

# ***ADT3D* Tutorial**

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***Karlsruhe, 19-20 March 2012***



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# 1. Introduction

Automated Diffraction Tomography (ADT) technique<sup>[1-4]</sup> is based on electron diffraction tilt series acquired through a step-wise tilt of a selected crystal around an arbitrary (non-crystallographic) axis. The so obtained non-oriented (off-zone) diffraction patterns cannot be analyzed directly. *ADT3D* software package is able to transform a stack of 2D off-zone diffraction patterns in a 3D diffraction volume, to determine the unit cell parameters through automatic clustering and manual routines and to extract intensities that can be used for structure solution by direct methods. Moreover the 3D diffraction reconstruction can be used for identifying special features as polycrystallinity, twinning, super-structure modulations, incommensurate reflections, etc...

## 1.1 Aims of *ADT3D* tutorial

This tutorial is thought for leading the student through the use of *ADT3D* software package. The idea is to simulate a real data analysis, starting from a raw ADT acquisition consisting of a stack of 2D off-zone diffraction patterns (acquired from a single nano crystal by a tilt series performed in our lab) and ending with 3D reconstructed diffraction volume, cell parameters and an intensity hkl file. Together with the installation file you find four exemplary *mrc* files: BaSO<sub>4</sub>, Na<sub>2</sub>Ti<sub>6</sub>O<sub>13</sub>, natrolite and Hemimorphite\_BeamStop. More information about the data sets are in § 5. You can start the analysis opening the BaSO<sub>4</sub> *mrc* file and go through the functions of the program.

More information is available within the software manual.

## 1.2 Standard work-flow

The following is a brief outline of the analytical steps that lead you from the uploading of a raw data stack up to the reflection intensity integration.

- Loading data. An *mrc* file containing a stack of 2D off-zone diffraction patterns is the standard input format. An *mrc* file can be created from a stack of *tif* images using *TIF2MRC* converter (not included here).
- Inspecting the 2D patterns.
- Shifting the diffraction patterns to a common center.
- Finding the tilt axis azimuth.
- Rotating the 2D patterns in order to have the tilt axis in vertical position.
- Binning the data to save memory space.
- Creating a 3D volume (that can be saved in *mrc* format).
- Visually inspecting the 3D volume.
- Finding the reflections.
- Determining the unit cell with automatic or manual routines.
- Extracting intensities and compiling a standard *hkl* file usable for structure solution.

### 1.3 *mrc* data type

Two types of *mrc* files are used by the program, **2D diffraction patterns stack** and **3D reconstructed diffraction volume**.

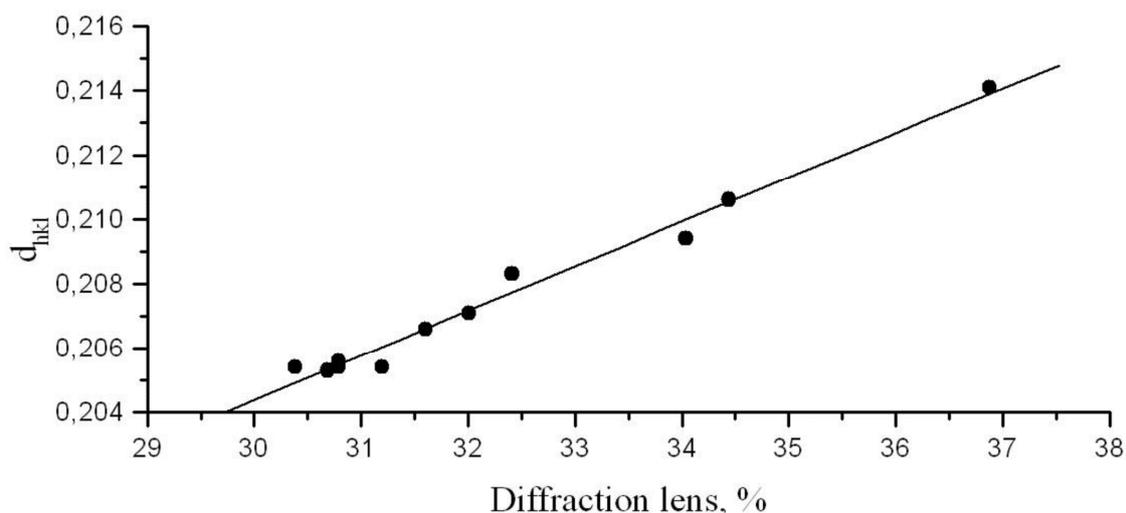
**2D diffraction patterns stack** is an *mrc* file which contains a series of off-zone electron diffraction patterns acquired during the tomographic tilt of a crystal. The  $x$  and  $y$  dimensions are the size of the image in pixels (all diffraction patterns within a tilt series must have the same size) and  $z$  is the number of patterns in the stack.

**3D reconstructed diffraction volume** is an *mrc* file containing a 3D diffraction volume in Cartesian coordinates. Usually this is the output of the *ADT3D* data analysis.

### 1.4 Fine diffraction patterns calibration

The calibration of a TEM camera length is normally valid only in selected area electron diffraction (SAED) conditions, i.e. when the electron beam is widely open and a fully parallel illumination can be assumed. In nano electron diffraction (NED) conditions, when the electron beam on the sample has a diameter of 100-30 nm, this assumption is not valid any more. In this regime a certain convergence of the beam is unavoidably introduced, and the resulting diffraction patterns appear out of focus. In order to have proper focused diffraction patterns, it is necessary to modify the diffraction lens current (normally this operation is referred as adjustment of the diffraction focus). This operation introduces deformations as strain and rotation. Moreover we observe a general expansion/contraction of the whole diffraction pattern. Therefore camera length and pixel size determined for SAED conditions (your normal calibration) are not accurate anymore in NED conditions and an error up to 30% can be introduced.

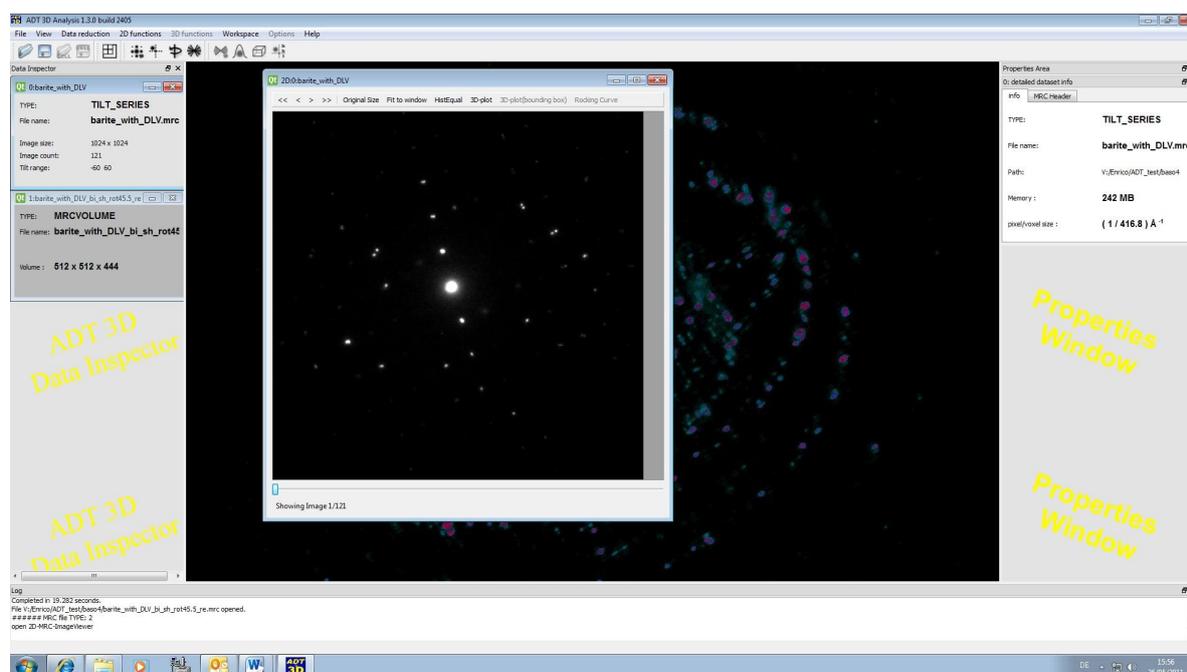
As many ADT data set come from nano electron diffraction one should take care of this error using a specific fine calibration. The value of the expansion/contraction of the diffraction patterns is in the first approximation linearly dependent with the diffraction lens current (Figure 1). In *ADT3D* it is possible to take care automatically of this fine calibration by the use of a small *txt* file where the gradient and the intercept of the regression line are reported (you can open the *fineCalibration.txt* file inside the folder *bin* in order to understand how this file is built). These parameters are different for different camera lengths. The diffraction lens current for any acquisition can be stored in and recalled from the *mrc* header.



