Collect and Reduce Intensity Data – Photon II

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 and 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.
5. A data collection strategy is calculated. 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 Quest Kappa-geometry goniometer with a Photon II cpad (counting pixel array detector) 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 ≤ 0.3 mm are best. Crystals should have no dimensions > 0.5 mm.

Specific Instrument Steps

1. Log into the computer next to the instrument using the xtal account. If the BIS and APEX3 programs are not running, then first start BIS. Starting BIS may also start the Measurement Server program. The Measurement Server program will show a diagram of the instrument; minimize this image. While the BIS program is loading, it will show a green progress bar along the bottom of the BIS window; minimize BIS when done. Open the APEX3 program. If the program asks for a username and password enter "guest" and "guest". Connect the APEX3 program to BIS by selecting Instrument > Connect and enter the value "bruker3".

2. In APEX3, open a new *project* by selecting 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 for that year. Check the log book if you are not sure what the next "*project*" should be. The directory should be set to c:\frames\project.

3. The crystal is centered on the instrument using the Set Up > Center Crystal option. Select "Center" is not already in a good position for adjusting the goniometer head. Select the "video" icon, a blue five-sided object with a green ">", and then select the round grid icon. Open the cabinet by clicking the lower button on the right side of the front instrument panel. Mount the goniometer head with crystal onto the instrument. Move the crystal horizontally and vertically to the center of the screen by adjusting the translational motions of the goniometer head. Rotate the sample 180° in φ. If the sample is not centered, then move the sample until it is ½ of the distance between its current position and the current "centered" position. Rotate the sample by 180° in φ to verify that the crystal is now centered on this new spot. If the crystal is not centered then again translate the crystal ½ of the distance between its current position and the current "centered" position, and repeat until the center of mass of the crystal does not move with a 180° rotation in φ. The center of mass of the crystal is the new "centering point". Then rotate the sample by 90° in φ to adjust the other translation of the goniometer head to the new centering point.

   Make measurements of the three crystal dimensions by first selecting the double-headed arrow icon. Drag the mouse between the two faces to measure the shortest distance between those faces. The distance appears just below the crystal display in the right side in units of micrometers. Multiply this distance by 1000 to convert to the value to units of millimeters. Repeat this step for the other two approximately perpendicular directions of the crystal.
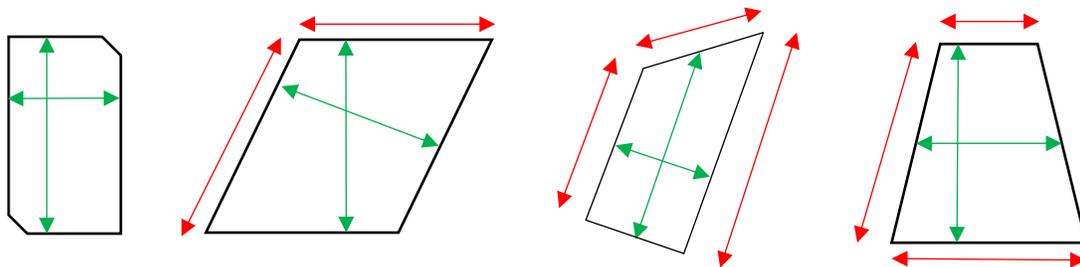
   The images above show how crystals should be measured. The green (inside) lines show where measurements should be made; the red (outside) lines show incorrect (large) measurements. When opposite faces are not parallel, select an average distance between the faces. Record these distances on a piece of paper or on

4. In the Set Up > Screen Crystal routine, set the top right values to: Distance = 40.0 mm, $2\theta = 0.00$, $\omega = 0.00$, ϕ = current value and χ = current value and hit "Drive". After the goniometer has stopped, then in the lower right panel 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 40°. 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 to step 5 below; if not mount another crystal and return to step 3 above.

