APEX 3 / APEXII DUO Quick Guide

➢ **Turning on the Low temperature (LT) device**

1. On the Cryostream Controller next to the APEX, press the **START** button (blue button/arrow in figure below).

2. Once the controller is done starting up, and the “TEMP” display shows a temperature, press **START** again to begin cooling to 100 K. Wait ~30 minutes for the temperature to reach 100K and stabilize.

3. If the crystal sample is no good and no one is going to be using the diffractometer after you, please press the **STOP** button (red button/arrow in figure below) to turn off the LT device.

![Cryostream Controller](image)

➢ **Login**

1. Click on **Sample** and select **Login**.
2. Enter username (lowercase group name) and password (pjc).

➢ **Create a New Sample**

1. Click ![File](image) or click **Sample** and select **New**.
2. Enter sample name (e.g. 1234a).

   **Note:** Check white board or website to determine # of sample for your lab.
3. Sample folder will be created in D:\frames\(username)\(sample name)\.

➢ **Centering the Crystal**

   **Note:** Take care when opening and closing the doors. **DO NOT SLAM THE DOORS.** When opening doors, an audible *click* should be heard when pressing the green “Open Doors” button. If the *click* is not heard, but the “Alarm” light is flashing, gently push in the handles until you hear the *click* then proceed to pull the handles and open the doors. If the doors still won’t open, contact your crystallographer.

![Cryostream Controller](image)
1. Using APEXServer (monitor in enclosure), click **Set Up** on the left-hand menu and
   click ![Center Crystal](image)
   .
2. On the bottom right, click **Center**.
   **Note:** If image is not moving, click ![in the VIDEO window](image).
3. Screw the goniometer head on making sure the notches are aligned.
4. Use the goniometer key to adjust the crystal until centered in the crosshairs.
5. On the right-hand side of the screen, click **Spin Phi 90** to rotate the crystal and continue to adjust the position until centered. Repeat three more times and close the doors.

➢ **Determine Unit Cell**

1. Click **Evaluate** on the left-hand menu.  
   ![Determine Unit Cell](image)
2. Click ![](image)  
3. Under “Manual Mode” on the right-hand side, click ![](image) and click **Collect** at the bottom right.
   **Note:** Spots should be well-defined circles and should not overlap. Samples that are twinned or multi-crystalline may have smeared or overlapping spots. If sample is stable, it may be worth finding another crystal.
4. Click ![](image) .
5. Change the **Min. I/σ(I) value** to 5 using the slider bar or manually entering the value.
6. Click **Harvest** at the bottom right.
   **Note:** After the spots have been harvested, inspect the “Expected Resolution” table (see figure below). Crystals with an expected resolution **greater than 1.0Å for 20 s/°** are generally too weak to give a publishable structure.
Note: Click ☺, and adjust the circle to check the best resolution spot at 10 seconds, please use your best judgement for exposure time (better to err on the side of caution and collect longer exposures).

7. Click Index, then click Index at the bottom right.

8. APEX3 will select the “best” unit cell. Click Accept at the bottom right.

   Note: If a method failed, two different unit cells were calculated or there are a large number of overlapping/unselected spots, use CELL_NOW to calculate the unit cell. (CELL_NOW instructions located at the end of the guide)

9. Adjust the Tolerance value to 5.00 or move the slider all the way to the left.

10. Click Refine until the Unit Cell parameters stop changing.

11. Click Accept.

12. Click Bravais.

13. APEX3 will suggest possible Bravais lattices. Always select Triclinic P, unless your crystallographer suggests otherwise or you are performing a quick screening.

   Note: The correct lattice type may not always be the highest symmetry lattice. Therefore, collecting in Triclinic P ensures a sufficient amount of data for any lattice option.

14. Click Refine.

15. Adjust the Tolerance value to 5.00 or move the slider all the way to the left.

16. Click Refine until the Unit Cell parameters stop changing.

   Note: Inspect the predicted overlay on the matrix frames. Make sure ALL the spots are picked/circled.

17. Click Accept.

18. Before setting up data collection...

   a. Inspect the “Expected resolution” table to determine the optimal exposure time for good data for resolution out to 0.77 Å.

   b. Check your unit cell versus known materials/starting materials using the X-ray facility website or the Cambridge Database.

