PETS

Process Electron Tilt Series User Manual

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A. INTRODUCTION

PETS is a computer program for the processing of a series of diffraction images. It is intended for electron diffraction and more specifically for electron diffraction tomography data (EDT), possibly combined with precession electron diffraction. In principle it could be used for x-rays or neutrons, too, but for these sources dedicated software is available with much more options and better interface.

The original version of PETS was a console-based program with limited graphical capabilities. This manual describes version 2.0 with fully developed graphical interface and with a number of new options.

This brief manual has – apart from the introduction – two parts. The first part describes how to quickly start with PETS through an example of a basic run. The essential options needed to understand the procedure are briefly explained when they appear in the example. The example proceeds from the format of the input file and the parameters that must be determined before PETS is started to the creation of the reflection file(s) that can be used in the software for structure determination or refinement. In the second part of the manual all the options of the program – basic or advanced – are described in detail.

PETS performs the following basic procedures: peak hunting, refinement of the position of the rotation axis, peak analysis (clustering and difference space generation), indexing, integration of intensities, integration of intensities for dynamical refinement, calculation of a 3D distribution of intensities in reciprocal space, and reconstructions of sections through the reciprocal space (also known as reconstructed precession images or unwarping).**CONTACT US:** PETS is under constant development and it can thus suffer from occasional bugs and weird behavior. Please contact us with bug reports and feature suggestions.

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B. QUICK START WITH PETS

1. Requirements

PETS is currently available as a Windows executable for 64-bit operating system. The graphic card must support OpenGL v3.0 or higher.

2. Installation

PETS does not need a special installation. Just unpack the zip you will find available at pets.fzu.cz/download/petswx.zip to a directory of your choice. The zip file contains the executable and the minimal set of dlls needed to run the program.

3. The input file

The input file for PETS is a free format ASCII file that specifies the parameters for the program. The name of the input file can be arbitrary, but I recommend giving it an extension „.pts“. AFAIK this extension does not clash with any standard extension, it is thus easy to locate the files, and, if you want, you can easily create a file association between pts files and PETS. The part of the name before the extension is used as the base for all files produces by PETS. The ***Fig. 1*** represents a basic input file using all necessary keywords.

The keywords are case sensitive. Their description follows:

- ⇨ lambda: relativistic wavelength of the incident electrons in angstroms. 100kV = 0.0370Å, 120kV = 0.0335Å, 200kV = 0.0251Å, 300kV = 0.0197Å.
- ⇨ Aperpixel: scale of the images given as the size of one pixel in reciprocal angstroms.
- ⇨ omega: orientation of the tilt axis of the sample holder with respect to the positive horizontal axis of the image. Must be estimated either by calibration, or by visual inspection of the series of images. Should be close to the correct value within +-20 deg. Can be refined later in PETS.
- ⇨ noiseparameters: two parameters for the determination of σ(I). The first value is Gγ, the second is ψ. The uncertainty on each pixel γ is then calculated as: σ2(p)=Gγp+ψ For more information see Waterman, D. & Evans, G. (2010). J. Appl. Cryst. 43, 1356-1371.

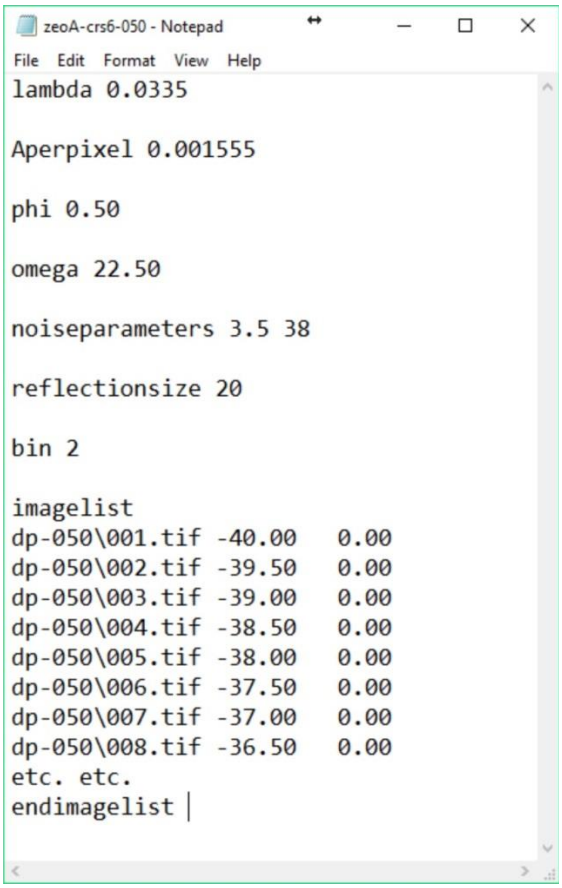


Fig. 1: basic input file.

⇨ phi: Precession angle in degrees used during the data collection. Can be zero, of course.

⇨ reflectionsize: Diameter of the spots on the images in pixels. Used for peak search and integration, should be large enough to encompass also the strong spots, but small enough to avoid overlaps of neighboring spots.

⇨ bin: binning of the images prior to any treatment. Saves time, makes nicer pictures, and seems to make the whole analysis somewhat more robust. bin 2 is a good choice from my experience for most data sets.

⇨ imagelist – endimagelist: multiline keyword. Each line contains one image. The format is: Name of the picture\alpha\ beta.

Name is the file name of the image (including possible relative or absolute path), alpha is the tilt angle in degree, and beta is the possible second tilt value of the double tilt holder. The last value should be zero, if you tilt only around one axis, but it must always be given. The only format PETS can read is 16bit unsigned TIF. If you have signed TIFs (the diffraction image appears grey, if you open it in a normal image viewer), add a line “background 32768” in the input file to subtract the false background that is created by reading a signed TIF as unsigned.

4. Basic data reduction procedure

The standard procedure is the following: Make peak search; refine the rotation axis (angle omega); analyze and process peak table; index the lattice and define the orientation matrix; integrate intensities and create hkl(m)/ I / σ(I) type output files needed to solve or refine a model in dedicated software (e.g. JANA2006).

We are going to go through all the steps presented on the ***Fig. 2***. This section presents the basic procedure to follow for most of the data without “complication”. Complications can be modulation,

obvious data/crystal distortions or twinned data, etc. For all these cases, additional options are needed during and after the indexation step and are explained and illustrated in the section C.

Back to the procedure! We will use the example of a zeolite compound. 120 frames were recorded with a 0.5 degree step on a zeolite crystal on a CM120 Philips TEM operating at 120 kV with a precession angle of 0.5 degree.

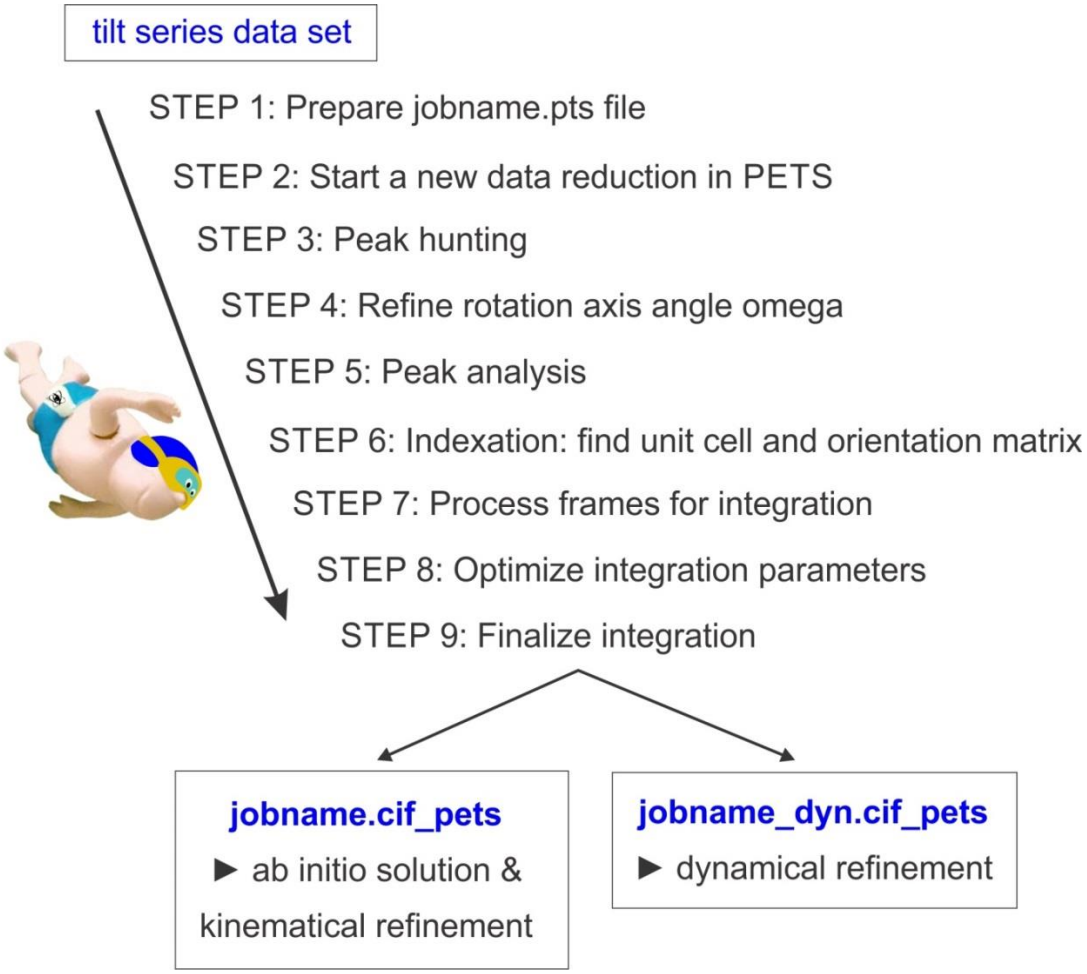


Fig. 2: basic data reduction procedure

► **STEP 1: set up the pts file**

According to your data set (see example *Fig. 1*)

► **STEP 2: start a new data reduction**

Start pets by double clicking “petswx.exe” executable file or the shortcut on the desktop you might create. Then, open the input pets file with File/Open/jobname.pts in the directory where the jobname.pts file is located. Another option is to associate the input file extension with PETS, and then double clicking the input file. In any case you will get the window of the ***Fig. 3***.

The graphical version of PETS is organized in three main panels:

- ⇨ The **command panel** with functions/parameters on the left. Each function is run by clicking the corresponding main button (action button). To set up parameters related to this function click the small arrow on the right side of this option.
- ⇨ The **main panel** with images and plots on the right: raw data, processing pictures or graphs produced during the procedure.
- ⇨ The **console** at the bottom window with details about the current calculation.

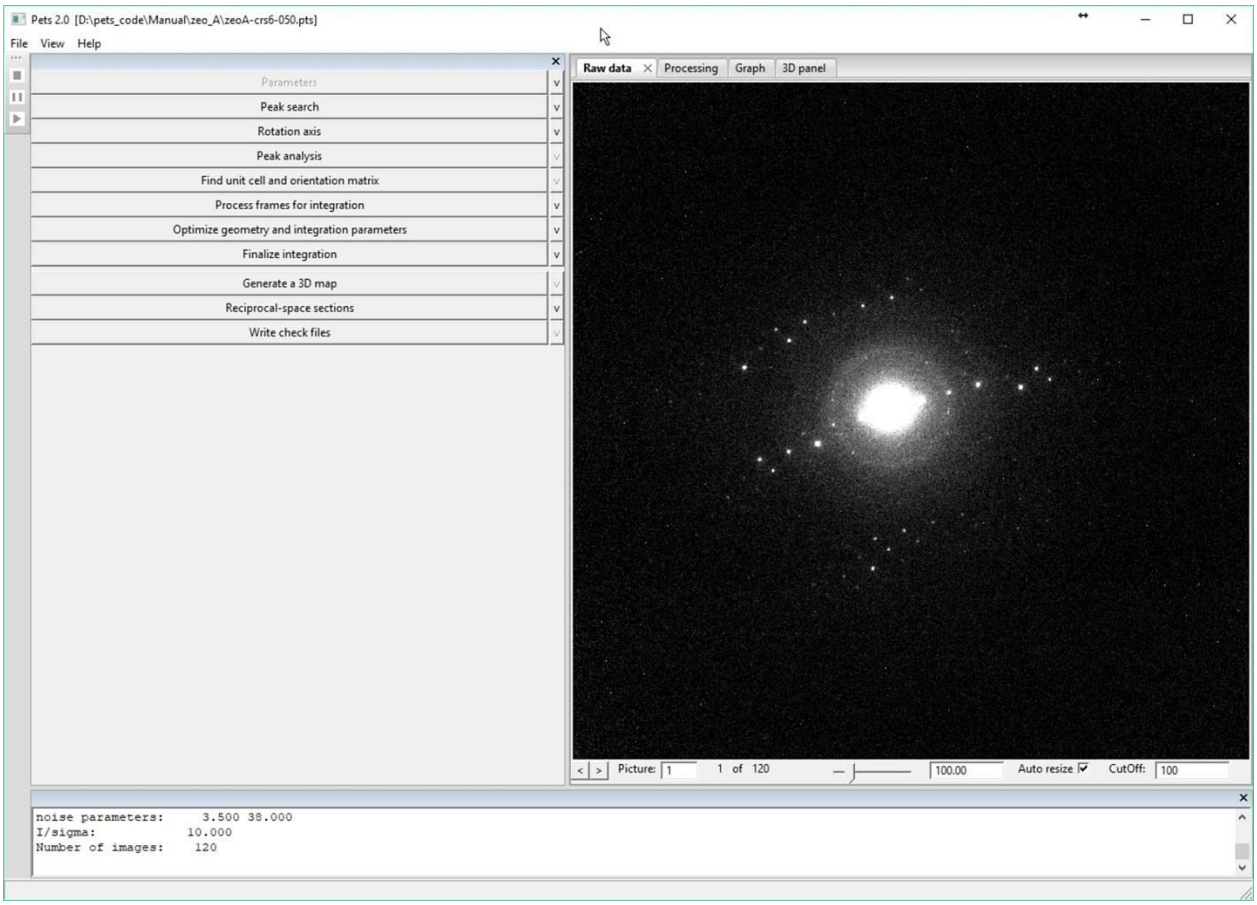


Fig. 3: main window.

