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 produced 2017 and upgraded 2022 and 2024. 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$_{Cu}$ 3.0" X-ray tube, HELIOS multilayer Montel 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 AD61 dry air unit.
- The instrument computer: This computer runs the Measurement Server, the Bruker Instrument Service (BIS), Diffrac.Maintenance and Apex5.

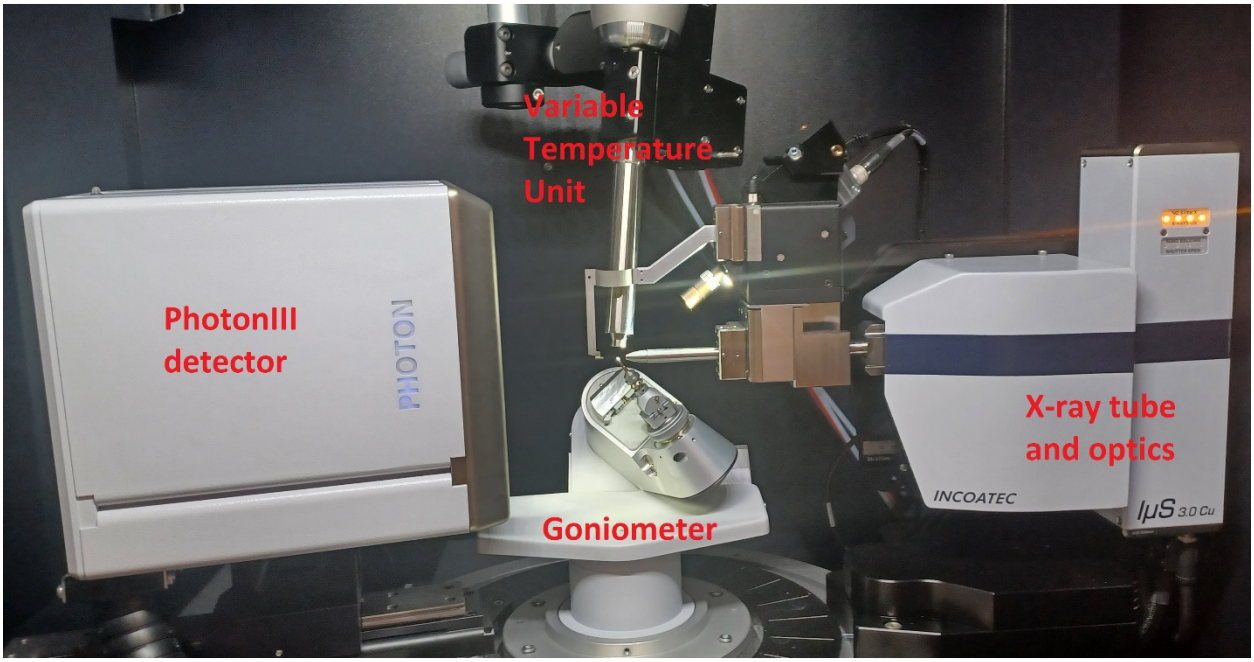


Figure 1, Inside of the Quest X-ray enclosure with microsource X-ray tube, PhotonIII Detector, Variable Temp Unit and Goniometer. Between X-ray tube and detector are the Optics (Multilayer Optics for monochromatization, Collimator), the Video Microscope (in back, partially hidden), and the Beam Stop. 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 Apex5 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 AD61 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 Apex5 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 Apex5 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.

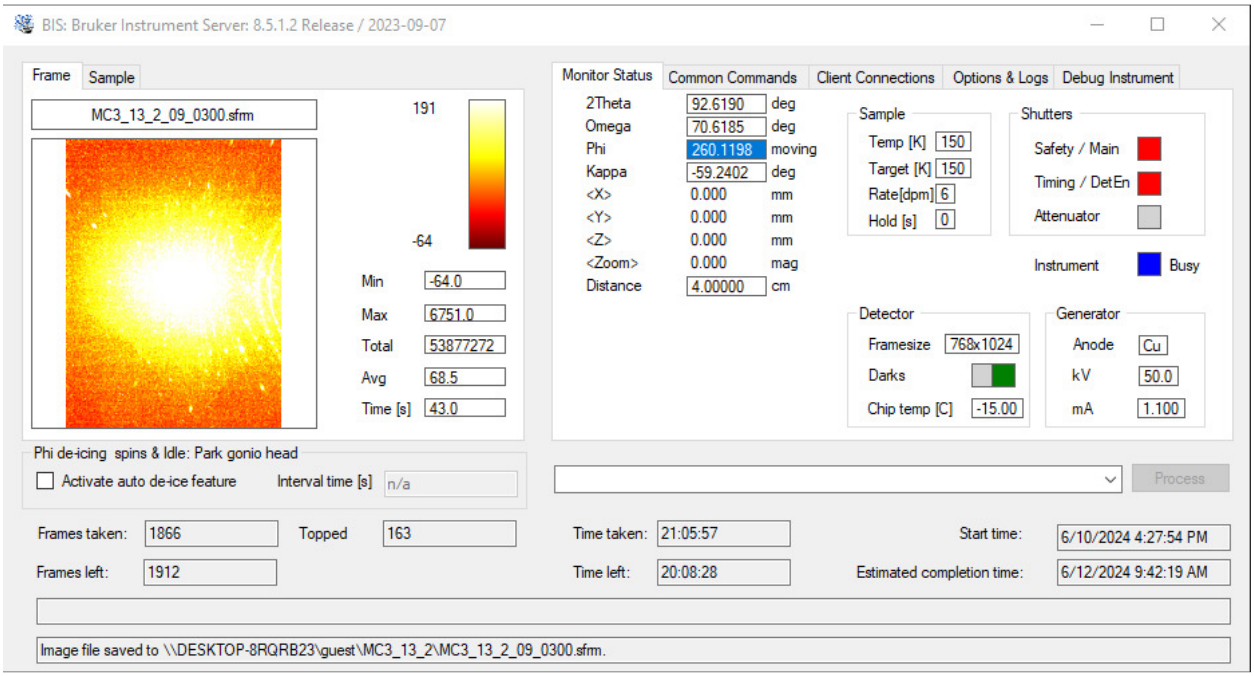


Figure 3, The BIS window

- 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 Apex5 and click the STOP symbol in the Apex5 interface.
- Minimize any sessions of Apex5 that might be open.
  - Start a new session of Apex5. 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*)

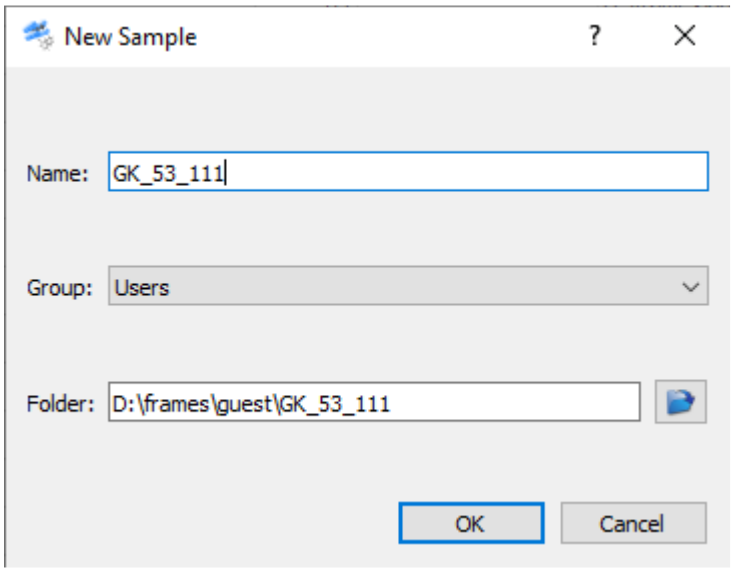


Figure 4, New Sample pop-up window

- 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 until the temperature has settled at the target value.

Unloading the Previous Sample

- Under <Set Up>, go to <Center Crystal>
  - The video feed will automatically open showing you a life picture of the mounted crystal.
  - 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, Parabar 10312 (previous name: Paratone oil), Fomblin oil (both are perfluorpolyether oils) or polybutene oil (a 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.

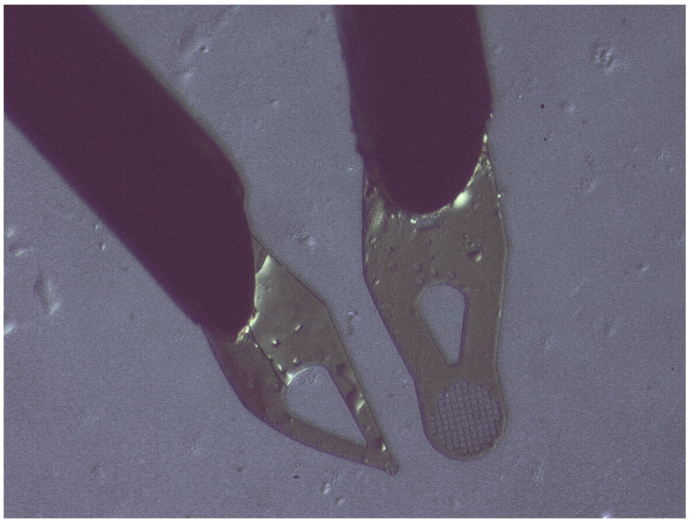


Figure 6, Tips of Mitegen Micromesh mounts as seen through a microscope (Left: small tipped mount with 15 μm openings for crystals 0.1 mm and smaller. Right: large mesh mount with 25 μm openings. Mesh is 0.4 mm across).

- 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).

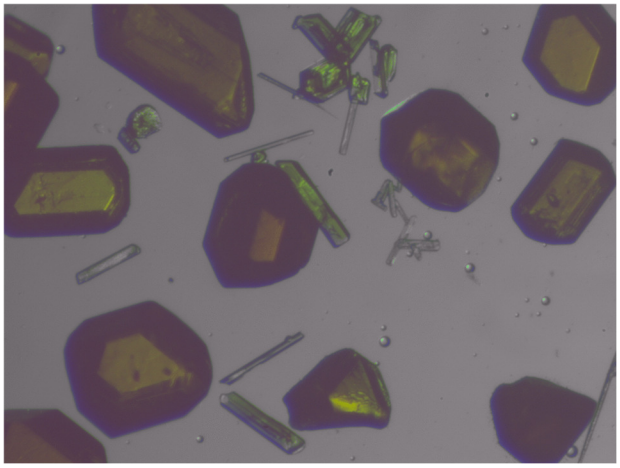


Figure 7, Crystals with well-developed faces on a microscope slide

- 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).
- Micromesh mounts are not single use. If necessary, they can be cleaned using e.g. chloroform (which readily dissolves most of the mounting oils and cryoprotectants)

Crystal Mounting and Centering

- Make sure exactly one copy of Apex5 is running (trying to open the crystal centering tab from a second copy of Apex5 can crash the video feed).
- 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.
- In Apex5, 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. Check the position of the mount by eye (is it aligned with the collimator and the beam stop? Using the crystal centering tool as described below adjust the position on the goniometer head by eye until the crystal can be seen in the live video feed.

