

CRYALIS^{PRO} USER GUIDE

❖ TURNING ON LT DEVICE AND X-RAYS

1. Launch CrysAlisPro (online) with the desktop icon.



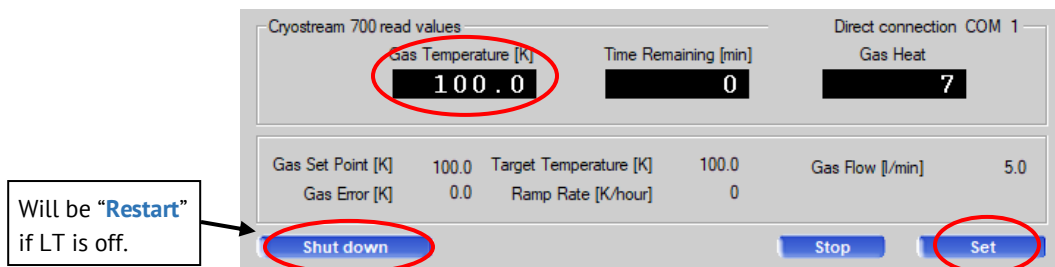
2. Wait for the instrument to initialize.



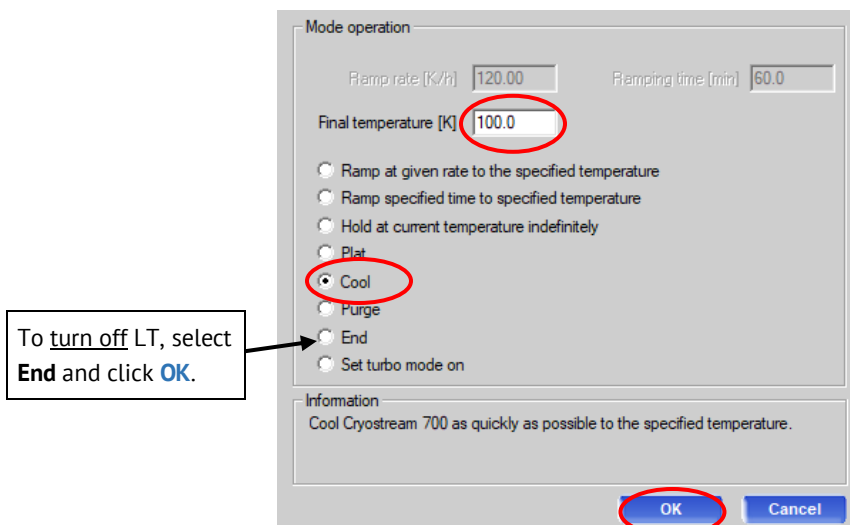
3. If the Oxford Cryosystem/LT unit is off, left click on **CRYO**.



- i. Click **Restart** if the LT is not turned on and wait for the Gas Temperature to show numbers instead of "??".
- ii. Click **Set**.



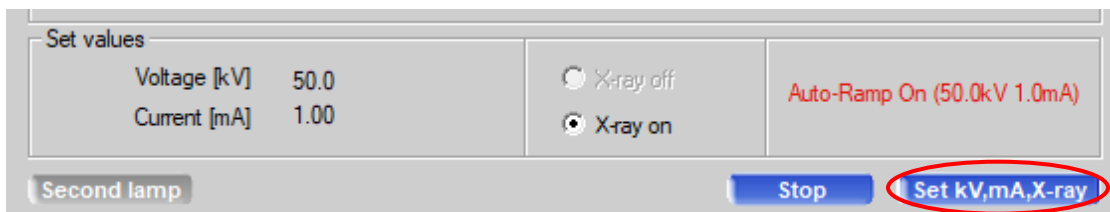
- iii. Change the mode to **Cool** and Final temperature to **100K**, click **OK** and close the Oxford Cryosystems window.



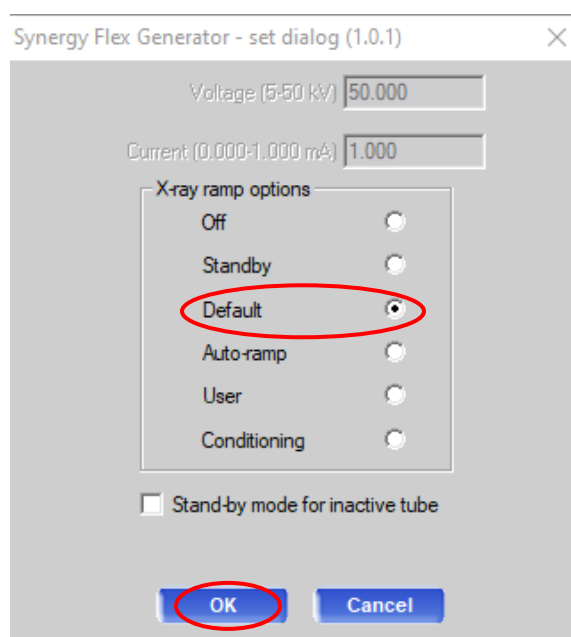


4. If the X-ray generator is off, left click on **X-RAY**.

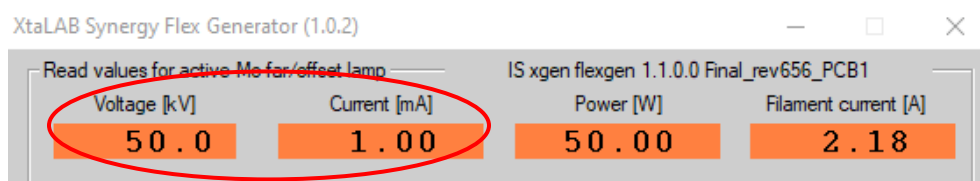
i. Click **Set kV, mA, X-ray**.



ii. Select **Default** and click **OK**.



iii. Close the window when Voltage is **50.0** and Current is **1.00**.



Note: The generator window cannot be closed while the X-rays are being ramped up.

