

## *Collect and Reduce Intensity Data -- APEX*

### *General Steps in Collecting Intensity Data*

Note that the steps outlined below are generally followed when using all modern automated diffractometers, regardless of manufacturer.

1. Mount crystal using a microscope.
2. The mounted crystal is *centered* on the different axes of the goniometer which places the sample in the path of the incident X-ray beam.
3. One or more preliminary images of the diffraction pattern of the sample are taken to verify the sample's crystallinity, mosaicity, and scattering power.
4. A preliminary unit cell is determined either before data collection begins or after data collection has started.
5. A data collection strategy is selected. Intensity data are collected.
6. Auxiliary information about the absorption correction or cell parameters may be collected.

The following specific instructions are for collecting intensity data using the department's Bruker D8 goniometer with an APEX ccd (charge-coupled device) detector using the APEX3 software.

### *Mount the Crystal*

The crystal should be selected and mounted using instructions in the "Microscope.pdf" handout. As a reminder, the crystal should generally be mounted on the end of a plastic loop, plastic support, or glass fiber using an appropriate adhesive. Crystals with all dimensions  $\leq 0.5$  mm are best. Crystals should have no dimensions  $> 0.8$  mm.

### *Specific Instrument Steps*

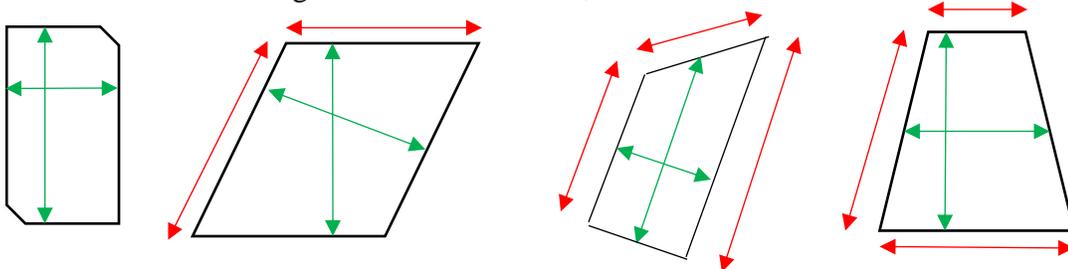
1. Place the goniometer head with the mounted crystal on the instrument. If a low-temperature device is used, the sample should be raised into the cold stream slowly (5-10 seconds) to cool the crystal. The goniometer head is screwed onto the instrument – just finger tight.
2. Log into the computer next to the instrument using the xtal account.
3. Open the BIS program, if it is not currently running. Check the distance between the sample and the detector by looking at the track of the arm of the detector. Usually the front of the detector is located 5 cm from the sample on the scale. Enter this number into BIS when requested.
4. In BIS check that the power of the generator is at 50 kV and 30 mA. If the generator is turned off or if the power is at lower settings, then exit BIS, start D8Tools, and connect D8Tools to the instrument by selecting "On Line Status" then select the left icon in the

lower row of icons. Select the X-ray Generator menu item in the left-side panel. If the generator is off, turn it on. At the top of the screen select Utilities > X-RAY > Set kV and mA. Set the kV to 50 and the mA to 30. When the screen shows this power level on the generator, exit D8Tools, and go back to step 3 above.

5. Open the APEX3 program. The username and password for the database used by the program are “guest” “guest” (without quotes). Select Instrument > Connect to “localhost”.
6. Open a new *project* by opening the Sample > New menu. The name of the *project* can be any unique value. In this lab, it is common to use a 5-digit number as the “*project*” where the first two digits are the last two digits of the current year and the next three digits are the next serial number of projects. Check the log book if you are not sure what the next “*project*” should be. The directory should be set to c:\frames\project.
7. The crystal is centered on the instrument using the Set Up > Center Crystal. Select “Right” to move the sample into a good position for adjusting the goniometer head. Move the crystal to the center of the screen by adjusting the translational motions of the head. Rotate the sample 180° in  $\phi$ . If the center of the sample is not centered, then move the sample until it is 1/2 of the distance between its current position and the centered position. Rotate the sample by 180° in  $\phi$  to verify this fact. Then rotate the sample by 90° in  $\phi$  to adjust the other translation of the goniometer head.

Note: if the crosshairs of the crystal-alignment display are not centered on the focal point of the diffractometer, then center the crosshairs using the procedure described in the Crosshair\_Alignment.pdf.