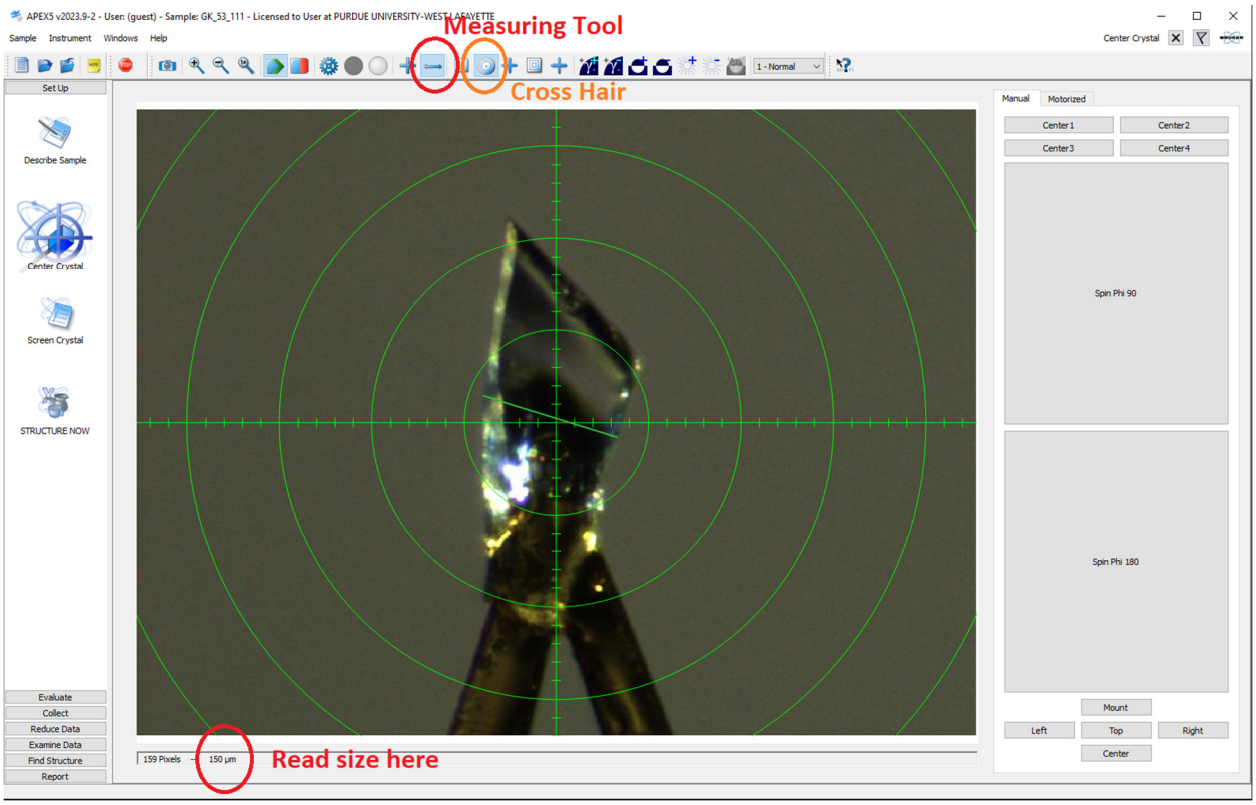


Figure 8, Crystal Centering Window and live video feed with mounted sample and cross hair.

- If the image is too dark to see the crystal well, click the <Center1> button or 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 Apex5) 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!).

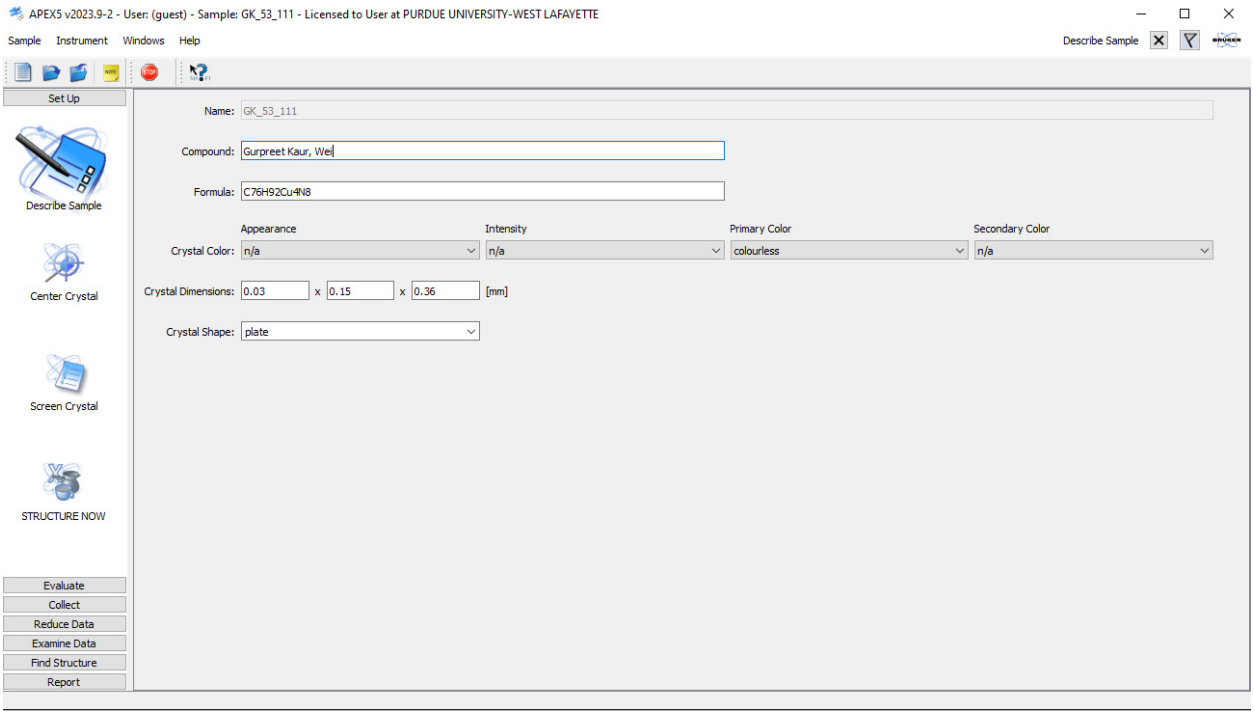


Figure 9, Crystal and compound description

Unit Cell Determination

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

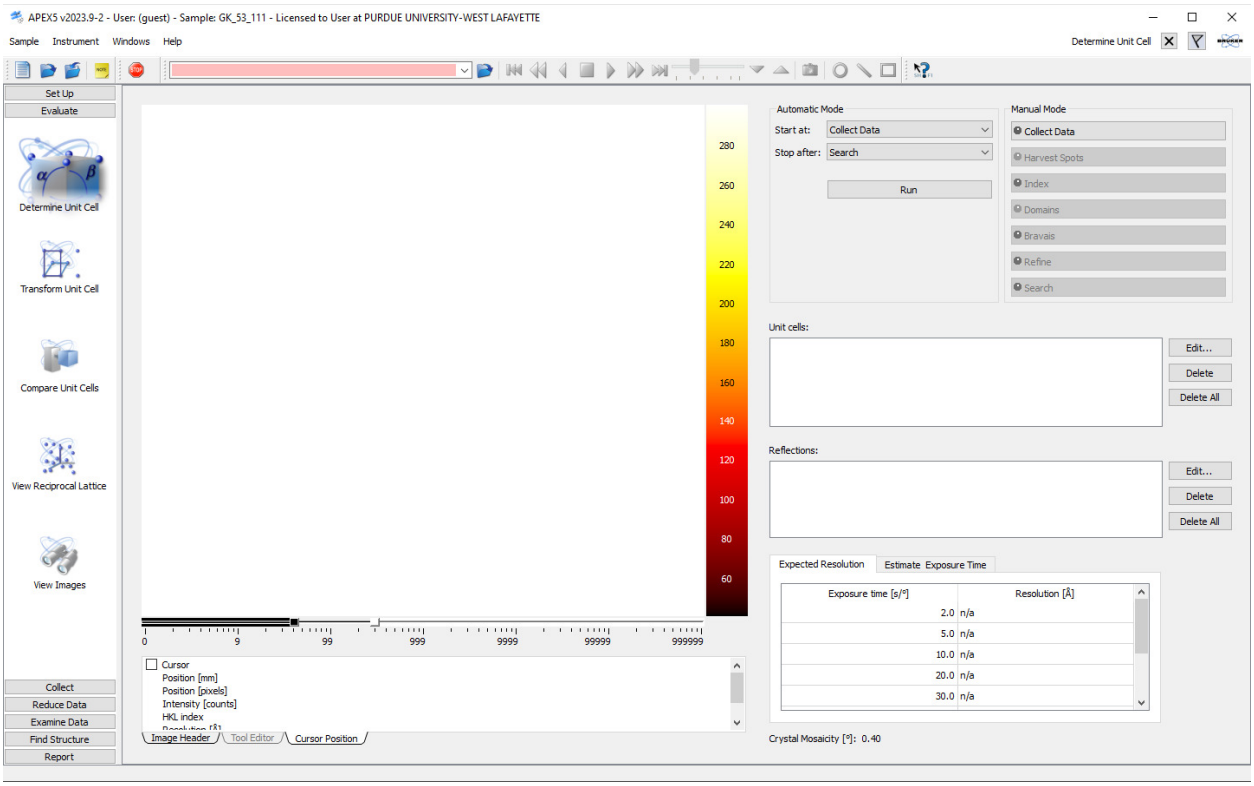


Figure 10, Starting Window of the Unit Cell determination

If you are a novice user, it is best to use <Manual Mode>. To do so click <Collect Data>, this will open the Unit Cell Data Collection Window.

- Choose an exposure time (default is 10 seconds). Click <Collect>.
- 3 × 20 frames will be collected which will be displayed in the frame window.
- By default, the window is scaled to fit the width of the frame. To see the entire frame, right mouse click on the window and select <Zoom>
- 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.

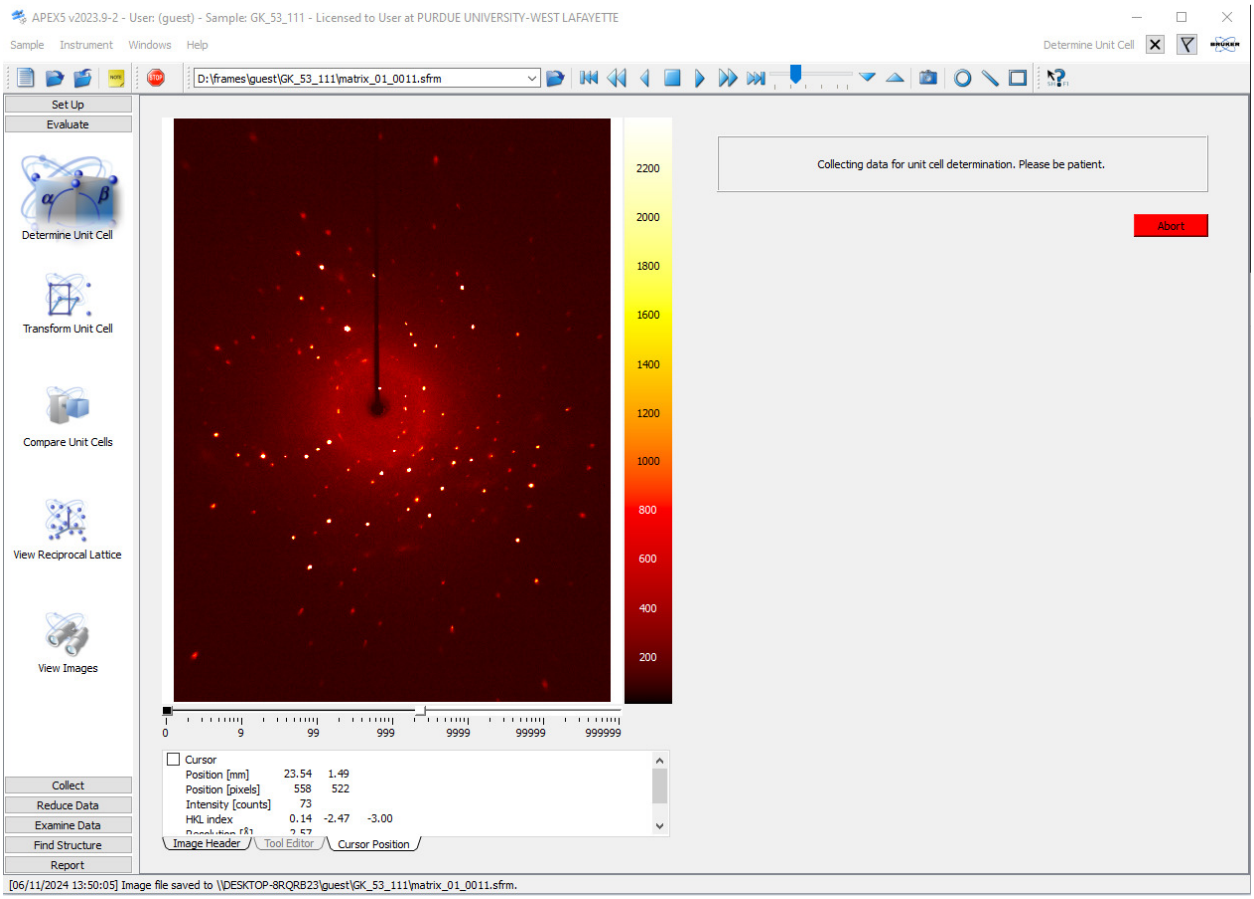


Figure 11, Unit Cell window with the first (low angle) sweep running.