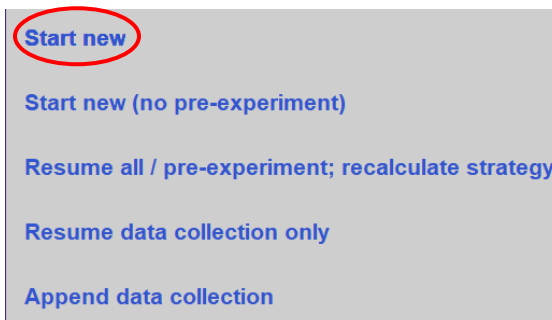
5. X-ray generator will turn off automatically after 6 hours. If you'd like to turn them off manually, click **Set kV, mA, X-ray**, select **Off** and click **OK**.

❖ GETTING STARTED (SETTING UP AN EXPERIMENT)

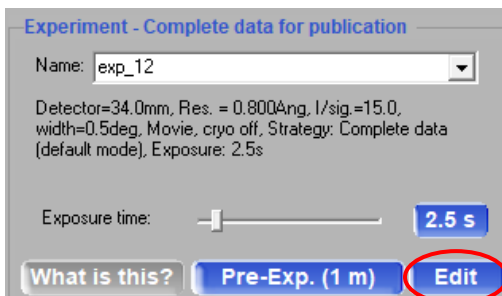
1. Click on **START/STOP**.



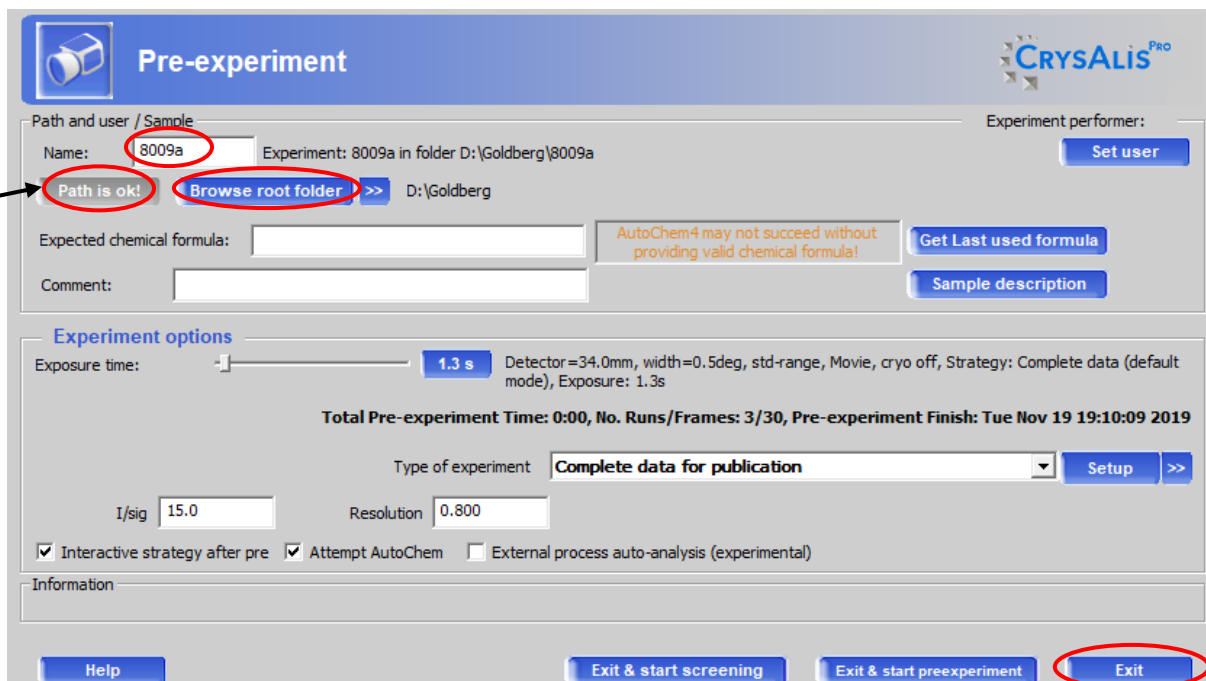
2. Click on **Start new**.



3. Click on **Edit** to setup experiment info.
4. Click on **Browse root folder** and locate your lab's folder in the D:\DATA drive and click **OK** (click **No** when asked to change root folder).
5. Change the name of the experiment (check Penn database or sign-up board) and click **Exit**.
e.g. 6874a (# differs between labs, letter is used if multiple crystals of the same sample are being tested)

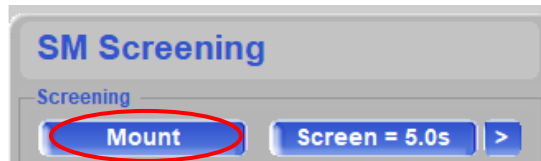


Will be "Clear Folder" if the name is taken. Please do not overwrite anything. Use the next letter.

