# Example: 13.3 α-glycine

**Last update 29.06.2023**

| Data | Topic | Level |
|---|---|---|
| Electron diffraction (continuous rotation) | Data reduction, structure solution, refinement | Basic |

## Input data

**Data**

Electron diffraction data were measured on a transmission electron microscope JEOL JEM-2100-$LaB_6$ (hybrid pixel detector from ASI, 200 kV acceleration voltage, $\lambda$ = 0.02508 Å).
164 frames, each covering a goniometer rotation of $\Delta\alpha$ = 0.3558°, exposure time = 0.3 s
Every 20$^{th}$ frame was recorded with a defocus to check for the crystal position.

**Input files**

Folder "tiff": contains measured diffraction patterns in TIF format
Folder "reference_cif_pets": reference PETS output files for structure determination

**Additional information**

glycine.pts as input file to PETS2:

```
lambda    0.025080          # wavelength
geometry continuous         # diffraction geometry: continuous-rotation
omega  230                  # orientation of goniometer axis
phi  0.18000                # semi-angle of the covered angular range Δα of one frame
virtualframes   7 5 1
# The idea of virtual frames is to assure that the dynamical calculation of rocking curves avoids that incomplete or partial intensities are determined. Only fully integrated intensities should contribute to the refinement. 7 frames are (virtually) combined to form one virtual frame. Two subsequent virtual frames have an offset of 5 frames.

aperpixel    0.005340
noiseparameters      2.5000      1.0000

center    234.87    233.17
beamstop no

dstarmax  1.800
dstarmaxps  2.0
i/sigma    7.00    5.00
reflectionsize  8.0

referencecell    5.08760    11.80920    5.46150    90.00000    111.99200    90.00000 1
#reference lattice parameters
```

## Keywords

Continuous rotation, in-situ crystallization

## References

For further information about the data processing in PETS2, see:
- L. Palatinus et al. Specifics of the data processing of precession electron diffraction tomography data and their implementation in the program PETS2.0. Acta Cryst. B 75: 512-522 (2019).
-PETS2 manual pets.fzu.cz/download/

For further information about the dynamical refinement and associated parameters:
- L. Palatinus et al. Structure refinement using precession electron diffraction tomography and dynamical diffraction: theory and implementation. Acta Cryst. A71: 235–244 (2015).
- L. Palatinus et al. Structure refinement using precession electron diffraction tomography and dynamical diffraction: tests on experimental data. Acta Crystallogr B71: 740–751 (2015)

## Highlights

None

# PART 1 - Data reduction in PETS2

## 1. *Peak search*

Start PETS2 by double clicking the executable.
Main menu bar: "File"→"Open"
Open the file glycine.pts
Open the "Parameters" menu by clicking on the arrow ("v")
*All important parameters are already set in the input file.*
Click the action button “Peak search”.

Notes

*The progress can be followed in the "Image data" panel.*
*In the console, for each frame the estimated position of the primary beam, the number of Friedel pairs and the number of significant peaks is given. If you cannot see the console, activate View → Panels → Console.*
*The process ends with the following message in the console:*

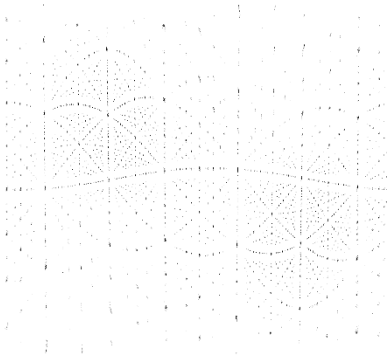
```
Minimum, average and maximum position of the primary beam:  234.74
236.10  236.63  |    232.08  233.24  233.65
Finished reading   3274 peaks from the file glycine_petsdata/glycine.rpl.
```

## 2. *Tilt axis and peak analysis*

Click on the action button “Tilt axis”.

Notes

*In the “Cylindric projection” panel, a cylindrical projection of the difference space of the extracted peak positions is displayed. A correct azimuthal angle refinement results in the image containing sharp peaks aligned on sinusoidal curves. This step provides a first estimation of data quality. The refined omega angle is 230.301 degrees.*



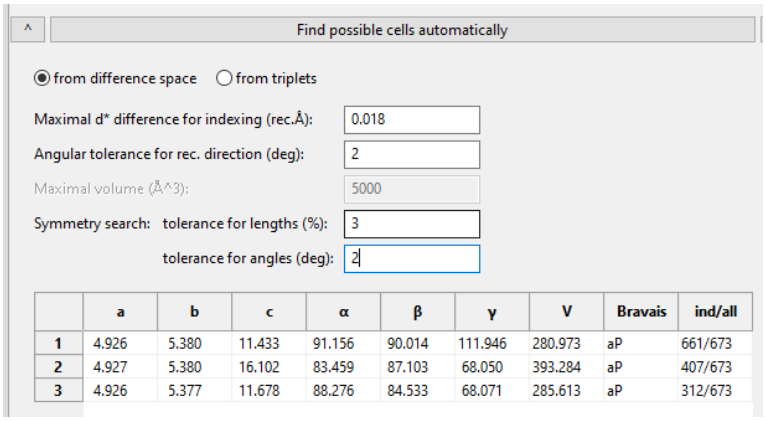
Click the action button “Peak analysis”.
Click “Peak analysis (continue)”
Click “Peak analysis (continue)”

## 3. *Find unit cell and orientation matrix*

Click “Find unit cell and orientation matrix”
Click on “Find possible cells automatically”

Notes

*A triclinic unit cell is found with α and β close to 90 degrees, and γ =11.946°. PETS did not suggest a monoclinic unit cell because the tolerance settings are too tight.*




*Increase "tolerance for angles" to 2 degrees*

Click on "Find possible cells automatically"

Notes

*Now the found unit cell is recognized as monoclinic.*

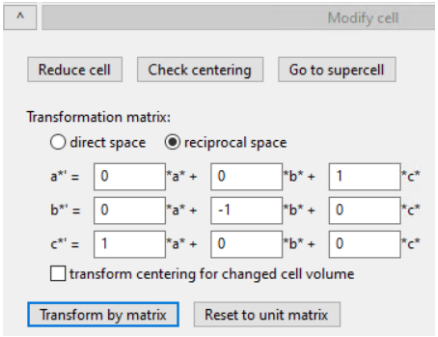
*However, α-glycine is usually described in a cell with a ≈ 5 Å, b = 11.4 Å, c = 5.4 Å.*