**Figure 1.** Dependency between a selected  $d_{hkl}$  distance and the diffraction lens current.

## 1.5 General ADT3D interface aspect

The program interface is shown in Figure 2. The **Main Menu**, on the top of the page, consists of two command lines. The first line contains drop-down submenus, while the second line contains main icons. In the **Data Inspector** on the left the loaded files are shown, together with some basic information. On the right the **Properties Area** delivers further information about the selected file, like the full file header and, when present, the cell vectors description. In the center of the page the **2D** and **3D Viewers** appear when called. Finally, at the bottom of the page there is a **Dialog Panel** where the work history and the alarm messages are stored.



**Figure 2.** Screen shot of ADT3D interface.

## 2. Data analysis: 2D diffraction pattern stack

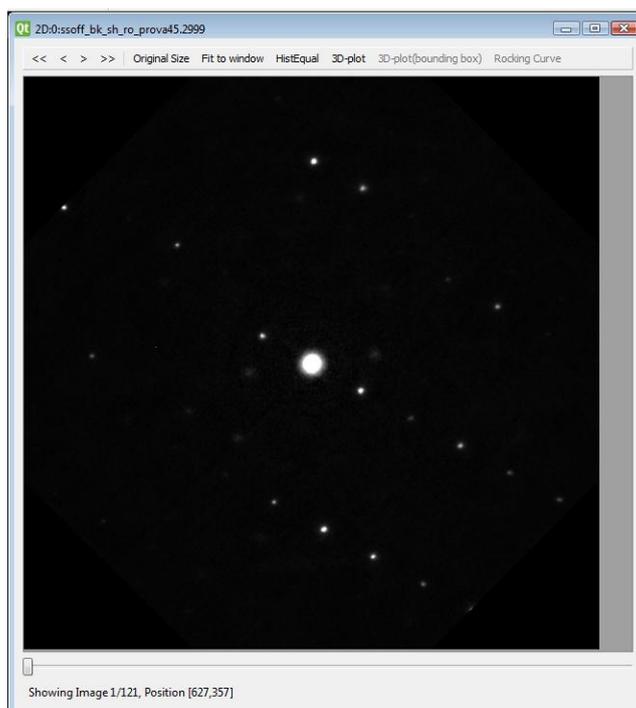
### 2.1 2D diffraction pattern stack input

To open an *mrc* file containing a 2D diffraction patterns stack, as delivered by an ADT acquisition or built by our *TIF2MRC* converter, click on the *Open MRC file* icon in the **Main Menu**. A dialog window allowing you to browse through your data will appear. Once the data is loaded, a window with information from the *mrc* header will appear in the **Data Inspector** (on the left side) showing the *TYPE* of the data (in this case *TILT\_SERIES*), the *File name*, the *Image size* (size in pixels of a single diffraction pattern), the *Image count* (the number of diffraction patterns in a stack), and the *Tilt range* (in °). On the right side in the **Properties Area** a second window will appear with more detailed information and the full file header.

Multiple *mrc* files can be loaded simultaneously. However, because an *mrc* file can be very large, the developers advise loading only few files at the same time. After every step of the data processing the new created files can be saved clicking on *Save MRC file* icon and the old file can be closed. This will help to avoid any memory capacity issue.

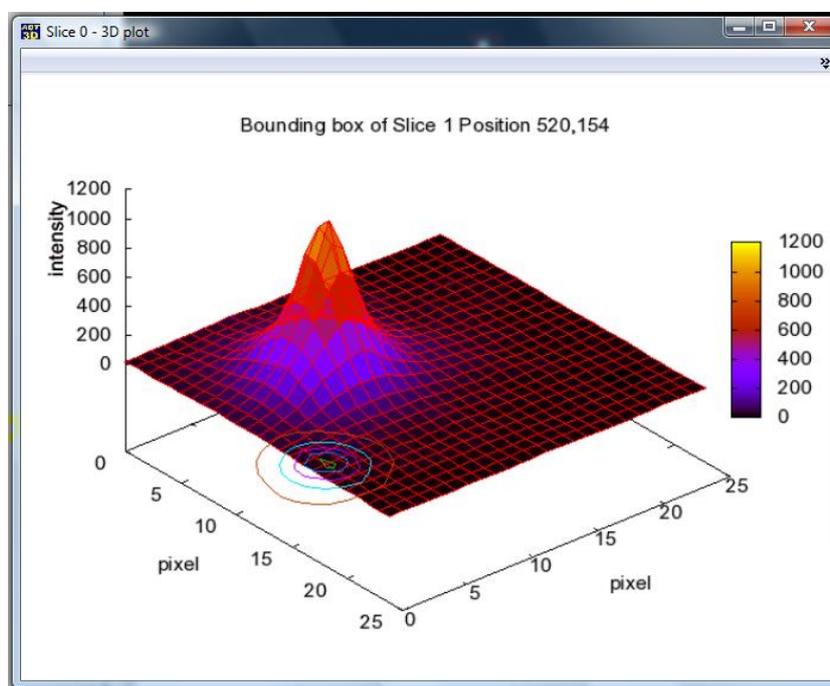
### 2.2 2D Viewer

The **2D Viewer** can be called by the corresponding icon in the **Main Menu**. The viewer allows the visualization of the recorded diffraction patterns (Figure 3). Within the **2D Viewer** it is possible to navigate through the stacked 2D diffraction patterns. Diffraction patterns are visualized in 8-bit scale. Histogram linearization (*HistEqual*) displays the image with the highest available contrast.



*Figure 3. 2D viewer.*

The *3D-plot* command opens an extra window with the current diffraction pattern presented as a surface plot. A particular reflection can be selected by clicking on the diffraction pattern. A bounding box is placed around the reflection. A 3D plot of the reflection within the bounding box can be visualized by clicking the *3D-plot(bounding box)* button (Figure 4).



**Figure 4.** 3D plot of pixel intensities for a single reflection.

Intensity variation of the selected reflection through the tilt series can be visualized by the *Rocking Curve* function. In the rocking curve plot, intensities integrated for subsequent patterns and within different bounding boxes size are shown.

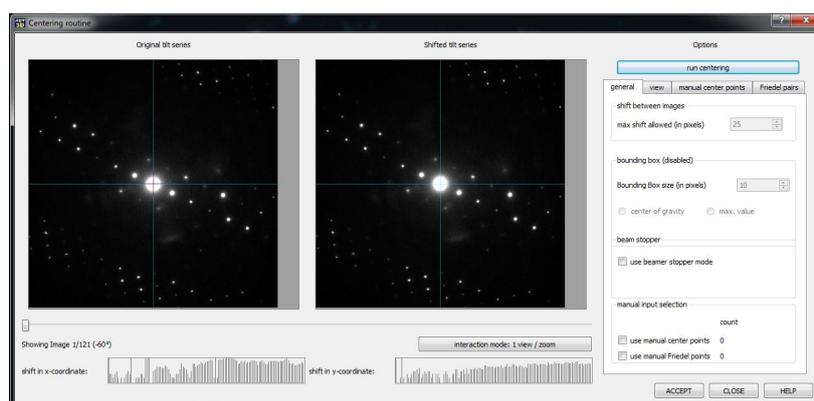
## 2.3 Data binning

Most of the time you do not need data with full (as recorded) high definition (a normal CCD has an area of 1028x1028 or 2056x2056 pixels). Binning means halving the dimension of the 2D diffraction patterns. The purpose of this operation is to save memory capacity while doing operations which do not require the full data definition. Binning can be done at the beginning of data analysis in order to speed all the following steps. The binning procedure creates a new *mrc* file with the suffix “*\_bi*”. The exemplary data sets available for this tutorial are already in a convenient size of 512x512 pixels. If you have memory problems with your laptop you can bin again the data.