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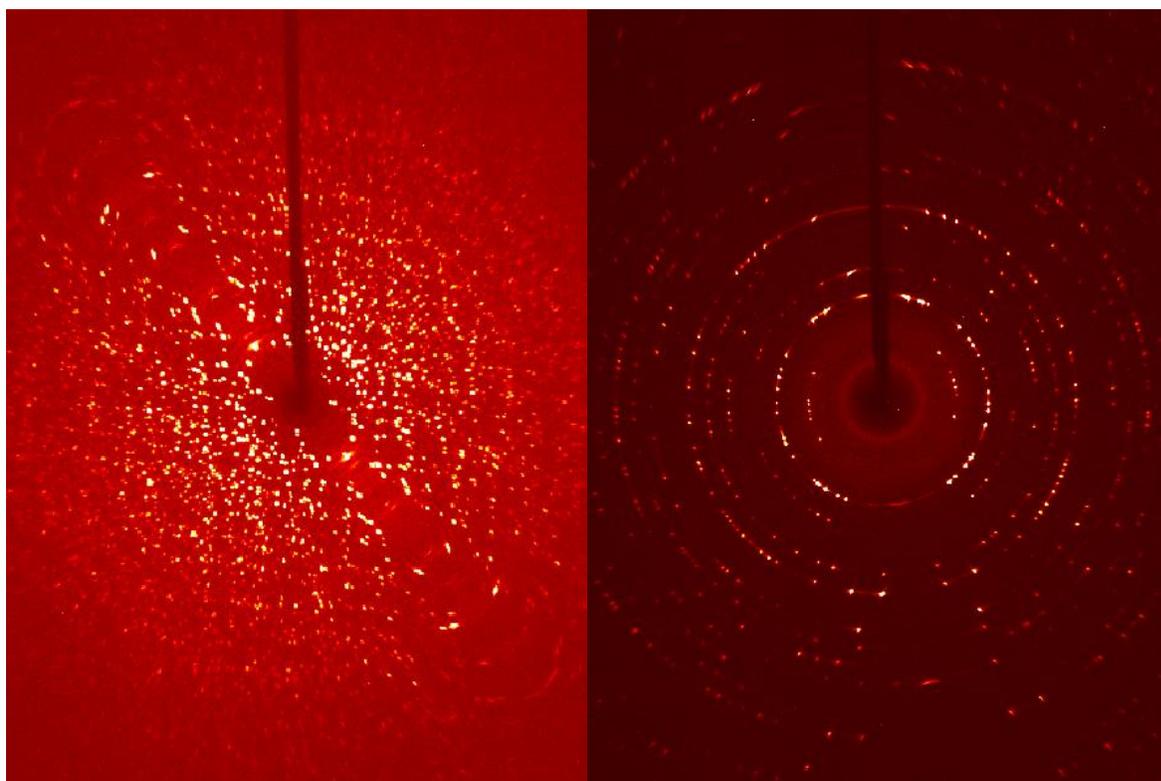
5. In the Set Up > Describe Sample menu, enter your sample name (usually your lab notebook reference), the chemical composition (without spaces), the primary crystal color, the crystal dimensions determined above, and a brief description of the crystal (often "needle", "plate", or "block" without quotes).

Data-Collection Strategy and Collect Data

Most data-collection strategies require that you first determine a preliminary unit cell. Intensity data are collected and peaks are "harvested" to determine their relative positions and intensities. Then the peaks are "indexed" to assign (*hkl*) indices to each spot and to determine an orientation matrix and initial, primitive (reduced) cell parameters. The cell is then checked for higher metric (Bravais lattice) symmetry and refined again with possibly 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 remember, the true symmetry of the sample can always be lower than the symmetry expected from the cell parameters alone.

There are three main methods to determine a data collection strategy. The method directly below is usually preferred. The 1st alternative method is best if time is short. The 2nd alternative method works best if you have very little set-up time, but plenty of time to collect data.

1. In the Evaluate > Determine Unit Cell menu select "Collect Data" on the right then click "Collect". If the spots appear in rows are too close to each other, cancel data collection, change the sample-to-detector distance to a larger value (e.g. 60 or more mm) and restart data collection. 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 by non-merohedry. Accept the cell and refine the cell parameters. In the first refinement pass, refine with a tolerance level of about 1.0. Hit the refine button several times until the volume stops changing. Follow this by refinements at a tolerance level of about 1.5. Accept the results, and in the next menu select the Bravais routine that only looks for possible higher-metric-symmetry cells. Accept the cell with the highest score (> 0.6). The program then directs you to refine the cell with constraints on the cell parameters.

2. The data collection strategy is determined from the Collect > Calculate Strategy menu. Select the required resolution (≤ 0.82 Å) of the data and the crystal symmetry, and click "Apply". Click the "Determine Strategy" button, set the sample-to-detector distance to $\geq$ 37.0 mm (larger values for a cell axis > 25 Å or a twinned sample), and set the "Redundancy" to at least ≥ 2 and click "OK". The program will calculate an optimal strategy for collecting the data. Then use "Scan Parameters" menu to select the scan width ($\leq 0.5°$) and count time (usually 10 seconds), and click "OK".