Open the "Modify cell" menu by clicking on the arrow ("v")

Define the following transformation matrix:

a*' = c*, b*' = -b*, c*' = a*

And Click "Transform by matrix"




Go back to the indexing menu and

Click "Refine Cell"

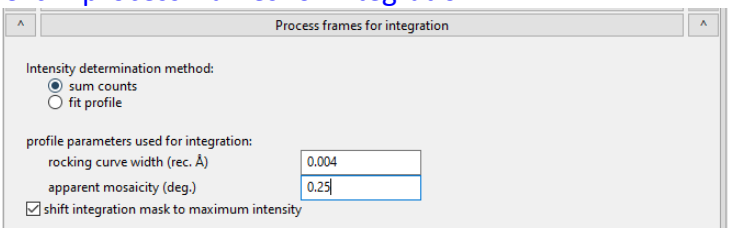
| | a | b | c | α | β | γ |
|---|---|---|---|---|---|---|
| cell: | 4.9262 | 11.4306 | 5.3792 | 88.830 | 111.945 | 89.995 |
| s.u.: | 0.0009 | 0.0052 | 0.0011 | 0.028 | 0.015 | 0.027 |

Leave the indexing panel by clicking "Finish"

## 4. *Integrate intensities*

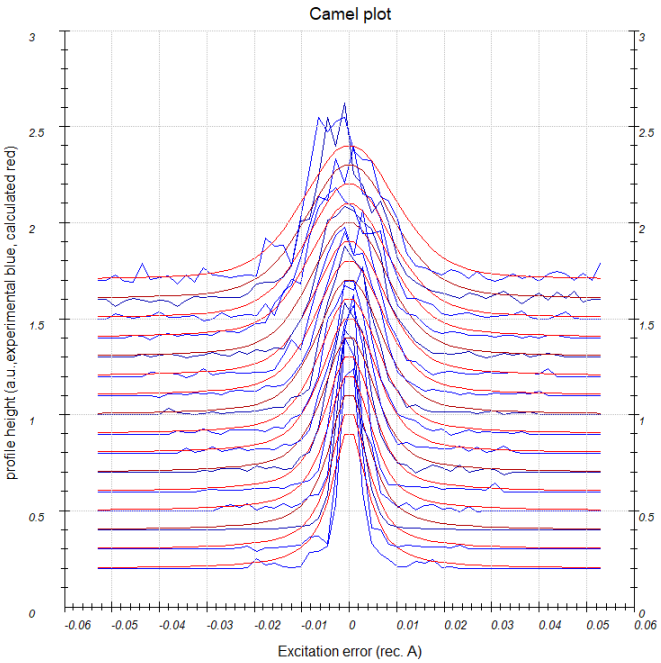
In the options of "Process frames for integration" accessible by clicking one of the side-arrows of the action button, increase RC width to 0.004 (rec. Å) and the apparent mosaicity to 0.25 (degrees).

Click "process frames for integration".



Notes

*We will assume an extremely broad rocking curve to make sure that reflections are integrated on all frames and later adapt the profile parameters.*




Notes

*The expected profile (red) is too broad. We first look at the lowest-resolution shell at the bottom. Reflections at this resolution are hardly affected by the mosaicity and thus are suitable to estimate the RC width.*

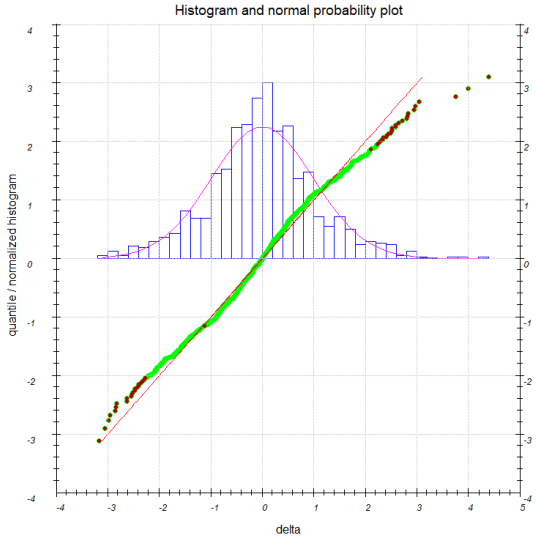
Open options for “Optimize reflection profile”.

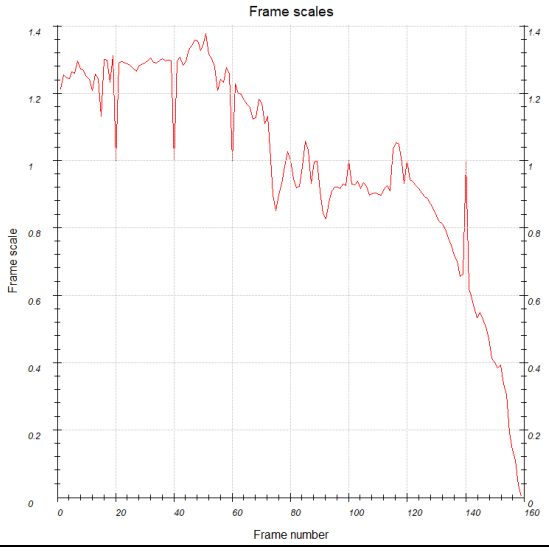
Run the refinement.

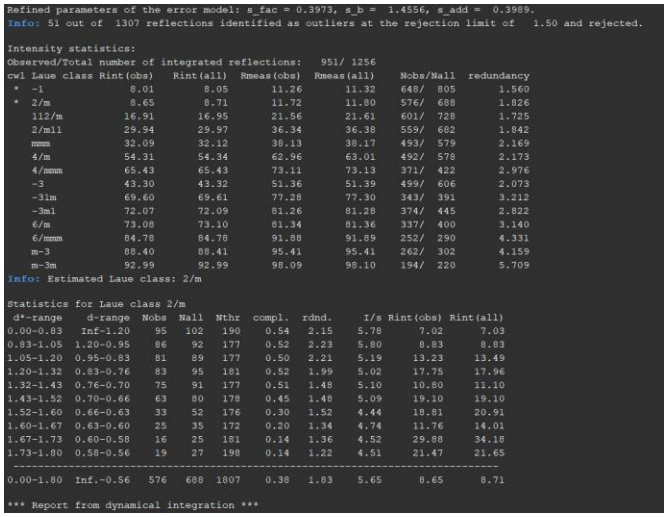
Click "Finalize integration" keeping all the parameters by default.