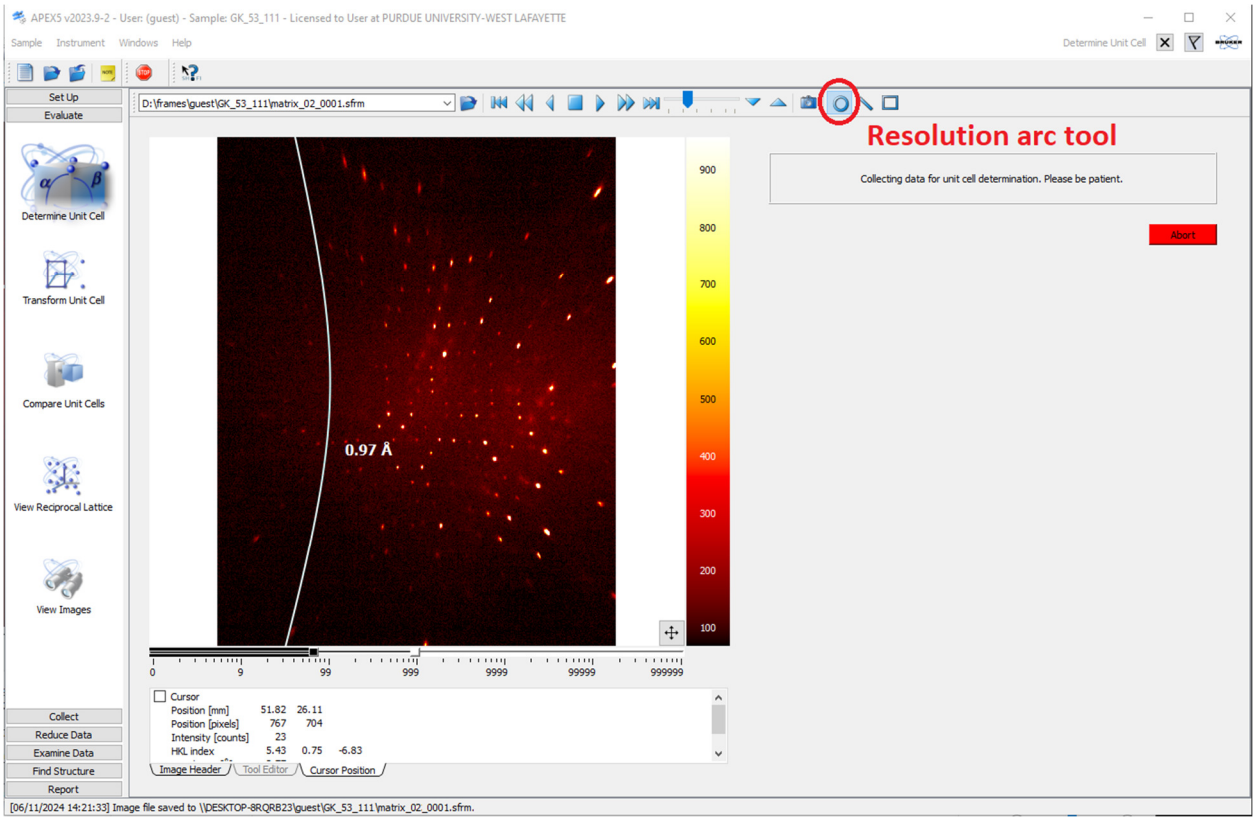


Figure 12, Unit Cell window with one of the high angle sweeps running and resolution arc tool.

- 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). Click on <Harvest>.

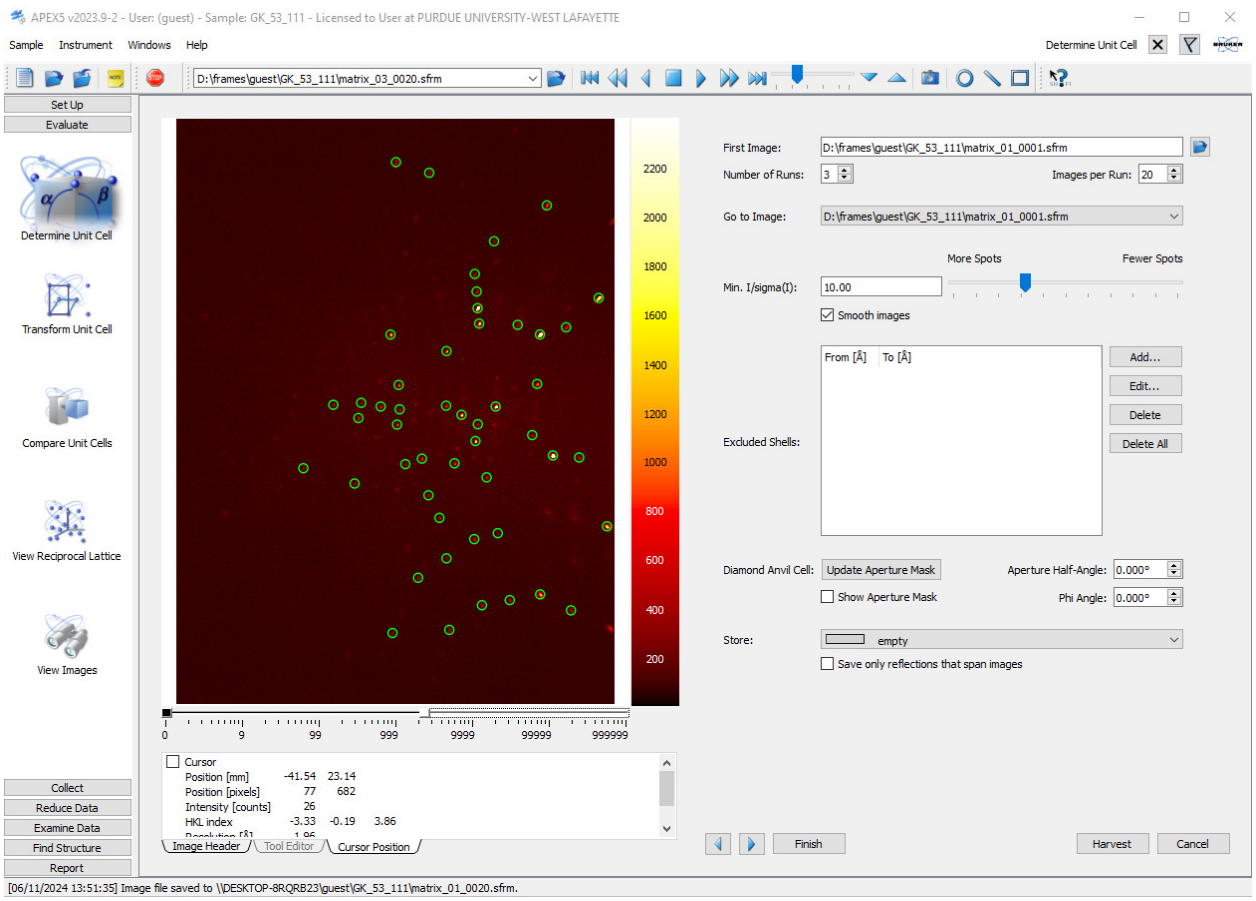


Figure 13, Unit Cell Harvesting Window

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

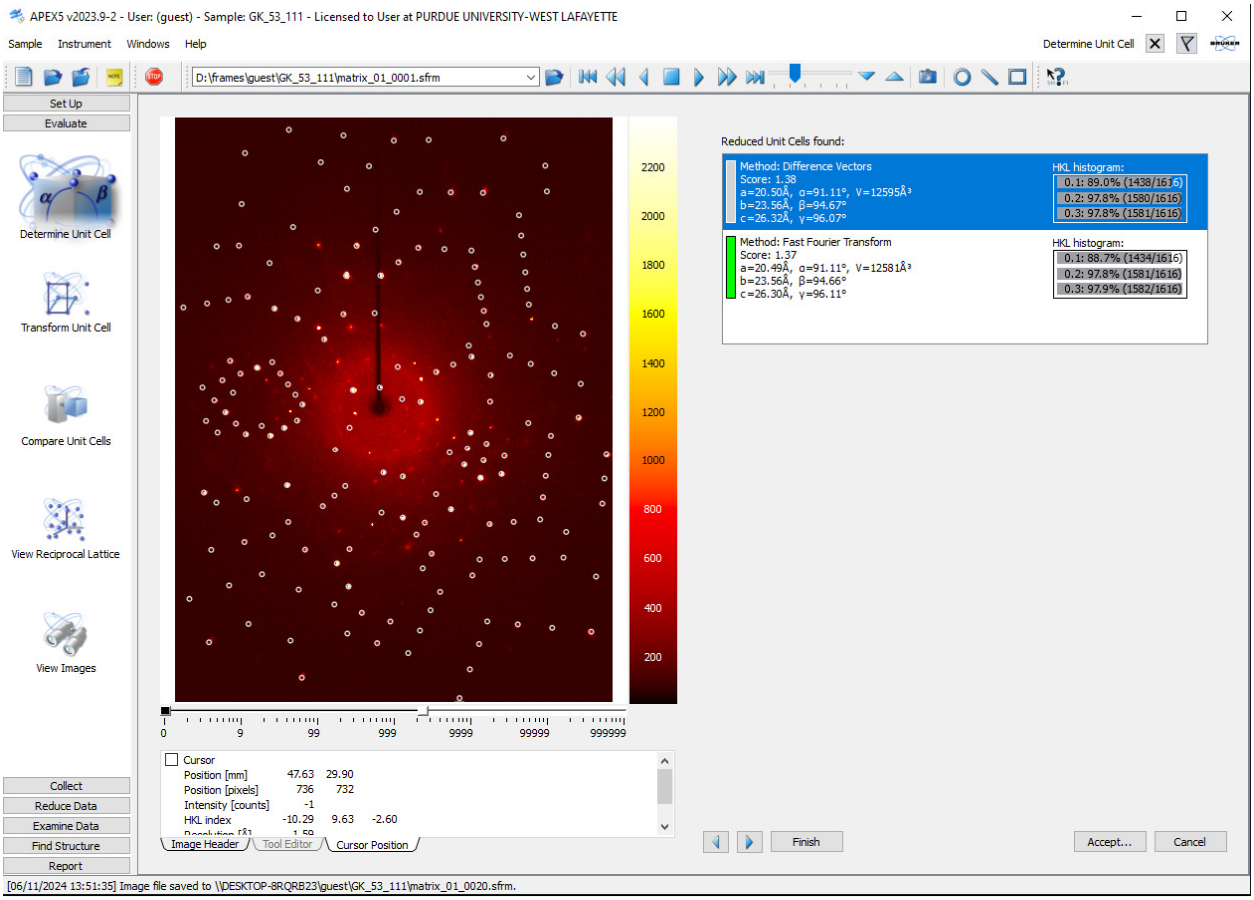


Figure 14, Unit Cell Indexing Result Window

- Check the unit cells obtained. The positions of the diffraction spots (ignoring natural decay events, recognizable as very intense but very sharp “spikes”) should agree with the predicted positions (circled in diffraction images).
- 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>.