► STEP 3: peak search

With this step, the program will extract all the peaks that are present in each frames of the data set.

In the first tab "Parameters" of the menu you can find the basic parameters used throughout PETS. These parameters are mostly read from the input file. In the order:

- ⇨ Reflection size: same parameter than in the input file but with binning included (value in pixel).
- ⇨ Pixel size: size of pixels in the 3D reconstruction of reciprocal space. In reciprocal angstroms. Values between 0.005 and 0.015 are usually reasonable (0.01Å^{-1} by default).
- ⇨ Resolution limits as d* in reciprocal Angstroms. Three distances are considered:

-max d* for peak search: maximum resolution used for the peak search, or distance from the center at which the program looks for peaks (default value 1.4 Å^{-1}) (blue circle on **Error! Reference source not found.**)
-min d*: minimum resolution, distance from the center beyond which the program considers reflections/peaks. This parameter avoids detecting fake reflections that are in fact part of the transmitted beam (red circle on **Error! Reference source not found.**) (default value 0.05 Å^{-1}).
-max d* for peak integration: maximum resolution for processing frames for integration (default value 1.4 Å^{-1}).

Most of the time these values can be kept at their default values

- ⇨ Beam stop yes/no: specifies if the beamstop was used during the data collection. **In our example we selected no**.

To define a beam stop manually or import a file containing the beam stop coordinates, see **section C23.**

- ⇨ Method to determinate the center of the frames: there are two methods but here, the best was to select "Friedel pairs" and the options "Always accept center" and "Center auto". For the data acquisition, the crystal was selected with a nano diffraction beam and not with a selected area aperture. So in this case the transmitted beam can show some asymmetry and the "Friedel pairs" method is more accurate than the "Central peak" one based on the circular symmetry of the center.
- ⇨ I/sigma I: Intensity over sigma ratio for peak search. Only peaks with integrated intensity over sigma larger than the given value are saved in the peak list. Default value is 10. Decrease this number, if you are seeing too few picked peaks, and increase it, if you see noise being picked in the images.

When all the parameters in the sections "Parameters" and "Peak search" are set up, you can run "Peak search" to start the peak hunting. The detected peaks are indicated on the frames by white circles and visible on the main panel in the tab **Processing** (***Fig. 4***). In the console you can see the information for each frame: the angles alpha and beta, how many peaks are collected and what are the estimated coordinates of the center. At the end of the procedure, in the tab "Graph" of the main panel, the curve represents the x and y coordinates of the center for all the frames (***Fig. 5***).

In this example, we obtained 5457 total peaks over the 120 frames that are listed in the file jobname.rpl.

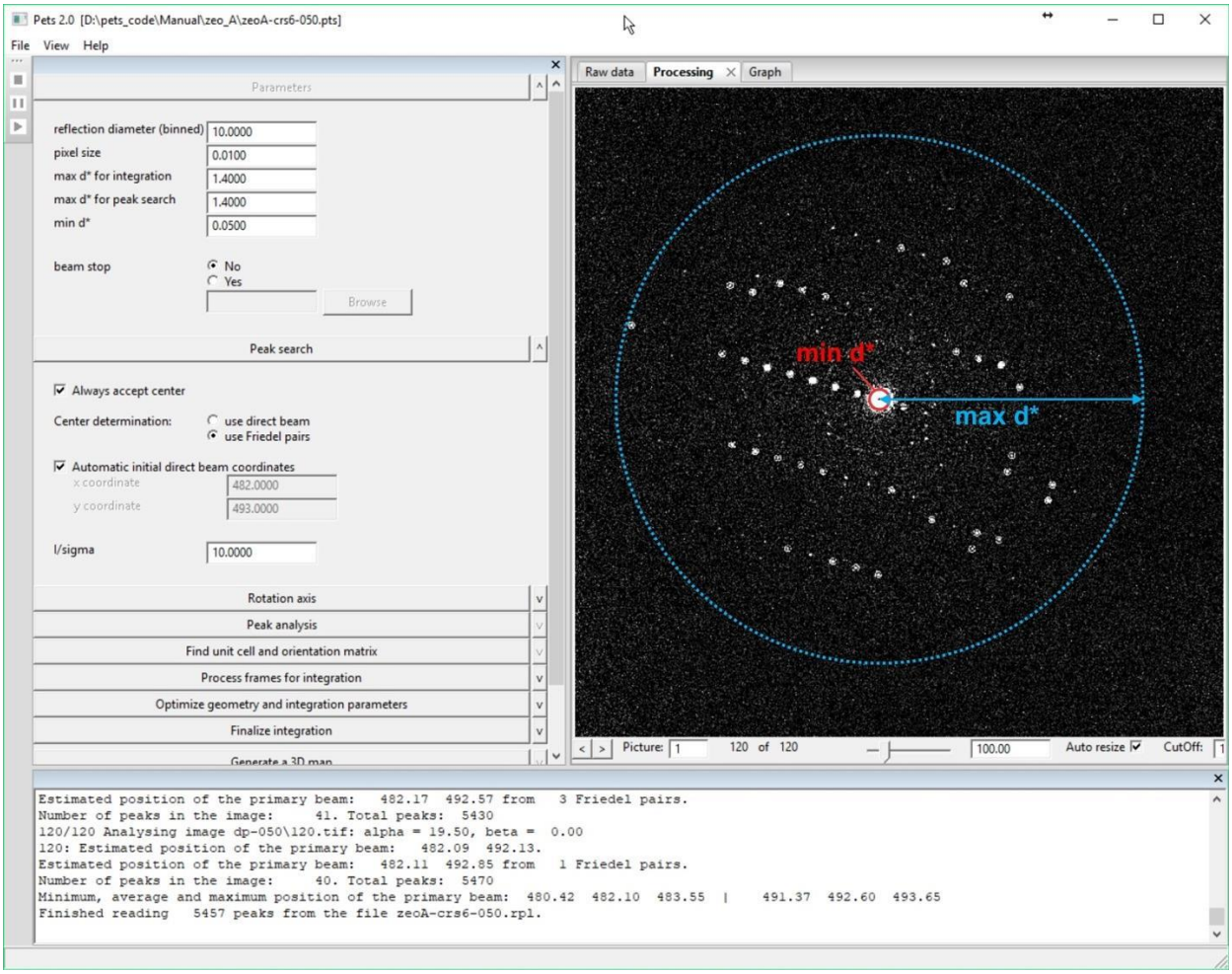


Fig. 4: peak searc (STEP 3).

► STEP 4: refine the angle of the rotation axis

The angle omega (angle between the projection of the tilt axis on the image and the horizontal axis) depends on the camera length and on the exact focusing conditions. Therefore it cannot be exactly calibrated, and must be refined for each data set. The details of how this works are beyond the scope of this tutorial, but you can easily asses the quality of the data and accuracy of the parameters by looking at the cylindrical projection of the peak positions that is shown by PETS during the refinement in the tab **Processing**. Below are two examples of such plots (**Fig. 6**). The first one is made with the correct omega (**Fig. 5**), the second with an omega that is off by 10 deg.

Note that the refined omega value replaces the initial one and is taken into account for the next steps of the data reduction as long as the project jobname.pts is open in PETS. The refined value does not replace the initial value in the input file. To save it, replace omega manually in the input file. Then you will not need to re-refine the parameters for the same data at next run of PETS.

In this example we know that omega is around 22.5 degrees and we select "Refine rotation axis position (omega)" in the option "Rotation axis" (**Fig. 6**). If you do not know this angle, you can evaluate the position of the axe of rotation by looking at all the frames or, select "Global search for rotation axis position" in "Rotation axis" (see section C8 for more explanations). To start the refinement, run "Rotation axis".

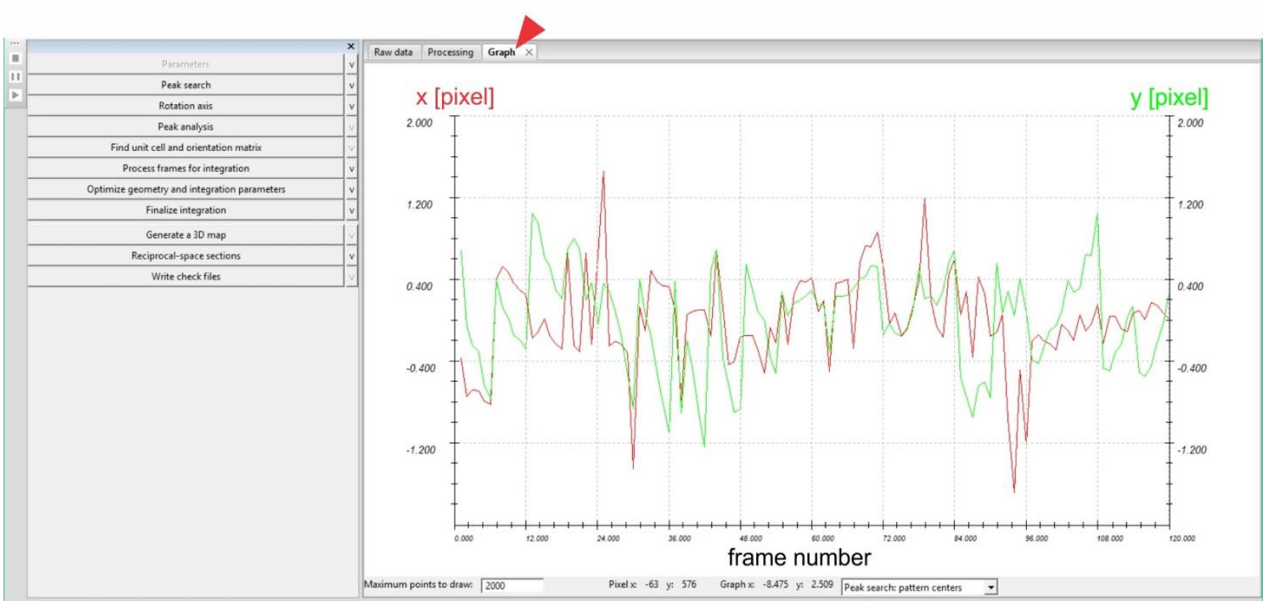


Fig. 5: deviation in pixels of the x and y center coordinates



*Fig. 6: refinement of the omega parameter and examples of two cylindrical projections with two values of omega **(STEP 4)**.*

► STEP 5: peak processing analysis

Run Peak analysis, then Peak analysis(continue) and again Peak analysis(continue).

The raw peak list is usually too noisy to be used for indexing directly. Therefore, PETS analyzes the peak list. This analysis proceeds in two steps. In the first step all reflections measured on subsequent frames are clustered, and the centers of the clusters are used instead of individual peak positions. For this step the limit for the clusters must be determined. To check that the program did this correctly, the program shows a plot of inter-peak distances sorted from the shortest to the longest (***Fig. 7* Step 1**). If everything works fine, you should see a clear jump in the distances accompanied by a peak in the derivative (red in the plot).

In the next step, PETS calculates difference vectors between the peaks. Then the same clustering procedure is applied to the group of difference vectors, and again a plot of distances is shown for checking the correctness of the choice. The jump in the second plot is usually even sharper than in the first plot (***Fig. 7* Step 2**).

At the end of this procedure three files are produced: jobname.xyz contains the "cleaned" peak list, where individual peaks are replaced by the cluster centers. jobname.diff contains the difference vectors, and jobname.clust contains the list of cluster centers from the difference vectors.

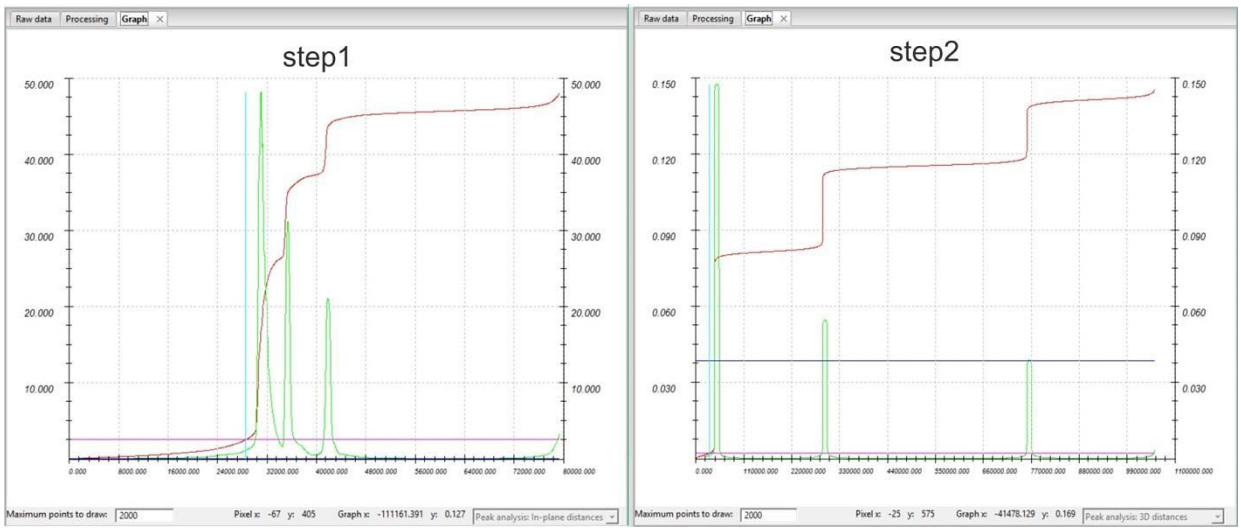


Fig. 7: peak analysis

► STEP 6: indexation-define the unit cell(s) and the orientation matrix

If you are used to data reduction software, the PETS interface should be fairly easy to understand, if you play a bit with the tools. In the indexation routine one or several unit cell(s) can be defined.

In the command panel, press "Find unit cell and orientation matrix" to access the indexation routine. By default, the jobname.clust is open and the cloud of reflections is displayed on the 3D panel. To work from the other 3D reconstruction files, you can select jobname.xyz (or .diff or .cor) in the menu (red arrow on the ***Fig. 8*)**. The indexation panel is organized with several parts:

-The upper panel with the basic information on the lattice parameters, the orientation matrix and the percentage of indexed reflections.