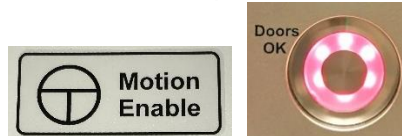


❖ CENTERING THE CRYSTAL

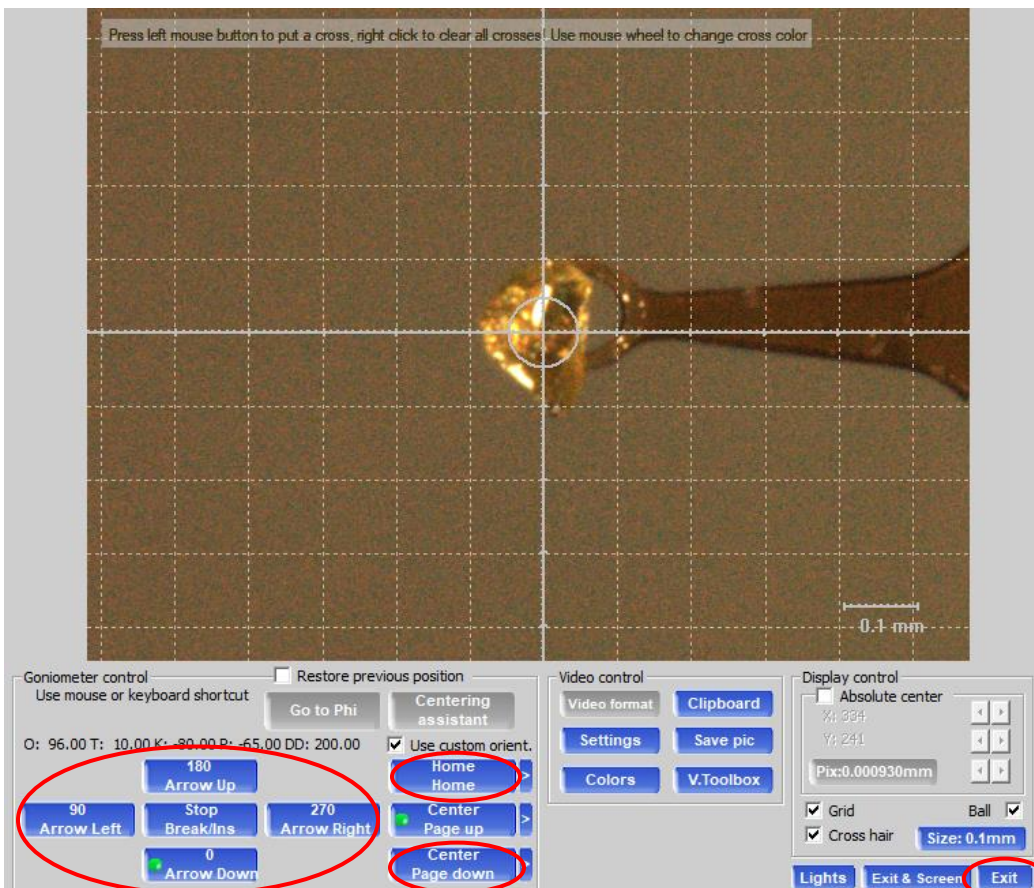
1. Click **Mount** or press  on the keyboard, this will open the centering interface.



Note: If the door is open, press and hold both “**Motion Enable**” buttons in the enclosure or the goniometer will not move. Alternatively, you can close the door and press the “**Doors OK**” button.



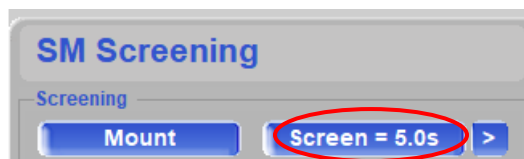
2. Click **Home** or **Center** and mount the goniometer head, do not overtighten. **Be careful** not to bump the beamstop, collimator or coldstream nozzle.
3. Click **Center** (either Center will work) to begin centering (**hold down** Motion Enable buttons to allow movement).
4. Cycle through **0**, **90**, **180** and **270** phi orientations while centering the crystal at each position. Use the knobs facing you on the gonio head to adjust the position shown on the camera.




5. Once centered, click **Exit**, **gently close the door** and press the **Doors OK** button.

❖ SCREENING, PRE-EXPERIMENT AND STRATEGY

1. Click the **Screen** button using default exposure time.



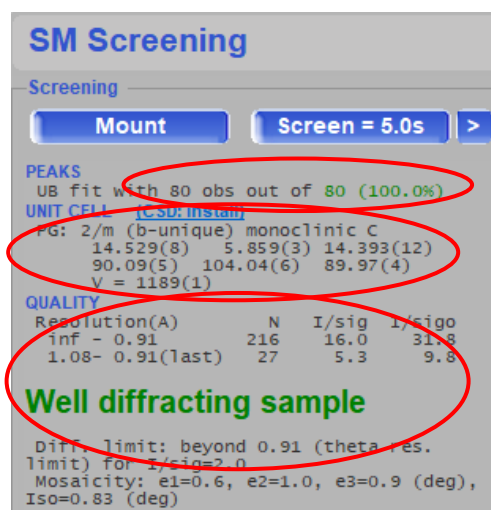
Note: If you have tried several crystals and they are all weak, you can increase the exposure time by clicking  and adjusting the value in the **Exposure time** box. It is beneficial to find a good sample BEFORE the pre-experiment.

2. Examine the images to ensure you have well-defined spots with no rings/streaks or overlaps. If you have trouble seeing spots, make sure the contrast is at **Level 1 (255)** and you can change the image color scheme as well.

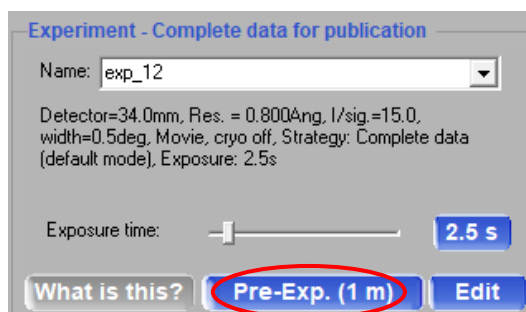


3. Once screening is complete, you should **check the results** before continuing to the Pre-Experiment.

- i. **High % fit** of predicted cell to observed spots
- ii. Reasonable unit cell (volume and lattice)
- iii. **Well diffracting sample** and good quality reflections
 - a) If you have a 50-70% fit, then you might have a twinned crystal, try another and if it persists, you may continue.
 - b) Check the density of your crystal sample ($d = MW/V \times 1.66 \times Z$), a reasonable density is 1.2 to 2.0 g/cm³.
 - c) If you have a **moderately diffracting** or **weakly diffracting** sample, try another crystal, if it persists but the reflections look well-defined, you may continue.



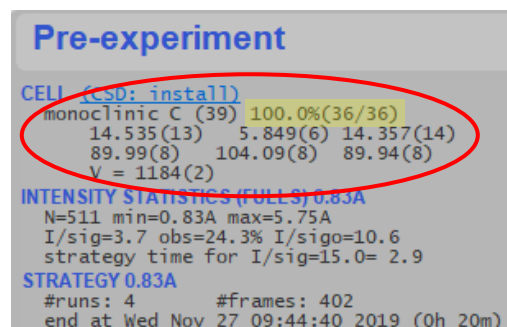
4. Click **Pre-Exp** to begin the pre-experiment with default exposure time (estimated from screening process).