## 2.4 Patterns centering

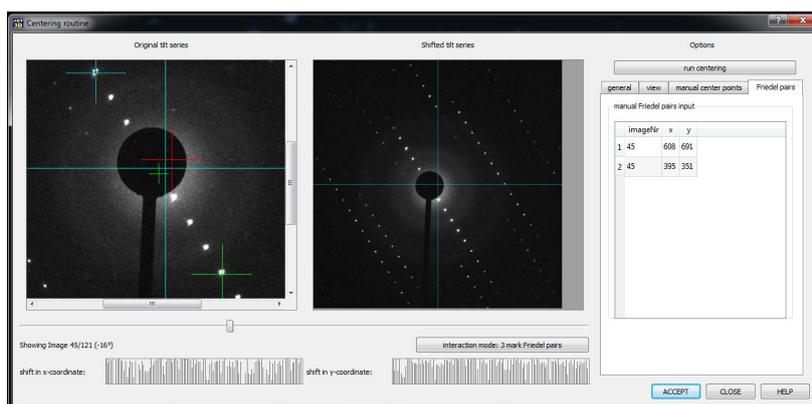
Despite your best effort, experimental diffraction patterns are never perfectly centered. Moreover, during the tilt series acquisition the position of the central spot, and therefore the complete diffraction pattern, may shift. The shift may occur for different reasons, but it is usually connected with an electron beam shift on the sample. In fact the beam shift usually occur working only in NED conditions, where the user has to open and close the beam at each acquisition and may need to follow with the beam the position of the crystal during the tilt. On the contrary, a good SAED acquisition should not show any shift between two consecutive diffractions. In this case the only problem should be a constant (small) misplacement of all the patterns from the center. Anyway, for a proper 3D reconstruction all the 2D patterns need to be perfectly centered.

The centering function is called by pressing the *Pattern centering* icon. This command opens the *Centering routine* panel (Figure 5). There are several options for centering the patterns. The default method is based on the analysis of the central beam. The routine is launched by clicking on the *run centering* button in the top right corner. After the patterns are centered, the shifted diffraction patterns are visible on the right side and can be visualized with the cursor on the bottom.



**Figure 5.** Centering routine panel.

If some patterns appear wrongly shifted by the automatic procedure, this can be corrected by manually setting the center position or selecting a convenient Friedel pair in the misplaced pattern. For selecting the center, click on the *interaction mode 1: view / zoom* bottom and therefore going into *interaction mode: 2 mark beam center* or into *interaction mode: 3 mark Friedel pairs*. You can zoom into the pattern of interest (left window) with the right mouse bottom (but don't be too fast!) and select the center or a convenient Friedel pair with the left mouse bottom. You can check what you selected into the *manual center points* and *Friedel pairs* panels in the upper right (Figure 6). Then you have to select *use manual center points* and/or *use manual Friedel points* options and launch again the *run centering* routine. All the slides will be centered according with the aforementioned routine except the ones for which you selected the center manually.



**Figure 6.** Centering routine panel: Friedel pair selection.

If during the data collection a beam stopper was used, the centering routine fails for all the slides and the user can only define the center by Friedel pairs. Assuming that the central beam shift is the same in all the slides (normal condition in SAED; usually you use a beam stopper in SAED conditions, but not in NED), you just need to select a single pattern where one Friedel pair is clear and select it. Then you have to select the *use beamer stopper mode*. This would make all your patterns to shift according to the designated center.

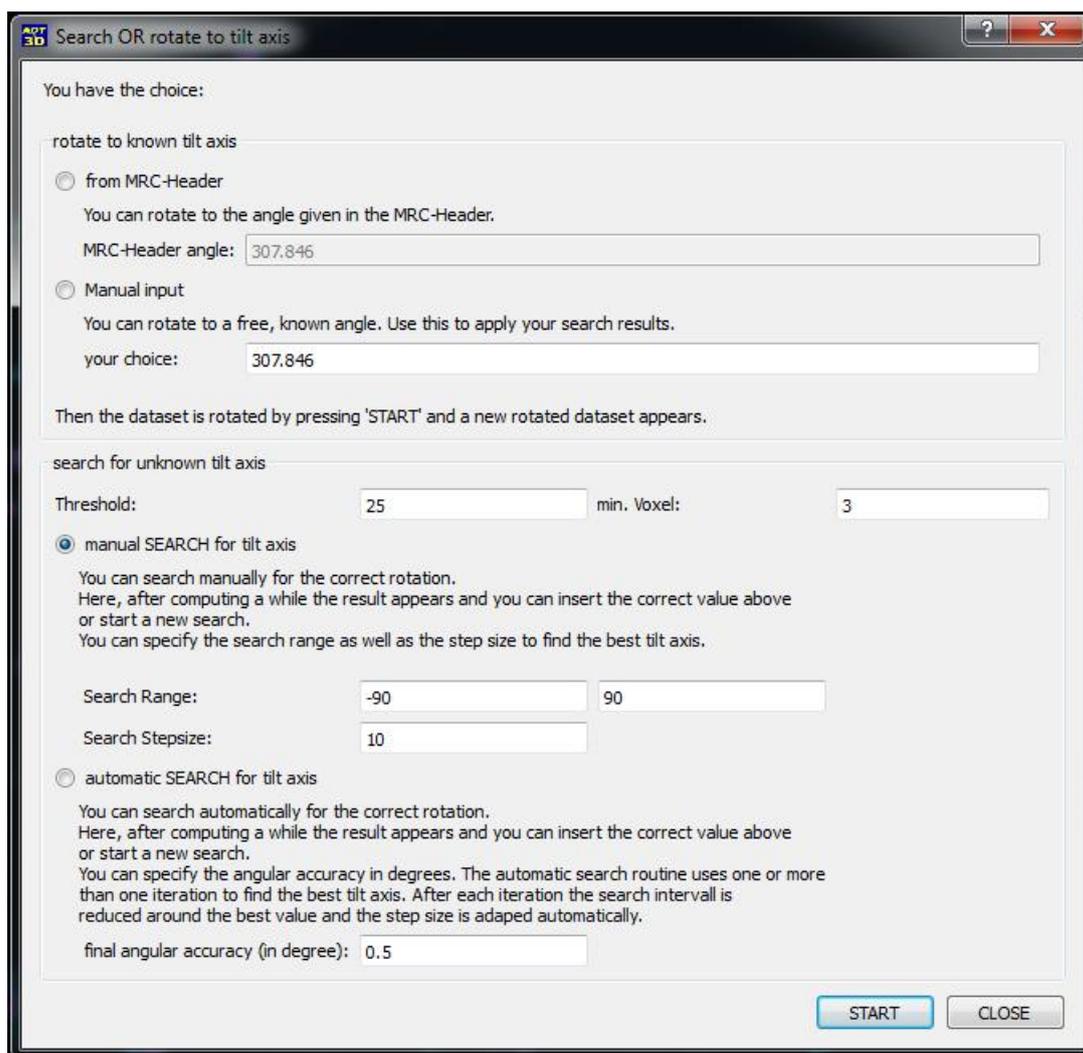
If at a certain point you observe a different pattern shift (not optimal situation, but unfortunately possible), you have to select a different center from this point on. If in a following slide a second different center is selected by another Friedel pair, then all the following slides will shift according with this last selection.

Once the user is satisfied with the centering, he can push the *ACCEPT* bottom for closing this panel and creating a new *mrc* file, whose name is extended by a “*\_sh*” suffix.