-Two tabs to find automatically or manually the unit cell.

-The tools to modify the unit cell in the direct or reciprocal space

-Refine cell

-Display options

-Finish button to finish the indexation and return to the command panel and the procedure.

As a rule, if everything works fine, the file jobname.clust is the best for indexing. At the end, however, the lattice should be checked against jobname.xyz to make sure nothing was missed or messed up during the clustering procedure. Two notable cases where jobname.clust will not work are twins and (strongly) incommensurately modulated crystals. Do not forget to check the xyz file and comparing with the lattice determined from the clust file. Only strong peaks are used for clusters, and there is a danger of missing some superlattice/weak reflections.

ON the 3D panel: To rotate the cloud in 3 dimensions, click inside the cloud. To rotate in the 2d plane of the panel click outside the cloud. You can also fix the rotation direction by selecting one of the *x,y* or *z* tab at the bottom of the 3D panel. Here the x,y or z directions are the directions of the screen (*x* horizontal, *y* vertical and *z* out of the screen). To adjust the magnification of the pictures use the scroll wheel on the mouse.

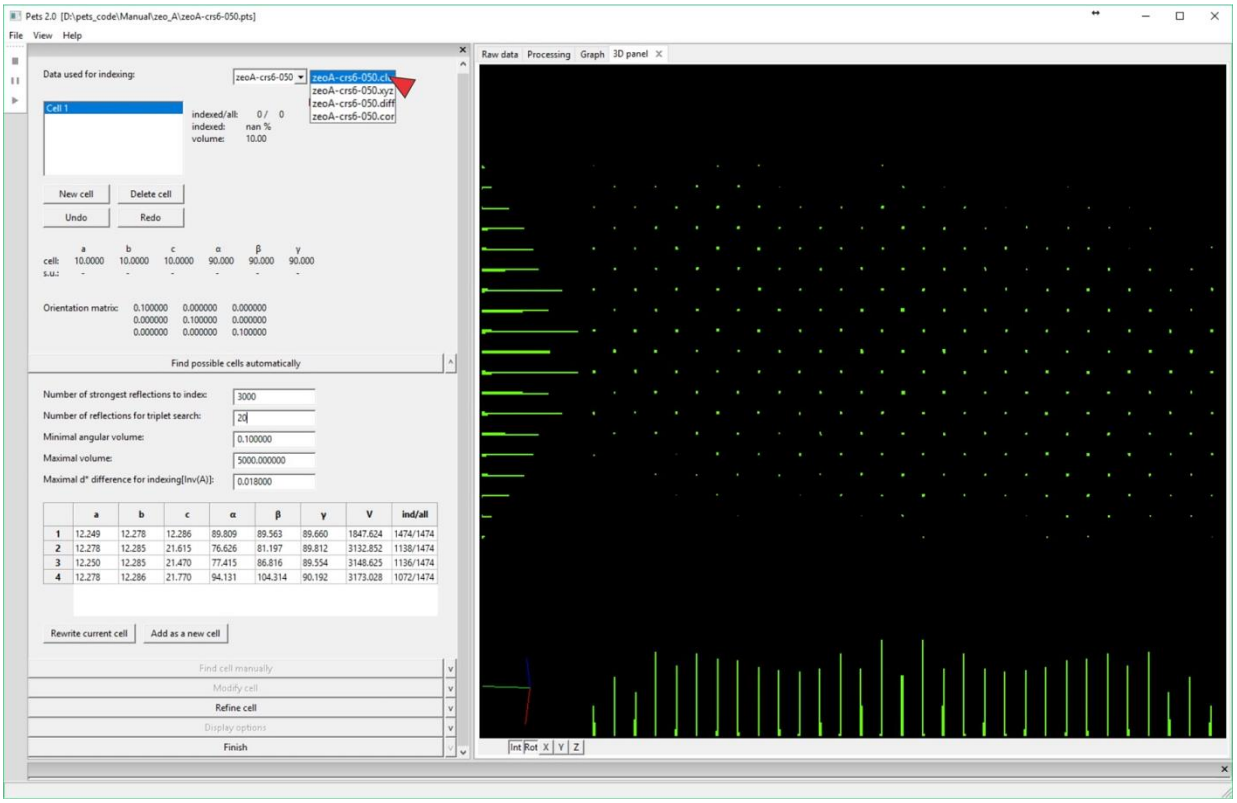


Fig. 8: indexation panel

FIND THIE UNIT CELL AUTOMATICALLY:

Press "Find possible cells automatically". The program searches and offers several possibilities that you can see in the table in the options of "Find possible cells automatically". To select one unit cell, click the line you want and then press "Rewrite current cell" or "Add as a new cell". On the 3D panel the axis x (a/ a*), y (b/ b*) and z (c/c*) are represented in red, green and blue respectively.

According to your data, you might change the defaults parameters for searching:

-number of strongest reflections to index (3000 by default),

-number of reflections for triplet search (default value : 20),

-minimal angular volume: to avoid unit cell with extreme angles (0.1 deg),

-maximal volume: maximal allowed volume of the unit cell (5000 $Å^3$),

-maximal d* difference for indexing = 0.018$Å^{-1}$: maximal allowed distance in the reciprocal space between the position of the lattice nodes and the exact position of the reflections (also the maximal error used in the unit cell refinement in the option "Refine cell").

FIND THIE UNIT CELL MANUALLY:

Using the cloud of points, it should be fairly easy to locate the special directions by rotating the cloud. Once you find a nice projection, you can switch to indexing mode in the option "Find cell manually" by pressing "Define direction" and selecting the axis you want to define. Then, drag the directions of special planes by mouse and enter manually the order of the interplanar distances to define the lattice parameters. To confirm the unit cell, press either "Rewrite current cell" or "Add as a new cell". To delete, click "Clear". You can also set specific projections of the cloud along the directions *a*,*b* or *c* (**Fig. 9**).

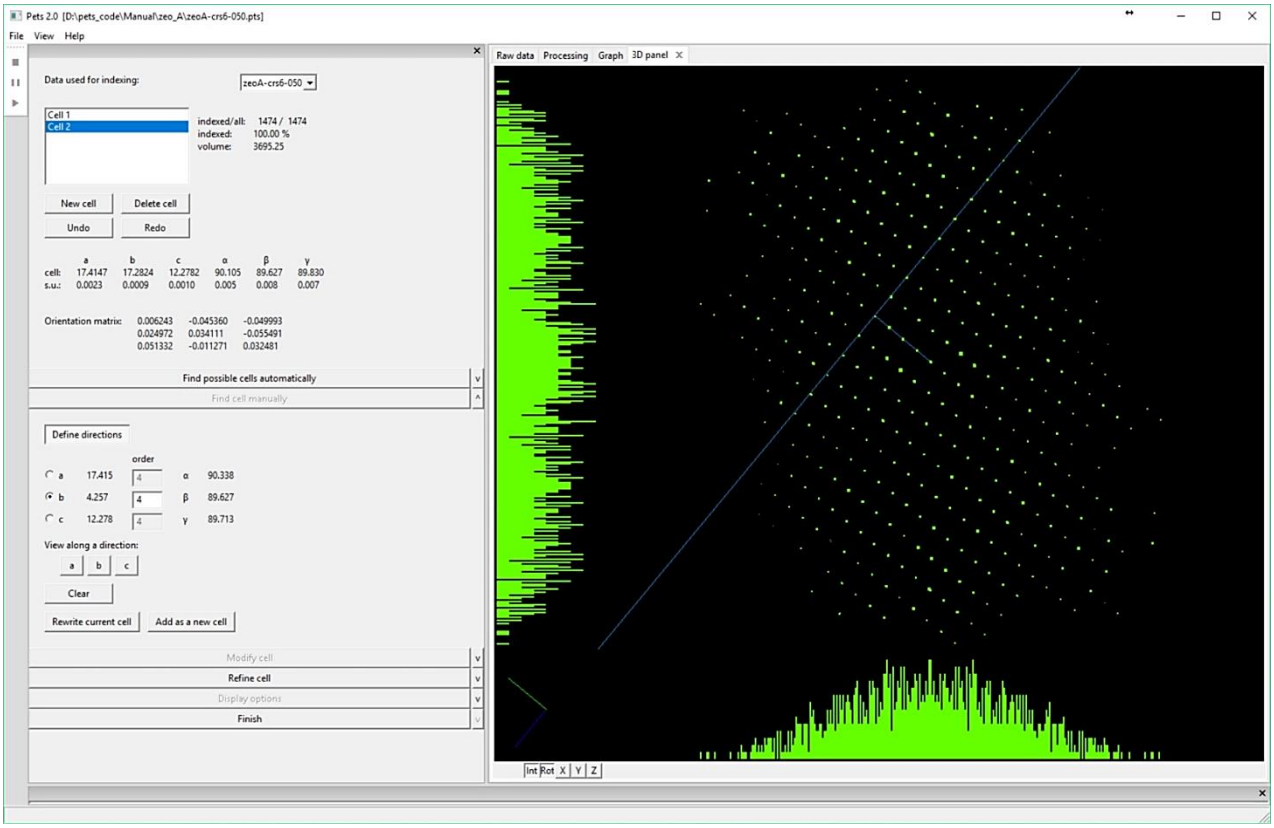


Fig. 9: manual indexation routine.

MODIFY THE UNIT CELL:

To modify the unit cell, select the option "Modify cell". You can reduce the cell and modify the parameters using a transformation matrix in the direct or the reciprocal space (***Fig. 10***).

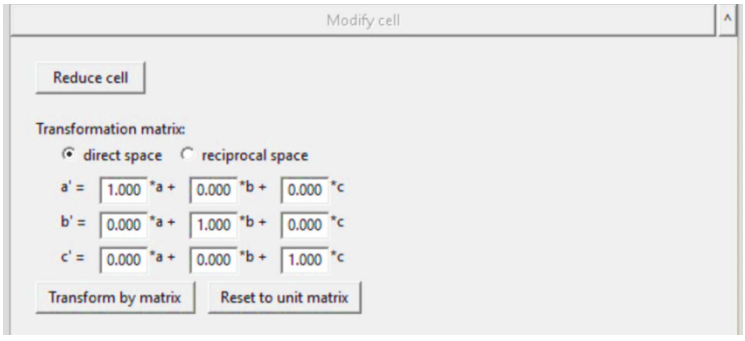


Fig. 10: "Modify cell" options.

REFINE CELL:

On the top of the indexation routine, select the Cell to refine if you defined several. Then, open the "Refine cell" menu. Two options are available to refine: Refine UB+cell (the orientation matrix and the lattice parameters are refined) and Refine cell from d (d-spacing). You can adjust the maximal d* difference for indexing and set a specific symmetry system. To run the refinement press "Refine cell".

If you defined several unit cells, you need to do the refinement one unit cell after the other after selecting the cell you want in the box (green box on the ***Fig. 11***). Once you are happy with the refinement, select the unit cell you want to continue with in the reduction procedure and press "Finish" to exit the indexing routine. At this point a file jobname.smr is created that contains the orientation matrix and the lattice parameters of the selected unit cell.

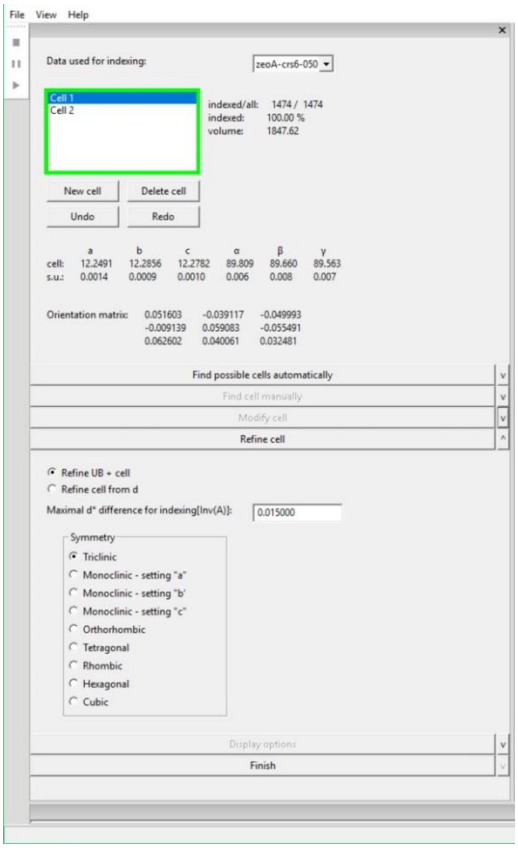


Fig. 11: refine unit cell options.

DISPLAY OPTIONS:

As shown on the ***Fig. 12***, you can display the axis (on the 3D panel) in the laboratory, reciprocal or direct bases. When the unit cell is defined, you can choose all the projections you want by defining the axis along with the view will be done. One specific option "Fold to cell" is use to project all the 3D reconstruction in only one unit cell or few of them. To visualize the lattice (red on the 3D panel), select "Show lattice".

