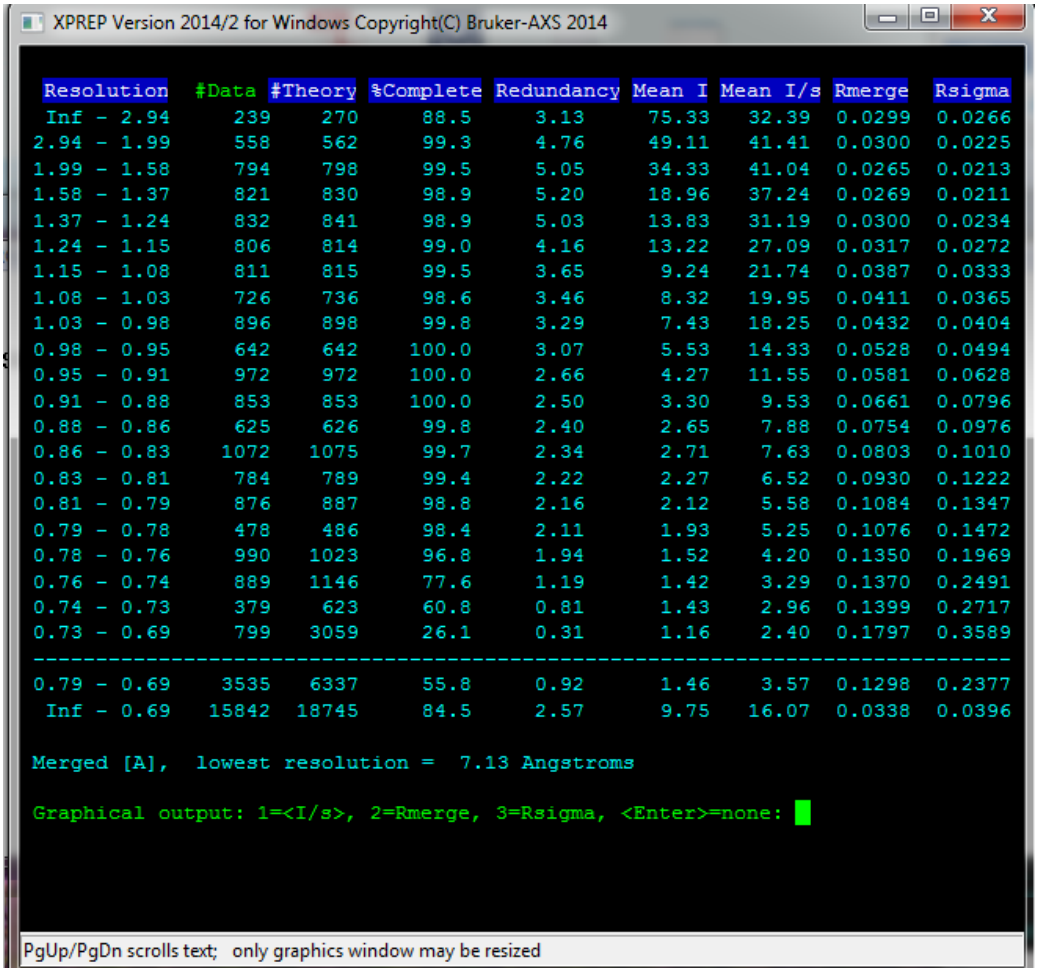


Figure 34, XPREP Window, intensity statistics

Have a look at the Completeness, Redundancy, Rmerge and Rsigma (Rmerge is the same as Rint in Shelxl and Sadabs). The completeness should be ideally close to 100 % down to a resolution of d = 0.82 Å. Rsigma and Rmerge should be ideally below 10 % down to d = 0.82 Å.

Hit the Enter key

- Select [E] to exit to the main menu, then [C] to define the unit cell contents.
- If no unit cell contents are given type the most likely molecular formula (element symbols and numbers only).
- If the given formula seems wrong, select [F] for new formula, and type the most likely formula.