## 2.5 Tilt axis determination / rotation of the patterns

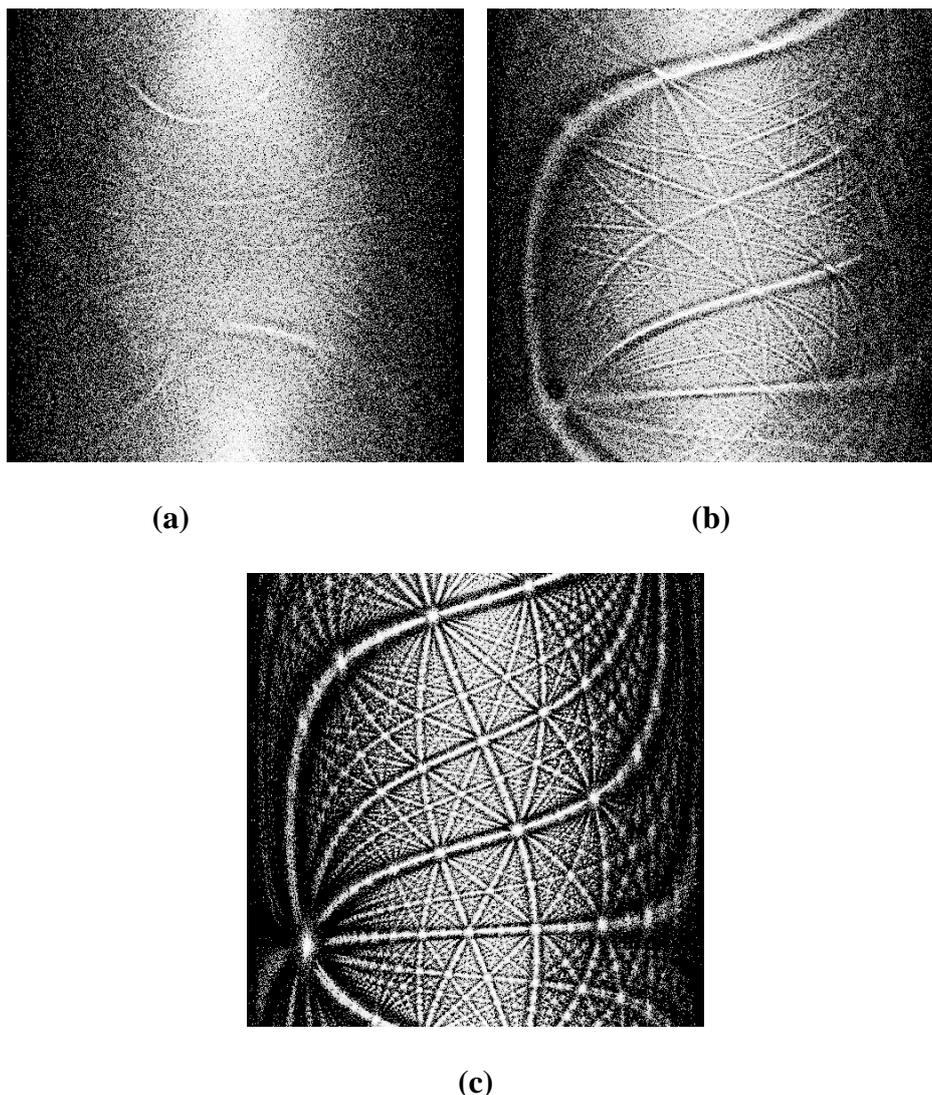
To ensure an accurate 3D reconstruction of the diffraction data the precise azimuthal position of the tilt axis is necessary. The procedure for determining the tilt axis angle is called by the icon *Refine Tilt Axis*. On the click a dialog panel *Search OR rotate to tilt axis* appears (Figure 7). The position of the tilt axis is measured from the horizontal in a standard Cartesian coordinate system where *x* is the horizontal axis, and *y* the vertical.

The tilt axis position can be stored and recalled from the *mrc* header. A user can also arbitrarily select a reasonable value for the tilt axis. When the exact position is not known or needs to be refined, an automatic search for the tilt axis can be used. Normally it is not necessary to scan the full turn, so the user can impose a defined search range. In the exemplary cases proposed for this tutorial the tilt axis is in the range 40/50° for BaSO<sub>4</sub>, Na<sub>2</sub>Ti<sub>6</sub>O<sub>13</sub> and natrolite, while it is in the range -40/-35° for Hemimorphite\_BeamStop. You can search it with a step of 0.5°. For the examples of this tutorial set the *Threshold* to 300.



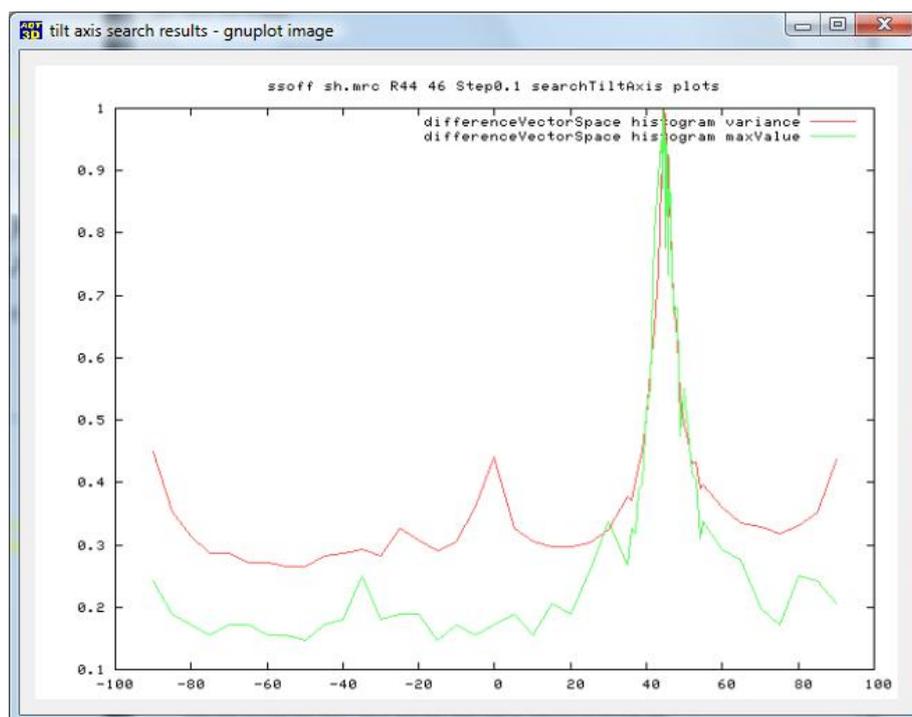
*Figure 7. Search OR rotate to tilt axis dialog panel.*

The search for the tilt axis is based on the assumption that the correct tilt axis value delivers a correct 3D diffraction volume, i.e. the reciprocal volume should contain a coherent 3D lattice. This coherent lattice should produce a clear well-defined difference vector pattern in the stereographic projection. If the position of the tilt axis is wrong, the 3D reciprocal lattice is twisted, and the stereographic projection is blurred. Therefore the “sharpness” and the “brightness” of the stereographic projection of the 3D reciprocal lattice can be used as criteria for the tilt axis search (Figure 8). More details of this procedure can be found in Kolb et al. 2011.<sup>[5]</sup>



**Figure 8.** Three stereographic projections of the 3D reciprocal space: (a) a blurred projection from a reconstruction with completely wrong tilt axis; (b) a blurred projection from a reconstruction with a tilt axis  $10^\circ$  away from the correct value; (c) a sharp projection from a reconstruction with the correct tilt axis.

You find all the stereographic projection images in *jpg* format in a folder called *FILE\_NAME\_tilt\_angle\_search* next to your *mrc* input file. The result of the search is shown in the plot of Figure 9. Actually the highest value of the variance profile is taken as the correct value for the tilt axis. If there is more than one peak in the plot or the maximum profile differs from the variance profile, it is better to start manual searches in all regions and compare the stereographic projections.



*Figure 9. Variance plot as result of the tilt axis search.*

After the tilt axis is determined, each 2D diffraction pattern can be rotated in order to have the tilt axis vertical. This makes the 3D reconstruction easier. A new *mrc* file with the suffix “\_ro\*\*\*” (where \*\*\* is the value of the rotation in °) is created.

## 2.6 Reconstruction of the 3D volume data

This step is the core of the procedure. The reconstruction of the volume is done just by pressing the *3D Interpolation* icon. An *mrc* file containing a 3D reconstructed diffraction volume is produced with the suffix “\_re”.

## 3. Data analysis: 3D diffraction volume

### 3.1 3D diffraction volume input

An *mrc* file containing a 3D diffraction volume file can be loaded by clicking on the *Open MRC file* icon (the same used for loading 2D diffraction pattern stacks), or reconstructed from a 2D diffraction pattern stack, as described above. As for 2D data, information coming from the file header is reported in the *Data Inspector* and in the *Properties Area*. The data *TYPE* is now MRCVOLUME. In this case the *Volume* indicates the 3D size of the volume in voxels. Multiple *mrc* files can be loaded simultaneously. However, because an *mrc* file, and particularly a 3D volume, can be very large, the developers advise loading only one file per time. The file can be saved clicking on *Save MRC file* icon.