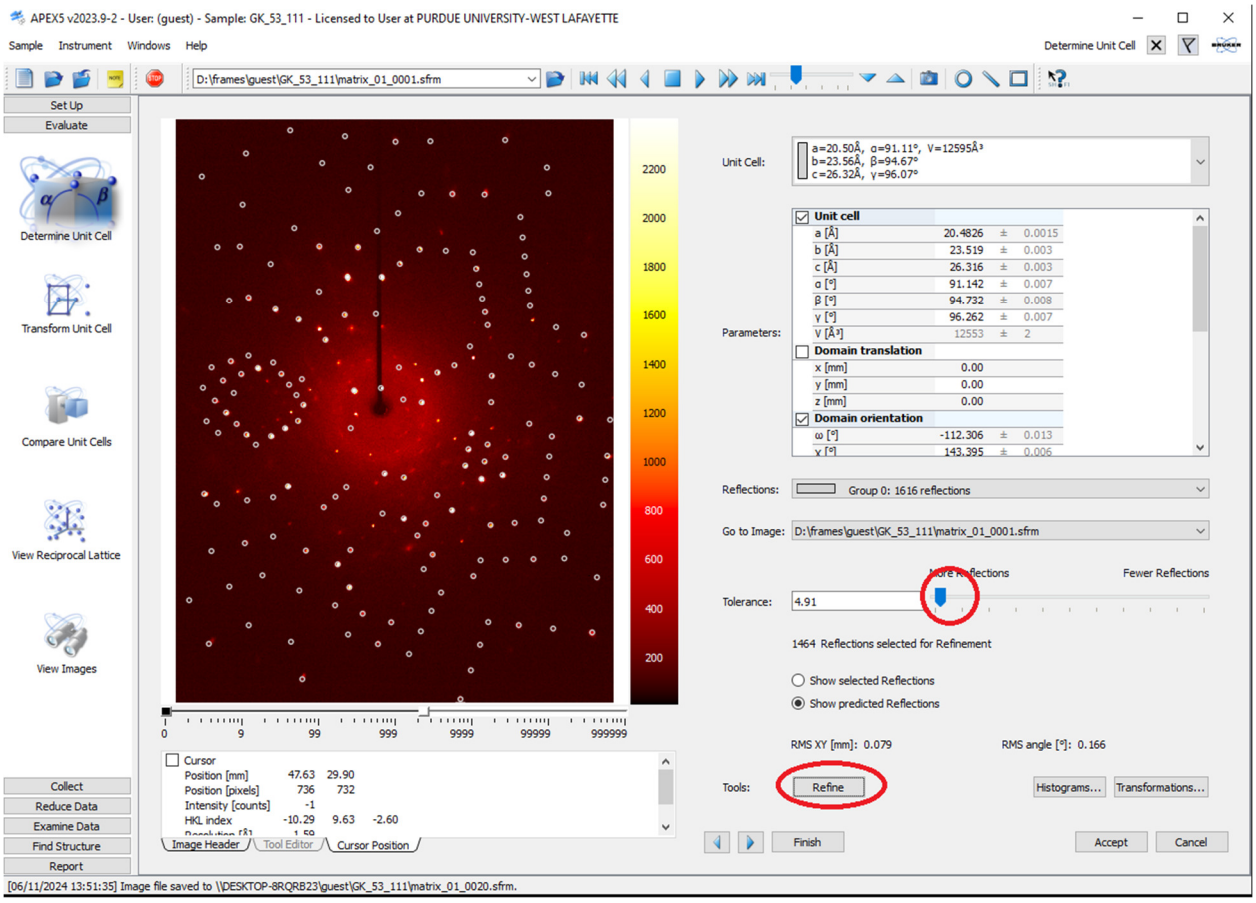


Figure 15, Unit Cell Refinement Window

- If there are excess spots not assigned to the cell a non-merohedral twin might be present, or the specimen is not a single crystal (multi-domain). **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. Depending on the case at hand there are two options:

A) The unit cell appears correct, but there are excess diffraction spots that indicate presence of a second domain: Proceed to <Domains>. Click the <Find Domains> button.

B) The unit cell appears wrong or makes no sense at all: First go back to <Index>. At the bottom of the Index window uncheck the options for <Difference Vectors> and <Fast Fourier Transform>. Select <Least Squares> instead. You can narrow down acceptable unit cells via the <Specific Cell Search> option. Click <Index>. This will use a brute force vector search algorithm based on the program "Cell Now" to identify a unit cell. If a reasonable cell is obtained but there are excess diffraction spots that indicate presence of a second domain: Proceed to <Domains>. Click the <Find Domains> button.

C) No reasonable cell is obtained via options A) and B): Export the data as a p4p file using <Sample>, then <Export> and use the program Cell Now to get a listing of multiple possible unit cells. Select the most likely correct cell (based on FOM, number of fitting reflections, cell volume, etc), export it as a p4p file and import it back into Apex5. Proceed to <Domains>. Click <Find Domains>.

- Additional details on twinning are not covered in this manual (yet). The remainder of this manual assumes the crystal not to be twinned: Proceed to <Bravais>. A list of possible Bravais lattices will be displayed with the software's choice highlighted in green.

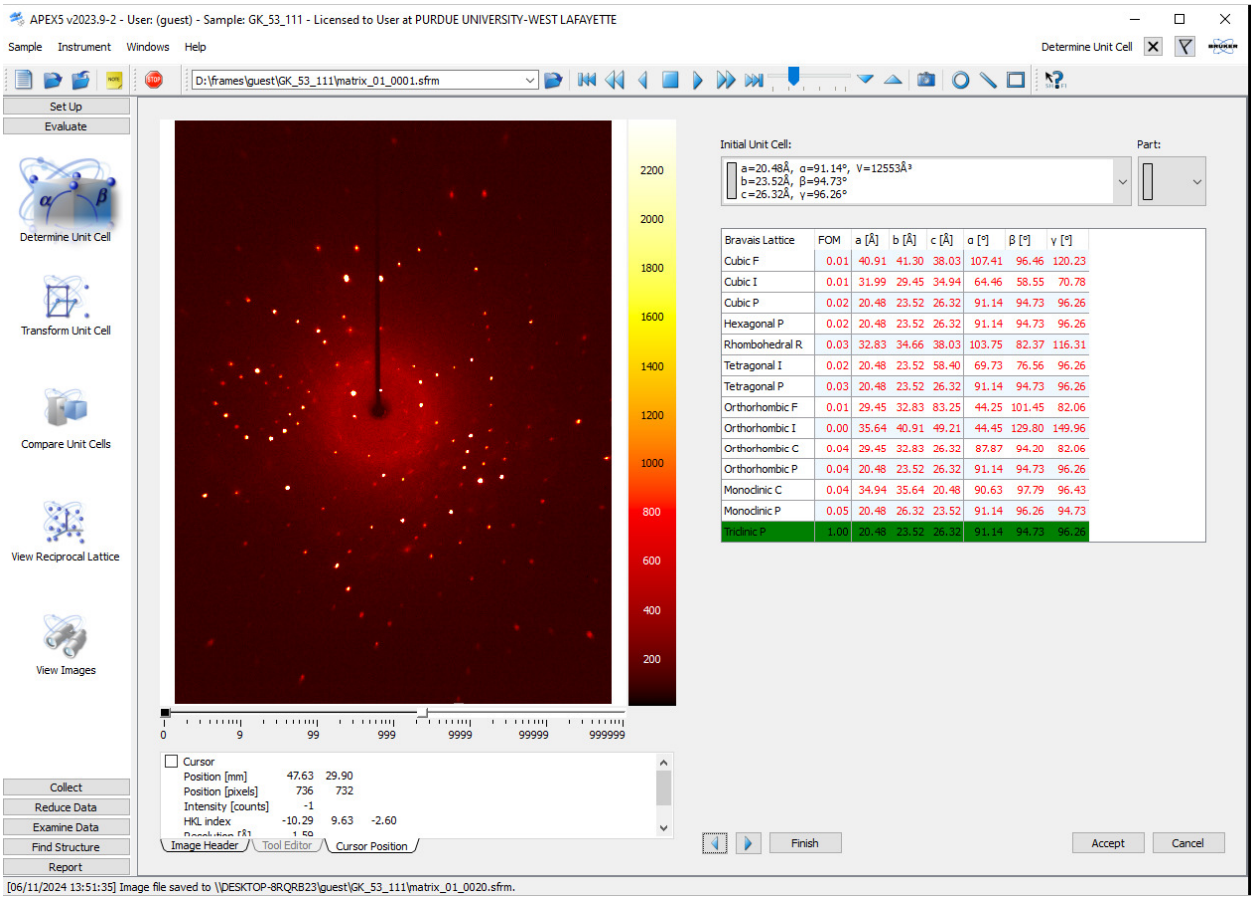


Figure 16, Bravais Result Window

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>.
- Make a note of the Expected Resolution vs Exposure Time.

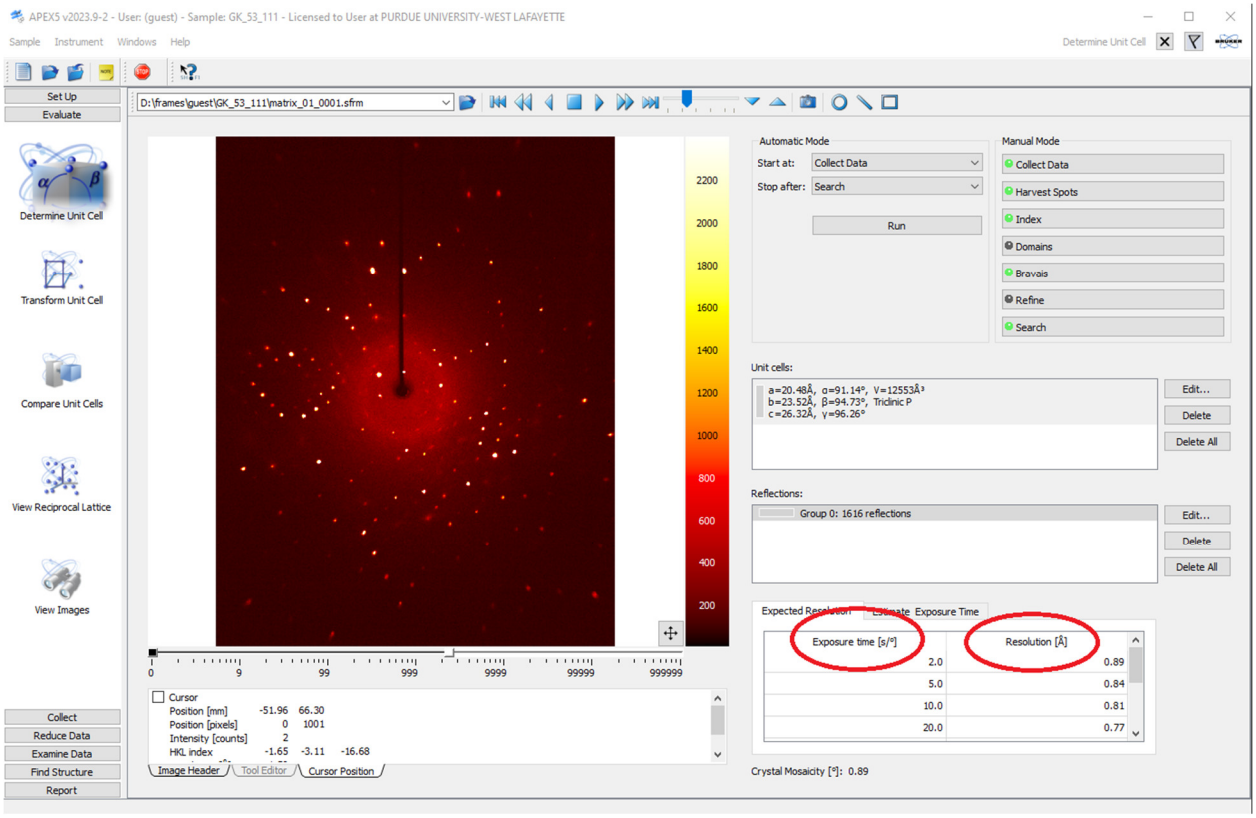


Figure 27, Expected Resolution vs Exposure Time

- This finishes the Unit Cell Determination.