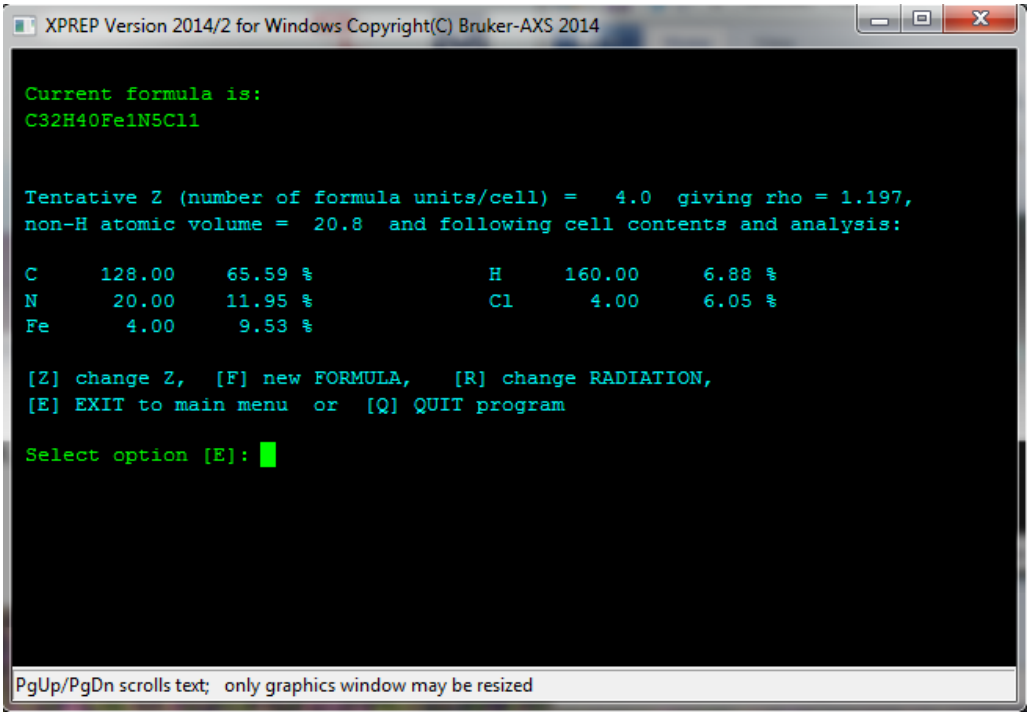


Figure 35, XPREP Window, formula definition

- If necessary, select [R] to change the radiation (Mo radn; λ = 0.71073; Cu radn; λ = 1.54178)

- If necessary, select [Z] to change the number of (symmetrically independent) molecules Z per unit cell.

- Select [E] to exit to the main menu.

Note: If you had been using SADABS for the generation of your *.hkl file, no absorption correction has to be applied. If you still need to apply absorption correction, this can be done here by choosing [A] (not covered here).

- Select [F] to setup the new hkl file. If you have changed the unit cell or its orientation in XPREP, the program will force you to choose a new name for the hkl file to avoid overwriting and losing the original data (change it back to your old filename after closing XPREP, or start a new project with the new hkl filename).

- Select [Y] at the prompt to generate an .ins file.