### 3.2 3D Viewer

The *3D Viewer* command opens four windows where the diffraction volume is projected along different directions (Figure 10). The volume shown in the upper right window can be rotated freely using the left mouse button. The images can be separately zoomed with the mouse wheel. The user can make any of them full screen by placing the mouse cursor on it and pushing the space bar.

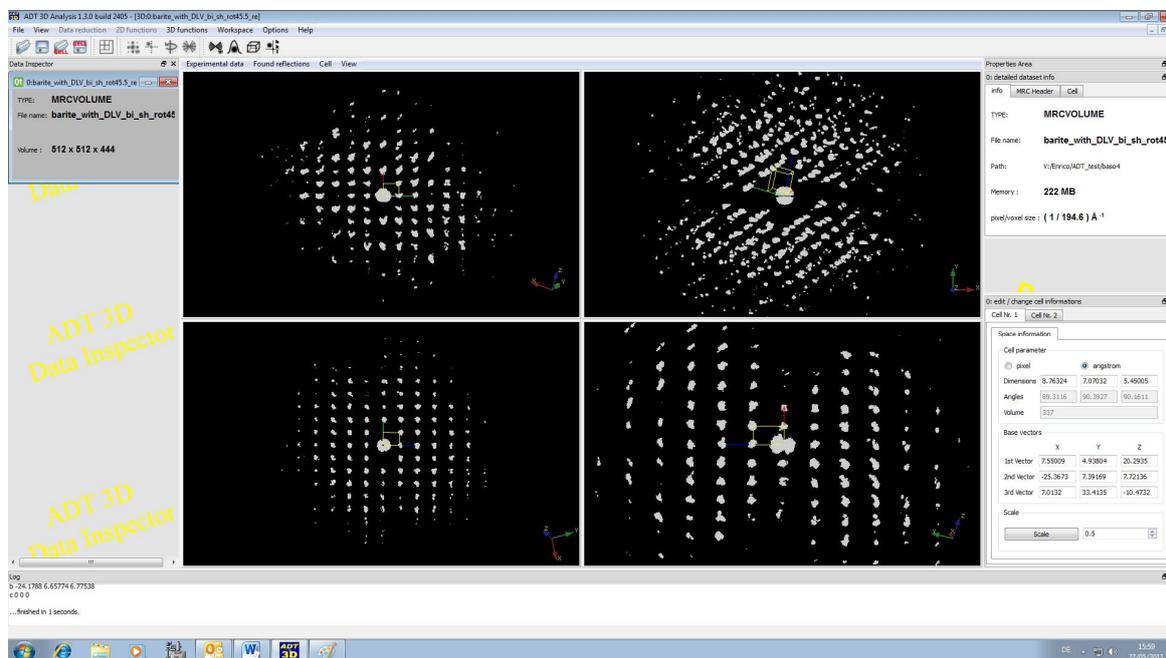


Figure 10. 3D viewer with unit cell superimposed.

### 3.2.1 Experimental data

The *Experimental data* drop-down menu offers visualization options for the 3D data. *ADT3D* supports a volume rendering which can be configured via the *Experimental data > Volume Rendering* menu. For each data set a transfer function (grey values or colored) can be saved, loaded or changed. The default transfer function is grey scale. Via *Volume Rendering > Edit - > Load / Save Transfer Function (TF)* you can load other transfer functions or save your own transfer function.

*Volume Rendering > Adjust > Transfer* allows you to change hue, saturation, value and alpha of the image.

*Volume Rendering > Adjust > Windowing* allows changing the view range of the data. On the top you see the complete range, while on the bottom you can set the visualization range, by default set to 0-255 (8 bit image). You can input values by hand, or use right and left mouse bottom to change the range and check the effect of your operations.

The option to visualize data by *Volume surface* is not implemented in this version of the software.

### 3.2.2 Found reflections

The *Found reflections* menu shows the reflections found during the automatic search for the cell parameters and used for intensity extraction. The use of these functions will be clear in the following, when the routines for reflection search are described.

### 3.2.3 Cell

A cell file can be loaded from the *File > Open CELL file* icon, or can be determined with the routines described in the following. The three reciprocal vectors are shown as red, green and blue vectors:  $a^*$ ,  $b^*$  and  $c^*$  (Figure 8). The unit cell parameters and the reciprocal vectors components are shown in the *Properties Area*. As default the 3D volume is now visualized along the three main reciprocal directions.

Within the cell panel the user can switch between pixels (in reciprocal space) and angstrom (in real space) representations. The components of the reciprocal vectors (cell parameters + orientation matrix) are also shown either in pixels or in angstrom.

The unit cell can be displayed within the diffraction volume data as a single cell, or as a lattice by activating the *MultiCell* option. As soon as an appropriate unit cell vector set is created it can be saved by clicking the *Save CELL file* icon in the *Main Menu*. Cell vectors are saved in a file with *cell* extension. You can open a cell file with *WordPad* and check which information it contains.

Cell parameters can be manually adjusted and redefined by free editing inside the cell panel in the *Properties Area*. The components of the reciprocal vectors ( $a_x, a_y, a_z; b_x, b_y, b_z; c_x, c_y, c_z$ ) can be changed independently. Otherwise the cell lengths can be directly changed and the components of the relative vectors are adjusted accordingly. Angles cannot be changed directly. The full cell can be scaled by using the *Scale* button. As a final option, in the free rotation window (upper right) of the *3D Visualizer* the user can select a specific orientation and set this orientation as a main direction pushing *shift+a*, *shift+b* or *shift+c*. The user can

freely modify the cell but for crystallographic computations it is preferably that the reciprocal vector basis is right handed (i.e. the determinant of the vector matrix is positive). When the matrix determinant is negative a warning appears in *Dialog Panel* at the bottom of the page.

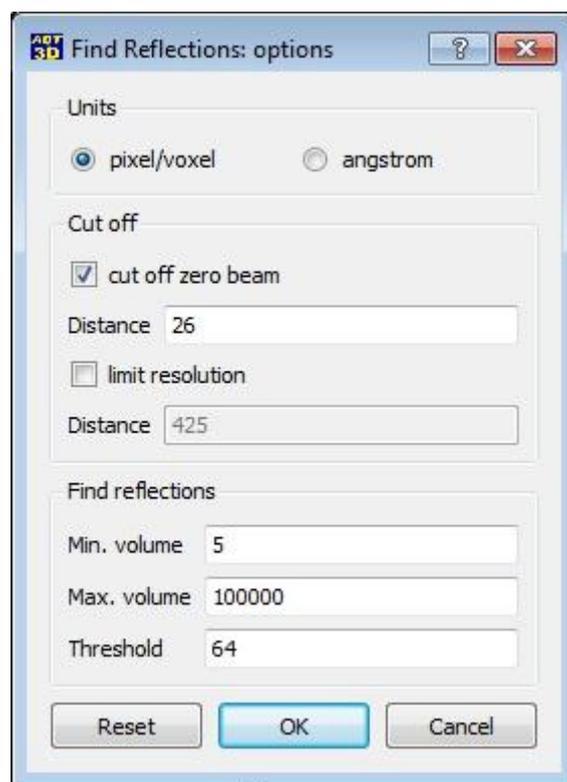
### 3.2.4 View

*Toggle Camera* changes the projection mode from parallel to perspective and vice versa.