Notes

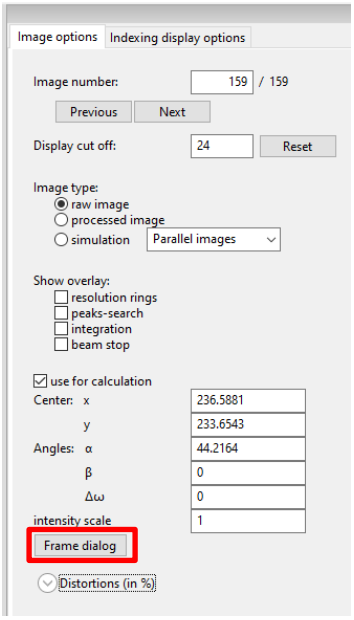
*In the console, a list appears summary of the integration. PETS2 automatically recognized Luae class 2/m, performed frame scaling using this symmetry and for kinematical integration it refined error model and rejected reflections with intensities too far from the group of the symmetrically equivalent reflections (5%). Inspect the “Frame scales” graph. The last frames have very low scale – remove them.*



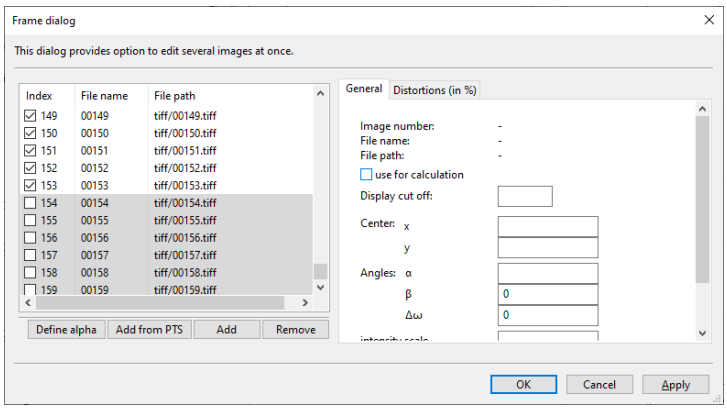






Open the frame dialog by clicking on the “Frame dialog.

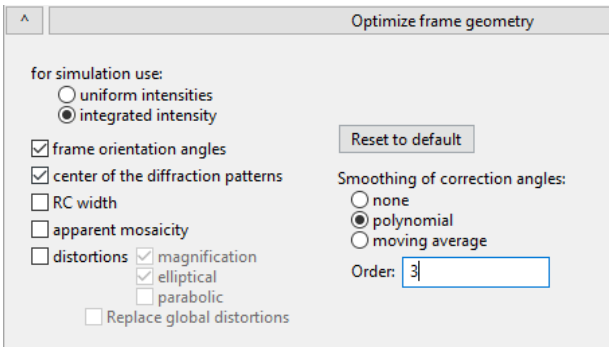



Mark frames 154 to 159
Deactivate the checkbox "use for calculation"
OK



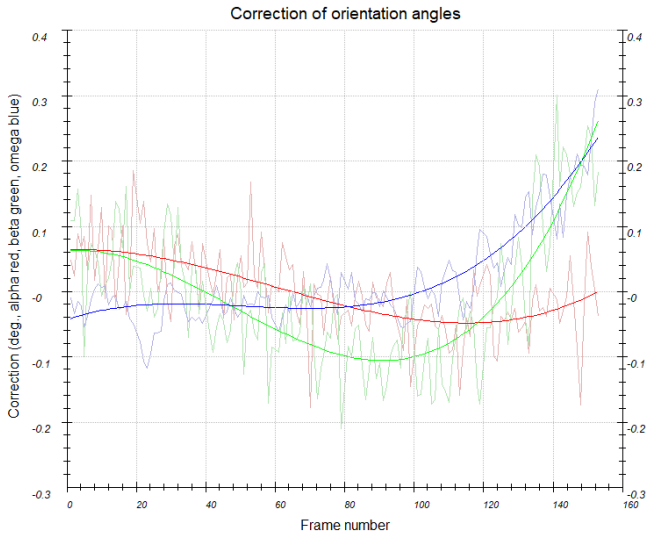

## 5. *Optimize frame orientation*

Open the "Optimize frame geometry" menu by clicking on the arrow ("v").
Set the options to match the image below.




Notes

*The frame orientation (alpha-tilt, beta-tilt and omega angle) and DP centers are optimized. You can evaluate the success and the progess of this optimization by looking at the following curves in the Graph tab:*
*-Tilt corrections: with Δalpha and Δbeta-tilt frame by frame. You should see a trend.*
*-Origin corrections: corrections applied on x and y coordinates of the center frame by frame.*




Open the "Find unit cell and orientation matrix" menu by clicking on the arrow ("v")
At the top "Data used for indexing": select "cor"
Click "Refine cell"
*This refined the orientation matrix taking the new frame orientations into account.*

| | a | b | c | α | β | γ |
|---|---|---|---|---|---|---|
| cell: | 4.9226 | 11.4504 | 5.3809 | 88.998 | 111.942 | 90.123 |
| s.u.: | 0.0003 | 0.0070 | 0.0005 | 0.018 | 0.006 | 0.016 |

Click "Finish"

## 6. *Remove suspicious frames*

*We only want to use reflections from frames that were properly measured.*
Go to the "Image data" panel
Select radio button "processed"
At the bottom menu activate "si" to see the integration masks
Set the "Display cut off" to 10
Set the "Frame" to 1, i.e. 1 of 159

Notes

*We can clearly see that on Frame 1/159 the bottom has a dark area with ~0 counts suggesting that something, probably a part of the TEM grid, blocked the electron path between the sample and the detector in this area. Though a part of the frame, it is easier to remove the complete frame.*

Set the "Frame" to 2, i.e. 2 of 159

Notes

*The not-illuminated area has moved a bit downwards, but still a significant fraction of the detector was not illuminated and thus the reflection intensities are not properly determined in this area. The same holds for frames 3, 4, 5, 6 and 7. On Frame 8 the bottom left corner shows still shadow, but the area is very small.*

Open the "Frame dialog" In the "Image options" tab
Mark frames 1 to 7 (using the SHIFT key and the mouse or up/down keys)
Deactivate the checkbox "use for calculation"
OK
Run again "Process frames for integration".