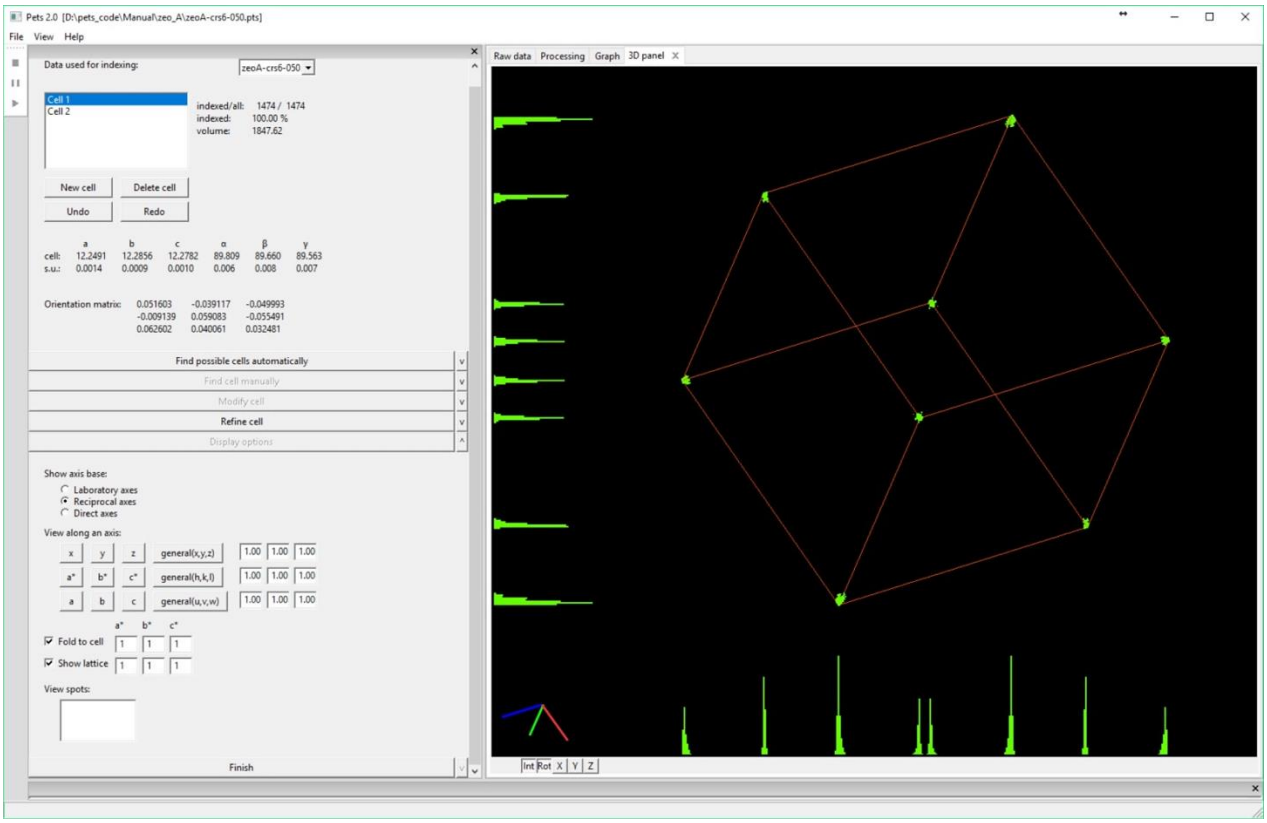


Fig. 12: Display options.

► STEP 7: Process frames for integration

The intensities are integrated on each frame using the current orientation matrix and the lattice parameters. In the option "Process frames for integration", leave the default parameters and run "process frame for integration" (***Fig. 13***).

For each frame the reflection positions are predicted using the current orientation matrix and the centering (if present, see **section C15**). The intensity at each predicted reflection position is determined. During the process, PETS shows an image in the "Processing" window of each frame with an overlay of circles and diamonds. A circle means the reflection is integrated, a diamond shows a reflection that is not integrated, but that is approaching or leaving the Bragg condition. A small circle inside the large circle means PETS found a significant intensity at that position. In such case the large circle is the predicted position and the small circle is centered on the real maximum in the image. At the end of the procedure a file jobname.dyntmp is created, where the intensities are written out in the format 3i4,2f10.2 (Fortran notation), plus some more information after the first five columns.

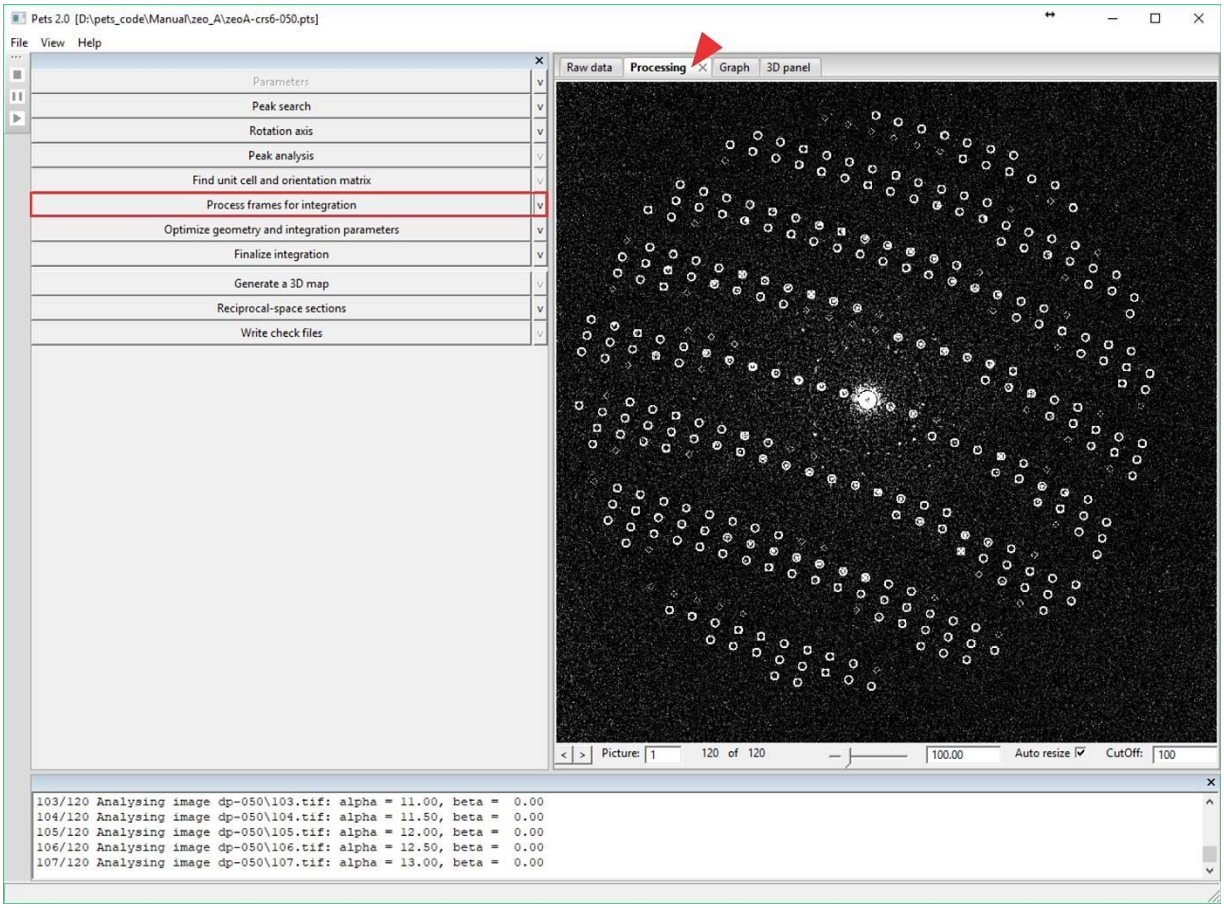


*Fig. 13: process frames for integration **(STEP 7)**.*

► STEP 8: Optimize integration parameters

These parameters need to be refined to allow a better estimation of the intensities and the angular size of reflections. This procedure is especially useful for precession electron diffraction data. It may also reveal hidden serious problems with the data.

The rocking curve of the reflections (the intensity as a function of the deviation from perfect Bragg condition) is a two-peaked curve with the separation of the peaks depending on the precession angle. Because of the specific shape we call the curve “the camel” and the plot of rocking curves “the camel plot”. As you can see in the example of the ***Fig. 14*** the calculated camel plot profile matches on default parameters (red) does not match well the data (blue). After refinement, the Camel plot fits better the data (***Fig. 14***). These parameters are used later in step 10.

How to run this optimization? Select Refinement using rocking curve profile (camel plot) and the two sub options RC width and apparent mosaicity. Then, run "Optimize geometry and integration parameters".

The default parameters are Rocking curve width = 0.003 Å-1 and moisaicity = 0.05. Only these two parameters are refined. The precession angle and the calibration constant cannot be refined, but they can be modified manually.

If you are happy with the refinement, you can re-Process frame for integration.

Note that if the refined moisaicity is too high (above 0.2), it is better to set up the default value to continue the procedure. Such high mosaicity usually means distortions in the data (rotating crystal, cracked crystal, jumps of the goniometer) rather than real mosaicity of the crystal.

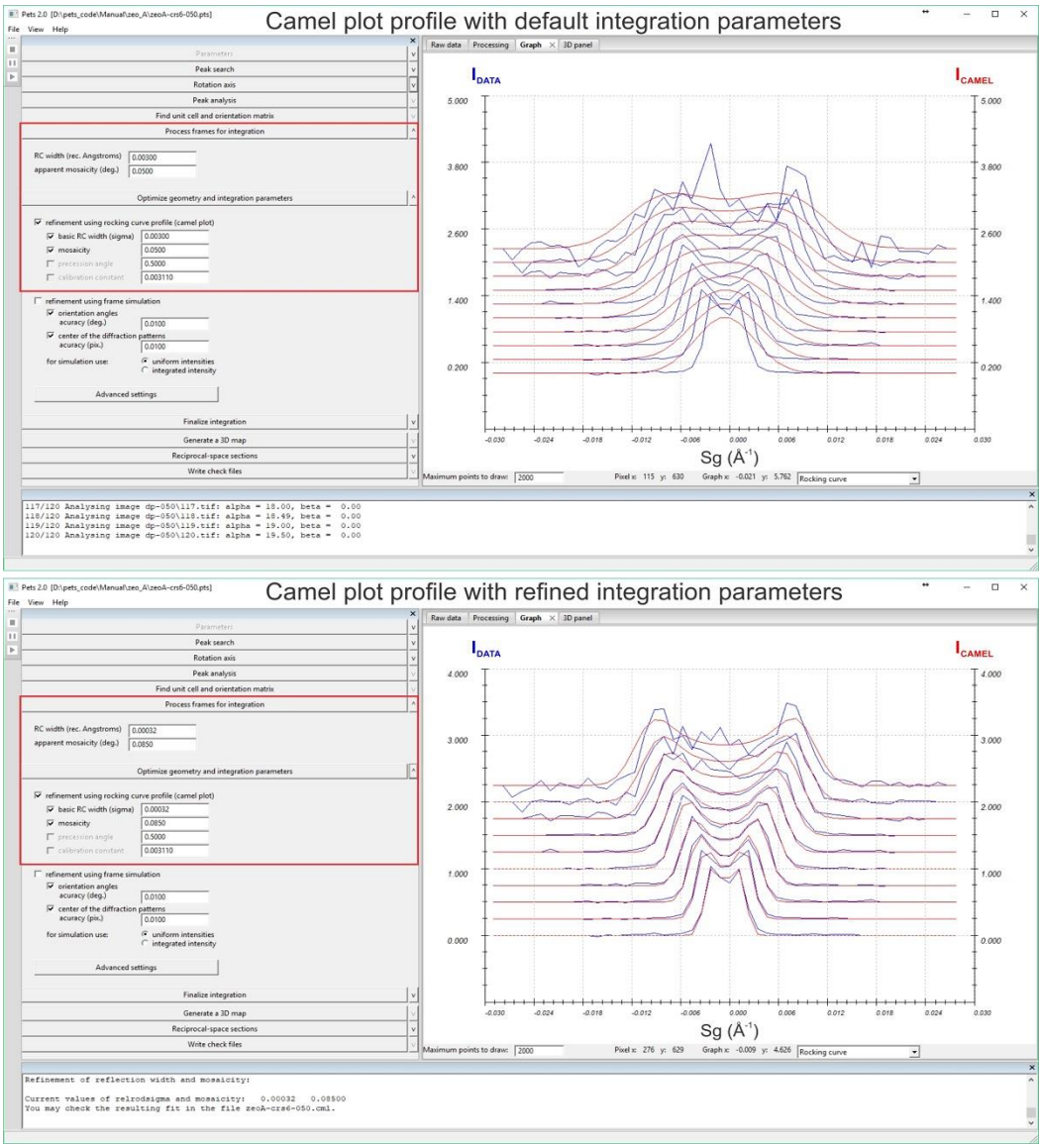


Fig. 14: Camel plot profile.

► STEP 9: finalize the integration

The climax of your efforts! The files for structure solution and kinematical refinement (jobname.cif_pets) and the dynamical refinement (jobname_dyn.cif_pets) can be produced at the same time by selecting both kinematical and dynamical check boxes and run "Finalize integration". The difference between these two types of integration is explained in the **section C12**.

KINEMATICAL:

As, you can see in the section "Finalize integration" several options and parameters need to be set up:

-the method used to estimate the intensities, mostly fit profil/integrate profil or in very rare and specific cases maximum intensity or Bragg position.

-two integration parameters: minimum number of reflection measurements and reflection width multiplier.

-the Laue class of the crystal for frame scaling.

In the **section C13** all these options are described and you will find clues to select the best estimation method for your data and set up the integration parameters.

Concerning the basic procedure:

The integration parameters were refined in order to use the option "fit profil" and get the most accurate intensities and coverage. Select frame scaling with the Laue class of your compound if it is known, otherwise you can select -1 for the first test (m-3m for the zeolite example). Keep the minimum number of reflection measurements and the reflection width multiplier parameter by default for the first run. Run Finalize integration.

This step can be long because of the frame scaling. At the end, in the console are the intensity statistics. Notably, the Observed/Total number of reflections and, for each Laue class, a list with Rint(obs), Rint(all), Nobs/Nall and the redundancy. In the main panel is the plot with the scale factors for all frames. You can try to obtain better Rint in increasing the min.samples parameter. This parameter set a threshold. Only reflections that are represented in the data set at least this number of time are included in the final file. By increasing this parameter, the Rint tend to be better because you include reflections that are better represented, so with more accurate intensities but you also might lose reflections. So you can make several tests and find a compromise between number of reflections (coverage) and Rint. In the example, the best compromise was found for min. samples = 4 and multiplier= 1 $Å^{-1}$(Error! Reference source not found.).

A file jobname.cif_pets is generated. This files contains one list of reflections *hkl* with their intensities and standard deviations σ(I). At the top of this file are the lattice parameters, the orientation matrix and the list of zone axis corresponding to the frames.

The jobname_.cif_pets is used in JANA2006 to solve the structure and refine it with the kinematical approximation.

DYNAMICAL:

The dynamical file is directly made from the list jobname.dyntmp previously created from "process for integration" but in a format that can be read by JANA2006 (jobname_dyn.cif_pets). In this file, you will find N lists for reflections *hkl* with their intensities and standard deviations σ(I) for the N frames. At the top of this file are the lattice parameters, the orientation matrix and the list of zone axis corresponding to the frames.