## 3.3 Automatic unit cell parameter determination

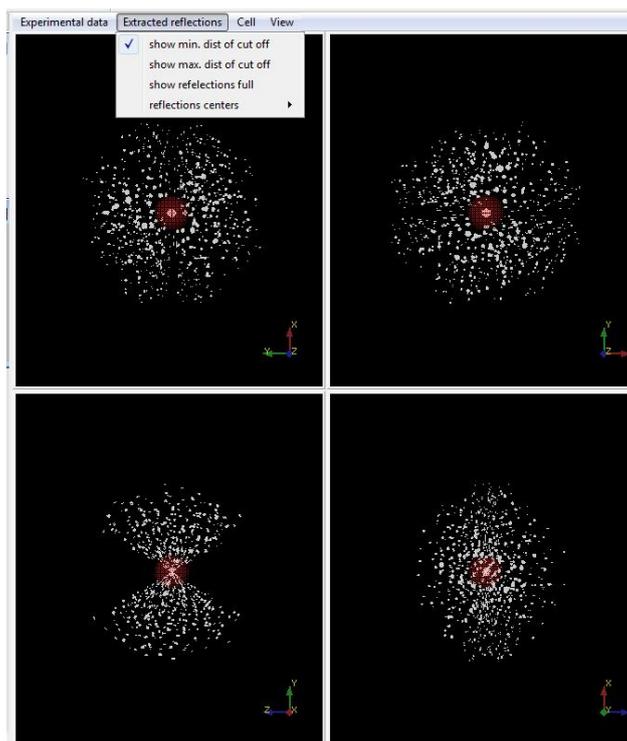
### 3.3.1 Reflection search

The automatic cell parameter determination procedure starts with the definition of the reflections in the 3D space. In order to correctly sample the reflections, appropriate parameters have to be set in the relative menu, called from the *Main Menu* by *Options > Find Reflections Parameters*. The corresponding panel is shown in Figure 11.



*Figure 11. Find Reflection Parameters panel.*

In this panel the parameters can be input either in pixels or in angstroms. The area around the central beam in electron diffraction patterns is usually not suitable due to the strong background and therefore it is advised to exclude this area from the reflection search using the *cut off zero beam* option. If the option *show min. dist of cut off* in the *3D viewer > Found reflection* drop-down menu is activated, the omitted area is marked with a semi-transparent red sphere (Figure 12).

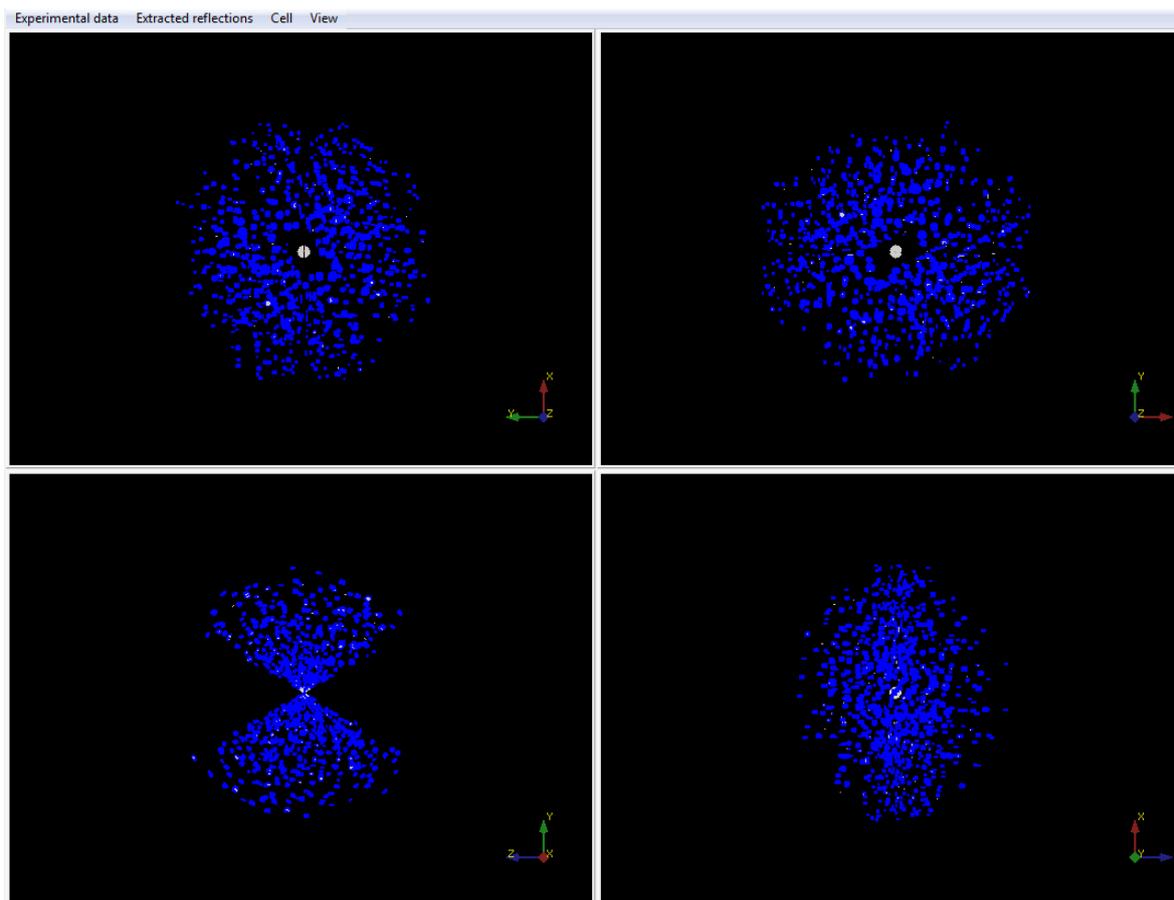


*Figure 12. 3D viewer with show min. dist of cut off option activated.*

Certain tasks may require excluding the high-resolution area for the reflection search. This is achieved by activating the *limit resolution* option. If the option *show max. dist of cut off* in the **3D viewer** > *Found reflection* drop-down menu is activated, the area inside the desired resolution is marked with a semi-transparent green sphere.

Reflections are defined as objects having more than *Min. volume* adjacent voxels with total intensity higher than a certain *Threshold*. Reflections bigger than *Max. volume* voxels are excluded, as they could represent disorder features as streaks or artifact due to reflection overlapping or camera blooming. When the options of the reflection search procedure are defined, the procedure can be started by clicking on the *Peak search* icon of the **Main Menu**.

In the **Dialog Panel** at the bottom of the page the number of found reflections is displayed. When the *show reflections full* option in the **3D viewer** > *Found reflection* drop-down menu is switched on reflections are shown in the 3D viewer with blue markers (Figure 13). Reflections should be sampled in order not to overlap but at the same time to be collected even if close to the central beam. You can tentatively change the *Find Reflections* parameters (better only one per time) and see what happens.



*Figure 13. 3D viewer with show reflections full option activated.*

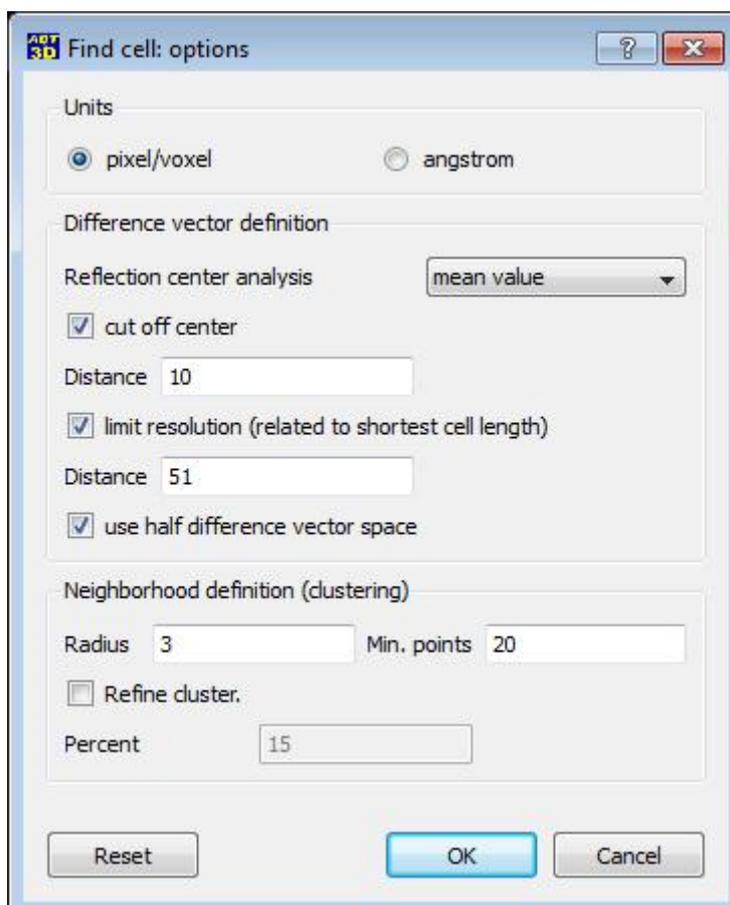
Reflections are therefore defined as sets of adjacent voxels all with intensity above a certain threshold. In principle there are different ways to calculate the coordinates of these reflections: just taking the voxel with the highest intensity out of the set, taking the geometrical mean value of the set, or taking the weighted (through the intensities) center of mass. In the *3D viewer* these different centers can be displayed using the options offered in *Found reflections > reflection centers* drop-down submenu. Normally, these coordinates do not differ significantly.