## 7. *Generate output file for structure determination*

Run “Optimize reflection profile”.
Click on “Finalize integration”

```
Refined parameters of the error model: s_fac = 0.3587, s_b =  3.4505, s_add =  0.1782.
Info: 55 out of  1210 reflections identified as outliers at the rejection limit of   1.50 and rejected.

Intensity statistics:
Observed/Total number of integrated reflections:   785/ 1155
cwl Laue class Rint(obs)   Rint(all)  Rmeas(obs)  Rmeas(all)    Nobs/Nall  redundancy
 *  -1            6.99        7.04        9.70        9.78    531/  748       1.544
 *  2/m           8.48        8.54       11.00       11.08    485/  662       1.745
    112/m        15.98       16.02       20.12       20.17    502/  687       1.681
    2/m11        29.51       29.52       35.56       35.59    465/  641       1.802
    mmm          31.99       32.01       37.65       37.68    424/  563       2.052
    4/m          51.36       51.34       59.69       59.68    420/  557       2.074
    4/mmm        60.88       60.86       68.10       68.09    318/  414       2.790
    -3           38.53       38.53       45.69       45.70    424/  575       2.009
    -31m         67.51       67.49       75.13       75.12    300/  376       3.072
    -3m1         68.56       68.54       77.08       77.07    318/  425       2.718
    6/m          71.89       71.86       79.93       79.91    290/  378       3.056
    6/mmm        84.01       84.00       91.10       91.09    220/  276       4.185
    m-3          84.91       84.91       92.48       92.48    242/  296       3.902
    m-3m         91.57       91.56       96.89       96.88    176/  216       5.347
Info: Estimated Laue class: 2/m

Statistics for Laue class 2/m
 d*-range     d-range  Nobs  Nall  Nthr  compl.  rdnd.     I/s Rint(obs) Rint(all)
0.00-0.83   Inf-1.20    86    94   190    0.49   2.07   13.22      5.25      5.26
0.83-1.05  1.20-0.95    82    88   178    0.49   2.11   12.07     14.86     14.87
1.05-1.20  0.95-0.83    73    87   172    0.51   2.07    8.79      9.05      9.16
1.20-1.32  0.83-0.76    70    96   187    0.51   1.85    7.20     12.00     12.39
1.32-1.43  0.76-0.70    56    85   172    0.49   1.51    6.89     10.08     10.40
1.43-1.52  0.70-0.66    49    77   180    0.43   1.38    6.93      7.18      7.42
1.52-1.60  0.66-0.63    24    52   175    0.30   1.44    4.36     18.89     22.35
1.60-1.67  0.63-0.60    20    34   167    0.20   1.38    5.26     18.15     18.58
1.67-1.74  0.60-0.58    15    26   188    0.14   1.27    4.09     31.15     32.62
1.74-1.80  0.58-0.56    10    23   197    0.12   1.17    4.32      8.38      8.38
 ------------------------------------------------------------------------------
0.00-1.80  Inf.-0.56   485   662  1806    0.37   1.74   11.41      8.48      8.54

*** Report from dynamical integration ***
```

Notes

*The Rint(obs) is now 8.48%. These stats confirm the point group 2/m.*
*Two output files (apart from the log files) are generated:*
*glycine.cif_pets is the list of reflections for structure solution and kinematical refinement.*
*glycine_dyn.cif_pets is the list of reflections for dynamical refinement.*

Click on “File” → "Save"

*All the refined parameters and graphs are saved also in the Log file, which are accessible after the job is again opened or you can access them in glycine_petsdata\log folder.*
Close PETS2

# PART 2 – Structure solution and kinematical refinement

## 1. *Create new structure*

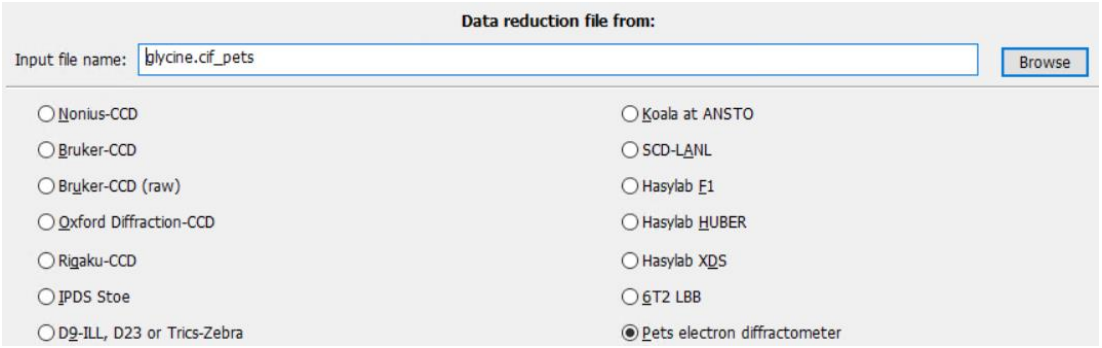
| Notes |
| --- |
| *Important! The data-processing procedure is almost never perfectly reproducible. Small differences in the indexing and cell refinement procedure may result in small differences of integrated intensities. If you want to be sure that you can reproduce the following part of the tutorial, it is recommended to use the file "glycine.cif_pets" in the folder "reference_cif_pets" provided with the tutorial files. Using your own cif_pets file is also possible, but your results may slightly differ from the results described in this tutorial.* |

Start Jana2020
Main menu bar: "Structure" → "New"
Enter “glycine” as filename; “open”

## 2. *Import Wizard*

| Notes |
| --- |
| *The data import is automatically started.*<br>*[On the screen: Specify type of the file to be imported]* |