Data Collection

- Click < Calculate Strategy> under <Collect>.
  - Change the resolution to d = 0.83 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. If you are not sure, use the "Estimate maximum resolution" button. Collecting to 0.78 rather than 0.83 Å increases the data collection time and is only recommended for samples that do indeed diffract all the way to the detector edge.

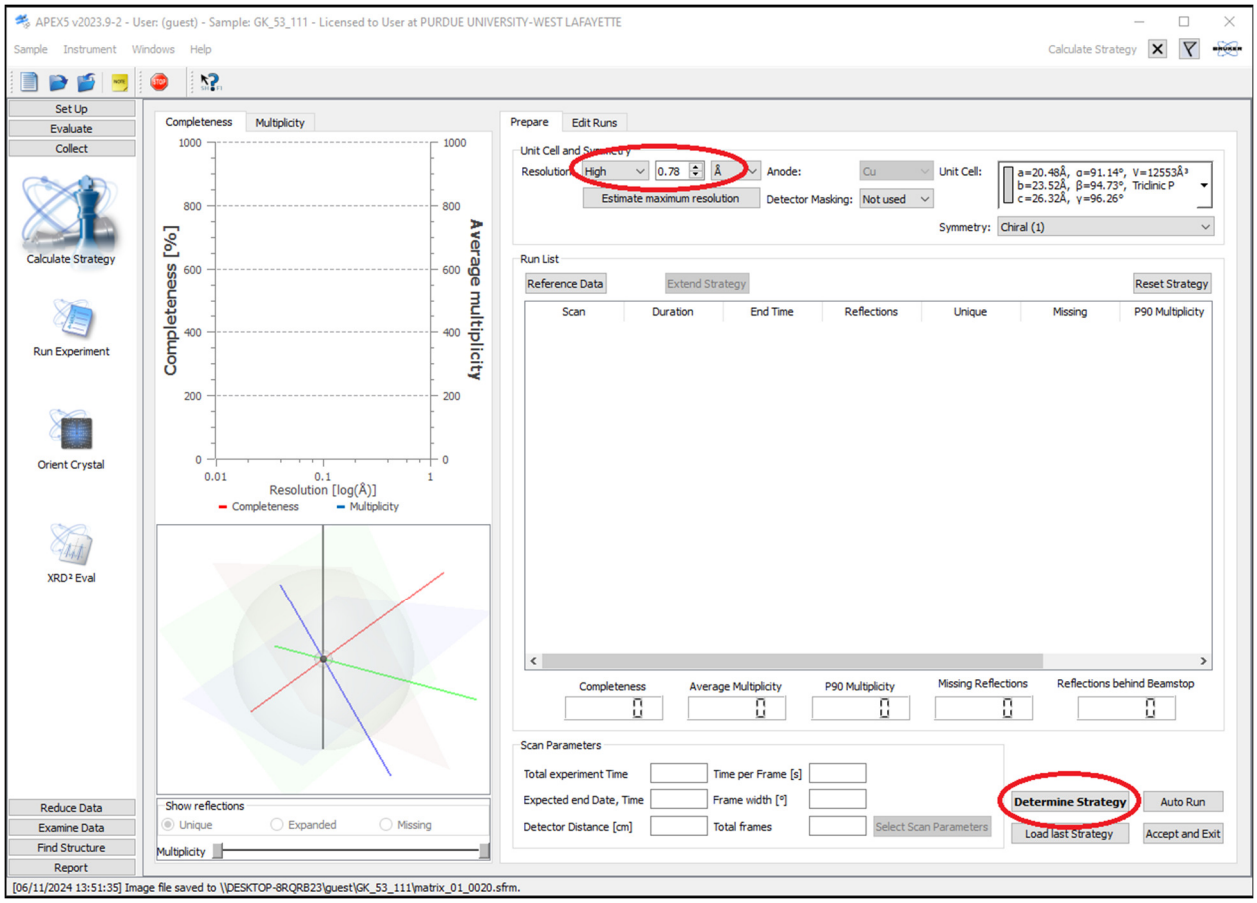


Figure 18, Strategy Starting Window

- Click <Determine Strategy> (lower right corner). In the pop-up window (Fig. 19), it is usually safe to use the default values. Click <OK>.

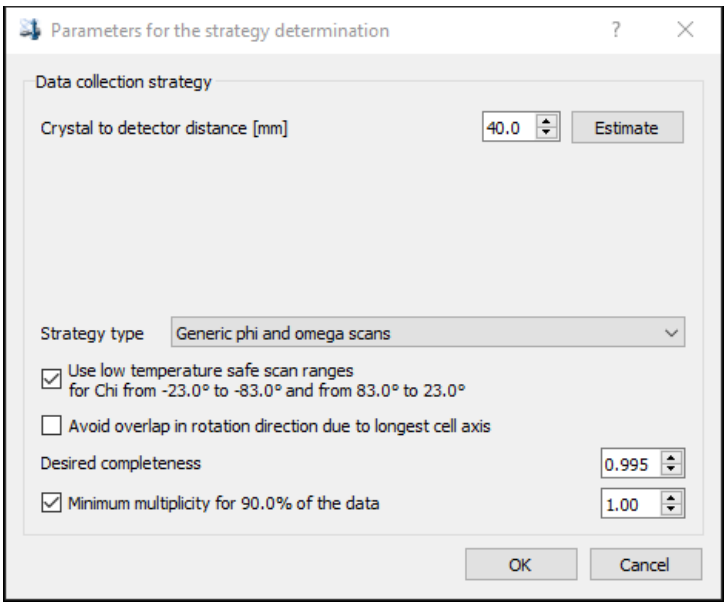


Figure 19, Determine Strategy popup window

- The Scan Parameters box will now be populated. Click the <Select Scan Parameters> button. In the popup window, click the two <Estimate> buttons for "Frame width" and "Frame time" to obtain suggested values (Fig. 20).

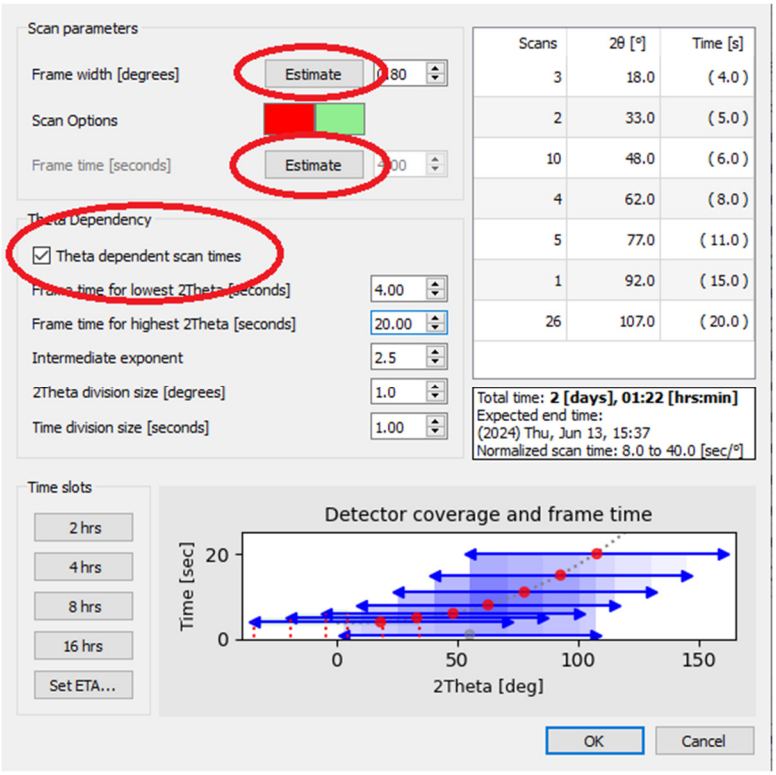


Figure 20, Strategy Scan Parameters sub-window

- The frame angle value can usually be accepted it is.
- Check the box <Theta dependent scan times> when using copper radiation. Check the suggested values for the <Frame time for highest 2Theta> and <Frame time for lowest 2Theta>. The values 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!! The values of Expected Resolution vs Exposure Time at the end of the Unit Cell Determination are often a good choice for the high angle exposure time.
- Adjust the <intermediate exponent> as required.
- Click <OK> to proceed.