Note: The pre-experiment is a more intensive screening where more frames are collected to better determine exposure times, unit cell and the orientation matrix.

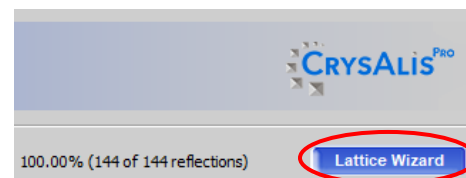
5. Once the pre-experiment is finished, the strategy module will open automatically.

Note: Double check the pre-experiment results are consistent with the screening results. (% of fit reflection and unit cell)

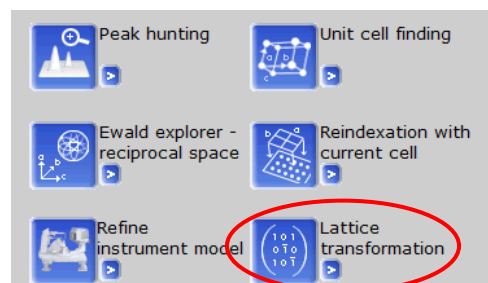


Note: If you closed the Experiment Strategy window, the only way to re-open it is to click **START/STOP**, then **Resume all/pre-experiment; recalculate strategy**, and then select the “pre_xxxx.run” file. If you want to **Screen** a different crystal, you must start over. Click **START/STOP**, then **Start New** and **change the name of the experiment** before continuing.

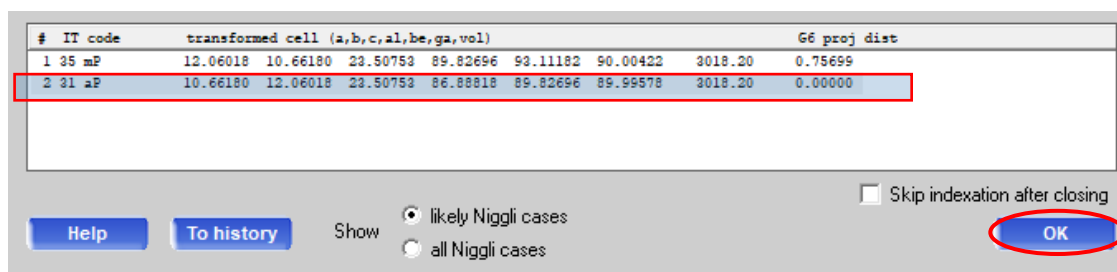
6. Click **Lattice Wizard** in the top right of the Experiment Strategy window.



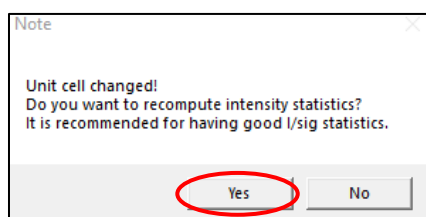
7. Click **Lattice transformation**.



8. Select **aP** for Triclinic P (should always be on the bottom) and click **OK**.



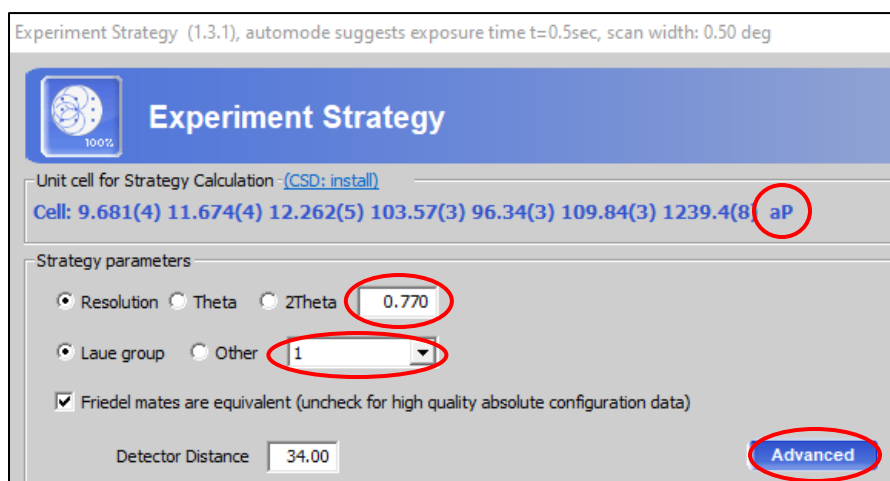
9. Click **Close** to exit the Lattice wizard. A window will pop up informing you of the unit cell change, click **Yes**. The strategy module will re-open with recalculated exposure times.



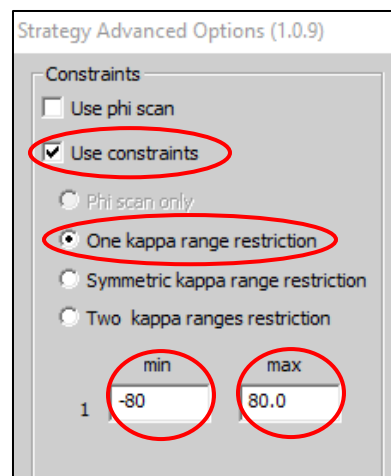
10. **aP** should be the new Cell and the Laue group should be **1**.

11. Under **Strategy parameters**, change the **Resolution** to **0.77** for heavy atom compounds (contains elements heavier than Cl) or **0.83** for light atom compounds (contains only Cl or lighter).

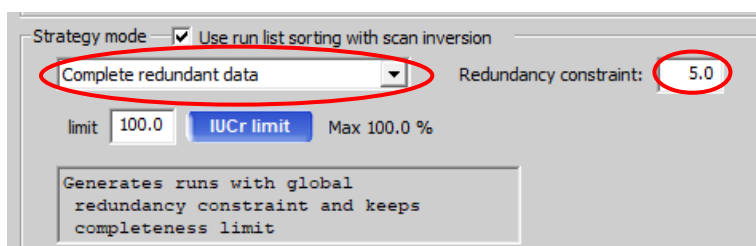
12. Click **Advanced** to open the Strategy Advanced Options window.