Select “known diffractometer formats”; NEXT
Select "Pets electron diffractometer"
Make sure that "Make the reflection file for dynamical refinement" is NOT checked
"Browse" for the file glycine.cif_pets; “Open”; NEXT




| Notes |
| --- |
| *[On the screen: Complete/correct experimental parameters]*<br>*The unit cell parameters, radiation type and wavelength are correctly set. The sample was measured at T = 100 K. The temperature has no effect on the structure solution or refinement.* |

NEXT

| Notes |
| --- |
| *[On the screen: Define the reference cell]*<br>*We do not want to change anything.* |

NEXT
*1155 input reflections were properly handled.*
OK

*[On the screen: Define parameters for absorption and scaling procedure]*

NEXT

Notes

*The import wizard is complete. As a next step you can import another or modify the previously imported ones.*

FINISH; OK

## 3. *Symmetry wizard*

Notes

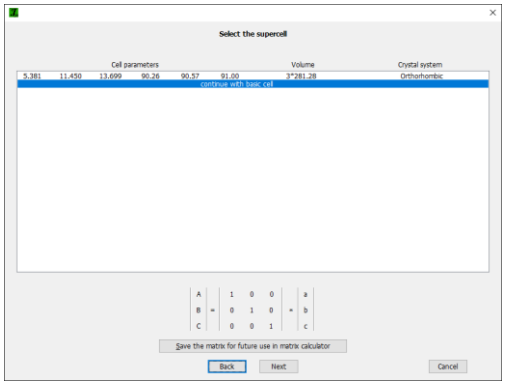
*The symmetry wizard starts automatically after the import wizard. You may alternatively start the symmetry Wizard by expanding "Reflection file" in the Command tree. There, double click on "Make space group test".*

NEXT

"Maximal deviation for cell angles in degs": 1.5

NEXT;

Continue with basic cell




Notes

*If the monoclinic point group is not shown it means that in the previous step the allowed deviations were too strict.*

Select the point group "2/m"; NEXT

Notes

*[On the screen: Select cell centering]*

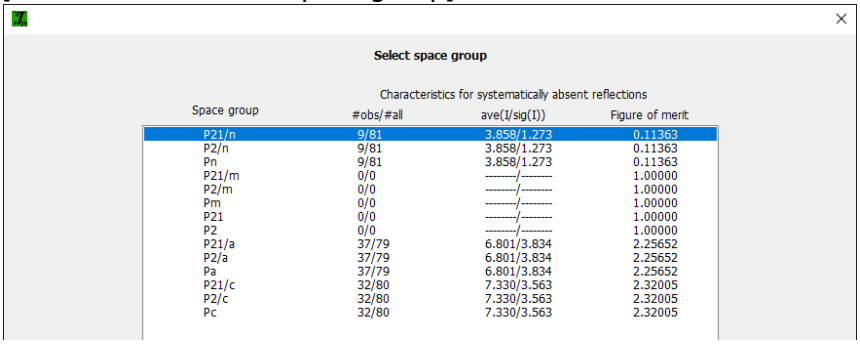
*We assume a primitive unit cell.*

Select the radio button "P"

NEXT

Notes

*[On the screen: Select space group]*




*The space group should be selected based on the analysis of reciprocal space. You may do so in PETS2 with the option "Reciprocal-space sections". From the reciprocal-space*

*sections, we could derive the space group P21/n. Space group determination is not part of this tutorial.*

Choose the space group “P21/n”; NEXT

*[On the screen: Final step of space group test]*

Leave “accept the space group transformed into the original cell”; FINISH

Notes

*[On the screen: Processing refinement reflection file for Block1]*

*In the next step the reflection file is generated from the hkl input file taking the determined symmetry into account.*

NEXT;

Notes

*784/1155 reflections read from input file*

*775/1074 reflections written to output file*

OK; OK;

(At the bottom) "Sigma(I(ave)) from": leave "Poisson"

Notes

*PETS2 provides uncertainties based on detector and counting statistics, which can be modified by error model refinement and outliers will be rejected. This was done during data processing so the expected sigmas should be close to normal distribution.*

NEXT

Notes

*Summary after averaging*

*Rint(obs/all) = 6.40/6.45 for 476/616 reflections …*

OK; FINISH;

OK to start the structure solution wizard

## 4. Structure solution

Notes

*The structure solution setup windows open automatically after the space group determination. You may alternatively start the structure solution by expanding “Structure solution” in the Command tree. There, double click on “Rund Superflip”.*

Enter the chemical formula: C2 H5 N O2

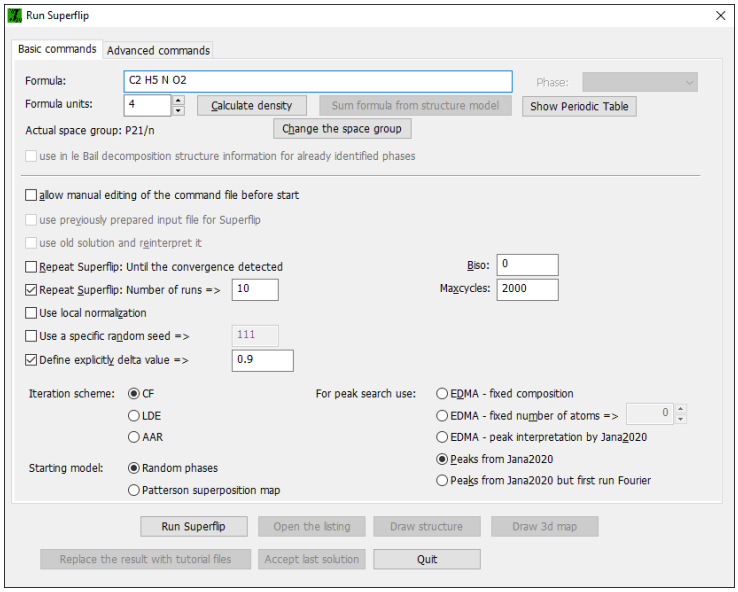
Formula units: 4

Activate “Use local normalization”