Note: the reflection search doesn't refresh if no one of the relative parameters has been changed in the *Find Reflection Parameters* panel. Therefore before a new search you **MUST** change one of the parameters.

### 3.3.2 Automatic primitive cell determination by clustering routine

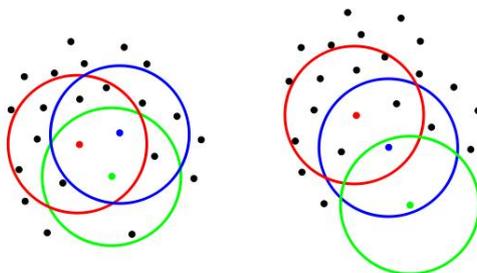
The automatic cell parameter determination routine is based on clustering in difference vector space. As the reflections are not distributed homogeneously, but sit mostly on the nodes of a 3D lattice, the difference vector space must have the same periodicity of the reciprocal space, but strongly enhanced, especially for short vectors. The three shortest non-coplanar vectors found in the difference vector space should correspond to the primitive unit cell vector set. Due to geometrical uncertainty of the reflection position, like slicing error during the data acquisition and the physical size of reflections, each node of the lattice is smeared out. Data clustering should group vectors into sets corresponding (ideally) to each node.

Clustering options can be set in *Options > Cell calculation Parameters* panel (Figure 14), where lengths can be expressed either in pixels or in angstroms. The user can use either the reflection centers defined by the maximal intensity, or the geometrical center, or the weighted center of mass. The *cut off center* option set the shortest difference vector that can be considered as a cluster. Very short difference vectors are usually omitted because they are normally noise or artifacts. Difference vectors are normally calculated between all reflections. Nevertheless some distances may be quite long and working with all of them is computationally intensive and unnecessary. Therefore, only difference vectors within a certain shell are considered for unit cell vectors calculation. The size of this shell is defined by the *limit resolution* distance. The option *use half difference vector space* reduces the time for the computation, but in some cases leads to error in the calculation; in general it's better to deactivate this option.



**Figure 14.** Cell calculation Parameters panel.

For the clustering routine two parameters are used in the definition of the groups: *Radius* and *Min. points*. In *ADT3D* package the clustering procedure is done using a density algorithm. Any difference vector belongs to a cluster if it has at least *Min. points* other different vectors inside a neighborhood defined (in pixel) by *Radius* value (Figure 15).

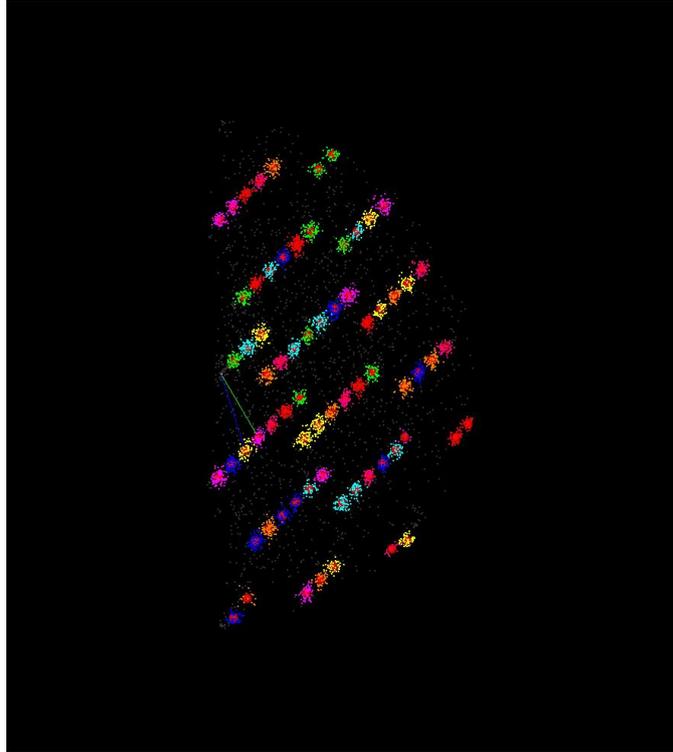


**Figure 15.** Clustering routine algorithm. On the left blue, red and green vectors belong all to the same cluster as they have many other vectors in their neighborhood (defined by related circles). On the right the green vector doesn't belong to the cluster as it has only one other vector inside his neighborhood.

Each data set may have different distribution of reflections, therefore *Min. points* and *Radius* should be adjusted for each data set. Using a too large *Radius* may lead to fusion of separate clusters; a too short *radius* may lead to cluster splitting. If *Min. points* is set to a too high value, it may happen that no clusters will be found at all as the cluster definition is not satisfied; if this value is too low, only a few large clusters will be found likely mingling several lattice nodes. It is strongly recommended to change *Min. points* and *Radius* one per time, as they strongly influence each other.

*Refine cluster* option can be used in order to make the cluster position more accurate once they are correctly found. Data points assigned to a cluster but placed on the outer shell of the cluster are omitted using the given threshold (in %). The new center of the cluster is then recalculated. This procedure improves the coherency of the cluster lattice especially when the clusters have irregular shape.

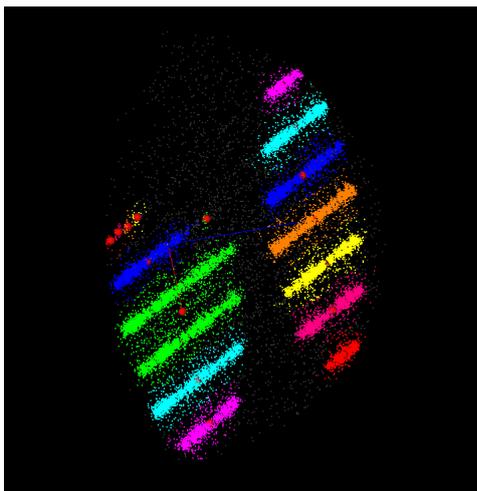
The *Find Cell* icon of the **Main Menu** starts the automatic unit cell parameters determination. After the procedure is finished, a *Difference vector space viewer* window opens, showing the found clusters as colored clouds of spots (Figure 16). The centers of the detected clusters are marked by small red spheres. These spheres represent *nodes* of the reciprocal lattice. Three shortest non-coplanar vectors towards cluster centers are found automatically and are marked as  $a^*$  (red),  $b^*$  (green) and  $c^*$  (blue). For more information about the *Difference vector space viewer* see § 3.4.



*Figure 16. Difference vector space with colored clusters and proposed primitive cell.*

### *Empirical clustering parameters tuning*

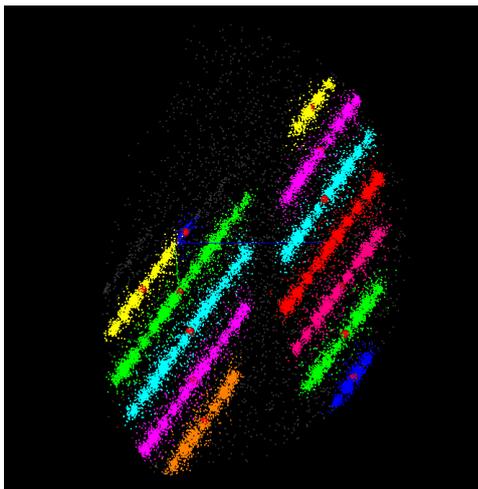
In the following is shown a case where the tuning of the clustering parameters is particularly critical. In this case the cell is characterized by extremely anisotropic cell parameters.



Starting parameters: *Radius 3, Min. points 20.*

The clusters along the reciprocal rods are not resolved. Moreover several rods are assigned to the same cluster.