With the jobname_dyn.cif_pets you are now ready to do the dynamical refinement of your structure!

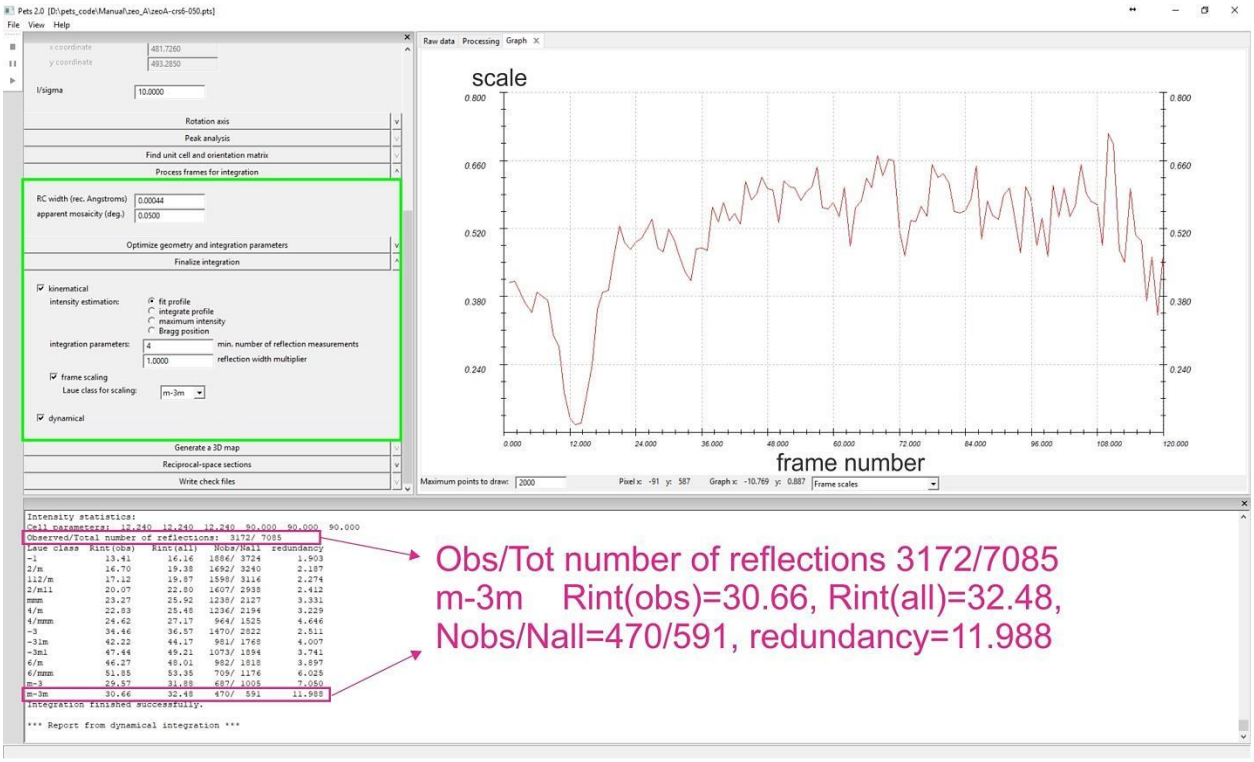


*Fig. 15: Results of the final integration with the scale plot on the right and the Intensities statistics in the console. A big decrease of the scale factor**s** is usually an indication of an oriented zone axis.*

C. PROGRAM OPTIONS (BASIC AND ADVANCED)

The basics options are presented in the order they appear in the procedure.

1. Reflection diameter

Diameter of the spots on the images in pixels. Used for peak search and integration, should be large enough to encompass also the strong spots, but small enough to avoid overlaps of neighboring spots. This parameter is found in the "Parameters" option and the value is binned.

2. Pixel size

Size of pixels in the 3D reconstruction of reciprocal space. In reciprocal angstroms. Values between 0.005 and 0.015 are usually reasonable (default =0.01Å-1).

3. Maximum and minimum distance for integration and peak search

See basic procedure

4. Beam stop options

This option specifies if the beamstop was used during the data collection. If yes you can either define manually the beam stop in PETS or import a file type ".xyz" with the beam stop coordinates and then adjust its position on the picture. This option is set in the first tab "Parameters" of the command panel.

► **Define the BS manually**

On the raw data panel, select the first picture. Press the two options **sbs** (show beam stop) and **bs** (define beam stop mask) (see the circled red options on the ***Fig. 16***a) and then click left on the picture to create point by point the shape of the beam stop. To remove a point, press **dbs** (delete beam stop) and then click the point you want to remove.

► **Import BS *xy* coordinates:**

Another option is to define the BS mask once by creating an external file containing the xy coordinates (express without the binning). You can define the polygon defining the beamstop using as many points as you need (***Fig. 16***b). Then, select beam stop "Yes" in "Parameters" and press "Browse" to import your file. To display the BS on the raw data picture, make sure the option sbs is activate. Then you can select mbs (= move beam stop), click left once on the picture to set the shape in motion (dashed shape). To set up the final position, click left again (***Fig. 16***).

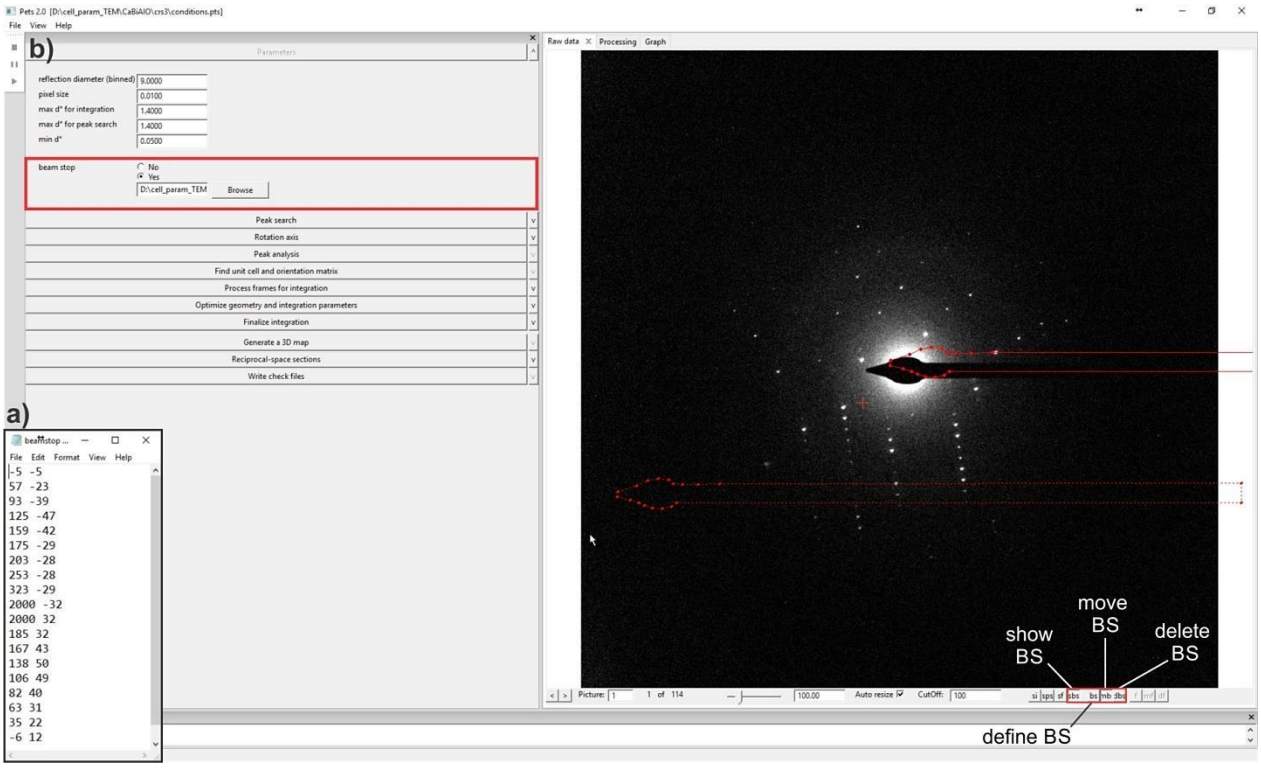


Fig. 16: a) beam stop panel, b) example of file with beam stop coordinates.

5. Center of the frame

It corresponds to the position of the primary beam on the image in pixels.

PETS can determine the center automatically by selecting “Automatic initial direct beam coordinate” in the option “Peak search”. This often works, but occasionally fails. In such case you have to give explicitly the coordinates of the center in the image, horizontal first, vertical second (binned value). It should be read on the image at the lowest tilt angle (the first image to be treated by PETS). The center is then refined for each image separately during peak search. The program offers two evaluation methods in the “peak search” menu.

-use direct beam: the center of each pattern is located from the strong primary beam. This method is usually less accurate than the second method for the Nano diffraction experiment because of the asymmetry of the primary beam.

-use Friedel pairs (default): Friedel pairs are used to locate the center of the image. The latter option can lead to problems, especially if only a few reflections are visible in the patterns. Manual intervention might be required by the program in such cases.

-Always accept center: the program will accept by default the center during the peak searching. In most of the case it works fine. Sometimes, because of the presence of too few visible reflections, the program

does a wrong estimation of the center or does not find it. In this case, you can restart the peak hunting without selecting this option. Then, during the peak search when fails in finding the center, the peak hunting stops and you will get the following message:

"The center could not be found. Do you want to keep the previous center? If yes, press the button "Peak search(continue)". If no, choose one or several Friedel pairs on the Raw data and then press the button continue (for remove Friedel pair press df)."

To define manually Friedel pairs, use the raw data window. The current pattern is displayed. Select **sf** =(show Friedel pairs) on the bottom menu and **f** to define new pairs. Then you can create the pairs on the raw frame (one or several) and then press "Peak search (continue)". To move or correct the Friedel pairs manually defined, use the options **mf** (=move Friedel pairs) and **df** (=delete Friedel paris).

6. Detection threshold for peak hunting

This parameter is the Intensity over sigma ratio for peak search and noted I/sigma(I) in the "peak search menu". Only peaks with integrated intensity over sigma larger than the given value are saved in the peak list. Default value is 10. Decrease this number, if you are seeing too few picked peaks, and increase it, if you see noise being picked in the images.

7. Noise parameters

These two parameters are used for the determination of σ(I).They are set in the input file. The first value is Gγ, the second is ψ. The uncertainty on each pixel γ is then calculated as: σ2(p)=Gγp+ψ For more information see Waterman, D. & Evans, G. (2010). J. Appl. Cryst. 43, 1356-1371.

8. Refinement of the rotation axis

The angle omega (angle between the projection of the tilt axis on the image and the horizontal axis) depends on the camera length and on the exact focusing conditions. Therefore it cannot be exactly calibrated, and must be refined for each data set. The details of how this works are beyond the scope of this tutorial, but you can easily asses the quality of the data and accuracy of the parameters by looking at the cylindrical projection of the peak positions that is shown by PETS during the refinement in the right window/Integration. Below are two examples of such plots. The first one is made with an omega that is off by 10 deg, the second with the correct omega appears sharper (***Fig. 6***). You can see the projection on left window in the Integration tab.

Note that the refined omega value replaces the initial one in the "omega" box and is taken into account for the next steps of the data reduction as long as the project jobname.pts is open in PETS. The refined value does not replace the initial value of the input file. To save it replace omega manually in the input file and you do not have to re-refine the parameters for the same data again.

In this example we know that omega is around 22.5 degrees and we select "Refine parameters" in the tab "Refine parameters" (*Fig. 5*). If you do not know the angle for your microscope, you can evaluate the position of the axe of rotation by looking at all the frames or, select "Global omega search" in "Refine parameters" (see *Fig. 17*). To start the refinement, click "Refine parameters".

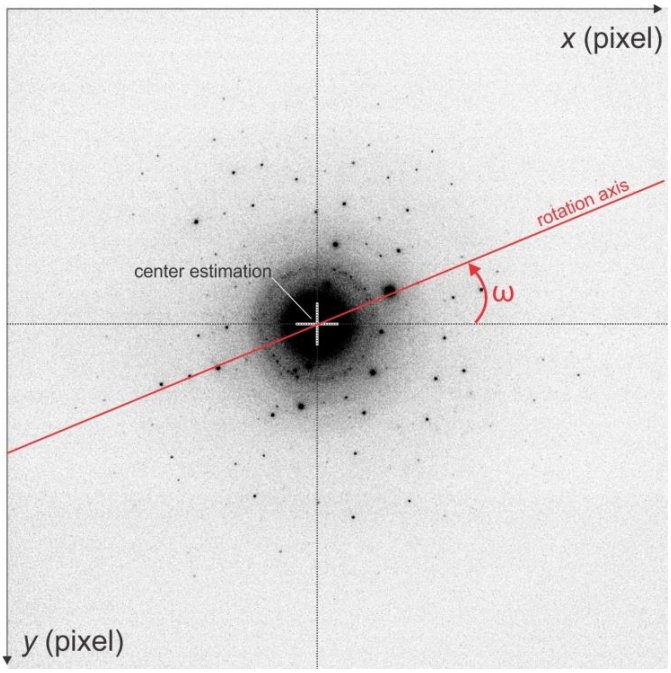


Fig. 17: estimation of the omega angle of the rotation axis.

9. Peak analysis

See example in the **section B4** STEP 5

10. Indexation routine

See **section B4** STEP 6

11. Process frame for integration

The intensities are integrated on each frame using the current orientation matrix and lattice parameters. In the option "Process frames for integration", leave the default parameters and run "process frame for integration" (***Fig. 13***)

For each frame the reflection positions are predicted using the current orientation matrix and the centering (if present, see advanced options) and the shape of the rocking curve is also take into account through the two parameters basic RC width and moisaicity. The intensity at each predicted reflection position is determined. During the process, PETS shows an image in the "Processing" window of each frame with an overlay of circles and diamonds. A circle means the reflection is integrated, a diamond shows a reflection that is not integrated, but that is approaching or leaving the Bragg condition. A small

circle inside the large circle means PETS found a significant intensity at that position. In such case the large circle is the predicted position and the small circle is centered on the real maximum in the image. At the end of the procedure a file jobname.dyntmp is created, where the intensities are written out in the format 3i4,2f10.2 (Fortran notation), plus some more information after the first five columns.