Iteration scheme: CF

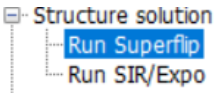
Starting model: Random phases

For peak search use: EDMA – fixed composition

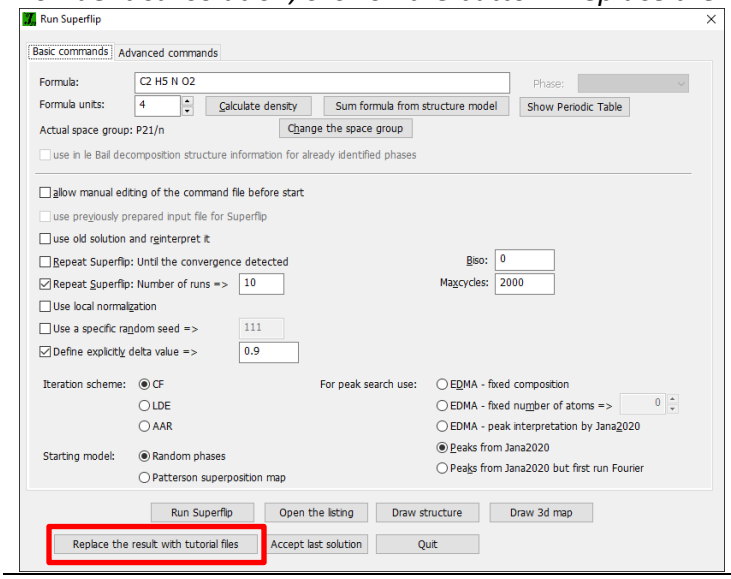



## Notes

"Run superflip"

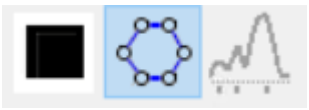



*The structure is solved by superflip. The solution is never the same in superflip as it starts from random phases. Therefore, there are files, where the solution for this tutorial is saved. For identical solution, click on the button "Replace the result with tutorial files".*



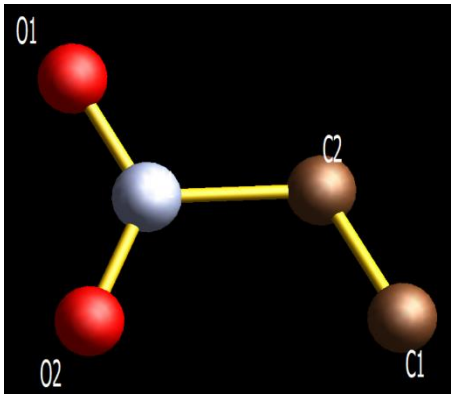

OK
"Draw structure"
"View along": b
Click on the red "X" to close Vesta
"Accept last solution"

Open JanaDraw

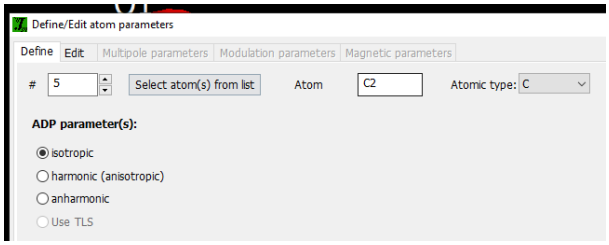


## Notes

*All the atoms in the solution (tutorial) are correctly assigned. If you did not use provided solution, assign the atoms correctly. The oxygen atoms should be bonded to a carbon (sp2) atom, and the terminating atom is nitrogen. To correct the atom types*

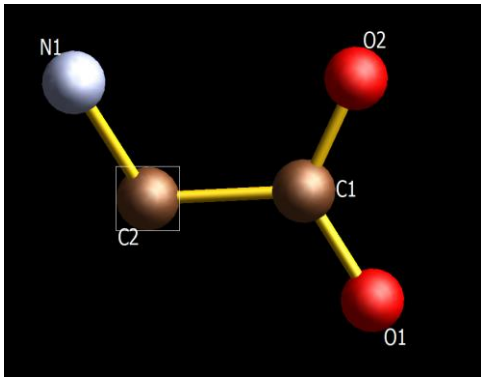



Double click an atom and change the “Atomic type”. Do not forget to correct also the labels. OK.



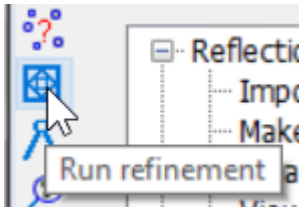

Click "Select all" to select all atoms of the list
Click "Action" -> "Rename atoms - atom_type+number"
OK; YES

*Now the structure should look like the one shown below.*




## 5. *Kinematical refinement*

Right-click the quick button “refinement” (on the left panel) to acess the “Refinement commands” window




Uncheck the checkbox "Refinement on F(obs)**2"
OK; YES+START
*The refinement converges with R(obs) = 19.45% and wR(all)= 26.38 %*
Click JanaDraw
Right-click the carbon atom C2 (which is bonded to another C atom and the nitrogen atom) -> "Adding hydrogen atoms – automatically"; OK;
"Run refinement"

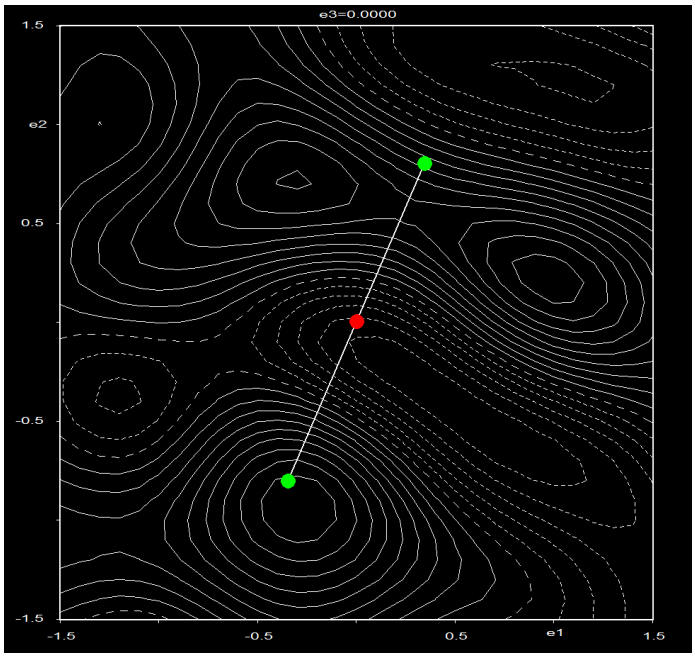
Notes

*The refinement converges with wR(all)= 26.14*
*The structure is not charge balanced yet and three hydrogen sites must be identified. The C-O distances are rather similar. If there is a hydroxyl group, there must be 2 hydrogens bonded to the terminal N1. It is well known that the hydrogen of OH of glycine molecules in the solid state migrate to the nitrogen atom, so that the terminating nitrogen is part of $(NH_3)^+$*