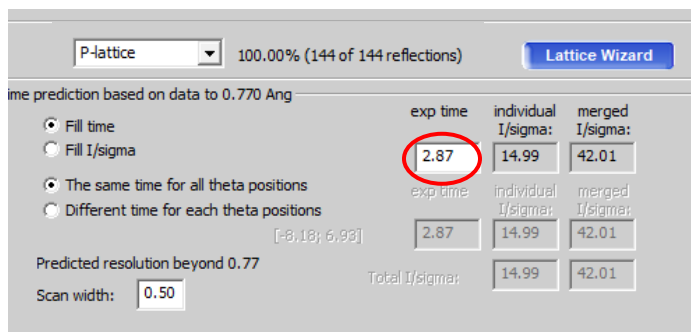
13. Select **Use constraints**, and **One kappa range restriction** and change the min to -80 and max to 80, click **OK**.
(kappa restriction prevents icing for long collections)



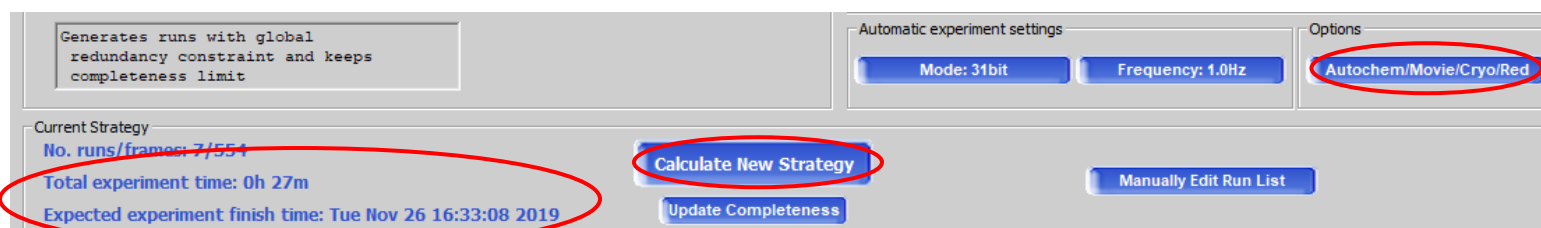
14. Under Strategy mode, use the dropdown menu to select **Complete redundant data** and change the value to 4 if the crystal may be higher symmetry than triclinic and 6 if the crystal can only be triclinic.



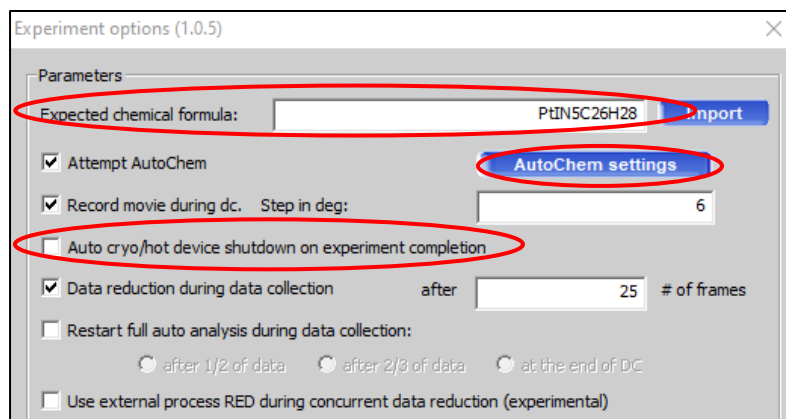
15. The checkbox for Friedel mates and Detector Distance value can be left as is. (the only reason to increase detector distance is if you have a twinned crystal with a lot of overlapping spots).
16. **Exp time** can be left as is or adjusted (we like to change it to a whole number, a half or an interval of 5).



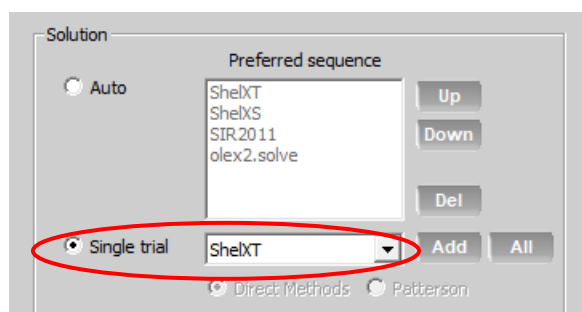
17. Click **Calculate New Strategy** (might have to change exp time again).
18. Make note of the **total experiment time** and the **experiment finish time**.
19. Click **Autochem/Movie/Cryo/Red**.



20. Enter the **Expected chemical formula**.
21. If the experiment will finish in the middle of the night or another user will not use the diffractometer after you, **check the Auto cryo device shutdown**.
22. Click **AutoChem settings**.



23. Select **Single trial** and use the dropdown menu to select **ShelXT**.



24. Click **OK**, then **OK** again to exit Experiment options.

25. Click **Start Experiment**.



26. CrysAlisPro will begin automatically processing the data every 25 frames and attempt a solution when the data completeness reaches ~40%.

Note: When the collection is finished allow it to run a final integration, scaling and AutoChem. You can find the AutoChem files (.res, .hkl and .cif_od) in the **struct** folder of your corresponding experiment.

- If the automatic data processing and solution are satisfactory, you can use the files in the **struct** to finish the structure. If you would like to manually process the data (we prefer to cutoff the resolution at 0.77Å or 0.83Å) then continue to the next section.



