APEX 3 / D8 Quest Quick Guide

➢ Turning on Low temperature (LT) device

1. Open **CryoConnector** by clicking  on the desktop or on taskbar.
2. Click **Restart** or **Start**.
3. Click **Cool to 100.0K** and wait until temperature is stabilized (~1 hour).
4. If the crystal sample fails and no one is going to use the diffractometer after you, turn off the LT by clicking **Stop**.

➢ Login

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

➢ Create a New Sample

1. Click  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: The door must be **closed** for the goniometer and detector to move into "Mount" or "Center" positions. Door can be left open during Phi rotations.

1. Using APEXServer (monitor in enclosure), click **Set Up** on the left-hand menu and click 

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2. On the bottom right, click **Mount** and close the door.
3. Screw the goniometer head on. The side marked with an arrow aligns with the notch.
4. Click **Center** and close the door.
5. Use the goniometer key to adjust the crystal until centered in the crosshairs.

   Note: If the camera is not on, click  .
6. 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.

➢ Determine Unit Cell

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

2. Click Determine Unit Cell.
3. Under "Manual Mode" on the right-hand side, click Collect Data 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 Harvest Spots.
5. Change the **Min. I/sigma(I) value** to **5**. If the crystal is very strong (bright spots seen at resolutions further than 0.77), leave the value at the default of 20.
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.

Expected resolution:

	Exposure time [s/°]	Resolution [Å]
1	2.0	0.96
2	5.0	0.90
3	10.0	0.86
4	20.0	0.83

> **Note:** If the Expected resolution table does not agree with a visual inspection (click  , and adjust the circle to check the best resolution spot at 10 s), 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 

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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 **0.77Å**.

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

    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/cm^3.

➢ Set Up Data Collection

1. On the left-hand menu, click **Collect**.
2. Click 

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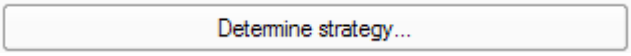
3. Adjust "**Resolution**" to **0.77Å** for a heavy atom structure (Cl or heavier) or **0.83Å** for a light atom structure (all atoms less than Cl).
4. Use the dropdown menu to adjust "**Symmetry**" to "**Centrosymmetric (-1)**" unless compound is known to be chiral. If compound is chiral, do not change original selection of "**Chiral (1)**."
5. Click 

and then click **OK**.

    Note: If crystal is possibly twinned or multi-crystalline, change "Crystal to detector distance" to 50-60mm.

6. Click **Select scan parameters...** at the bottom.

7. Adjust "**Frame angle** [degrees]" to **0.50**.
8. Adjust "**Frame time** [seconds]" (exposure time) according to "Expected resolution" table from Unit Cell Determination. (Max exposure of 30 seconds)
9. Click **OK.** Take note of the "Expected end time" listed at the bottom of the page.
10. 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**.
11. Click 

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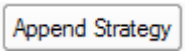
12. In the 1st row, use the dropdown menu under "Operation" and select **Fast Scan**.

    Note: If data collection "frame time" is longer than 10 seconds, adjust "Time" of fast scan to be 10-20% of collection time. If data collection "frame time" is less than 10 seconds, leave fast scan "time" at 1 second and increase "Width" to 2 or 3 degrees.
13. Click 

in the bottom left corner.
14. 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.
15. Click 

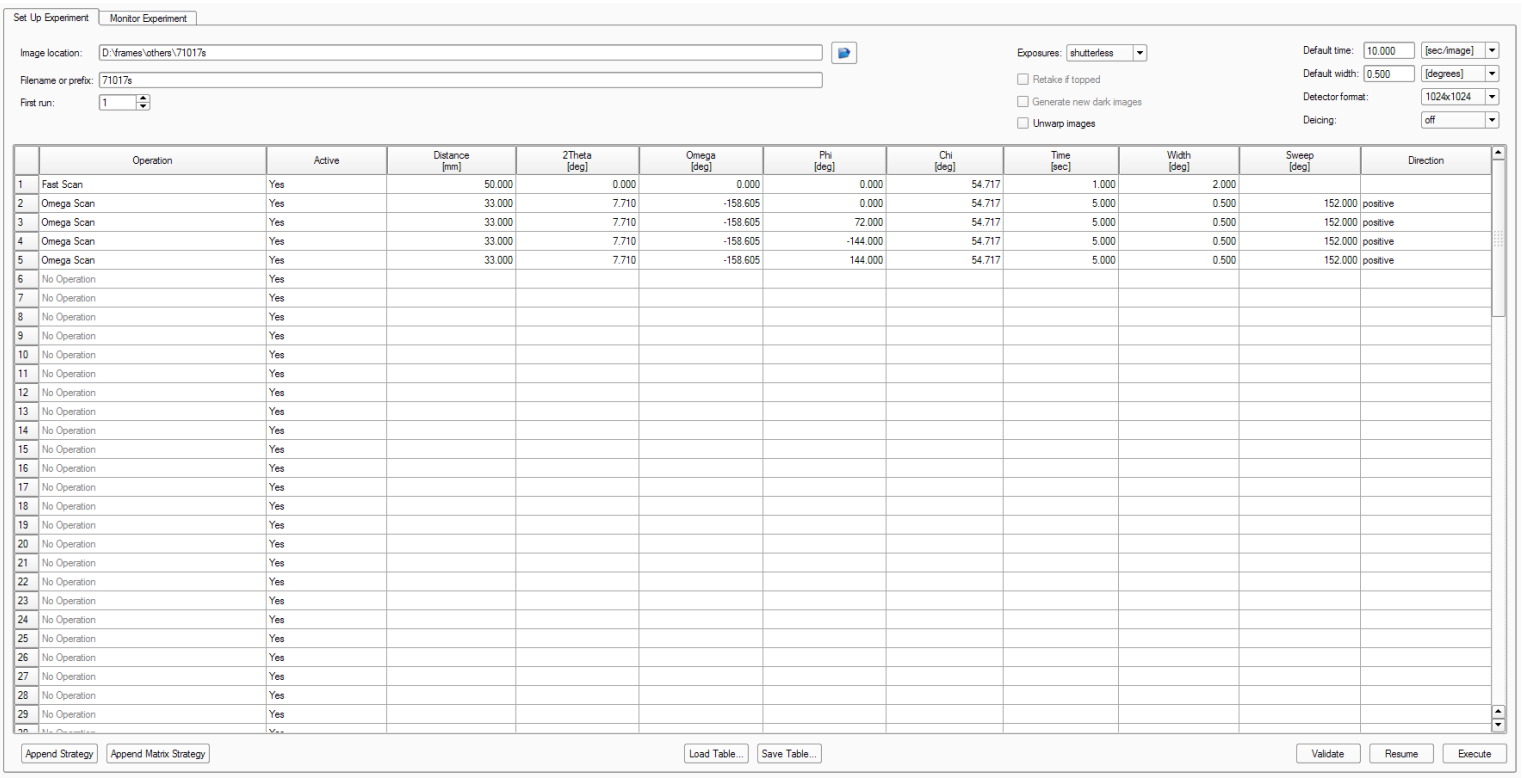
in the lower right corner.