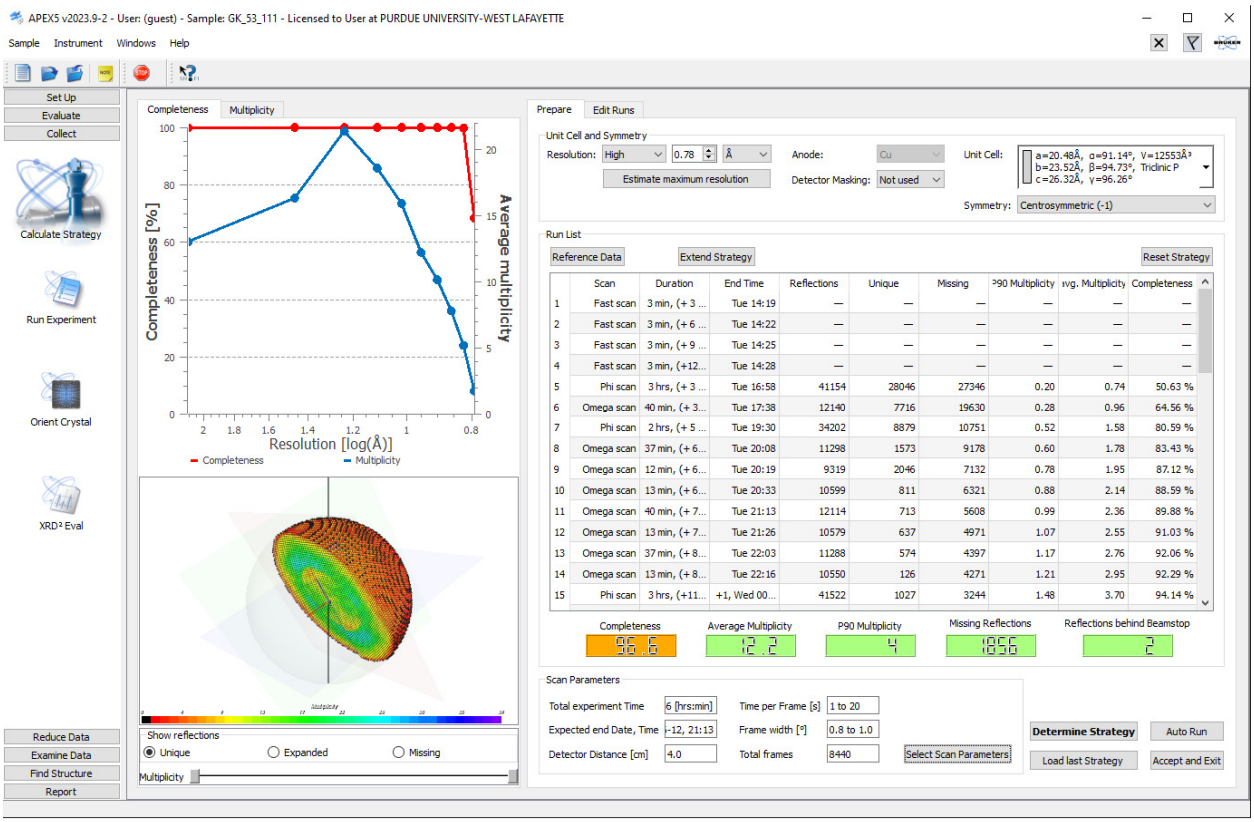
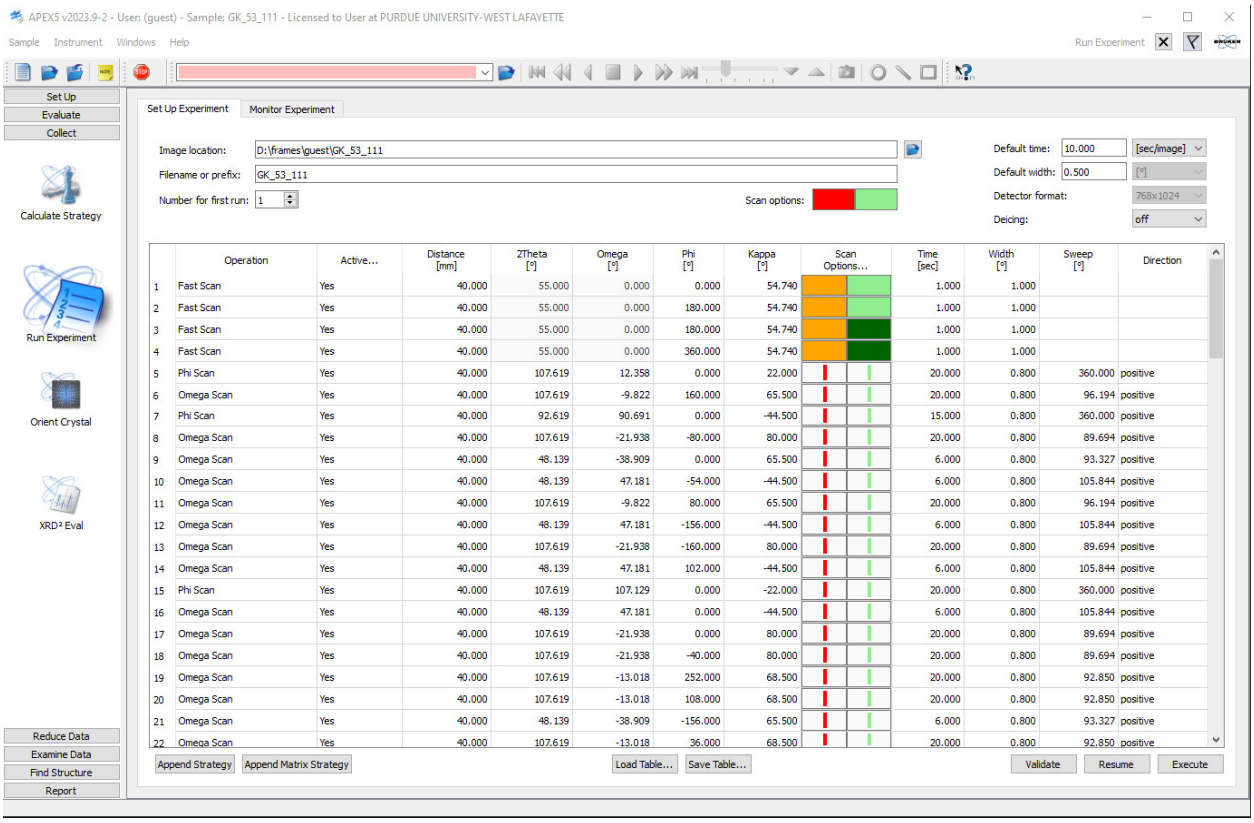


Figure 21, Final Strategy Window

- 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.
  - Apex5 did automatically add two <Fast Scans> (for triclinic: four), one with 1 second exposure per degree, using dynamic mode (orange colour code in Scan Options). One uses an attenuator (a thin metal foil automatically moved into the beam path during the measurement to further diminish extremely intense reflections; dark green colour code in Scan Options). Fast Scans (and especially the attenuated fast scan) are often 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 Fast Scans, edit the exposure time for the fast scan to be about ten times less exposure time / degree than for the other low angle runs. The fastest scan rate is 1 second per degree. If a faster rate is needed, keep the fast scan that uses the attenuator.



	Operation	Active...	Distance [mm]	2Theta [°]	Omega [°]	Phi [°]	Kappa [°]	Scan Options...	Time [sec]	Width [°]	Sweep [°]	Direction
1	Fast Scan	Yes	40.000	55.000	0.000	0.000	54.740		1.000	1.000		
2	Fast Scan	Yes	40.000	55.000	0.000	180.000	54.740		1.000	1.000		
3	Fast Scan	Yes	40.000	55.000	0.000	180.000	54.740		1.000	1.000		
4	Fast Scan	Yes	40.000	55.000	0.000	360.000	54.740		1.000	1.000		
5	Phi Scan	Yes	40.000	107.619	12.358	0.000	22.000		20.000	0.800	360.000	positive
6	Omega Scan	Yes	40.000	107.619	-9.822	160.000	65.500		20.000	0.800	96.194	positive
7	Phi Scan	Yes	40.000	92.619	90.691	0.000	-44.500		15.000	0.800	360.000	positive
8	Omega Scan	Yes	40.000	107.619	-21.938	-80.000	80.000		20.000	0.800	89.694	positive
9	Omega Scan	Yes	40.000	48.139	-38.909	0.000	65.500		6.000	0.800	93.327	positive
10	Omega Scan	Yes	40.000	48.139	47.181	-54.000	-44.500		6.000	0.800	105.844	positive
11	Omega Scan	Yes	40.000	107.619	-9.822	80.000	65.500		20.000	0.800	96.194	positive
12	Omega Scan	Yes	40.000	48.139	47.181	-156.000	-44.500		6.000	0.800	105.844	positive
13	Omega Scan	Yes	40.000	107.619	-21.938	-160.000	80.000		20.000	0.800	89.694	positive
14	Omega Scan	Yes	40.000	48.139	47.181	102.000	-44.500		6.000	0.800	105.844	positive
15	Phi Scan	Yes	40.000	107.619	107.129	0.000	-22.000		20.000	0.800	360.000	positive
16	Omega Scan	Yes	40.000	48.139	47.181	0.000	-44.500		6.000	0.800	105.844	positive
17	Omega Scan	Yes	40.000	107.619	-21.938	0.000	80.000		20.000	0.800	89.694	positive
18	Omega Scan	Yes	40.000	107.619	-21.938	-40.000	80.000		20.000	0.800	89.694	positive
19	Omega Scan	Yes	40.000	107.619	-13.018	252.000	68.500		20.000	0.800	92.850	positive
20	Omega Scan	Yes	40.000	107.619	-13.018	108.000	68.500		20.000	0.800	92.850	positive
21	Omega Scan	Yes	40.000	48.139	-38.909	-156.000	65.500		6.000	0.800	93.327	positive
22	Omega Scan	Yes	40.000	107.619	-13.018	36.000	68.500		20.000	0.800	92.850	positive

Figure 22, 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. The instrument status is shown on the right where you can check variables and completion time. You can also check many of these items via the BIS interface.

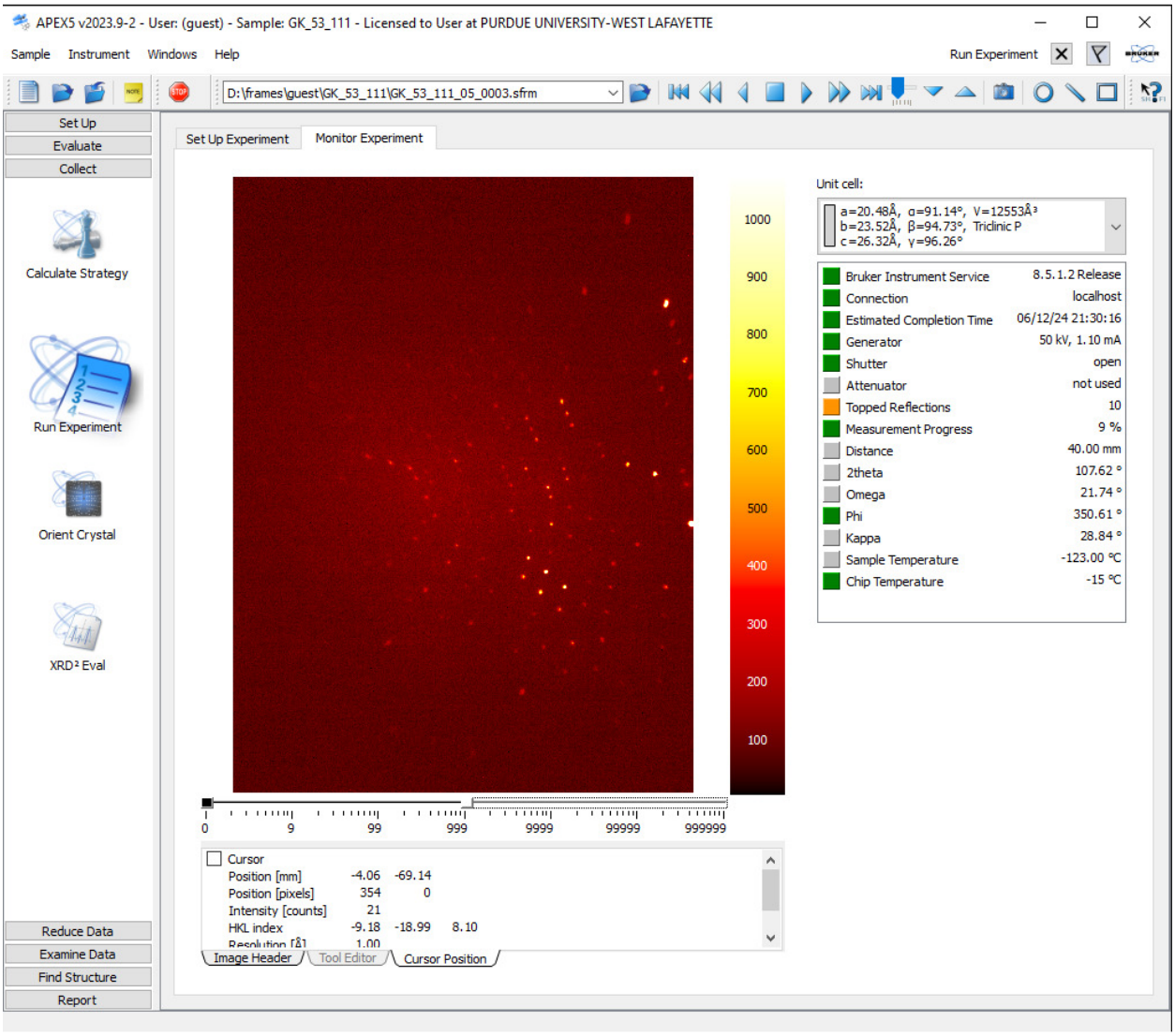


Figure 23, Data Collection Window with active data collection

- 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 better.
- Click on <Find Runs>, check the runs you would like to integrate (leave out the Matrix runs).