Experiment Strategy

Strategy Overview



1 Click and go to "Lattice Transformation" to change unit cell to triclinic P (aP).

Unit cell for Strategy Calculation - (CSD: install)

Cell: 9.681(4) 11.674(4) 12.262(5) 103.57(2) 95.84(2) 108.9(3) 1239.4(8) aP

Strategy parameters

☒ Resolution ☐ Theta ☐ 2Theta 0.800

☒ Laue group ☐ Other 1

☒ Friedel mates are equivalent (uncheck if data is anomalous)

Detector Distance 34.00

5 Only increase detector distance if crystal is twinned and there are a lot of overlapped spots.

Strategy mode ☒ Use run list sorting with

Complete data (default mode)

limit 100.0 IUCr limit Max 99.86 %

Generates runs that reach completeness limit

6 Change to **Complete redundant data** and change value to 4 if possible higher symmetry or 6 if actually triclinic.

8 Click Calculate New Strategy.

Current Strategy

No. runs/frames: 7/760

Total experiment time: 0h 07m

Expected experiment finish time: Tue Nov 19 19:37:06 2019

9 Make note of total experiment time and when the experiment will finish.

Calculate New Strategy

Update Completeness

Time prediction based on

☒ Fill time

☐ Fill I/sigma

☐ The same time for all theta positions

☐ Different time for each theta positions

7 Adjust exp time to a whole number or interval of 5 if you have OCD. OK to leave as is.

exp time	individual I/sigma:	merged I/sigma:
0.51	15.01	28.08

exp time	individual I/sigma:	merged I/sigma:
0.51	15.01	28.08

Total I/sigma:	15.01	28.08
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Predicted resolution beyond 0.80

Scan width: 0.50

10 Click Autochem/Movie/Cryo/Red to enable cryo shutdown and change Autochem settings.

Automatic experiment settings

Mode: not supported

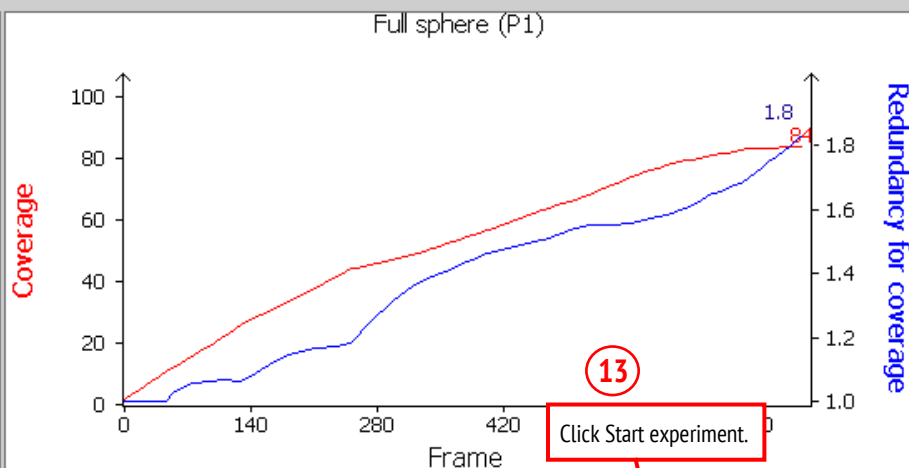
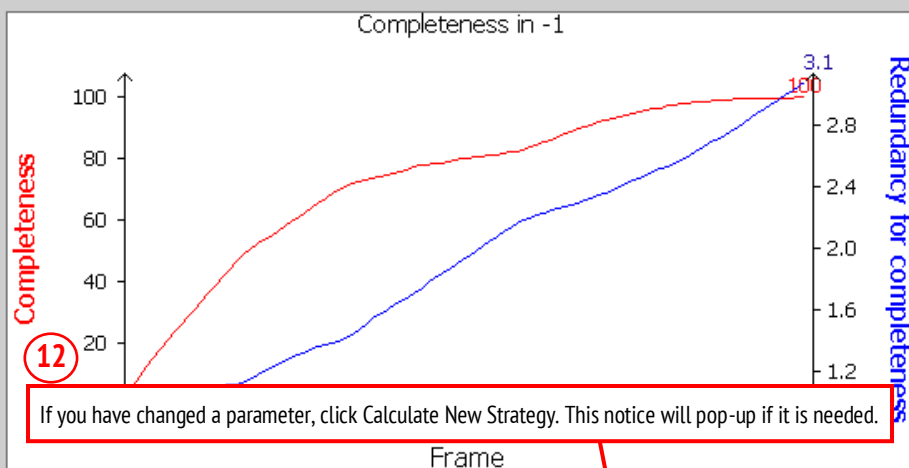
Frequency: no slicing

Options

Autochem/Movie/Cryo/Red

11 Click and enter a value if you'd like to see the "pile up" of data.

☒ Completeness/Coverage curves ☐ Completeness/Coverage tables ☐ Completeness/Predicted resolution



You have changed parameters, please click 'Update Completeness' or 'Calculate New Strategy'

Help

Start named experiment

Start experiment

Cancel

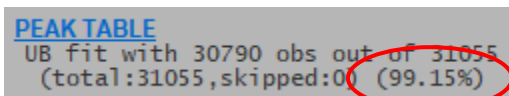
❖ MANUALLY PROCESSING THE DATA



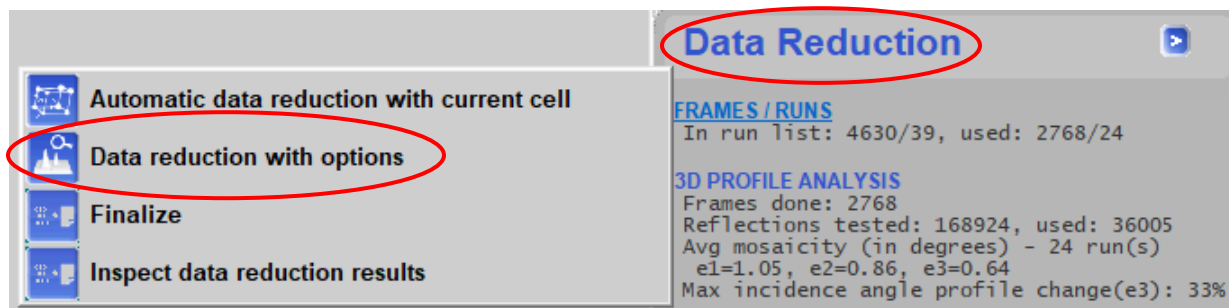
1. Launch CrysAlisPro (red) with the desktop icon.
2. Locate the experiment you are trying to open. Select it and click **Open selected**. (If it's not automatically listed, click **Browse experiment**, locate the folder, click on the **xxxx.par** file, click **Open**, and then **Open selected**.)
3. Left-click or move cursor on **Crystal RED**, then click **Full auto unit cell finding**.