    Note: If error occurs, resolve error. Typically, increasing detector distance resolves issue.
16. Click 

in lower right corner.

    Note: Click on the BIS window and make sure no fast scan frames result in topped spots. If so, increase the "Width" of the fast scan to 2 or 3 degrees.

Example experiment setup:



	Operation	Active	Distance [mm]	2Theta [deg]	Omega [deg]	Phi [deg]	Chi [deg]	Time [sec]	Width [deg]	Sweep [deg]	Direction
1	Fast Scan	Yes	50.000	0.000	0.000	0.000	54.717	1.000	2.000		
2	Omega Scan	Yes	33.000	7.710	-158.605	0.000	54.717	5.000	0.500	152.000	positive
3	Omega Scan	Yes	33.000	7.710	-158.605	72.000	54.717	5.000	0.500	152.000	positive
4	Omega Scan	Yes	33.000	7.710	-158.605	-144.000	54.717	5.000	0.500	152.000	positive
5	Omega Scan	Yes	33.000	7.710	-158.605	144.000	54.717	5.000	0.500	152.000	positive
6	No Operation	Yes									
7	No Operation	Yes									
8	No Operation	Yes									
9	No Operation	Yes									
10	No Operation	Yes									
11	No Operation	Yes									
12	No Operation	Yes									
13	No Operation	Yes									
14	No Operation	Yes									
15	No Operation	Yes									
16	No Operation	Yes									
17	No Operation	Yes									
18	No Operation	Yes									
19	No Operation	Yes									
20	No Operation	Yes									
21	No Operation	Yes									
22	No Operation	Yes									
23	No Operation	Yes									
24	No Operation	Yes									
25	No Operation	Yes									
26	No Operation	Yes									
27	No Operation	Yes									
28	No Operation	Yes									
29	No Operation	Yes									

Note: The above figure is an example, not all strategies will have four omega 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 

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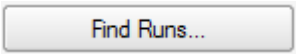
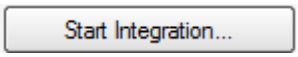
3. Change "Resolution Limit" value in top right corner to **0.77Å** for heavy atoms structures or **0.83Å** for light atom structures.
4. Click 

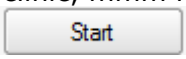
in the bottom right. Make sure the correct scan sets are selected and click **Choose**.
5. Click 

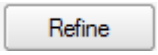
Note: Pay attention to the "Spot Shape Correlation" graph. The average value should be around 0.6 or higher for usable data. Sudden drops in the graph suggests something occurred during collection. Inspect the frames and consult your crystallographer. In addition, pay attention to the "Spot Shape Profile."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

➢ Scaling Data

1. Click 

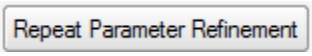
in left-hand menu.

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 

in lower right corner.
4. Click 

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. Check the "Fast Scan" box next to the appropriate scan # and click 

and repeat Steps 4 and 5.

   Note: APEX3 will attempt to preselect Fast Scans during the refinement, if it has been selected already, there is no need to repeat the refinement.

7. Click **Finish** in the bottom right corner.

➢ Generate .INS File

1. In left-hand menu, click **Examine Data**.
2. Click 

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3. 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.
4. Click **OK**.
5. In XPREP GUI window, press <Enter> on keyboard to accept default options.
   a. Make note of the **R(sym)** 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 **Rmerge/Rsigma** values.

Note: Completeness should be > 95% for publishable structure.

6. 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.

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

8. 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.

➢ Structure Solution

- **AUTOSTRUCTURE Method**

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

2. Click AUTOSTRUCTURE .

3. Click  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**

1. Click Solve Structure .

2. Click  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 

.
4. Adjust the **Min. I/sigma(I) value** to **2.50** 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  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 

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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 

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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**.