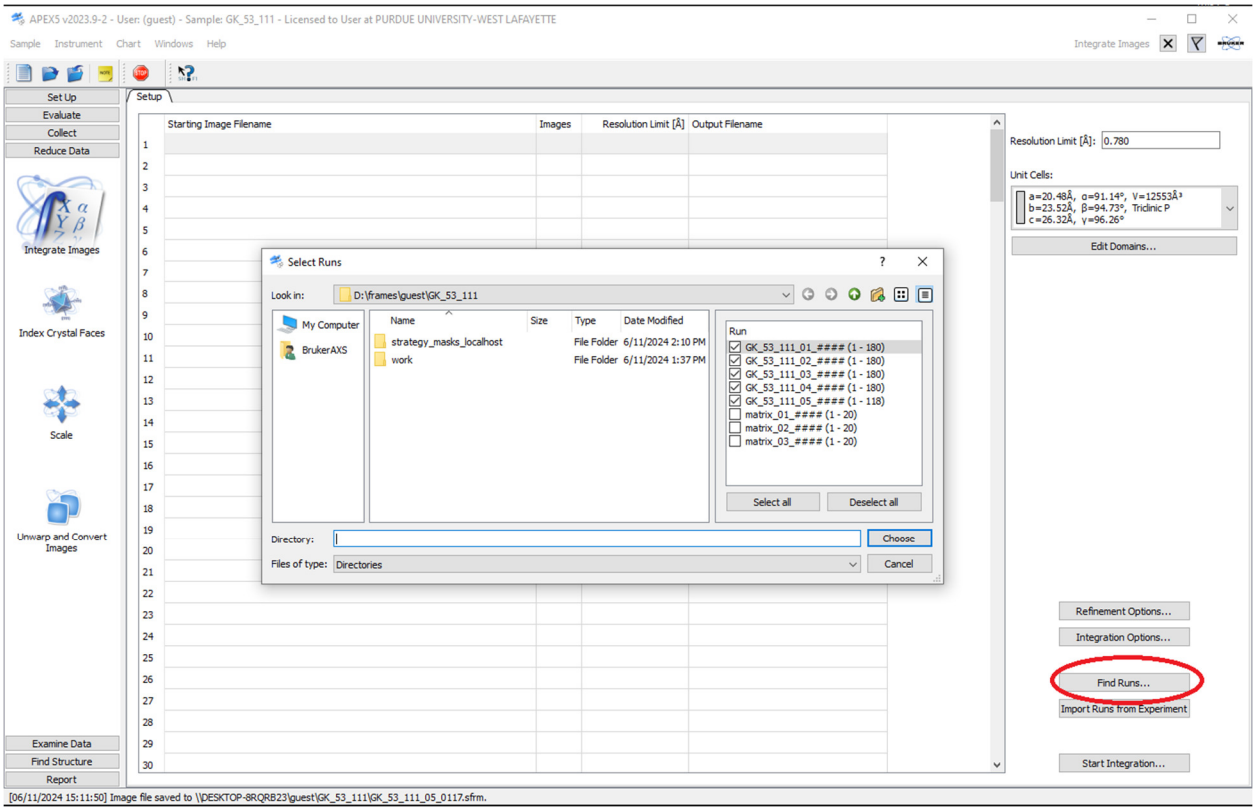


Figure 24, Integration Start Window with <Select Runs> Window open

- 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.

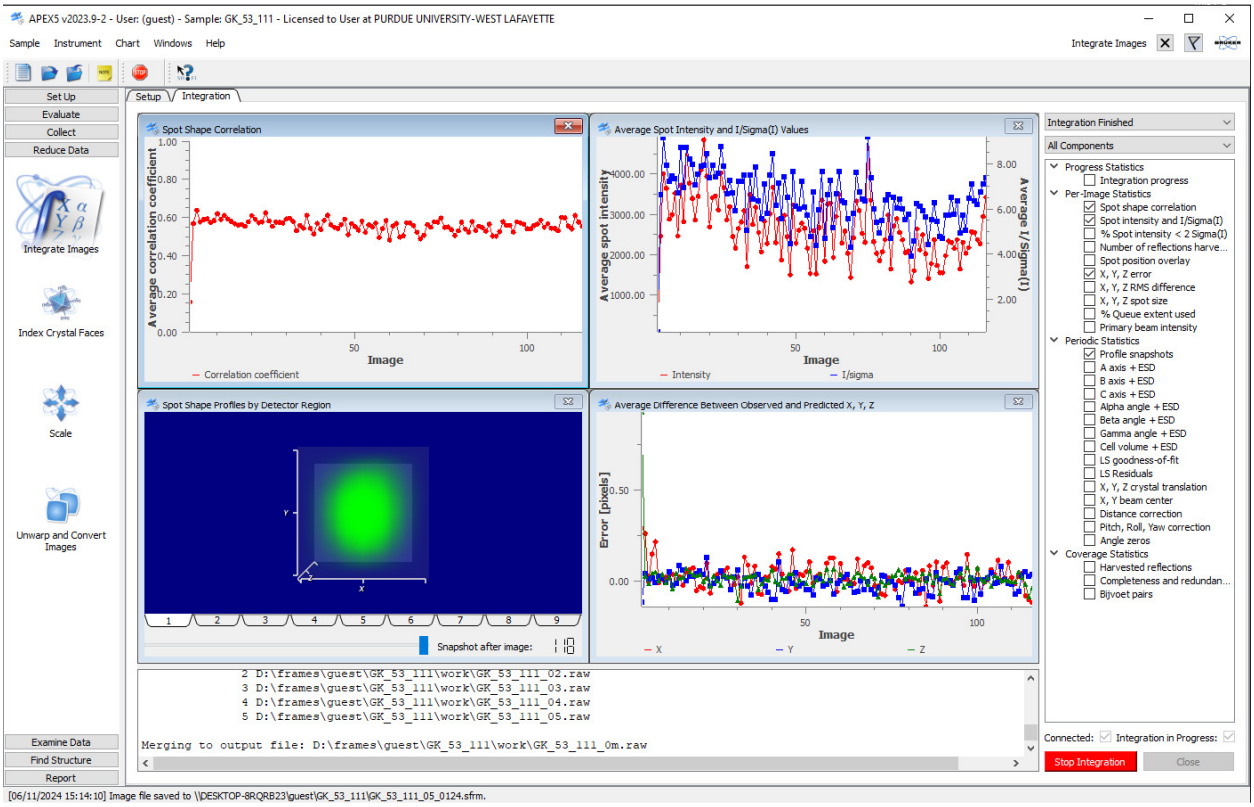


Figure 25, Integration Window with active Integration running

- Spot Shape Correlation for good crystals 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 can 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.
- **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 final integration.

Scaling and Absorption Correction

- Under <Reduce Data> Click on <Scale>

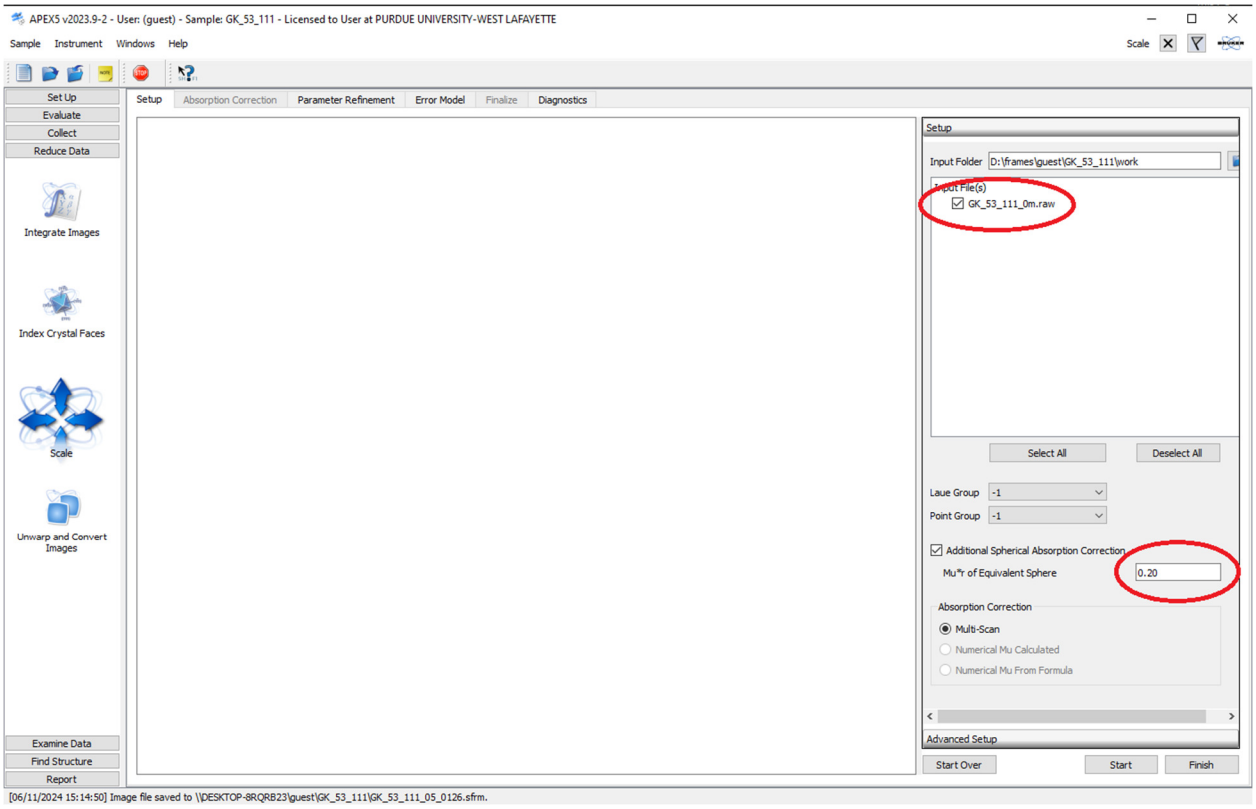


Figure 26, Absorption Correction and Error Model (<Scale>) Starting Window

- 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>.

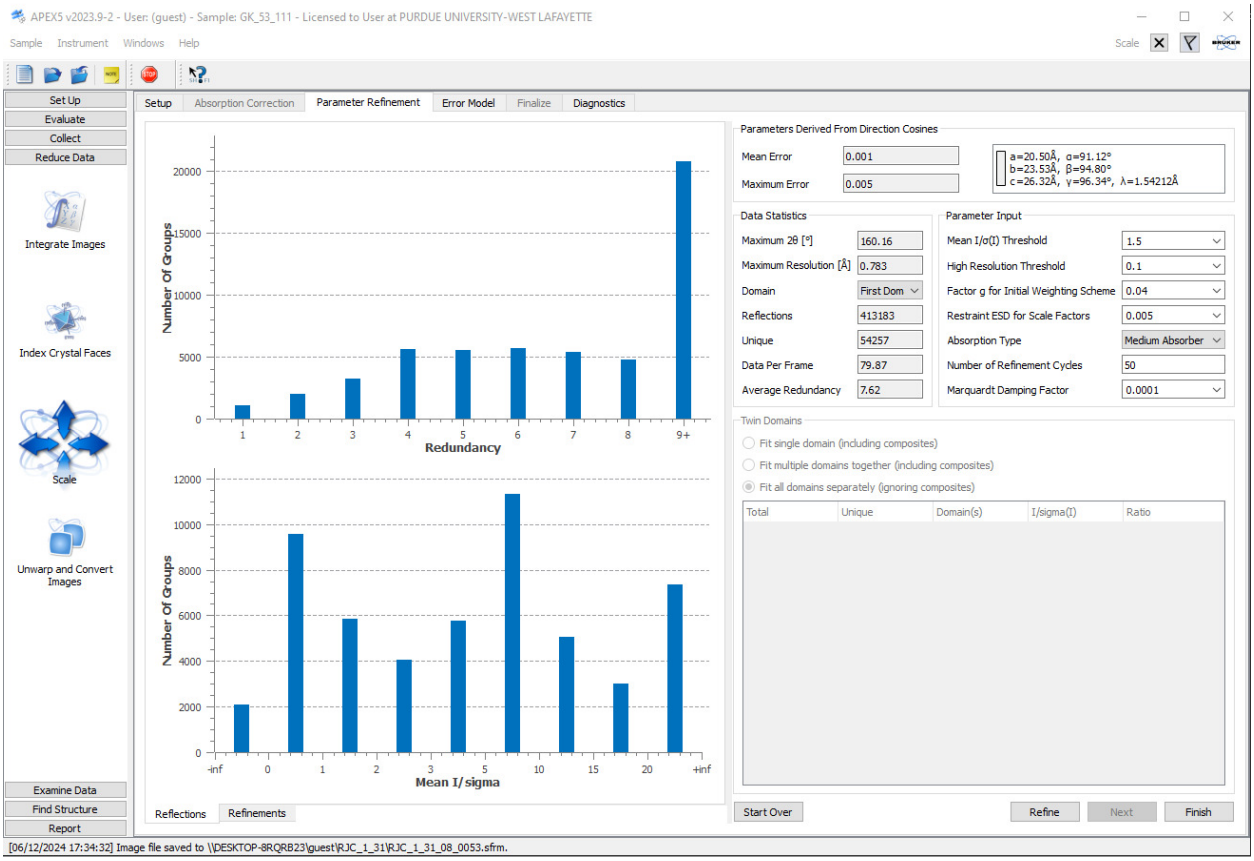


Figure 27, Absorption Correction window (second Scale Window) before refinement

- The parameter refinement window will open (Fig. 25). 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 workup.
- 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. 29) 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.