8. Make measurements of the three crystal dimensions in the Set Up > Screen Crystal routine by first selecting “Current” and then “Drive”, then move the  $\phi$  slider and press “Drive” until the crystal is oriented with a pair of faces perpendicular to the viewing screen. Drag the mouse between the two faces to measure the shortest distance between the faces. The distance appears just below the crystal display in unit of micrometers. Multiply this distance by 1000 to convert to the value to millimeters. Repeat this step for the other two approximately perpendicular directions of the crystal. When finished measuring all three sets of faces, select “Zero” and “Drive” to move all

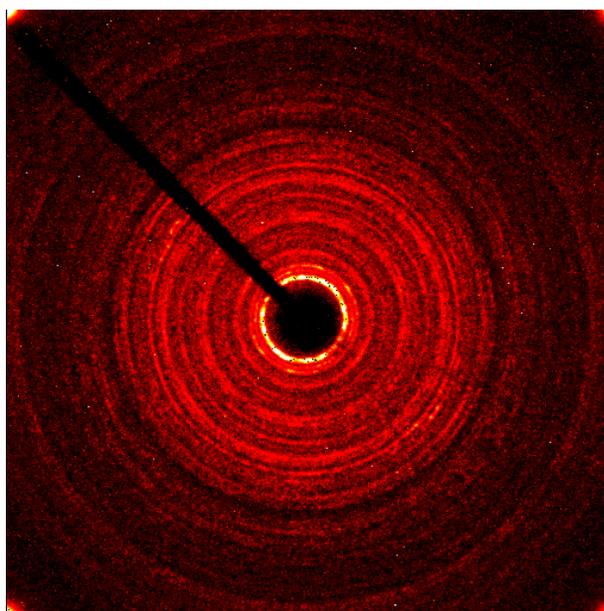
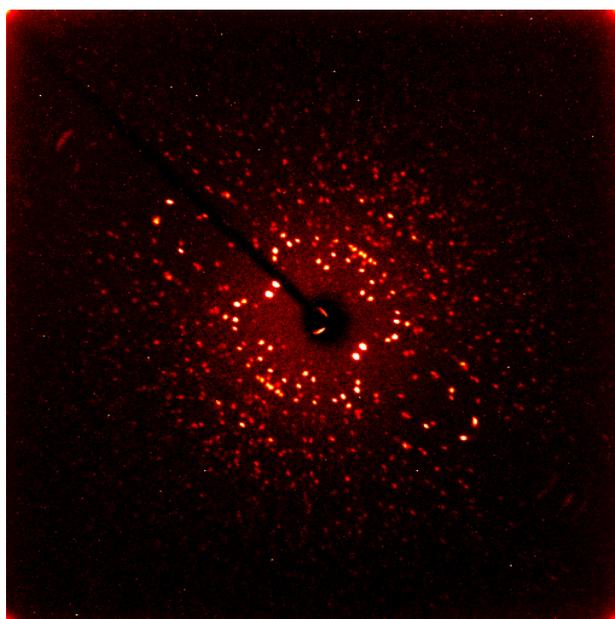


axes to the 0° position.

The images above show how crystals should be measured. The green (inside) lines show where measurements should be made; the red (outside) lines show incorrect measurements.

9. In the Set Up > Screen Crystal routine, select “360° Phi” and then “Drive & Scan” to record a rotation image. After about 1 minute an image should appear on the screen. The resulting image should show two distinct mirror planes of symmetry in the spots. In the example below left, note that the mirrors are offset from the horizontal by about 58°, the  $\chi$  angle of the fixed  $\chi$  arm. The shadow appearing on the center of the image and extending to the edge is the shadow of the beam stop and its support arm. The mirror planes have nothing to do with the symmetry of the sample and are an artifact of the type of image collected (full-rotation image). Note that if the mirror planes are not observed in the rotation image, then either the sample is not correctly centered on the instrument or the instrument itself is not correctly aligned. Some spots may be overlapped, but most should be isolated. Remember, the sharper the spots then the smaller the mosaicity of the crystal (usually a good thing). If the pattern is OK, continue below; if not mount another crystal and return to step 7 above.
10. To estimate the data collection count time, determine the relative distance of the weakest spots from the center of the image to the edge of the rotation image. If the spots extend to only  $\frac{1}{4}$  of the distance, then use count times of 60 sec/frame or greater. If the spots extend to about  $\frac{1}{2}$  of the way to the edge, then use count times of 30 sec/frame. If the spots extend about  $\frac{3}{4}$  of the way to the edge, then use count times of 10 sec/frame. If the spots extend to the edge of the image, use 5 sec/frame or less.