      Note: If a full dataset is collected, you will be charged, regardless of whether the structure has been done before.

   c. Calculate the density of the crystal sample.

      i. Use the formula: density = MW/V x 1.66 x Z, where MW is molecular weight and V is unit cell volume. Z can equal 1, 2, 4 or 8 depending on the Bravais lattice. A reasonable density should be between 1.2 and 2.0 g/cm³.
Set Up Data Collection

1. On the left-hand menu, click Collect, then Calculate Strategy.
2. Adjust “Resolution” in the top-right to 0.77Å for a heavy atom structure (Cl or heavier) or 0.83Å for a light atom structure (all atoms less than Cl).
3. Use the dropdown menu to adjust “Symmetry” to “Centrosymmetric (-1)” unless compound is known to be chiral. If compound is chiral, do not change original selection of “Chiral (1).”
4. Click and then click OK.
   Note: If crystal is possibly twinned or multi-crystalline, change “Crystal to detector distance” to 50-60mm.
5. Click Select scan parameters... at the bottom.
6. Adjust “Frame angle [degrees]” to 0.50.
7. Adjust “Frame time [seconds]” (exposure time) according to “Expected resolution” table from Unit Cell Determination. (Max exposure of 30 seconds)
8. Click OK. Take note of the “Expected end time” listed at the bottom of the page.
9. Pay attention to the “Average Multiplicity” value. If collecting as triclinic, but a higher symmetry Bravais is possible, an “Average Multiplicity” of ~4 is acceptable. If crystal system is actually triclinic, click Determine strategy... and increase “Minimum multiplicity for 90% of the data” value until “Average Multiplicity” is greater than 6 (usually 3.50 works).
10. Click Run Experiment.
11. Click Append Strategy in the bottom left corner.
12. If the collection will end at night or over the weekend with no new sample to be mounted after, add “Thermostat Off” to the next available row.
13. If exposure times are 5 seconds or less, change default “Exposures” from correlated to uncorrelated using the dropdown menu.
14. Click Validate in the lower right corner.
   Note: If error occurs, resolve error. Typically, increasing detector distance resolves issue. If error still occurs, contact your crystallographer.
15. Click Execute in lower right corner.
Example experiment setup:

**Note:** The above figure is an example, not all strategies will have the same number of scans.

- **Integration of Data**

  **Note:** Twinned and multi-crystalline samples require extra steps when processing data (integration, scaling, etc). Consult your crystallographer for further data processing.

1. On left-hand menu, click **Reduce Data**.

2. Click **Integrate Images**.

3. Change “Resolution Limit” value in top right corner to **0.77Å** for heavy atoms structures or **0.83Å** for light atom structures.

4. Click **Integration Options...** in the bottom right.

5. Click **More Options**.

6. Change “Generate Mask with Fractional Lower Limit of Average Intensity” to **0.550** and click OK.

7. Click **And Runs...** in the bottom right. Make sure the correct scan sets are selected and click **Choose**.
8. Click [Start Integration...]

**Note:** Pay attention to the “Spot Shape Correlation” graph. The average value should be around 0.6 or higher for usable data. Sudden drops in the graph suggests something occurred during collection. Inspect the frames and consult your crystallographer. In addition, pay attention to the “Spot Shape Profile.” The spot should be well-defined and spherical.

➢ **Scaling Data**

1. Click Scale in left-hand menu.

2. Pay attention to the “Input File,” which should be *xxxx_0m.raw*, and “Laue Group” and “Point Group,” which should match the Bravais lattice. (-1 for triclinic, 2/m for monoclinic, mmm for orthorhombic, etc.)

3. Click Start in lower right corner.

4. Click Refine in the lower right corner.

**Note:** Pay attention to the “Mean Weight” and “R(%).” Both graphs should plateau by the end of the refinement cycles. Mean Weight values >0.90 are good and the lower the R(%), the better. If graph does not plateau/converge, increase “Number of Refinement Cycles” to >50. If it still does not converge, then the Bravais chosen is incorrect.

5. Click Next in lower right corner.

6. Click Finish in the bottom right corner.

➢ **Generate .INS File**

1. In left-hand menu, click Examine Data, then click .

2. Files for XPREP should be automatically selected, if not, browse for the *mo_xxxx_0m.p4p* and *mo_xxxx_0m.hkl* files in the “work” folder.

3. Click OK.

4. In XPREP GUI window, press <Enter> on keyboard to accept default options.
   a. Make note of the $R(s)$ values for determining higher symmetry, lower values are better.
   b. Pay attention to the CFOM values as well as systematic absence values for space group determination.
   c. When merging data sets, make note of the Completeness, Redundancy, Mean I/s values, and $R_{merge}/R_{sigma}$ values.

   **Note:** Completeness should be > 95% for publishable structure.
5. When prompted, enter **formula** of compound. (Case and text sensitive)
   
   **Note:** XPREP will generate a Z value and calculated density based on formula. Reasonable density should be between 1.2 and 2.0.

6. When prompted, enter output file name (no spaces). Default name is acceptable, or a custom name if testing multiple space groups.

7. Continue pressing <Enter> until XPREP quits and window closes.

   **Important!!:** After the proper space group has been determined using XPREP, make sure to go back to “Determine Unit Cell” to change the Bravais to the **correct** symmetry lattice, then **re-integrate, re-scale** and **re-generate** the .INS file.