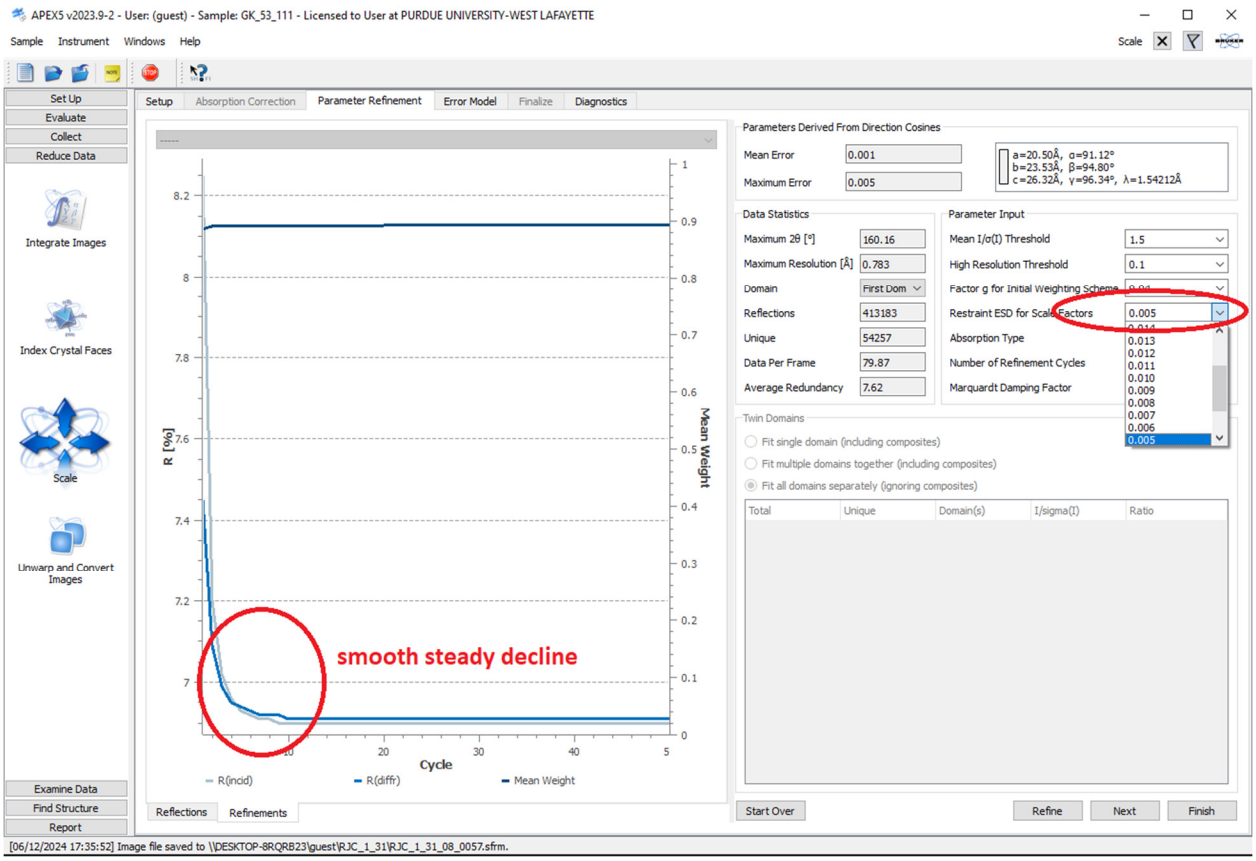


Figure 39, Absorption Correction window (second Scale Window) after refinement.

- 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).

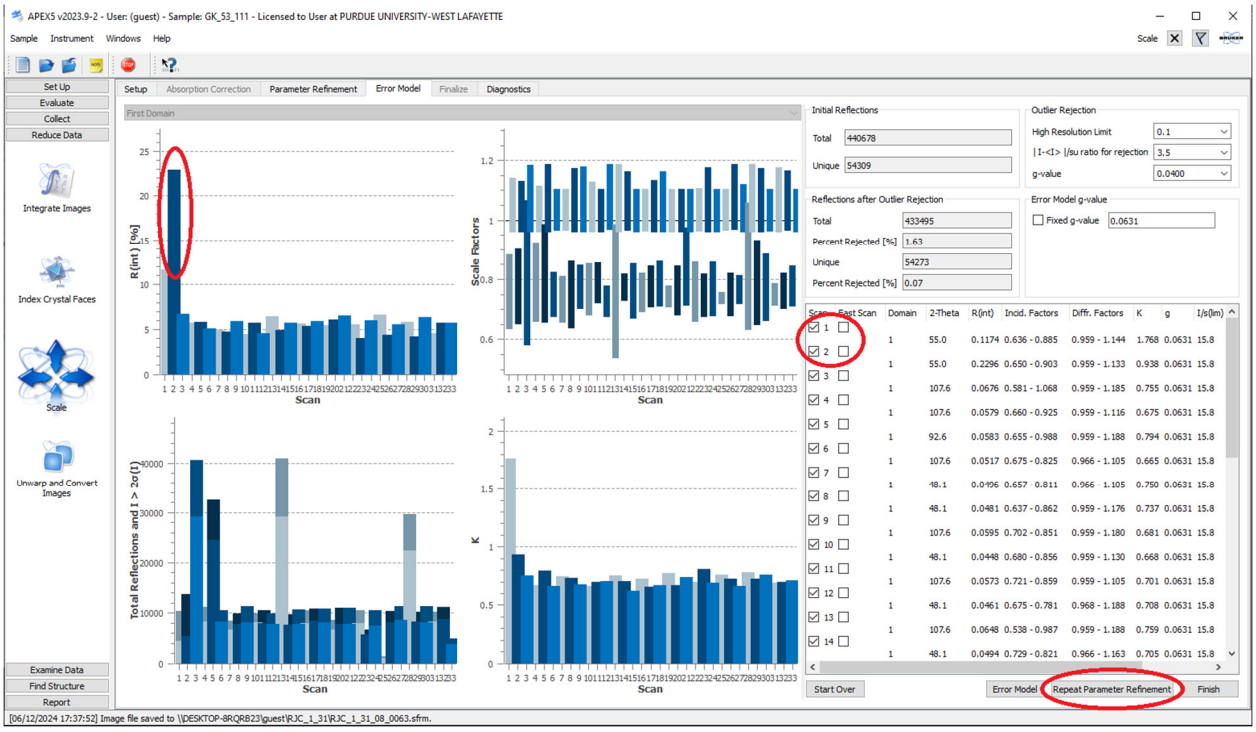


Figure 31, Error Model Window (third Scale Window).

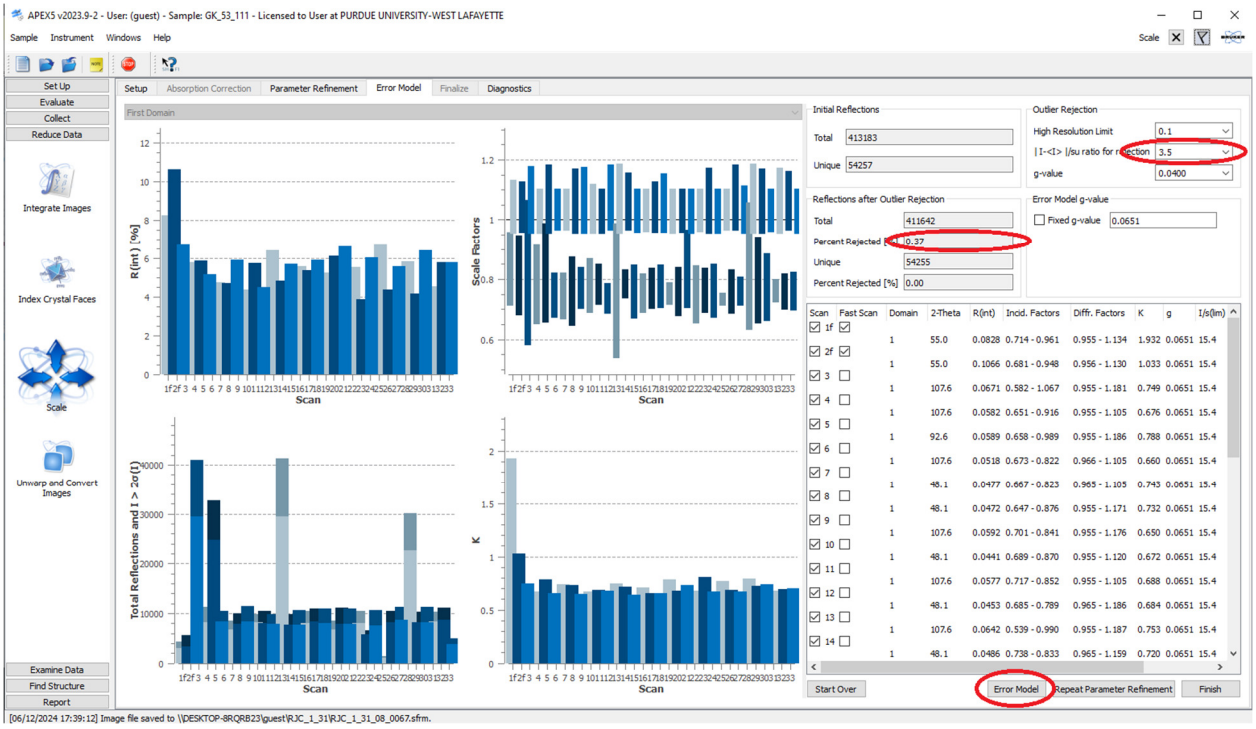


Figure 32, Error Model Window (third Scale Window)

- 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 3.0). If you do so, click <Error Model> to update the outlier rejection.
- 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. GK_53_111_0m.hkl
GK_53_111_0m.p4p

• For publication purposes and if you use FinalCif to make your cif you will also need copies of the *.abs and the *._ls files:

e.g. GK_53_111.abs (A copy of what you did in SCALE, SADABS, or TWINABS, contains the ratio of Tmin/Tmax).

GK_53_111_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

• Use the hkl and p4p files and the program XPREP to assign a tentative space group and create an .ins file. Use the programs XS, XM or XT to solve the structure, and refine using Shelxl.exe and the graphical interface of your choice (e.g. ShelXle).