In JanaDraw, click on N1 so that only this atom is selected
Right-click the terminal N1 -> "Adding hydrogen atoms – interactively"; OK;
*By default, Jana expects N to be bonded to one non-H neighbor (C2) and two hydrogen sites*
Click "Locate positions in map"

Notes

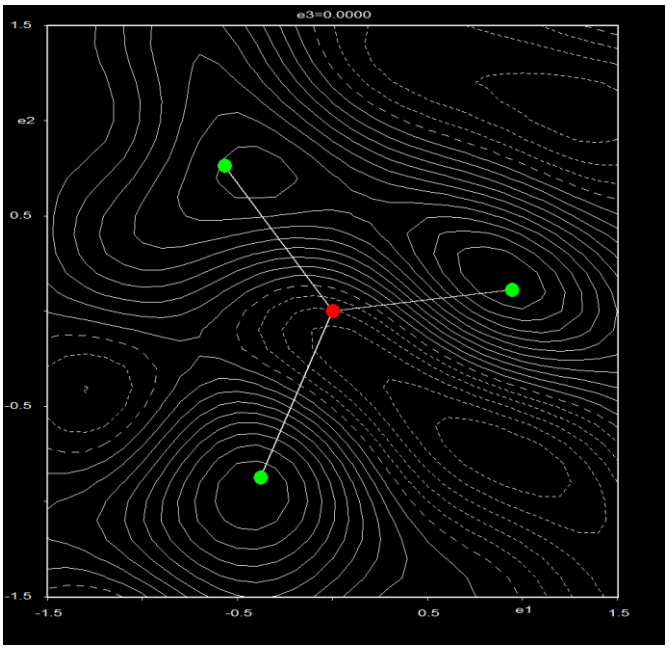
*The difference Fourier map shows the nitrogen site (red) and the two calculated hydrogen sites (green). The map only fits well to the calculated hydrogen site at the bottom, but not so well in the upper part.*




Close the Fourier map by clicking the red X
*[On the screen: Adding "hydrogen" atoms for "N1"]*
Select the radio button "Tetrahedral"
Click "Locate positions in map"

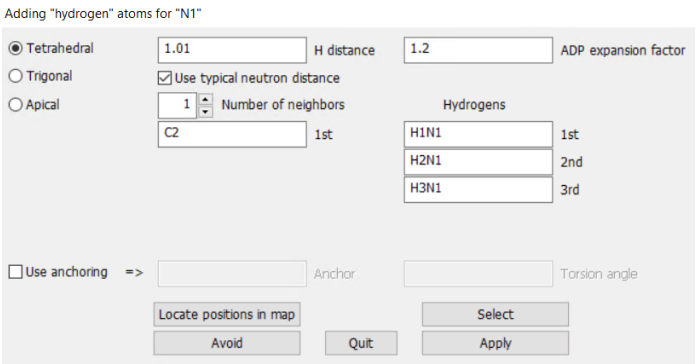
Notes (Cookbook normal)

*The difference Fourier map shows the nitrogen site (red) and the 3 calculated hydrogen sites (green). This map fits much better to the expected hydrogen coordinates. Given that it is a kinematical refinement based on one (rather incomplete) data set, this match is very good and confirms the (expected) $(NH_3)^+$ terminal group.*




Close the Fourier map by clicking the red X

*[On the screen: Adding "hydrogen" atoms for "N1"]*




APPLY

Notes

*Constraints were automatically written. You can see them by opening the M50 file with an editor or in Refinement -> Refinement commands -> Restraints/Constraints -> Keep commands*

Run refinement

Notes

*The refinement converges with wRall= 21.91*

With the left CTRL key pressed, select all C, N and O atoms

Right click anywhere except on atoms -> Define/Edit atoms

ADP parameter(s): "harmonic (anisotropic)"; OK;

At the bottom menu of JanaDraw click on "Draw Ellipsoids" button 

"Run refinement"

*The ADPs are not acceptable. This is due to the dynamical effects.*

Reset the ADPs to isotropic
In the top menu bar: "Structure" → "Save as"
File name: "gylcine_dyn"; SAVE
*[ On screen: Do you want to continue with the new structure?]*
YES

## 6. Data import for dynamical refinement

Expand "Reflection file" -> "Import/modify reflection file" (double click)
Click "Delete"; OK;
"Reflection file" -> "Import/modify reflection file" (double click)


Select "Single crystal": "known diffractometer formats"; NEXT
Select "Pets electron diffractometer"
Click "Browse"; Locate "glycine_dyn.cif_pets"; OPEN
Check "Make the reflection file for dynamical refinement"; NEXT

| Notes |
| --- |
| *[On the screen: Complete/correct experimental parameters]*<br>*You may set the Temperature to 100 (Kelvin). This has no influence on the refinement.* |

NEXT; NEXT;
*All 3134 input reflections were properly handled.*
OK; NEXT; FINISH; OK

| Notes |
| --- |
| *[On the screen: Processing refinement reflection file for: Block1…]*<br>*Note that "Make the reflection file for dynamical refinement" is checked.* |

NEXT;
`2424/3134 reflections read from input file …`
OK; OK; FINISH

## 7. Dynamical refinement setup

In the command tree (left), expand “Edit structure parameters” → “Edit parameters for electron diffraction” (double click)

| Notes |
| --- |
| *[On the screen: many options starting with orientation matrix]*<br>*In the top section on the left:* |