Two rotation images are shown below. The image on the left shows a good quality crystal. Note that because of the extent of scattering, a count time of 20-30 seconds per frame should be used. The image on the right shows the diffraction rings of a polycrystalline material.



11. In the Set Up > Describe menu, enter your sample name (often your lab notebook reference), the chemical composition (without spaces or parentheses), the primary crystal color, the crystal dimensions determined above, and a brief description of the crystal (usually “needle”, “plate”, or “block” without quotes).

### Data-Collection Strategy

There are two main ways to determine or to set up a data-collection strategy. The first method is to collect some preliminary data, determine a unit cell, and then run the strategy routine. The second method is to simply input a strategy from a data file and determine the unit cell after 40 or more frames have been collected.

In the first method, intensity data are collected and peaks are “harvested”. Then the peaks are “indexed” to assign (*hkl*) indices to each spot and to determine an orientation matrix and primitive cell parameters. The cell is then checked for higher metric (Bravais) symmetry and refined again with the new cell constraints. The orientation matrix is then passed to the strategy program to select an optimized set of runs to collect data. The Laue symmetry is presumed from the cell parameters but the Laue symmetry can always be lower than expected from the cell.

1. In the Evaluate > Determine Unit Cell menu select “Collect Data” then select the time to measure some preliminary data and click OK. After the data are collected, harvest the spots and select “Index” at the top of the screen and then “Index” at the bottom of the next screen. A unit cell is usually acceptable if the “Score” for a given cell is > 1.0 and > 90% if the peaks are indexed. If no acceptable cell can be determined, then see the notes below about handling a twin lattice. Accept the cell and refine the cell parameters. In the first refinement pass, use a tolerance level of about 1.0. Follow this by refinements at tolerance levels of about 1.5 and 2.0. Accept the results, and in the next menu select the Bravais routine which only looks for possible higher metric symmetry cells. Accept the cell with the higher score (> 0.6). The program then directs you to refine the cell with constraints on the cell parameters.

\*If an insufficient number (<20) of spots are harvested, then delete the spots already saved and collect a set of data using the “matrix” strategy (see next item). Harvest spots from the new runs and proceed with indexing as described above.

2. The data collection strategy is determined from the Collect > Calculate Strategy menu. Select the required resolution (typically in the range 0.65 – 0.82 Å) of the data and the crystal symmetry. Click the “Determine Strategy” button and set the “Redundancy” to at least 4 and click OK. The program will calculate an optimal strategy for collecting the data. Then use “Scan Parameters” menu to select the scan width (usually 0.5°) and count time and click OK. On the Collect > Run Experiment menu, select “Input Strategy” to upload the strategy determined earlier. If any  $\phi$ -scan is listed before all  $\omega$ -scans are done, rearrange the order of the runs to list all  $\phi$ -scan runs as the last runs in the series.

In the second strategy-determining method, a pre-defined set of runs is loaded into the data collection program. This method is preferred if the time available is < 15-20 minutes required to collect data and optimize the data-collection strategy. The strategy is loaded using the Load button at the bottom of the Collect > Run Experiment menu. Several possible strategies are saved in the c:\frames directory. If in doubt about which pre-set strategy to choose, select the “stand-triclinic.exp” file. If the detector has been moved back to 7 cm, then choose the “7cm.exp” file.

### Data Collection

After inserting the data collection strategy in the Collect > Run Experiment menu, select a Default count time, and, if the intensity data are being collected at low temperature, the time to “de-ice” the sample is set to be repeated every 30 minutes. If a count time is selected that has not been collected recently, then check the box to collect a new dark current correction. The data are collected automatically by selecting “Execute”.

### Cell Parameters

After collecting at least 40 frames it is often possible to determine cell parameters, if this has not been done previously.

Select the Evaluate > Determine Unit Cell menu. On this menu start with the “Harvest Spots” option and choose the first frame of the first omega-scan data run. Adjust the number of runs to include all omega-scan runs desired. Do *not* use phi-scan runs to index the crystal. Change the number of frames to some number > 20 of the frames on all selected omega-scan runs. Adjust the  $I/\sigma(I)$  minimum to be around 10-12, and click “Harvest”. After a short time, the program will list the number of peaks located. Continue with Indexing, Cell Refinement, Bravais lattice check, and Refinement as described earlier.