The two parameters responsible for the shape of the rocking curve are:

- ⇨ The basic Rocking Curve width (sigma): standard deviation in reciprocal angstrom. (***Fig. 14***).
- ⇨ The mosaicity: It represents how sharp/spread is the profile around the bRC width (in deg).

RC width = basic RC width + mosaicity. g. with g the length of the diffraction vector.

At this stage of the development, the software gives a 2D picture of a 3D Camel plot. The x-axis is the distance from the Bragg condition (Sg in $Å^{-1}$), the y-axis represents the intensity. The rocking curves are given at different resolution ranges.

12. Difference between kinematical and dynamical integration

In the kinematical integration, each reflection *hkl* is integrated on all the frames where it is present but also across these frames. The intensities are evaluated according to two main methods (see section below). At the end of the procedure the files jobname.hkl and jobname.cif_pets are created, where the intensities (hkl, I, σ(I)) are written out in the format 3i4,2f10.2, plus some more information after the first five columns. The "cif_pets" file is directly readable by Jana2006 because it contains the lattice parameters and the orientation matrix. At the end of the kinematical integration each reflection appears only once. The dynamical integration option integrates the reflection intensities frame by frame (i.e. no integration across frames) because the refinement will consider each frame as an independent data set. The result of the integration is a file jobname_dyn.cif_pets with integrated intensities suitable for dynamical refinement (well, if your sample is suitable for this purpose, of course).

For more information,

-L. Palatinus, et al. Structure refinement using precession electron diffraction tomography and dynamical diffraction: theory and implementation. Acta Cryst. A, (2015) 71, 235-244.

-L. Palatinus, et al. Structure refinement using precession electron diffraction tomography and dynamical diffraction: tests on experimental data. Acta Cryst. B, (2015) 71, 740-751.

13. Kinematical integration options

► Options

The software offers four options to estimate the intensities of the reflections.

_fit profile: this option corresponds to the red dashed area on the ***Fig. 18***. The intensity of an *hkl* reflection is defined with the calculated Rocking curve profile on all the resolution range according to the data and the two parameters (sigma and moisaicity).

_integrated profile: The intensity of one reflection *hkl* is determined by the area (volume in 3D) defined by the all the measurements for this reflection found in consecutive frames (blue area on the ***Fig. 18***).

_maximum intensity: the value of the measurement with the highest intensity for one given *hkl* reflection is use as a global intensity for this reflection (yellow measurement on the ***Fig. 18***).

_Bragg position: The intensity of the *hkl* reflection is estimated from the closest measurement from the Bragg position (green measurement on the ***Fig. 18***).

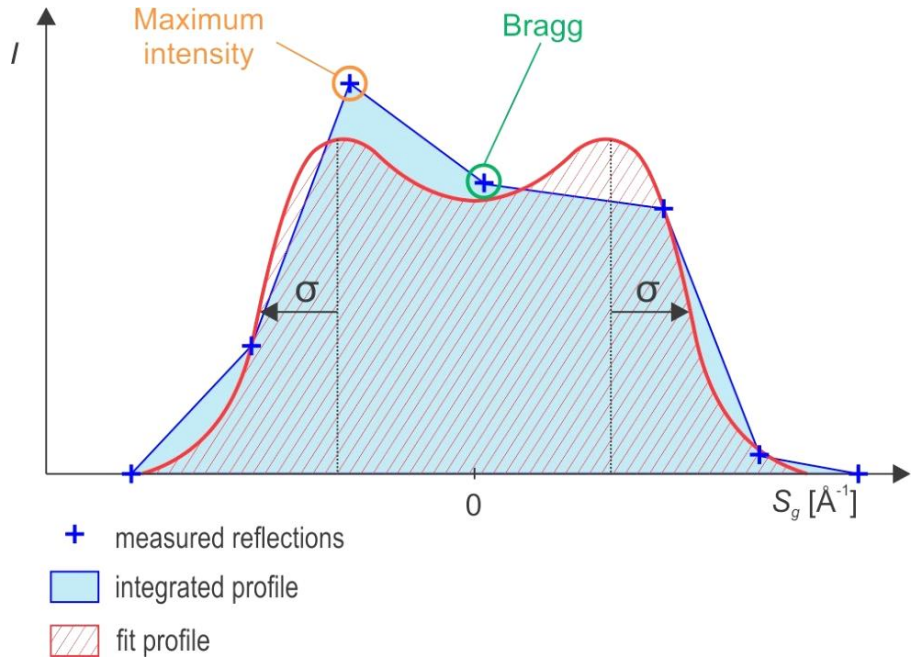


Fig. 18: integration options illustrated with a basic Camel plot profile drawn for one arbitrary resolution.

► **How to choose one or another option?**

In the previous version of PETS, the intensity estimation was done considering the integrated profile. The fit profile option gives more accurate intensities. Now, if the optimization of the integration parameters succeeds, the best results are always obtained from the fit option (better Rint). In general it seems that even if the optimization of the integration parameters fails, the default parameters for the RC width and the mosaicity are accurate enough to use the fit profile option rather than the integrated one. If the optimization of the moisaicity fails, the best procedure is to set the default value for moisaicity and try both fit/integrated profile options and compare the intensity statistics.

If you work with precession data, you have no reason to consider the maximum intensity and the Bragg position options. And if you are working without precession data, it is still unlikely that the two last options give the best results. The maximum intensity estimation is the method used with the electron rotation data (RED) (without precession and a very small rotation step). The Bragg position estimation could be considered with kinematical data collected without precession giving a Rocking curve profile with one sharp maximum…it never happens with electron data.

14. Integration parameters

► Min. number of reflection measurements

This parameter sets how many times an *hkl* reflection has to be measured in the entire data set to be considered in the integration. On the Error! Reference source not found., it is easy to see that if an *hkl* reflection is not enough represented in the data (too few points on the RC profile), its intensity profile and then the estimated intensity will be defined with less/no accuracy giving higher Rint. This parameters is used to avoid those reflections that are not enough measured. In the same time, by increasing this parameter the number of reflections decreases. It is up to you to find the best compromise.

► Reflection width multiplier

It represents how far from the maximum intensity on the Camel plot profile, the integration is extended. The value indicates how many times the sigma (RC width) is used to extend this integration. A meaningful value is usually between 0 and 2 with a default value of 1. For a value close to 0, a lower completeness will be obtained but with higher accuracy. For a value set to 2, a better coverage is reached with lower accuracy. You can consider doing the integration twice, one with a higher completeness to solve the structure, and another with a better accuracy to refine the model.

15. Define lattice centering condition

The centering condition is used in the integration process to skip reflections absent due to the centering. All standard symbols are acceptable: A, B, C, I, F, Robv, Rrev (***Fig. 19***)

A user-defined centering is also possible especially for modulated structures.

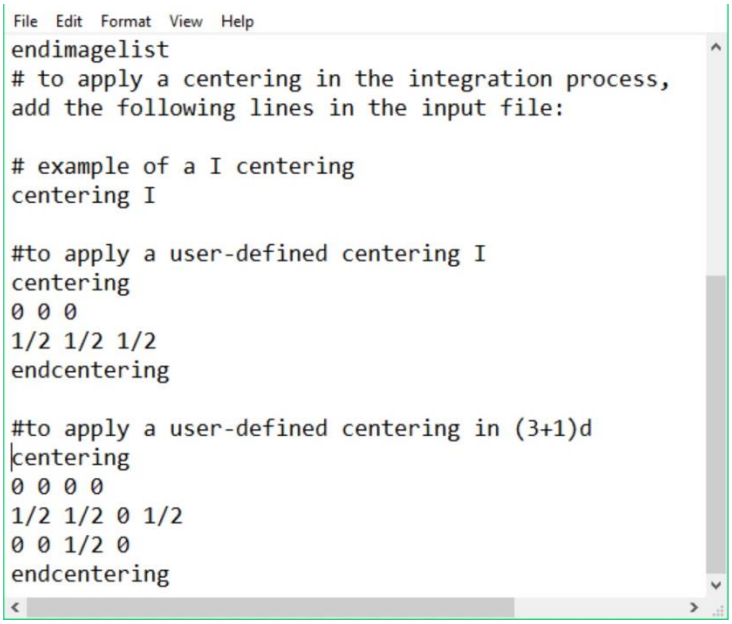


Fig. 19: centering condition in the input file.

16. Generate reciprocal space-sections

With this option you can generate the sections of the reciprocal space (***Fig. 20*** and ***Fig. 21*****)**. The standard set is *hk0*, *hk1*, *hk2*, *hk3, h0l*, *h1l*, *h2l*, *h3l*, *0kl*, *1kl*, *2kl*, *3kl*. If you want to define specific sections you can do it in the input file or in the "Define layer" window opened when you run the "Reciprocal space-sections"(***Fig. 20*****a**).

How to define your section? Multiline keyword. Each line contains a specification of one layer in the reciprocal space that should be reconstructed by PETS. The format is filename basvec1 basvec2 origvec, where each vector is given by three reciprocal-space coordinates. The first two vectors are the two vectors defining the orientation of the plane, and the third vector defines the origin of the plane. You can determinate a specific set of sections in the graphical interface (*Fig. 20*a) or directly in the input file using the keywords "reconstruction" and "endreconstruction" (***Fig. 20*****b**).

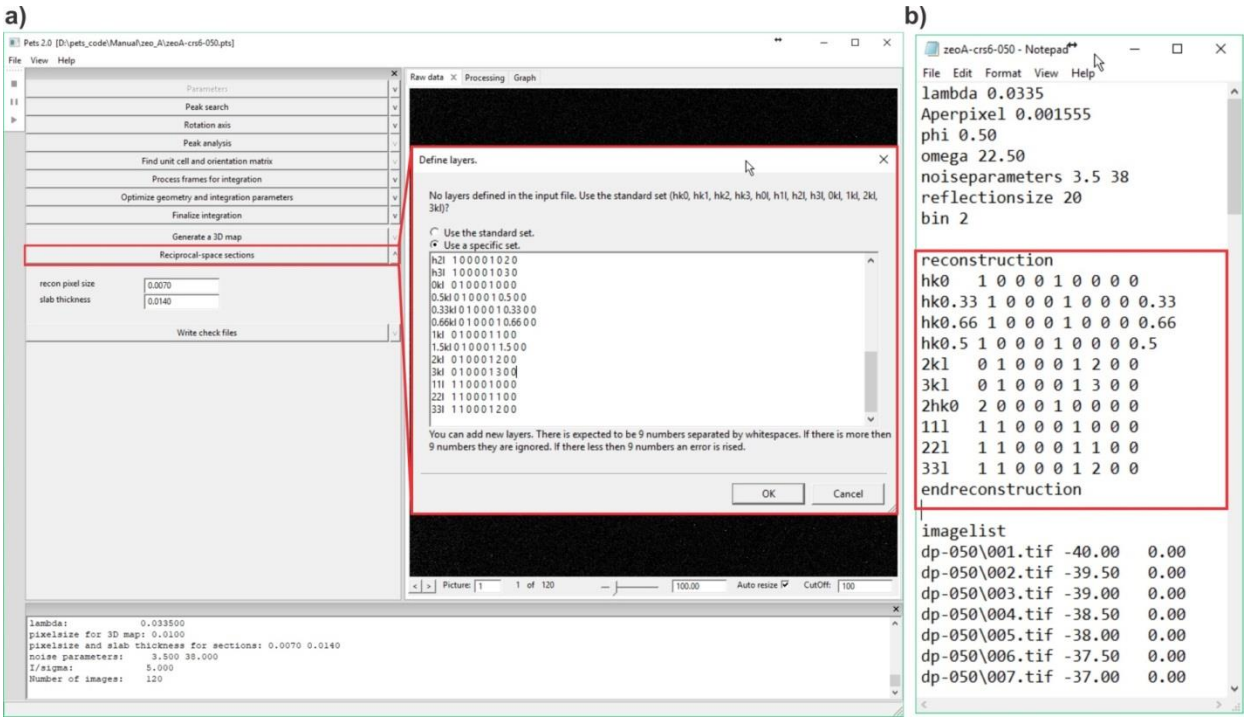


Fig. 20: reciprocal space-section panel to set a non-standard set of sections in a) the graphical interface or b) the input file.

Two parameters are linked to the reciprocal space layers reconstruction. The two values are express in reciprocal angstroms. The first is the pixel size (recon pixel size) in the reconstruction; the second (slab thickness) is the half-thickness of the slab around the exact layer position that is projected onto the layer. Taking a too small pixel size and/or too small slab thickness leads to "holey"reconstructions, where some pixels do not have any intensity. The default value for pixel size is equal to the value of the pixelsize keyword, default for the slab thickness is 2*pixelsize.

17. Generate 3D map of the reciprocal space

Another useful option for getting an overall picture of the reciprocal space. The program will take all images and calculate the intensity distribution in cube with the dimension 2*dstarmax. The data are exported in the format jobname.xplor (large file!) that can be viewed in 3D by software VESTA or UCSF Chimera (***Fig. 22***).