Set “Maximal diffraction vector g(max)” to 1.8

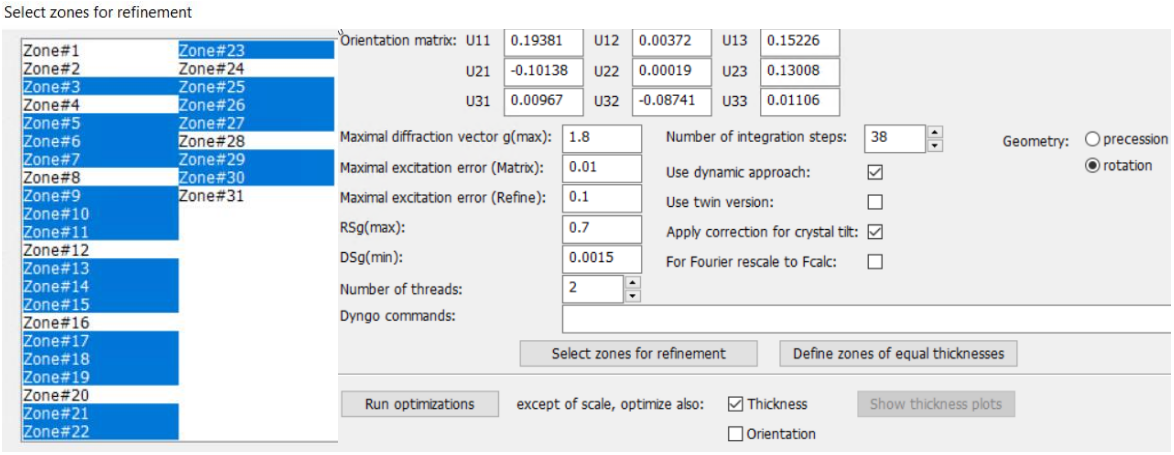
| Notes |
| --- |
| *This g(max) is related to the resolution of the dynamical calculations, which should in general be a bit higher than the resolution of the highest reflections used in the refinement.* |

Set “RSg(max)” to 0.7
Leave “DSg(min)” at 0 $Å^{-1}$

| Notes |
| --- |
| *RSg(max) and DSg(min) filter out reflections for which an incomplete or unreliable rocking curve integration is expected, e.g. reflections that lie on or are close to the goniometer rotation axis.* |

Set “Number of threads” to the number of physical cores of your processor
*In the top section on the right:*

Set “Number of integration steps” to 38
Set “Geometry” to “rotation”
*In the section in the middle:*
"Select zones for refinement"
Unselect zones 1, 2, 4, 8, 12, 16, 20, 24, 28, 31 (using the CTRL key + mouse click)




| Notes |
| --- |
| *These are the virtual frames of which at least one frame was deactivated during the data reduction.* |

“except of scale, optimize also”: check “Thickness”
Click “Run optimizations”

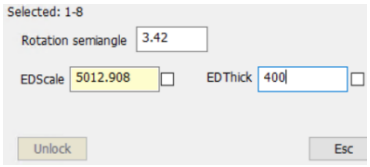
| Notes |
| --- |
| *The dependence of R factors of individual virtual frames on the thickness is determined. The initial thickness estimation is necessary to get a stable starting point for the dynamical refinement and to avoid getting stuck in a local minimum.* |

Click "Show thickness plots"

| Notes |
| --- |
| *You can look at the dependence of the R factor on the thickness for the different frames. The best R factors are typically found with a thickness of about 400 Å or in the range 200 to 600 Å.* |

Close the plot (red X)
Click on “Select zones for editing”; “Select all”; OK.

| Notes |
| --- |
| *The EDThick box is yellow and locked because the parameter is not the same for all selected zones.* |




Click EDThick box; click Unlock and change the value to 400.
*This changes EDThick for all zones to 400 Å.*
Click on “Select zones for editing”; “Refresh”; OK
*By changing number of zone by “Zone#” textbox, we can see that all zones have EDThick 400 Å*
Uncheck the checkbox “Thickness”
Click on “Run optimizations”
*This will optimize the scale factor for each frame based on a thickness of 400 Å.*

OK; YES to save m42 file.

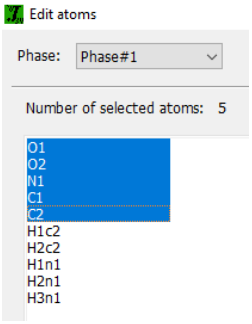
## 8. Dynamical refinement

"Refinement" -> Refinement commands
Check for convergence: stop if 0.5 in 1 consecutive cycles.
Deactivate "Refinements on F(obs)**2
OK; YES+START

| Notes |
| --- |
| *The refinement converges with wR(all) of 12.11%.* |

## 9. Refinement with anisotropic ADPs

Set ADPs of the non-hydrogen atoms to be harmonic.
In Edit atoms choose the atoms of interest.
Press Define/Edit button.
Choose harmonic (anisotropic) for ADP parameter(s).




OK; OK; Yes.
Open JanaDraw.
Refine the structure.
*Refinement converged to wR(all) equal to 10.90%.*

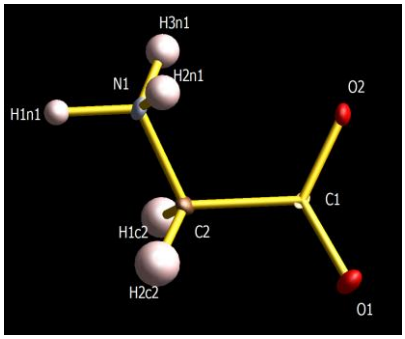
## 10. Refinement without constraints

In the Command tree, expand "Refinement "
"Refinement commands" (double click) -> Restraints/Constraints
Click "Keep commands";
"Refresh"; "Select all"; "Disable"; OK;

| Notes |
| --- |
| *Note that the button "Keep commands" is now labelled "Keep commands [0+4!]", indicating that there are 0 active "keep" commands and 4 disabled "keep" commands.* |

OK; YES+START

| Notes |
| --- |
| *Refinement converges to R(obs) 7.72% and wR(all) 10.17%.*<br>*All hydrogen atoms refined freely while maintained the expected geometry.*<br>*For comparison, you may also try an unconstrained kinematical refinement as continuation of step 5. Or you may try to locate the hydrogen sites from the difference Fourier map in a dynamical refinement after removing the hydrogen sites.* |


H3n1
N1
H2n1
H1n1
O2
C1
H1c2
C2
H2c2
O1