### Indexing a Twinned Lattice

If attempts to index the cell do not use > 90% of the data, then the crystal is probably split or twinned. If the spots are relatively sharp, then perform the following steps to determine the cell. First be sure that most, if not all, of the data are collected. If spots were harvested earlier, delete those harvested spots. Harvest spots from many frames in a single, omega-scan run (usually all frames in that run). Save those spots in a *project.p4p* file using the Sample > Export menu. Open a command prompt window. Change the working directory to the directory that contains the \*.p4p file (usually “c:\frames\project\work” directory). Run the “cell\_now” program. Enter the name of the \*.p4p file when prompted. Take most of the defaults for other variables. If you have some idea what the cell parameters could be, then be sure that the minimum and maximum cell lengths bracket the probable cell lengths.

Several cells will usually be given with a hint as to the Bravais lattice type in the far right column. Select the most likely cell. Check that the cell is the best cell by the following. (1) open another command prompt window, (2) start the xprep program without a project name, hit enter when prompted for the data file name, (3) enter the cell parameters and presumed lattice type given by cell\_now, (4) reduce the cell, check for higher metric (Bravais) symmetry. If the cell from xprep is the same as the cell from cell\_now, then simply proceed in cell\_now to find

other orientations of this cell in the intensity data. If the cells are different, then in cell\_now first select cell, then enter "R" to reorient the cell and type in the transformation matrix from xprep. The transformation matrix looks like "1 0 0 0 1 0 0 0 1" but will not be the values shown here.

In cell\_now, the cell is saved to a new file such as "temp1.p4p" Accept the spots that agree within "0.10" to "0.15" of the selected cell. Then select "S" to search for that cell in another orientation. Other cell orientations are saved to files such as "temp2.p4p" for the second cell, "temp3.p4p" for the third cell, etc. When the "remaining spots" are a very small number or when the "uniquely indexed" spots are a small number compared to the "remaining spots" then exit cell\_now. Copy the final cells (orientation matrices) to the APEX program using the Sample > Import menu and selecting the last "temp*n*.p4p" file.

### Reducing Intensity Data

This paragraph describes the general steps followed by data reduction programs for all area detector data sets.

1. The background in different regions of reciprocal space is determined.
2. From the orientation matrix(matrices), the locations of all peaks on each frame are determined.
3. From the selected box around each peak, the peak is integrated in that 2-dimensional slice and the backgrounds are subtracted.
4. The peaks from successive slices are summed for each (*hkl*) to get a total intensity for each peak.
5. The peak intensities are corrected for Lorentz and polarization effects and numerous anomalies in the detector and incident beam.
6. The peaks from all frames are sorted based on (*hkl*) values.
7. Selected statistics about the data are prepared, and the cell parameters are optimized from a selected set of peaks.

Data reduction may be started any time after about 1/3 of the unique intensity data are collected to verify the Laue symmetry of the data and to solve and partially refine the model of atoms.

Multiple data reduction runs should be performed, each time optimizing the orientation matrix and the spot size. After the first data reduction run, that is used to solve and partially refine the model, import a better orientation matrix by reading in the *project\_0m.p4p* file in the Sample > Import menu. Also examine the *project\_0n.\_ls* (where *n* is the run number) files to get an idea of better X, Y, and Z values for the integration box.

The IUCr requires that at least 98% of all unique data be collected to at least 0.82Å resolution for small-molecule crystals.

### Specific Steps to Run the Data Reduction Program in APEX - Saint

1. Before data reduction, create a *project.p4p* file in the APEX program by choosing Sample > Export, then change the *project.p4p* name if desired and click the Save button. This saves both the initial cell and the crystal description information.
2. Because the computer used to collect data is so slow, intensity data that are collected on the computer \\bruker2 are transferred to \\xray8\frames3\$ directory for data reduction. The entire directory should be transferred using the file explorer program on \\bruker2. On the \\xray8 computer, the files are now in the C:\frames directory.