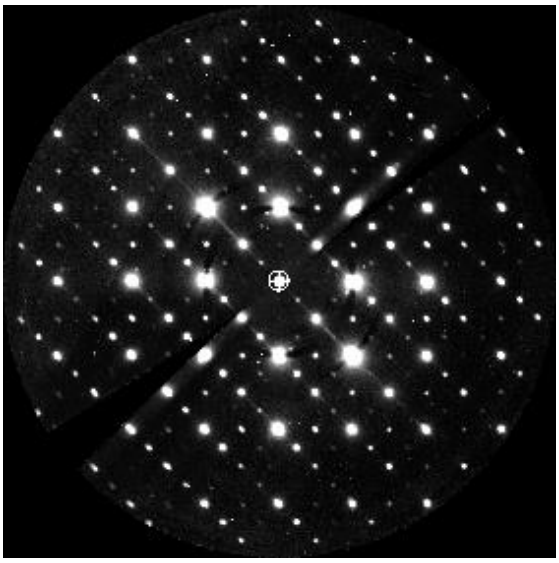


Fig. 21: example of (h0l) section.*

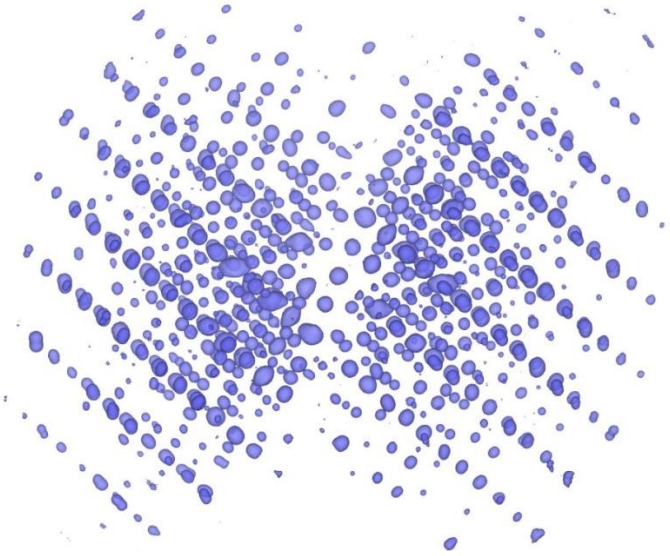


Fig. 22: example of a reciprocal space 3D map.

18. Optimize geometry (STEP 9)

This procedure is a way to correct part of the distortions to improve the integration. As far, this step appears after the indexation (see the complete procedure ***Fig. 23*****)**. So, it does not correct the distortions seen on the 3D reconstruction of the reciprocal space and it does not improve the lattice parameters. The aim of this optimization is to give access to the most accurate intensity and coverage. The most accurate position of the reflections process to integrate properly the reflections together with the optimization of the integration parameters (bRC width and moisaicity). The parameters involved are the alpha, beta, omega angles, the coordinates of the center for each frame, the scale and the RC width (the moisaicity is not refined). For a good quality data set, the improvement will be small but, for specific cases, you need it even to solve the structure: for instance, if you collected data on a bended crystal. In that case the integration will be very inaccurate because the positions of the integration boxes are predicted according to the orientation matrix, the unit cell and the center of the frame. So, even if you integrated most of the reflections of the data set, only few are observed. Indeed their intensities will be too small regarding the σ(I) because the integration is not done on all the intensity profile.

Limitations: data set with too few reflections, small lattice parameters.

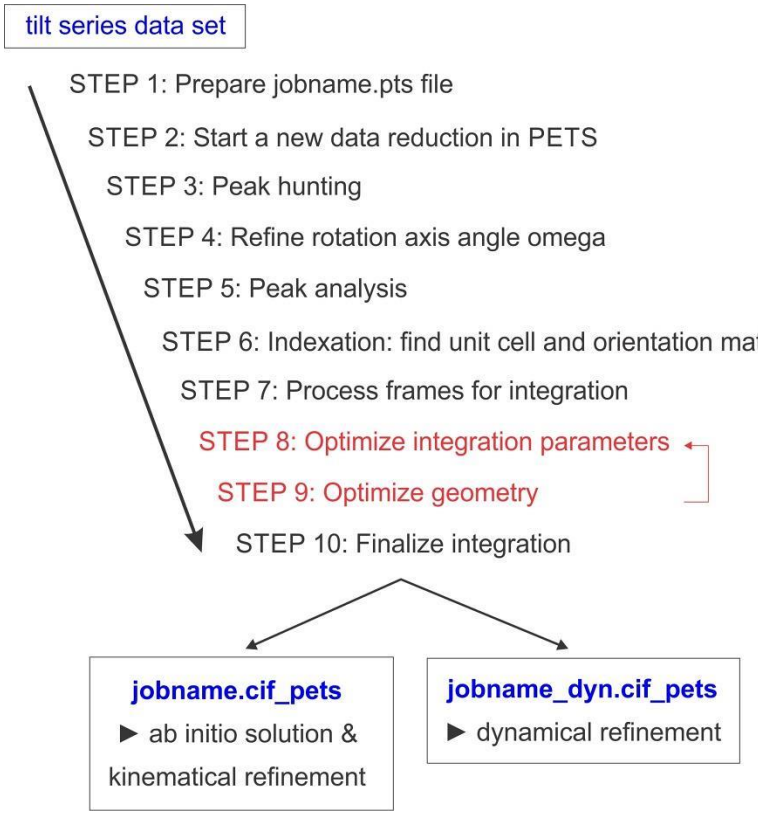


Fig. 23: complete data reduction procedure.

Procedure: The geometry optimization comes after the Optimization of the integration parameters (***Fig. 23***). However, if you have very distorted data, the STEP 8 won't be successful especially the value of the mosaicity (huge values). The Camel plot profile won't look nice too. So, in all the cases, you need to try the step 8 and if it fails, set up back the default value of the mosaicity before starting the STEP 9. The STEP 8 is re process with more accuracy after the step 9 when some of the distortions are corrected.

Let's go back to the nice zeolite example and restart for the STEP 8 "Optimize integration parameters". The optimization is successful, the values of the moisaicity, the RC width are reasonable and the optimized Camel plot profile is improved (***Fig. 14***). Here, it seems that no huge distortions are present and the STEP 9 is optional but we can try to get even better results.

In the menu "Optimize geometry and integration parameters", unselect the "refinement using RC profil" (STEP 8) and select "refinement using frame simulation". Keep the default parameters for the orientation angles accuracy (deg.) and for the center of the diffraction patterns accuracy (pixel).

For the simulation, you can use:

- ⇨ **The uniform intensities**
- ⇨ **The integrated intensities**. If you want to use the integrated intensities to optimize the geometry, you need first to "Finalize the integration" (STEP 10) because the file that is created from "Process for integration" is not dedicated to this purpose (jobname.dyntmp). The file

jobname.dyntmp (STEP 7 on *Fig. 23*) is a list of reflection frame by frame and the optimization needs a global position of the reflection in 3D, so a kinematical integration (jobname.cif_pets).

Which is the best route? For good data that gives nice CAMEL plot profile and integration parameters, you can either use integrated or uniform intensities. For distorted data or in general if the STEP 8 fails, it is better to use uniform intensities because otherwise you will base the optimization on wrong information. If you have a doubt, choose the uniform intensities.

To start the optimization, run "Optimize geometry and integration parameters". This step takes time…

Note that the option "Advanced settings" is only for development purposes, you do not need to use it.

What is happening in the process? In the console, you will see the result of the optimization frame by frame. On the **Graph** panel, the tilt correction of alpha, beta and omega graph is built point by point during the optimization (***Fig. 25***). In the window **Processing** for each frame are the observed pattern after processing (left) and the simulated one taking into account of the geometry corrections (right) (***Fig. 24***). When the geometry optimization works, the two patterns are very similar. If they are different, this is the indication that the process likely failed for this frame. On the plot of the ***Fig. 25*** the tilt correction are expected to be smooth with a general trend. If random big jumps appear, this is likely that the procedure failed for this frame. It happens more often for oriented or almost oriented patterns like in our zeolite example and for data with high precession angle.

In the console (***Fig. 26***), for each frame are the current values for alpha, beta, domega, xcenter, ycenter, RC width, the moisaicity, the reflection width in image, and the initial value of the minimization function. The Final parameters are given at the end of the process for each frame. 3 files are created at the end of the optimization: jobname.cenlocopt (optimized *xy* coordinates of the center), jobname.petsopt (pts file with optimized values) and jobname.ptsoptlist (summary of the calculations for all the frames).

- ⇨ If the optimization succeed, run again "Optimize integration parameters" (STEP 8) (new Camel plot ***Fig. 27***), then re"Process frame for integration" (STEP 7) and finish with the STEP 10 "Finalize integration" (***Fig. 28***). More observed reflections should be obtained after this optimization. It is not very obvious for this example, but for another distorted data set (see ***Fig. 29***) the improvement is important and the procedure was necessary.

Note: At the current stage of the development, all the optimized values are taken into account as long as the jobname.pst is open. If you want to remove the geometry correction for some frames where the procedure failed, there is not easy way to do! You need to go in the OPT file, correct manually the values, then, copy the list of the optimized angles/values in the input file, restart PETS, and restart the procedure… (Good luck).

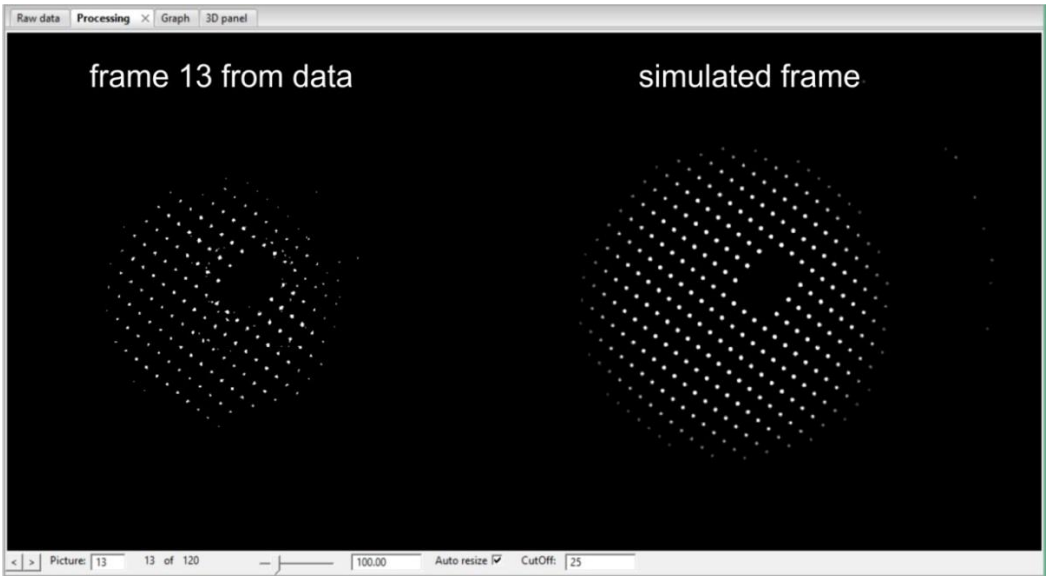


Fig. 24: example of one zone axis and the corresponding simulated pattern with optimized geometry parameters and uniform intensities.

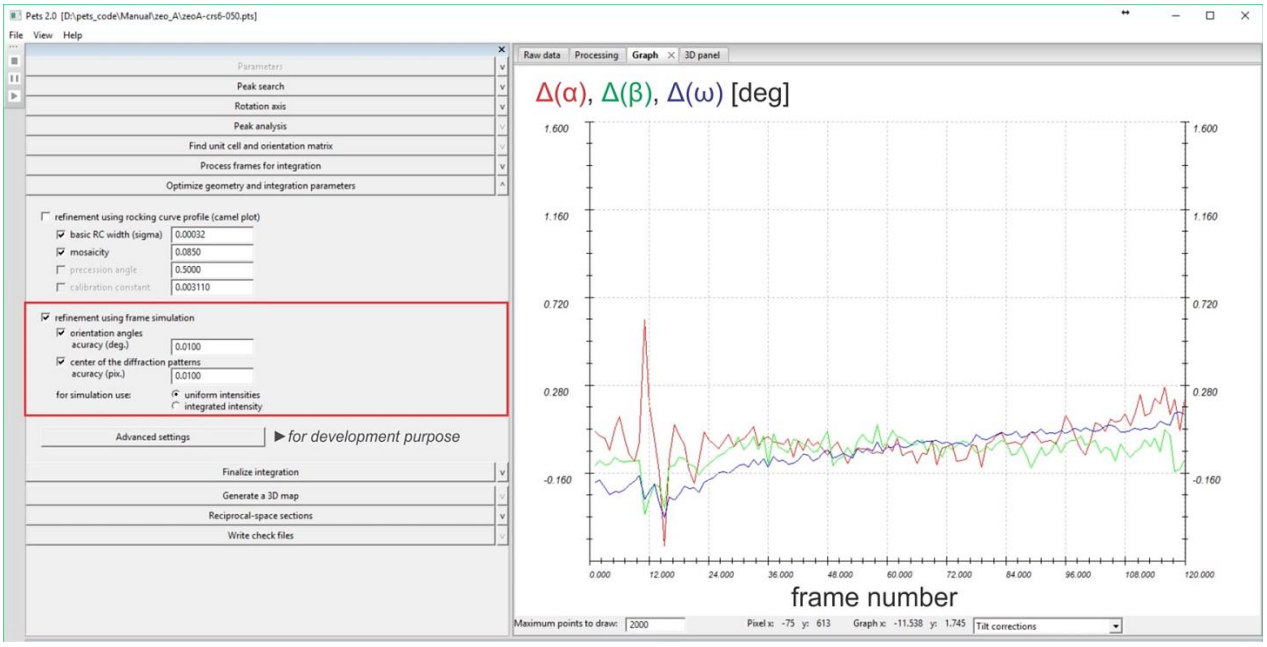
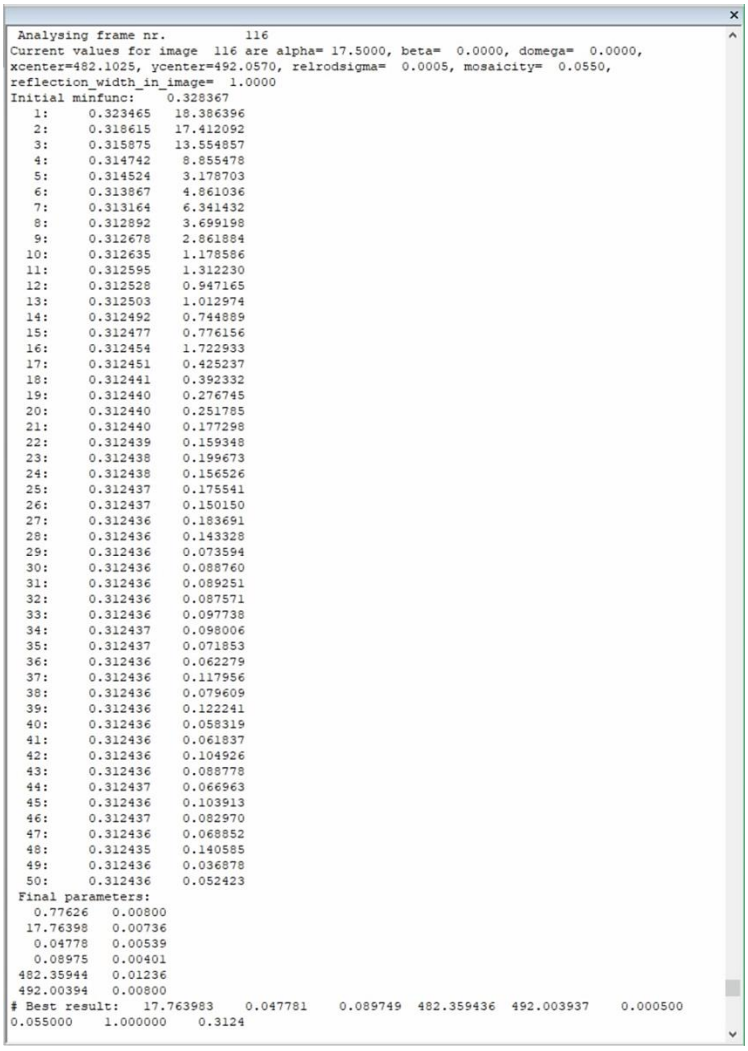