4. Check that the **PEAK TABLE** has a good fit (80-100%). If not, you might have a twin and will need to process the data as a twin. This is done using the **Ewald explorer – reciprocal space** interface in the **Lattice wizard**.



5. Click **Data Reduction**, then move cursor over **Data Reduction**. Then select **Data reduction with options**.



6. The Proffit interface will open. Check that the selected cell and lattice extinctions match. Click [Next >](#).

Proffit: CrysAlisPro data reduction assistant (1.0.29)

Profile fitting data reduction

Step 1: Orientation matrix for data reduction

UB - matrix:

```

-0.008414  0.044896 -0.008842 ( 0.000002  0.000001  0.000001 )
 0.014892  0.017724  0.022602 ( 0.000001  0.000001  0.000001 )
 0.061712  0.001841 -0.002198 ( 0.000002  0.000001  0.000001 )
 11.25270 ( 0.00031 ) 14.68214 ( 0.00038 ) 29.56885 ( 0.00066 )
 89.99092 ( 0.00166 ) 100.16227 ( 0.00206 ) 90.00009 ( 0.00216 )
 4808.85

```

Selected cell (from UM xx/UM ttt/UM f):

23 11.2527 14.6821 29.5689 89.9909 100.1622 90.0009 mP

Lattice extinctions (filter Bravais lattice extinctions):

☒ Don't use filter (P-lattice)

☐ Use filter for:

Incommensurate structures:

☒ Normal data reduction (HKL)

☐ Single q-vector

☐ Other (reduction list)

Twinning/Multi crystal (activated by UM TWIN entries)

☐ Use automatic twin/multi crystal data reduction with the following components: ☐ Multi crystal

Components to use:

7. Step 2 is useful if you need to change the start or end frame (due to icing or other issues). Click [Next >](#).

Proffit: CrysAlisPro data reduction assistant (1.0.29)

Profile fitting data reduction

Step 2: Experiment run list for data reduction

Run list: D:\Goldberg\8010a\8010a

Image dir: D:\Goldberg\8010a\frames

#	type	start	end	width	exposure	omega	detector	kappa	phi	start	end
1	o	-9.00	70.00	0.50	5.00	-	5.07	37.00	120.00	1	158
2	o	-23.00	39.00	0.50	5.00	-	5.07	-99.00	150.00	1	124
3	o	-23.00	26.00	0.50	5.00	-	-5.38	-99.00	-90.00	1	98
4	o	41.00	84.00	0.50	5.00	-	5.07	-178.00	0.00	1	86
5	o	-22.00	15.00	0.50	5.00	-	-5.38	-99.00	-180.00	1	74
6	o	-57.00	-4.00	0.50	5.00	-	-5.38	-19.00	-150.00	1	106
7	o	-19.00	71.00	0.50	5.00	-	-5.38	37.00	-180.00	1	180
8	o	-13.00	13.00	0.50	5.00	-	-5.38	-99.00	30.00	1	52
9	o	-85.00	5.00	0.50	5.00	-	-5.38	-19.00	-30.00	1	180
10	o	-69.00	-38.00	0.50	5.00	-	-5.38	178.00	-150.00	1	62

By default the whole experiment will be evaluated. To modify this behaviour edit the run list -->

8. Click **Clear data from previous run** and **Clear all data from tmp** to delete all info from previous processing runs. Then click **Edit special pars**.

Profile fitting data reduction CRYSTALIS^{Pro}

Step 3: Basic algorithm parameters

Reflection position prediction

- ☒ Auto select optimal prediction approach on run basis
 - ☒ Follow model changes on frame by frame basis (moderate sample wobbling)
- ☒ Follow significant sample wobbling (2-cycle 3D peak analysis)
- ☐ Follow sudden (discontinuous) changes of sample orientation

Orientation search range (max 10 deg) Search steps/deg (max 10)

Edit special pars

Data from previous run of 'dc proffit'

3d profile information and/or integration results on the disk

Clear data from previous run

Scratch all tmp data of previous proffit run

Empty tmp folder from all analysis files including background

Clear all data from tmp

< Back Next > Finish Cancel Help

9. In the special parameters window, check **Use resolution limits** and click **Edit limits**.

3D intensity integration

- ☐ 2D profile fitting (recommended only for very strong diffraction data)
- ☒ 3D profile fitting (improves weaker data, default option)

Reflection positioning and integration

- ☐ Single wavelength only (recommended exclusively for data up to 1.5 Ang, i.e. large molecules)
- ☐ HKL check in 3D peak analysis (recommended when reflections are very close to each other)

Skip filters

Lorentz min = 0.0500 **Edit Lorentz min**

☐ HP cell opening reject 0.00 **Edit DAC angle**

☒ **Use resolution limits** **Edit limits**

d-value (Ang): inf- 0.77
2theta (deg): 0.00- 54.85

☐ Reject reflections with bad profiles (e.g. for HP data)

&

Exinction rules

No extinction rules specified **Show rules**

HINT: You can use DC EXTINGT to add extinction rules and DC CLEAREXTINGT to remove selected or all rules from the list

Extra corrections

- ☐ Apply inverse float correction (f.ex. undo flood field correction)
- ☐ Apply float correction (f.ex. additional flood field correction)
- ☐ Apply pixelwise absorption correction (prepared by DC ABSTORUM)
- ☐ Apply monitor renormalization ☐ Use file for monitor values

DC JETSHADOW (to visualize beforehand use 'beamstop mask')

☒ Use JetShadow **Edit parameters**

alpha: 30.00, beta: 0.00, jet_width: 13.00, jet_distance: 6.00

Profile fitting

Override integration mask size (generally not recommended, but smaller mask can be useful for strongly overlapping reflections e.g. twins) of original size

- ☒ Follow profile size changes with incidence angle
- ☐ Adjust masks according to prediction uncertainty (for high angle data)
- ☐ Print average profiles to history window

OK Cancel

10. Click **Edit high limit** and change to **0.77Å** for heavy atom compounds and **0.83Å** for light atom compounds (no element heavier than Cl). Then click **OK** and **OK** in the special parameters window.

