

Chemical Crystallography Laboratory	OUCB-CCL-5
Department of Chemistry and Biochemistry	Version 004
University of Oklahoma	March 30, 2017
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## Data Collection Using APEX3

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Chemical Crystallography Laboratory

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## Distribution

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## Revision Record

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The following laboratory users have read this manual.

Name	Signature	Date
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### **A. Scope and Availability**

This procedure outlines intensity data collection using the APEX3 program with a Bruker APEX diffractometer. A copy of this manual will be publicly available on the Laboratory's web site.

### **B. Summary of Method**

This procedure describes the steps necessary to collect good quality intensity data using the APEX3 program.

### **C. Responsibility**

Trained lab users may use this procedure. Instrument malfunctions are to be reported to the lab manager.

### **D. Safety and Training**

This instrument produces ionizing radiation that is potentially harmful to anyone near the instrument. Anyone that uses or maintains this instrument must be trained in the properties of X-ray radiation.

To produce the radiation, parts of the instrument operate at high voltages (20-50 kV). The internal components of the instrument should only be serviced by people trained to handle high voltages.

All laboratory users must be trained annually in radiation safety as specified by the Radiation Safety Office (<http://www.ouhsc.edu/rso/>) at the University of Oklahoma. Further training in the safe use of this instrument will be provided by the lab manager.

### **E. Equipment and Supplies**

- Bruker D8 goniometer with APEX detector.
- Sample mounted on a goniometer head.

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## F. Procedure

Check the detector-to-sample distance. This is read at the front edge of the detector mount on the goniometer. If this is not at the 5 cm mark, then loosen the two screws holding the detector to the 2theta arm and slide the detector until the front of the support is at the 5 cm mark. There is usually a pin in the 2theta arm that stops the detector from being moved closer to the sample. Press the back of the detector support towards you and tighten the two screws that were loosened earlier.

If the instrument is turned off, then follow the procedures to turn on the instrument in the “APEX Instrument Operating Instructions” manual, OUCB-CCL-SOP-1.003.

The sample should be mounted using the instruction in “Mounting Samples” manual, OUCB-CCL-SOP-3.003.

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 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 APEX program. The username and password within this program are “guest” / “guest” (without quotes). Select Instrument > Connect and connect to “localhost”.
6. Open a new *project* by opening Sample > New menu. The name of the *project* can be any unique value. In this lab, it is common to use a 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*.

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- 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 located at the previous position, then move the sample until it is ½ of the distance between its current position and the previous 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 on the CCL web site.

- 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 and measured the distance will appear just below the crystal display in the right side 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.
- 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.
- In the Set Up > Describe menu, enter your sample name (usually 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).
- To estimate the count time to use during data collection, 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 ¼ of the distance, then use count times of 60 sec/frame or greater. If the spots extend to about ½ of the way to the edge, then use count times of 30 sec/frame.

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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.

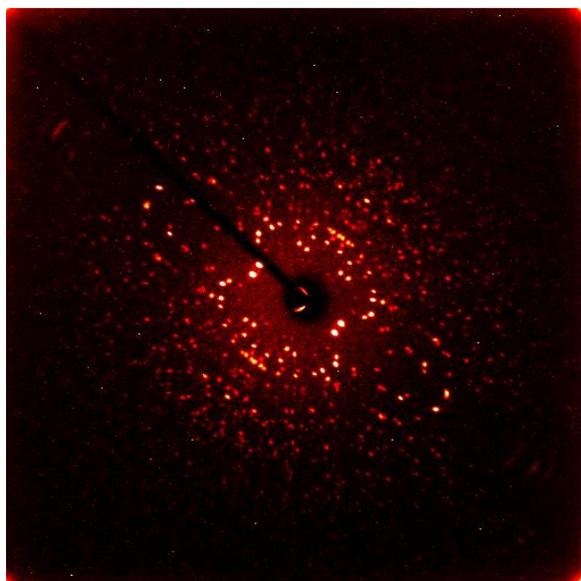


Figure 1. Typical rotation image.

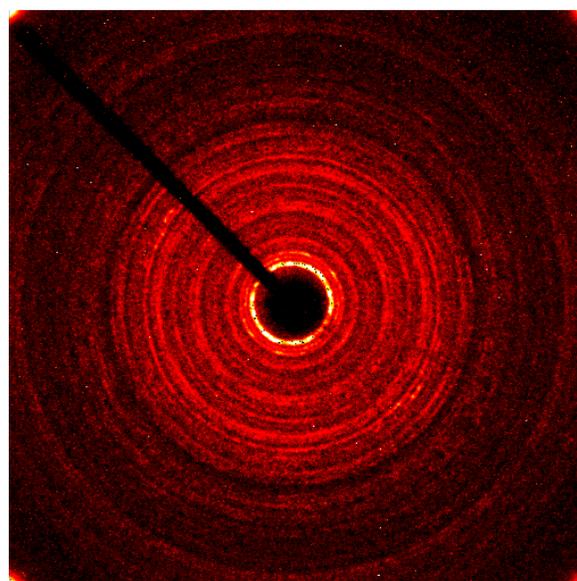


Figure 2. Polycrystalline sample.

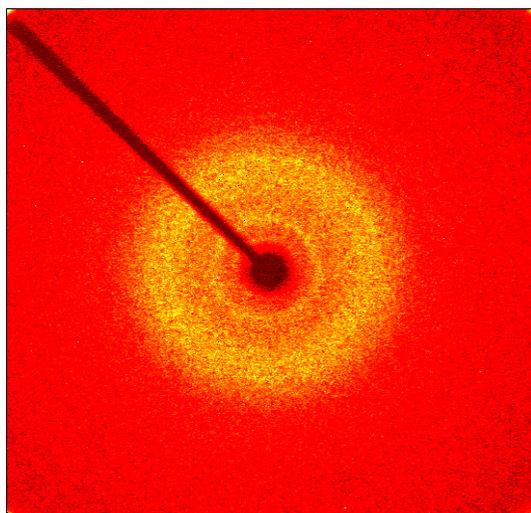


Figure 3. Glass sample.