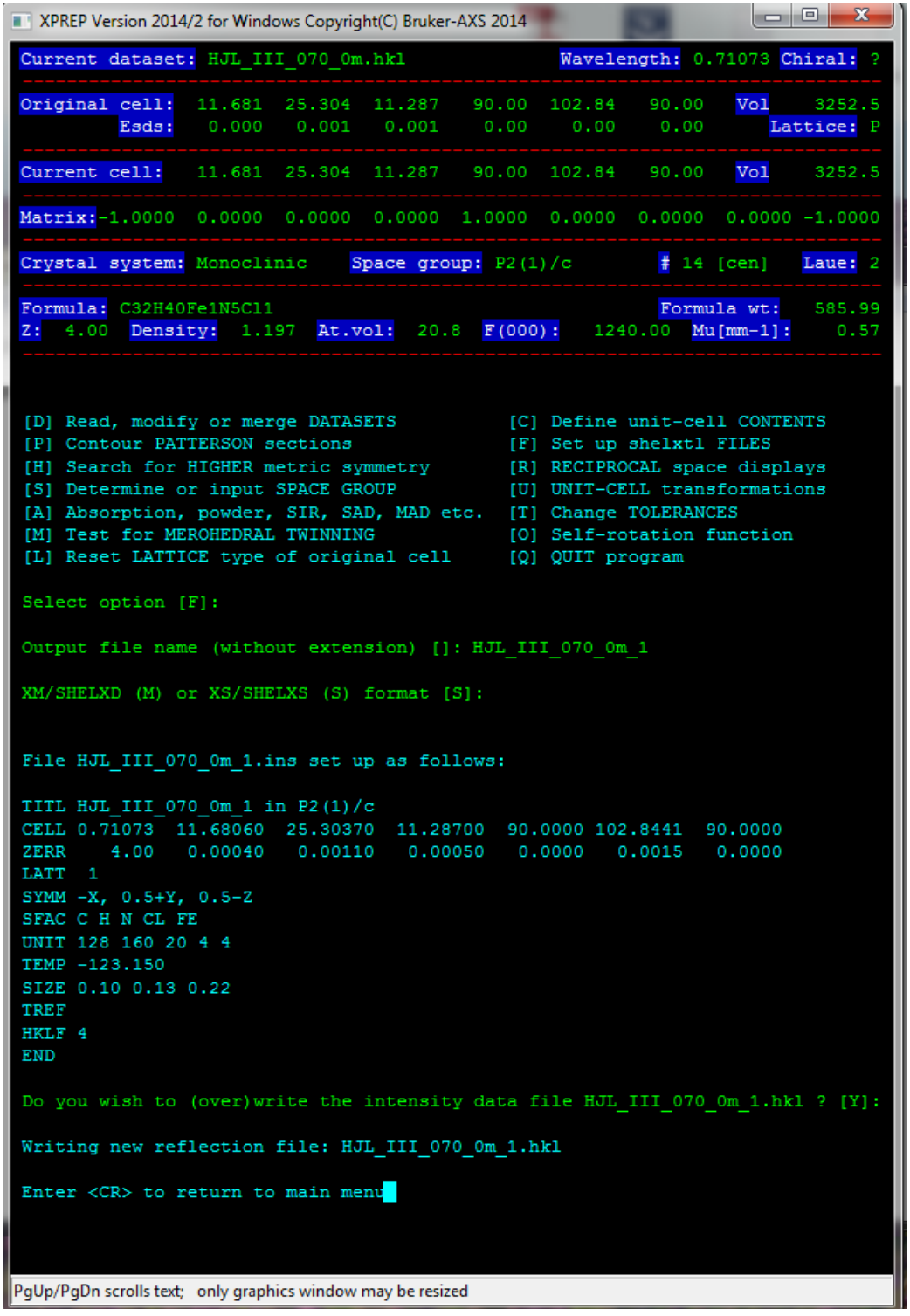


Figure 36, XPREP Window, ins and hkl file setup

- Select [Q] at the prompt

The Initial INS File

The initial *.ins file written in XPREP, together with the *.hkl file, contains all the information to get started solving and refining your crystal structure. The *.ins file can be opened and edited in any text editor. Open it via <Edit> and <Edit .ins> in the SHELXTL menu.

The initial ins file may look like this:

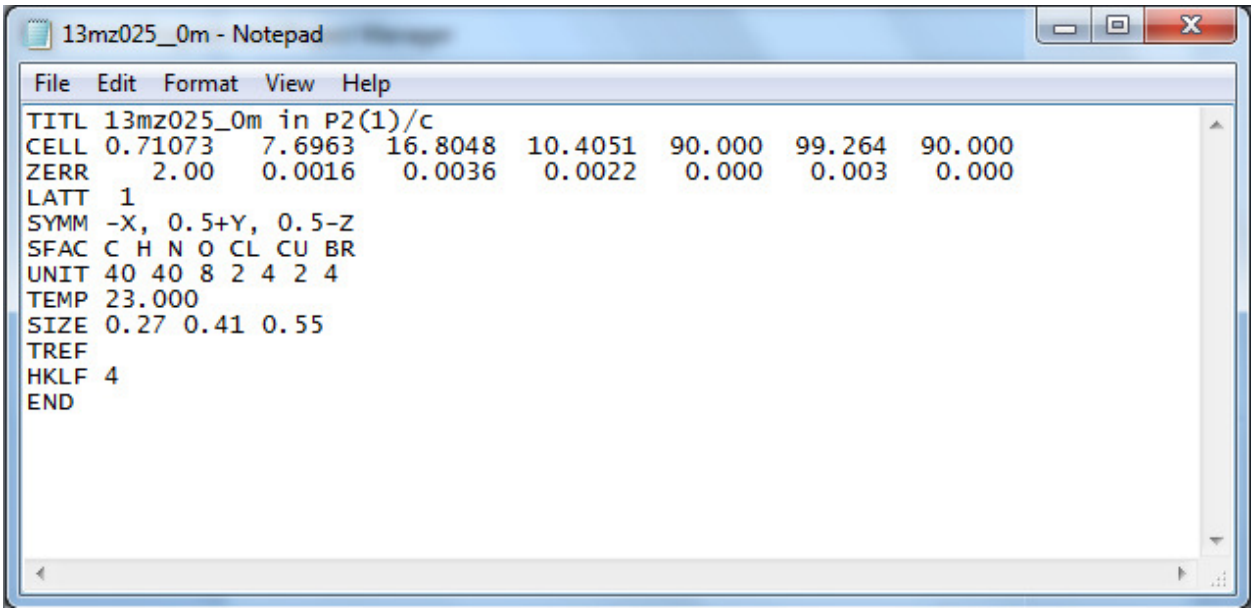


Figure 37, typical initial INS file

The TITL line gives the initial name of the *.ins file and the space group from XPREP.

The cell line gives the wavelength used, then the unit cell parameters a, b, c, α, β, γ.

The ZERR line gives the Z value (number of molecules in the unit cell as given in XPREP) and the estimated standard deviations for a, b, c, α, β, γ.

LATT gives the lattice type (see SHELXTL manual).

The SYMM line(s) give the symmetry operators of the space group that create symmetry dependent atoms (these lines define the actual space group, not the TITL line).

The SFAC line gives the atom types and defines the atom structure factors to be used by the program.

The UNIT line gives the number of atoms per unit cell as listed in the SFAC line.

The TEMP line gives the temperature in degrees Celsius. If needed, change the value to the actual temperature used in your experiment.

The TREF line defines the type of structure solution method to be used (see below).

The HKLF 4 line defines the type of refinement to be used (HKLF 4 is the standard single crystal refinement for a not non-merohedrally twinned dataset).
The END lines defines the end of the file.

Select <file> and then <save>.

Solving Structures using XS and XM

To perform the refinement of your structure, you will first need an initial guess (a starting point). Using the *.ins file provided by XPREP, the programs XS or XM will provide you with this initial guess (other programs and methods can be used, such as ShelXT, DirDiff, charge flipping, etc). In the program XS there are two methods to choose from: Direct methods (indicated by the line TREF in the INS file), and the Patterson method (indicated by the line PATT in the ins file). Direct methods can be used for all datasets with better than atomic resolution. Patterson methods can only be used for structures with at least one heavy atom (i.e. sodium or heavier). Direct methods usually provide a more complete initial guess (more atoms already found), but they tend to fail more often than Patterson methods and they have occasionally problems to place inversion centers correctly. XM is utilizing direct methods originally written for macromolecular data, but can also be used for small molecule data. It is more likely to work where XS/TREF fails, but requires more computing resources.

- To use XS, select <XS> on the toolbar and the computer begins to process data. The software tries in this step to find an “initial guess” for the atom positions. This can only be successful if the atoms listed in the formula are correct. The software will select the solution with the smallest value for CFOM (combined figure of merit) and the initial atom positions are written to the *.res file. The res file cannot be opened while XS is running.