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**Structure Solution**

- **AUTOSTRUCTURE Method**

  1. In the left-hand menu, click **Find Structure**.

  ![Find Structure](image)

  2. Click **AUTOSTRUCTURE**.

  3. Click ![Select File](image) and select appropriate .hkl file generated from XPREP.

     **Note:** Even though an .hkl file is loaded, you still need to reselect the latest .hkl file to update the unit cell parameters and lattice type determined from the .ins.

  4. Make sure the formula is correct (to the best of your knowledge) or the program will have difficulty generating a solution.

  5. Click **Start**.

- **Solve Structure Method**

  ![Select Files](image)

  1. Click **Solve Structure**.

  2. Click ![Select Files](image) and select the appropriate .hkl/.ins files.

  3. Select the method for solving the structure and click **Solve Structure**.
a. **Intrinsic Phasing**

   **Note:** Be sure that the unit cell and Bravais type match those from the XPREP and not the initial unit cell and lattice group from Determine Unit Cell.

   i. Uses XT to solve structure. Resulting .res has all atoms isotropic
   
   ii. May generate **more than one .res file**, each solving the structure in a different, but related space group.
   
   iii. Select the appropriate .res file based on R values and Flack parameter (avoid 0.50 Flack value).
   
   iv. Be sure to select corresponding .res/.hkl files. The output files will have “_a”, “_b”, and “_c” attached to file name.

b. **Direct Method**

   i. Uses XS, reads original space group determined in XPREP and recorded in .ins file.
   
   ii. Output .res file will have some heavy atoms labeled, but all other atoms will be Q-peaks.

c. **Patterson Method**

   i. Only recommended for difficult structures that did not work with previous methods.
   
   ii. Uses original space group determined in XPREP and recorded in .ins file.
   
   iii. Output .res file will only have heavy atoms assigned and Q-peaks present.

   **Note:** If the molecule has disorder, AUTOSTRUCTURE will have trouble producing a sensible solution. Be sure to try the other solution methods and look for features in the Q-peaks such as phenyl rings, t-butyl groups, etc.

4. Lastly, use the appropriate .res and .hkl files to refine the structure using the program of your choice. Have fun!
Determine Unit Cell using **CELL_NOW**

1. Click **Evaluate** on the left-hand menu.

2. Click **Determine Unit Cell**.

3. Click **Harvest Spots**.

4. Adjust the **Min. I/σ(I) value** to **1.75** by using the slider bar or manually entering the value.

5. Click **Harvest** at the bottom right.

6. Click **Sample** in the top-left corner of APEX3, then **Export**, then select **P4P file**...

7. Under “Export For:”, select **CELL_NOW**.

8. Leave the default .p4p name (*samplename*.p4p) and click **OK**.

9. Click **Sample** in the top-left corner of APEX3, then click **Run Command**...

10. Type “cell_now” and press <Enter>.

11. Type in the name of the .p4p file, default is “*samplename*.p4p” and press <Enter>.

12. Accept default .cn file name by pressing <Enter>.

13. Begin the initial unit cell search by pressing <Enter>.

14. Accept the default “superlattice threshold” by pressing <Enter>.

15. Accept the default “minimum and maximum values for cell edge” by pressing <Enter>, unless cell lengths are less than 5 or greater than 45.

   a. A table of possible unit cells will be generated and ordered by calculated Figure of Merit (FOM).

16. Press <Enter> to accept default “Maximum deviation from integer index.”

17. Press <Enter> to accept the best FOM unit cell. If a different cell is wanting to be selected, type the corresponding number from the list.

18. Type “cell#a.p4p” as the first domain file name and press <Enter>.

19. If a large number of “reflections not yet assigned to a domain” remains, press <Enter> to continue search for another domain and name as “cell#b.p4p.”

   Note: Make note of the FOM of the additional cells that are calculated (should be greater than ~0.50).

20. Type “Q” and press <Enter> to quit.

21. Click **Sample** in the top-left corner of APEX3, then **Import**, then **P4P/SPIN file**.

22. Click **Import** and select the last .p4p file made (e.g. cell1d if four domains were calculated) and click **Open**.
23. Select **Import all** and click **OK**.

   **Note:** The lattice type from CELL_NOW is not transferred when importing the .p4p file. If Bravais does not recognize the cell as the same type calculated in CELL_NOW (e.g. P, C, etc.), then click **Edit...** next to the “Unit cells” box and manually select the lattice type. You can skip the Bravais step if this is the case.

24. Click .

25. APEX3 will suggest possible Bravais lattices. Always select **Triclinic P**, unless your crystallographer suggests otherwise or you are performing a quick screening.

   **Note:** The correct lattice type may not always be the highest symmetry lattice. Therefore, collecting in Triclinic P ensures sufficient data for any lattice option.

26. Click .

27. Adjust the **Tolerance** value to 5.00 or move the slider all the way to the left.

28. Click **Refine** until the Unit Cell parameters stop changing.

   **Note:** Inspect the predicted overlay on the matrix frames. Make sure ALL the spots are picked/circled.

29. Click **Accept**.