3. On the Collect > Run Experiment menu, select "Append Strategy" to upload the strategy determined earlier. Add a "Fast Scan" run to the run list. Add a "Position" task to the run list and set the 2Theta value for the position task to "0.000".

4. Select the "Execute" button to start data collection.

1st Alternative Data Collection Path

1. In Collect > Run Experiment, set up a fast scan with Chi = 40.00 °. If the rotation image shows diffraction to the edge of the image, set to the count time 0.5 seconds. Execute the data collection of the fast scan.

2. When finished, go to Evaluate > Determine Cell to harvest all spots collected in the fast-scan run and determine the cell parameters as described above.

3. Go to Collect > Determine Strategy to determine an optimum strategy. Select the appropriate point group symmetry and resolution (0.40 < 0.82) and click "Apply". Then select "Determine Strategy" and select the appropriate sample-to-detector distance ($\geq$ 37.0 mm). Larger distances should be selected if one or more cell lengths > 25 Å or if the crystal is twinned. Select redundancy ($\geq$ 2) and click "OK". Set the count time to 10 seconds or less and set the scan width to $\geq$ 0.5 °.

4. On the Collect > Run Experiment menu, select "Append Strategy" to upload the strategy determined earlier. Add a "Fast Scan" run to the run list. Add a "Position" task to the run list and set the 2Theta value for the position task to "0.000".

5. Select the "Execute" button to start data collection.

2nd Alternative Data Collection Path

1. In Collect > Run Experiment hit the Load Table button and select the file "c:\frames\stand-tric.exp". Adjust the count times from the rotation image. If spots are too close, increase the sample-to-detector distance.

2. Select the "Execute" button to start data collection.

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 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"). Run the "cell_now" program. Enter the name of the *.p4p file when prompted. Take 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 likely cell lengths.

Several cells will usually be given with a hint as to the Bravais lattice type in the far right column. Set the tolerance to 0.15, and 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 select the first 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.

The cell is saved to a new file such as "temp1.p4p". Then select "S" to search for that cell in another orientation. Use the same tolerance of 0.15 for all other cells. 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 instruments.

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.

Best results can be achieved by performing multiple data reduction runs, each time optimizing the orientation matrix and the spot size. After the first data reduction run(s), that is(are) 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_*0*n.*_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 crystal structures.

Specific Steps to Run the Data Reduction Program in APEX - Saint
1. In the Reduce Data > Integrate Images routine select the data runs using the "Find Runs" command. Select those runs that have a significant number (> 25) of frames. A "Fast scan" run may be used to initially solve and refine the structure to check for correct Laue symmetry. However, after all data are collected, omit all "Fast scan" runs until the very last run as described below.

2. In the Refinement Options menu, uncheck the boxes for "Enable box size refinement" and "Enable orientation refinement". Adjust the size of the box to resemble the average spot size. If the values for X and Y are < 0.5, change the X and Y values to 0.95 and to

not change the Z value. You may improve these in later data reduction iterations. In the Periodic Refinement panel, **_always_** set the symmetry to "Triclinic". In the Global Refinement panel, set the symmetry of the cell to "Triclinic" for initial runs. Close this menu.

3. Select the "Integration Options" menu. In the "Background Subtraction" panel select "Use Best Plane Background". 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 this menu.

4. Begin data reduction with "Execute". Once integration has completed, check any statistics desired, then close this integration run with the "Close" button.

5. 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*_0*n*._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. Execute data reduction. Repeat this step until no large shifts are seen in the parameters.

6. In the penultimate data reduction pass, check the "Enable orientation refinement" box, and, in the Global Refinement panel, set the symmetry to the expected crystal symmetry for the cell. After executing this pass, *rename* the *project*_0m.* files to the *project*.* files.

7. In the final data reduction pass, use the Find Runs option to add the "Fast scan" run(s) to the integration. Execute data reduction without making any changes to the other options. At the end of this data reduction run, *copy* the *project*_0m.raw file to *project*.raw, overwriting the file prepared in the last pass. Note these files will be *project*_0m.mul and *project*.mul for integrations of a twinned sample.

The penultimate run gives the best unit cell information. The final run gives the best *.raw or *.mul file for the absorption correction program.

Indexing Faces of a Crystal

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 by 360° in ϕ. The images are stored in a zipped file with the name *project*.vzs. After 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 below the crystal image. 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 the crystal's 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 *project*f.p4p file using Export > P4P file.

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-merohedral 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.

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. If faces of the crystal were indexed, be sure to include the file with the faces and have the program determine the best value for the linear absorption coefficient of the sample.

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.