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Note that the spots on a rotation image should show *mm* symmetry with the mirrors rotated 54.8° from the horizontal axis. These mirrors are only an artifact of the type of image and not indicative of the symmetry of the sample. If these mirrors are not present in the spots, then either the sample is not centered on the instrument or the instrument is not properly aligned; recheck the sample alignment and if necessary ask the lab manager to check the instrument alignment.

If the diffraction pattern shows rings similar to the image in Figure 2 below, the sample is polycrystalline (powder) and should be abandoned. If there are no diffraction spots in the rotation image, similar to the pattern in Figure 3 below, then the sample is an amorphous glass or gel and should be abandoned.

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 > 20 frames have been collected.

In the first method, intensity data are collected and peaks are “harvested”. 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. 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.

#### *Determine Preliminary Cell Parameters*

1. In the Evaluate > Determine Unit Cell menu select “Collect Data” then select the time to measure some preliminary data and click OK.
  - a) 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.
  - b) Accept the cell and refine the cell parameters. In the first refinement pass, use a tolerance level of about 1.0.
  - c) Follow this by refinements at tolerance levels of about 1.5 and 2.0.
  - d) 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).
  - e) The program then directs you to refine the cell now with constraints on the cell parameters.

\*If an insufficient number (<20) of spots are harvested, then collect a set of data using the “matrix” strategy. Delete the earlier matrix runs and the harvested spots from those runs, then harvest the frames from the new set of runs and proceed with indexing above.

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If the program has trouble indexing the spots, then a) save the spots to *project.p4p* file using the Sample > Export menu b) open a command prompt window c) set the current directory set to c:\frames\*project* and d) start the program “cell\_now”. In cell\_now enter the data file name as *project.p4p*. If the spots were very far apart, then change the extreme cell lengths to 3, 45. If the spots were very close together, then change the extreme cell lengths to 4, 60. A series of possible cells will be output. If < 90% of the spots fit the most likely cell, then the sample is probably a twin. If the sample is probably a twin, decide if there is likely to be a better crystal or if data collection should proceed on this sample. Save each cell as some series of \*.p4p files such as temp1.p4p, temp2.p4p, etc. The cell that was saved in cell\_now may be read into APEX using Sample > Import > tempn.P4P.

### *Data Collection Strategy*

The data collection strategy is determined from the Collect > Calculate Strategy menu. Select the required resolution (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. All omega-scan runs should be listed first, then phi-scan runs. If a 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.

If the cell parameters are to be determined after

Estimate the volume of the chemical formula ( $18 \times \#$  of non-hydrogen atoms). The unit cell volume should be an integer number times the volume of the chemical formula. This integer should be related to the presumed symmetry of the unit cell. If no reasonable integer can be obtained, either the chemical formula is wrong or there may be some solvent incorporated into the lattice of the crystal.

### *Data Collection*

1. Estimate a reasonable time to count each image in the range of 5 to 120 seconds. This often is the time selected in step 1 above. Do not exceed 120 seconds without discussing your project with the lab manager.
2. Collect a new dark current correction frame using the Detector > Dark Current command. Set the count time to your desired value from above. Save the correction to the file “1060L####\_dk” with #### replaced by the count time with 0’s prefixed to the time if the time is < 100. The program will ask you to confirm that the electronics are operating at 400 MHz.

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3. Set up data collection conditions in the Acquire > Multi-Run menu. Use the values in the table below

#	2Theta	Omega	Phi	Chi	Axis	Range	#	Time
1	-28	170	0	54.8	3	-0.3	608	#
2	-28	-28	45	54.8	2	-0.3	608	#
3	-28	-28	135	54.8	2	-0.3	608	#
4	-28	-28	225	54.8	2	-0.3	608	#
5	-28	-28	315	54.8	2	-0.3	608	#
6	-28	170	0	54.8	3	-0.3	608	#

The time should be the value selected above. If the mosaicity of the sample was large, then set the Range value to a larger value such that each spot is measured on only 2-4 frames. Range should always be a negative value. If the range is changed, then recalculate the # of frames according to:  $\#_{\text{new}} = (608 * 0.3) / |\text{Range}_{\text{new}}|$  (rounded to the nearest integer). If the detector was moved to 7 cm then change the -28 values in 2Theta and Omega columns to -26. If the detector was moved to > 7 cm, then set the data collection conditions according to the procedures in Multiple Count Times manual.

4. Collect data with Acquire > Multi-Run. Set the Job Name to *projectcode* determined above.
5. Enter data collection information into the log book beside the frame buffer computer.

### G. Records Management

Data are collected to the frame buffer computer for later processing. Each experiment is recorded in the log book.

### H. Quality Control and Quality Assurance

Appropriate count times, sample-to-detector distances, and data collection runs assure that precise, redundant data is properly collected.

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## I. References

<http://www.ouhsc.edu/rso/>

Web site for the Radiation Safety Office of the University of Oklahoma.

APEX Instrument Operating Instructions, OUCB-CCL-1.002, Chemical Crystallography Laboratory, Department of Chemistry and Biochemistry, University of Oklahoma.

Center the Sample on the Instrument, OUCB-CCL-4.002, Chemical Crystallography Laboratory, Department of Chemistry and Biochemistry, University of Oklahoma.