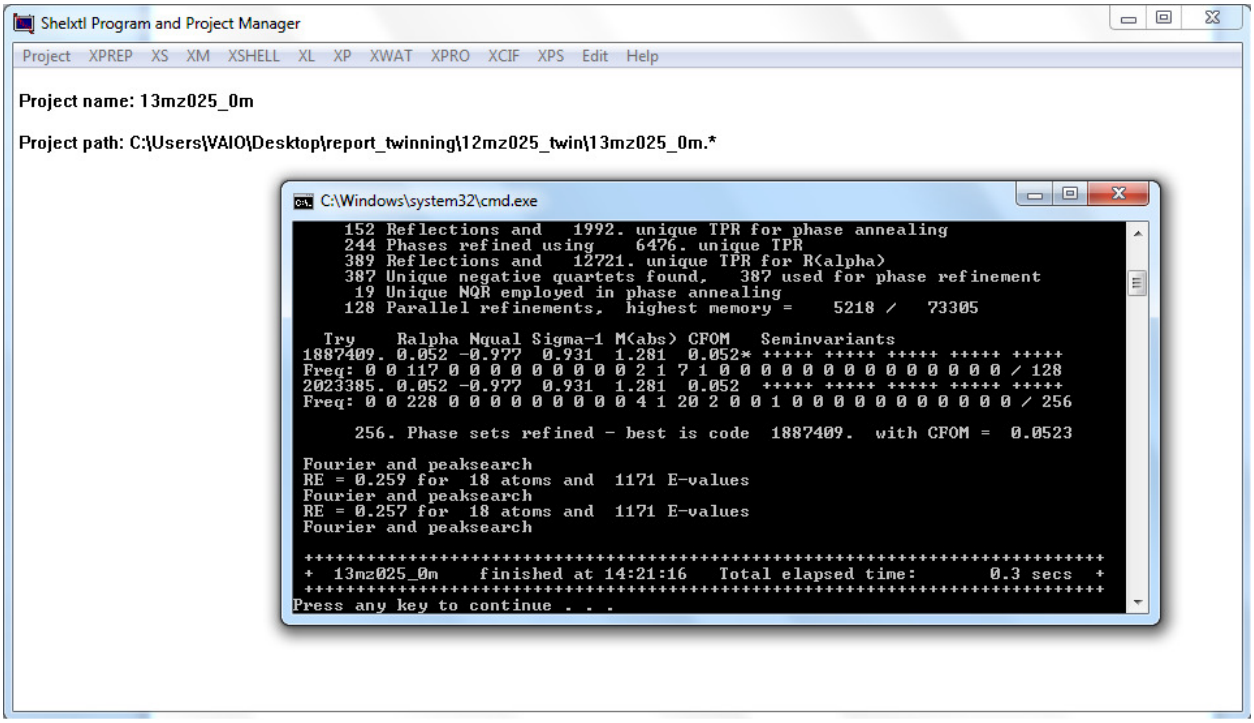


Figure 38, the XS window

- For good quality data the correct solution is normally “falling out” when continuing with Shelxle, Olex or Crystals graphical refinement interfaces.

If this is not the case, you can change the settings for XS in the ins file. Go to <Edit> on the toolbar and select <edit .ins>

Either, change TREF to TREF 2000 (or even higher) to run more iterations, or, for compounds with at least one atom heavier than sodium, use PATT instead of TREF. Select <file> and then <save> and run XS again.

If XS fails using both PATT and TREF methods, try using <XM>. In the *.ins file, replace the TREF line by the following four lines:

FIND 58

PLOP 77

MIND 1.0 -0.1

NTRY 1000

Save the ins file and run Click <XM> in the in the SHELXTL menu. The software will run 1000 tries (or the number specified in the NTRY line) and selects the solution with the highest correlation coefficient, CC. The initial atom positions are written to the res file. This will take substantially longer than XS (a fast computer may be required), but solutions can be analyzed while XM is still running. If a valid solution is found, XM can be aborted.

XM does not write all required lines to the *.res file. Before running the first refinement cycle, add the following lines:

FMAP 2

L.S. 4

PLAN 20

Solving Structures using ShelXT

In the rare case that XS (TREF or PATT) and XM both fail, try using XT (ShelXT). ShelXT uses a combination of Patterson and Direct methods and will try a number of space groups compatible with the metric symmetry. The output name of the res file will be appended by a, b, c etc (depending on the number of solutions created) and a new hkl file will be written. You can use XT from within Apex3, or via command line. ShelXT has an extensive array of options, but these are rarely needed and simple execution via “XT.exe filename” does usually work (filename = name of the .ins file, type without file extension). The output res and hkl files can be used as they are (but check if the input unit cell has been changed in case you are still processing data and plan to update the hkl file).

ShelXT can also be added as an external program to Shelxle via its “Extra” dropdown menue.

Structure Refinement

Open the result of the structure solution in a graphical interface such as Shexle, Olex or Crystals. See separate manuals for details of the refinement procedure, and for writing and validating the crystallographic information file.