Resolution limits

Parameter to enter: d-value (Ang)

Edit low limit **Edit high limit**

d-value (Ang):	inf	0.770
Theta (deg):	0.000	27.425
2 Theta (deg):	0.000	54.850

OK Cancel

11. Select **Smart background** and click **Next >**. This is used if the X-ray background is high and/or irregular throughout the dataset. Automatic integration uses **Average background**.

Proffit: CrysAlisPro data reduction assistant (1.0.29)

Profile fitting data reduction

Step 4: Background evaluation

Background for 3D centroids
For an accurate evaluation of integrated intensities a good background determination is essential. Two parameters control this evaluation: The evaluation range Re and the repeat frequency Fr.

Re = 50 [Edit Re](#) Fr = 50 [Edit Fr](#)

Binning may reduce the memory requirements for the background evaluation. Default is 1. You may use 2 or 4 in case of lack of physical memory on your machine (risk of swapping)!

☐ 1 ☐ 2 ☐ 4 ☒ Reduce background accumulation to SHORT type (saves memory)

Required disk/memory space for background evaluation: 42.7/18.9 Mb

Background for 3D integration

☐ Average background from 3D centroid evaluation (good for stable & low background, fast)

☒ Smart background (combination of local and average background computation, good for weaker data with high background and locally varying features, e.g. protein data, slower)

Frame range = 1 [Edit range](#) Memory usage: 4.0 Mb

[< Back](#) [Next >](#) [Finish](#) [Cancel](#) [Help](#)

12. Check the outlier rejection and make sure it matches previous lattice selections. Click **Next >**.

Proffit: CrysAlisPro data reduction assistant (1.0.29)

Profile fitting data reduction

Step 5: Outlier rejection

CCD data sets usually contain more than the unique data required for the structure determination. This redundant data can be used to check for measurement outliers. The rejection is based on R. Blessing (1997), J. Appl. Cryst. and additional CCD specific criteria.

Outlier rejection

☐ Don't use outlier rejection

☒ Use outlier rejection:

☒ 2/m (b-unique)

mP 11.25270 14.68314 29.56885 89.99092 100.16327 90.00086

☒ Use Friedel mates as equivalent

[< Back](#) [Next >](#) [Finish](#) [Cancel](#) [Help](#)

13. Click **Change output name** and enter a new output name (e.g. 8010a_v1), Click **OK**. This is important so no files will be overwritten.

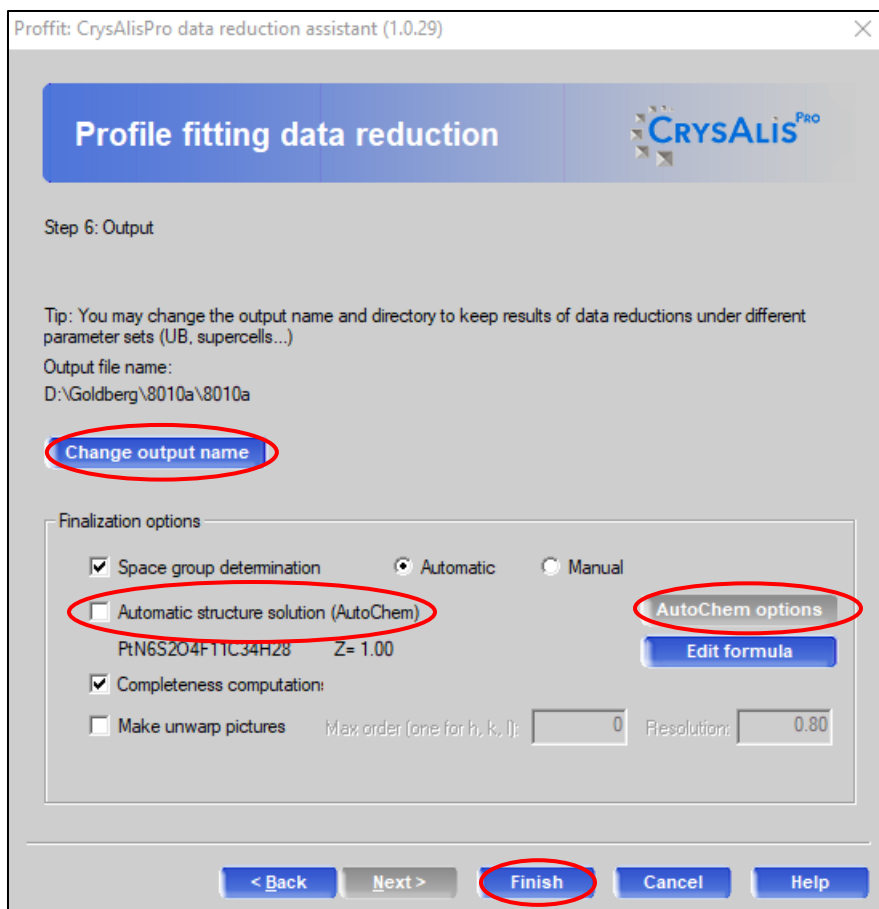
14. Check the **Automatic structure solution** box. This will generate a new **struct** folder with your new output .res and .hkl.

Note: If you forget to select this, only an .ins and .hkl will be generated in the primary experiment folder. Open the .ins in Olex2 and SOLVE with ShelXT.

15. Click **AutoChem options** and select **Single Trial** and **ShelXT**, then click **OK**.

16. Click **Finish**. CrysAlis^{Pro} will integrate, determine the space group and scale.


17. If the resulting .res and .hkl files in the corresponding **struct** folder are satisfactory, you may finish refining the structure.



18. To further inspect the data collection and reduction results, click in the left toolbar.

❖ USEFUL NOTES AND COMMANDS



- To go to Home position, click  in the left toolbar. Make sure CCD tab is selected and type “gt a 0 0 0 0 180” (goto all omega# theta# kappa# phi# detector distance#)
- If you forget to select Auto cryo device shut down during the Strategy setup, you can do so after the experiment has started. Click **Data Collection** and click on “**Off: Autocryo Shutdown**”. It should now read “On: Autocryo Shutdown.”