3. After transferring the data, log into the \\xray8 computer using an appropriate account, eg., xtal, and open the APEX program. The username / password to login to the APEX database is “guest” / “guest” (without quotes). A new project must be created in the Sample > New menu using the same *project* name as before and placing the files in the C:\frames\project directory. Finally, the project information must be transferred using the Sample > Import routine locating the *project.p4p* file and selecting the “Import All” box, then “OK”.
4. In the Integrate > Integrate Intensities routine select the data runs using the “Find Runs” command. Note that you need to move one directory back from where the browser is originally set to locate the data files. Select those runs that have a significant number (> 50) of frames.
5. In the Refinement Options menu, the Periodic Refinement cell (described on the left) should always be set to “triclinic” symmetry. The size of the box should be adjusted to resemble the average spot size. A good first guess is to set the X and Y values to 0.95 and to not change the Z value. You may improve these in later data reduction iterations. The symmetry of the right-side cell panel should also be set to “triclinic” for initial runs and to the appropriate Laue symmetry for the final run. Deselect the boxes for “Enable box size refinement” and “Enable orientation refinement”.
6. Select the “Integration Options” menu. Set the “Active Image Queue Half-Width (Images)” to at least “12”. For twinned samples select “Additional Parameters” and change the overlap factor to “15.0 – 20.00”. Close the menu.
7. Begin data reduction with “Execute”. Once integration has completed, check any statistics desired, then close this integration run with the “Close” button.
8. New integration runs are improved by the following. Use Sample > Import to update the cell parameters using the *project\_0m.p4p*. Also examine the *project\_0n.\_ls* files to determine better values for the X, Y, and Z parameters for the integration box, adjusting these parameters in the Refinement Options menu. Check the “Enable box size refinement” box. Repeat data reduction. In a later data reduction run, check the “Enable orientation refinement” box. Continue this iteration until the average integrated intensities do not seem to change.
9. Before the last round of data reduction, go to the Refinement Options menu and set the symmetry of the right-hand cell to the expected crystal symmetry for the cell.

### Absorption Correction or Scaling

There are two programs that were written to provide these corrections, *sadabs* and *twinabs*. The program *sadabs* is used for regular (untwinned) data sets; the program *twinabs* is used for data sets showing non-merohedric twinning or splitting that required more than one orientation matrix for integration. Both programs follow a similar path to determine appropriate absorption correction. Note that this correction is generally not needed to solve untwinned data for structure solution; however, *twinabs* must always be run to work with twinned/split data sets. The final data set must be corrected for absorption before publication.

For strongly-absorbing, untwinned samples, a routine in APEX3 is available to help index the faces of a crystal. The Reduce Data > Index Crystal Faces > Acquire New menu will allow you to collect 360 images of the crystal, 1 image per degree as the sample is rotated in  $\phi$ . The images are stored in a zipped file with the name *project.vzs*. Once all of the images have been collected, an image of the crystal is displayed. Below the image set the Maximum Miller index to 6. Rotate the crystal by moving the small inner circle around the outer circle. Select faces by moving the T-tool until the top of the “T” is along the edge of the crystal. By hitting enter, the indices of the face and the distance from the face to the center of the crystal are added to the list at the right. Continue rotating the sample and identifying faces until the black outline around the crystal is a reasonable approximation of its shape and the “Closed” box in the lower right of the screen says “Yes”. Hit the “Remove Invisible Faces” button below the list of faces. Save the faces to the *projectf.p4p* file using Export > P4P file.

One of the first questions asked by the program is the name for the new listing file describing all steps taken by the program. Typically, this file should be named “*project.abs*”. The program will ask for the symmetry of the crystal – on the first pass through this program, be sure to select the proper Laue symmetry. If the Laue symmetry is not the point group of the space group, and most of the data has a redundancy > 6 then rerun the absorption correction later using the correct point group. The name of the data files is typically input as “*project\_0m*”. From this point forward, default responses can typically be chosen.

In the *sadabs* program, the output file is usually *project.hkl* that is in SHELX hklf-4 format. In the *twinabs* program, it is possible to output both an “hklf-4” format file and an “hkl-5” format file (see the refinement program for details). Typically the hklf-4 file is saved as *project.hkl* and the hklf-5 file should be saved as *project.hkl5*. Note that the hklf-4 file from *twinabs* will typically only use the strongest twin domain of the crystal, and the hklf-5 file should be created from all domains. For twinned data sets, it is common for the combination of integration and scaling programs to effectively “detwin” the intensity data for structure solution and refinement.