Fig. 25: Geometry optimization options (command panel), and plot with the tilt corrections for alpha, beta and omega (main panel).



```
 Analysing frame nr.          116
Current values for image  116 are alpha= 17.5000, beta=  0.0000, domega=  0.0000,
xcenter=482.1025, ycenter=492.0570, relrodsigma=  0.0005, mosaicity=  0.0550,
reflection_width_in_image=  1.0000
Initial minfunc:    0.328367
   1:     0.323465   18.386396
   2:     0.318615   17.412092
   3:     0.315875   13.554857
   4:     0.314742    8.855478
   5:     0.314524    3.178703
   6:     0.313867    4.861036
   7:     0.313164    6.341432
   8:     0.312892    3.699198
   9:     0.312678    2.861884
  10:     0.312635    1.178586
  11:     0.312595    1.312230
  12:     0.312528    0.947165
  13:     0.312503    1.012974
  14:     0.312492    0.744889
  15:     0.312477    0.776156
  16:     0.312454    1.722933
  17:     0.312451    0.425237
  18:     0.312441    0.392332
  19:     0.312440    0.276745
  20:     0.312440    0.251785
  21:     0.312440    0.177298
  22:     0.312439    0.159348
  23:     0.312438    0.199673
  24:     0.312438    0.156526
  25:     0.312437    0.175541
  26:     0.312437    0.150150
  27:     0.312436    0.183691
  28:     0.312436    0.143328
  29:     0.312436    0.073594
  30:     0.312436    0.088760
  31:     0.312436    0.089251
  32:     0.312436    0.087571
  33:     0.312436    0.097738
  34:     0.312437    0.098006
  35:     0.312437    0.071853
  36:     0.312436    0.062279
  37:     0.312436    0.117956
  38:     0.312436    0.079609
  39:     0.312436    0.122241
  40:     0.312436    0.058319
  41:     0.312436    0.061837
  42:     0.312436    0.104926
  43:     0.312436    0.088778
  44:     0.312437    0.066963
  45:     0.312436    0.103913
  46:     0.312437    0.082970
  47:     0.312436    0.068852
  48:     0.312435    0.140585
  49:     0.312436    0.036878
  50:     0.312436    0.052423
 Final parameters:
   0.77626   0.00800
  17.76398   0.00736
   0.04778   0.00539
   0.08975   0.00401
 482.35944   0.01236
 492.00394   0.00800
# Best result:   17.763983    0.047781    0.089749  482.359436  492.003937    0.000500
0.055000    1.000000    0.3124
```

Fig. 26: geometry optimization console for one frame.

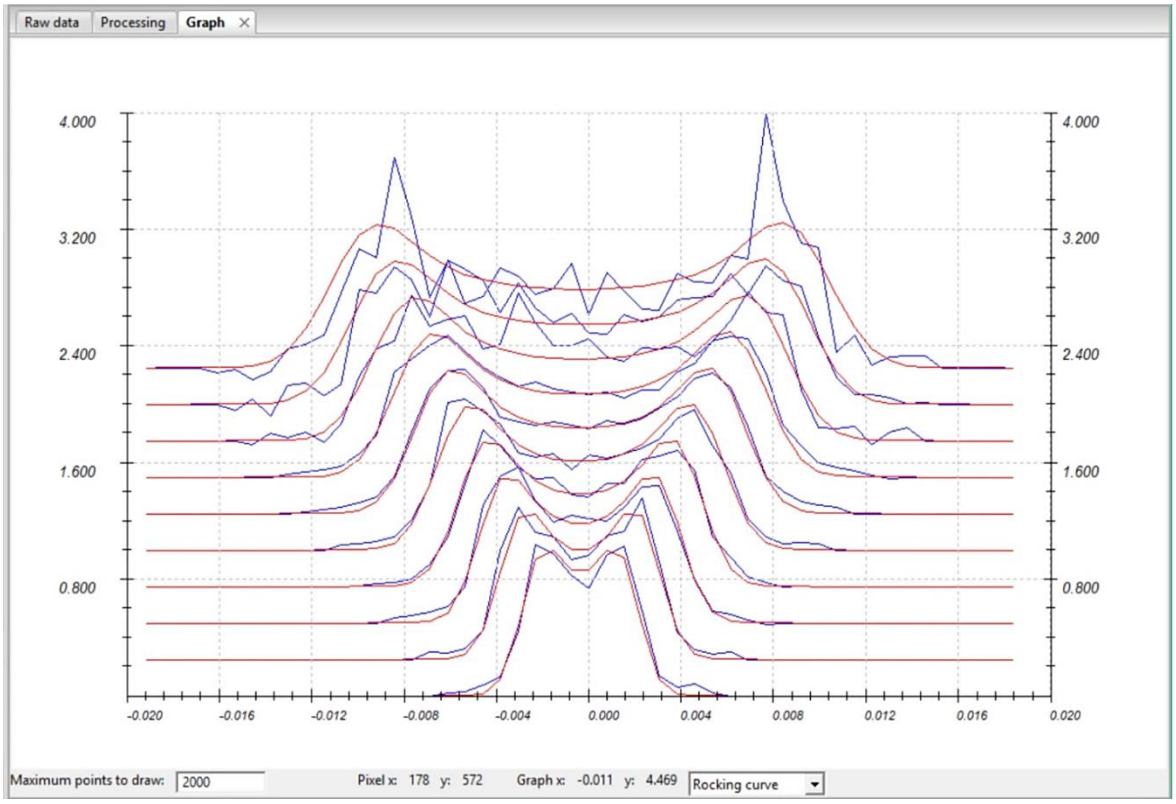


Fig. 27: New Camel plot profile after geometry optimization (to compare with Fig. 14) .

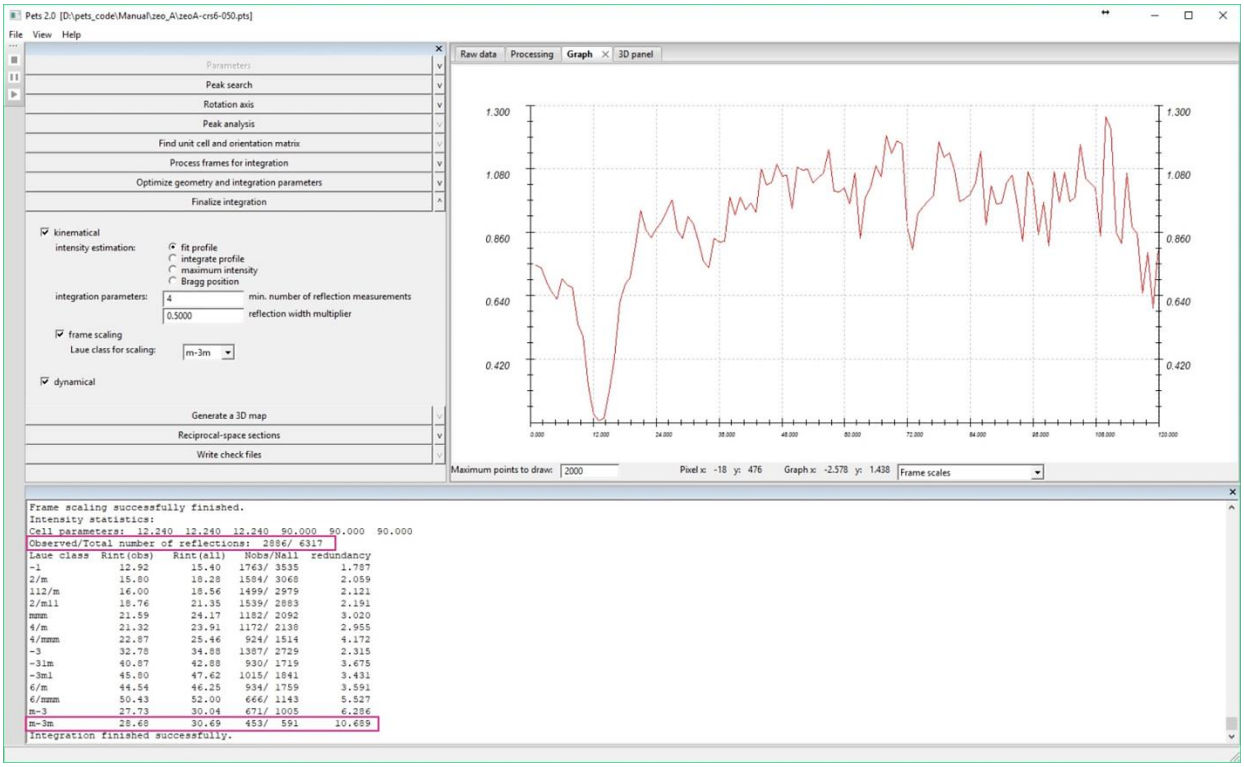


Fig. 28: Last integration results after all the optimizations.

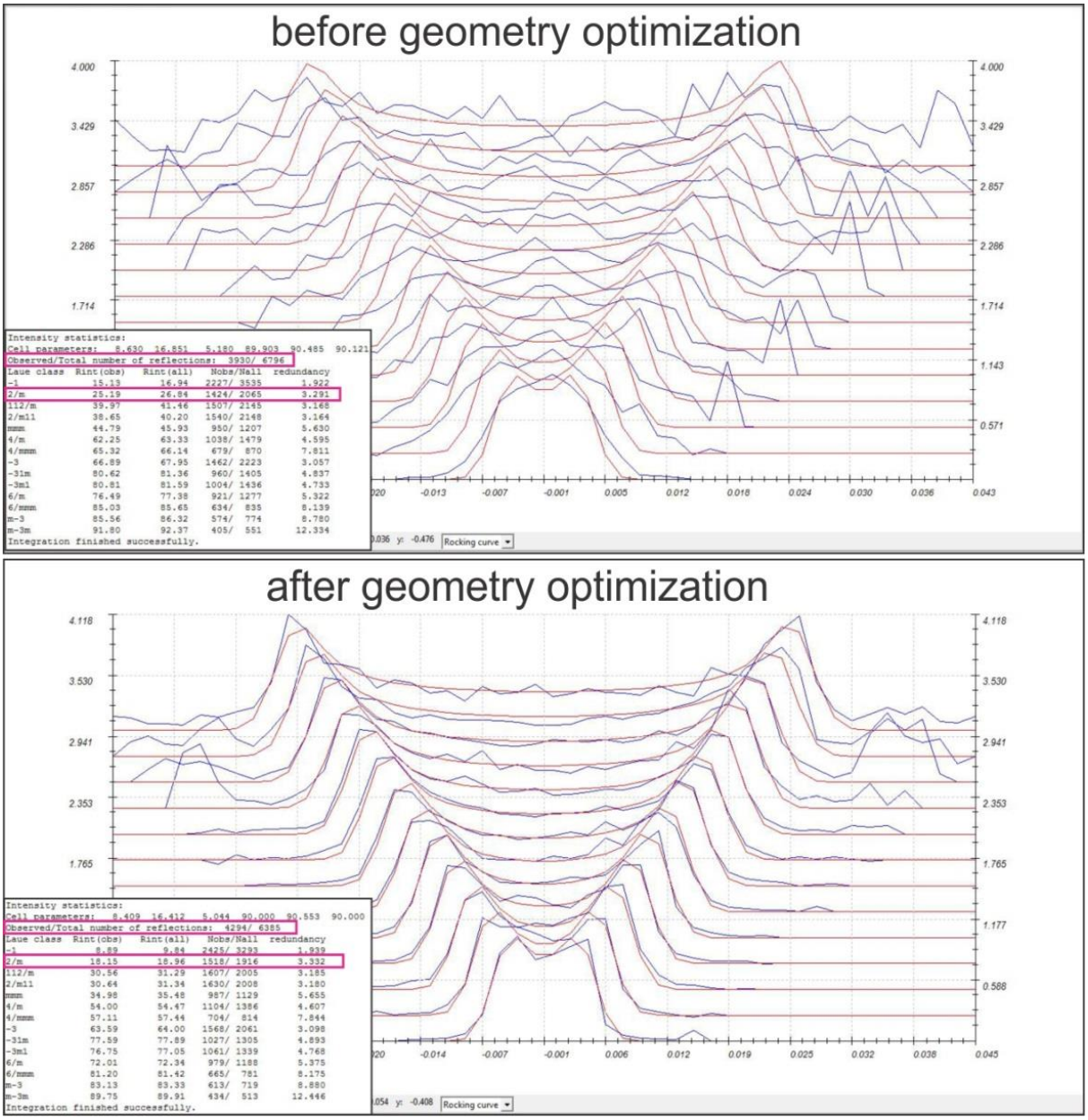


Fig. 29: example of Camel plot profiles and integration results before and after the geometry optimization for a data set with huge distortions.

19. Modulated structures

- **Index satellite reflections: soon**
- **Integration of satellite reflections**

To integrate satellite reflections and indicate the maximum order of satellites, the following line needs to be added in the input file:

```
maxindex 2 # to integrate satellites up to order +/-two
```

20. Twinned crystal

- **Indexation tools : soon**
- **Integration: soon**