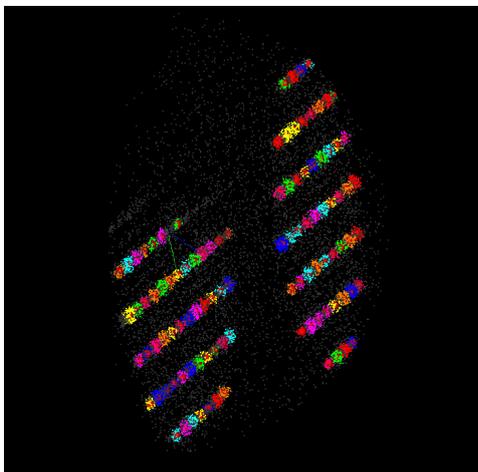
Solution: first reduce *Min. points* to separate the rods.



Parameters: *Radius* 3, *Min. points* 40.

The rods are clearly distinguished, but all the vectors belonging to a rod are assigned to the same cluster.

Solution: reduce *Radius*.



Parameters: *Radius* 1, *Min. points* 40.

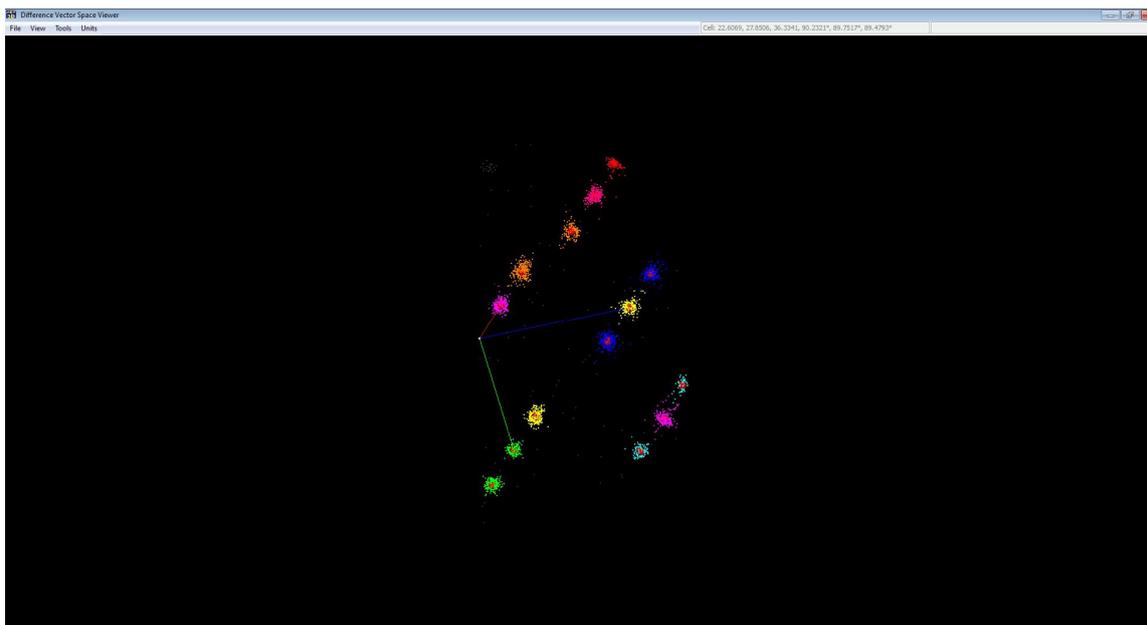
All the clusters are now clearly distinguished by the program and the user can proceed with the cell parameters determination.

### ***Clustering parameter tuning through a systematical approach***

The clustering parameters can also be tuned using a systematical approach based on statistic. The effect of different parameters can be visualized calling the panel *Options > Visualization options* from the **Main Menu**. Three different plots can be used for the tuning. This approach is not recommended for beginner users and is reported here only for completeness.

## **3.4 Difference Vector Space Viewer**

The ***Difference Vector Space Viewer*** visualizes the part of the difference vector space used for the unit cell vectors determination (Figure 17). Difference vectors are shown as grey points if they are not assigned to a cluster and treated as noise, and colored if they are assigned to a cluster. Three automatically defined unit cell vectors connecting the origin with the three closest clusters are shown as red ( $a^*$ ), green ( $b^*$ ) and blue ( $c^*$ ) lines crossing at the coordinate origin.



*Figure 17. Difference Vector Space Viewer with clusters and cell vectors.*

The *Text Field* on the top of the page shows the unit cell parameters defined by current cell vectors. The vector lengths are shown in pixels for the reciprocal space vector and in angstroms in direct space vector if *Units* are set to *angstrom*. The *File* drop-down menu offers the possibility to load or save the active cell, or and send the cell to **3D Viewer**.

In the *View* drop-down menu the user can select different visualization options: *cluster points* shows the difference vectors assigned to clusters; *noise points* show the other difference vectors, not assigned to clusters; *cluster* shows the centers of the found clusters as small red spheres; *additional cluster* visualizes in green extra clusters created by the user (this cluster can be added by the *Tools* menu); *cell* visualizes the cell vectors

In the *Visualization Mode* of **Difference Vector Space Viewer** the volume can be rotated using left mouse button. Pressing *Escape* key on the keyboard leads from the *Visualization Mode* to the *Editing Mode* (the mouse icon changes from a rotation double arrow to a normal pointer). In *Editing Mode* the clusters can be selected by the mouse and deleted by keyboard *Delete* key. The mode can also be changed by *View > toggle mode* command.

When a certain cluster is selected a 3D box is placed around it. It is easier to handle the clusters when only cluster points and noise points are not visualized. Keeping *Shift* keyboard key pressed several clusters can be selected. When two clusters are selected an additional text box appears in *Text Field* showing the distance between the two selected clusters and the angle between the corresponding vectors. When a third vector is selected, the parameters of the unit cell defined by these three vectors are shown. Any three selected vectors can be defined as a new basis set by using *Tools < set cell* command. The user is generally free to select any cell, but for crystallographic computations it is preferably that the reciprocal vector basis is right handed (i.e. the determinant of the cell vectors matrix is positive).

The *Tools* menu can be best used in the editing mode. The origin of the difference vector volume can be shifted to any cluster by selecting the cluster and choosing *Tools > centring >*

*set to center*. The back shift of the origin to the original position can be called by *Tools > centring > center to origin* command.

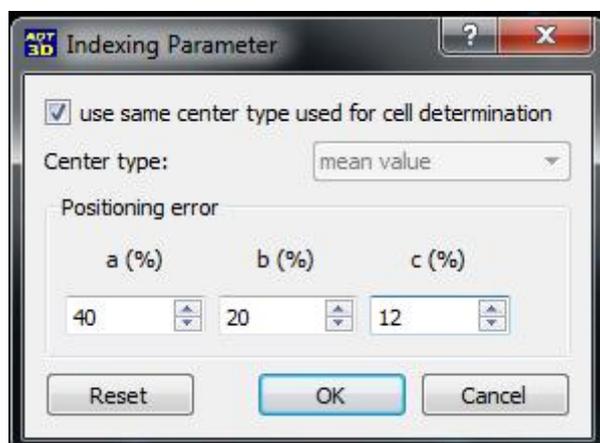
A list of options allows adding reciprocal lattice nodes to the existing cluster set. All these functions are available from *Tools > add clusters* submenu. This function can be used for finding lattice centering or “manually” define weak clusters not found by the automatic routines. *halve* creates a node in the middle of two selected existing nodes (if only one cluster is selected, the new cluster will be in the middle between the selected cluster and the origin); *thirds*: creates two nodes between two selected existing nodes spaced equidistantly; *double* creates a node with a double distance from the lattice origin along the given direction; *invert* creates a node with the opposite coordinates; *add family*, used after an additional node has been selected, adds a full node set with the same law; *undo all* removes all the additional created nodes.

The automatically determined cell is always primitive. In order to check the possibility of lattice centering the *Tools > analysis > conventional setting* menu can be used. In the relative panel any line corresponds to a different centering setting. The first number of any line defines a quality parameter of the setting. If this number is below 2 the setting should be considered as possible.

Pressing the *Tools < find cell* button recalls the automatic cell determination routine for the available cluster. If no additional cluster was added and the origin has not been moved, the original cell set will be redefined.

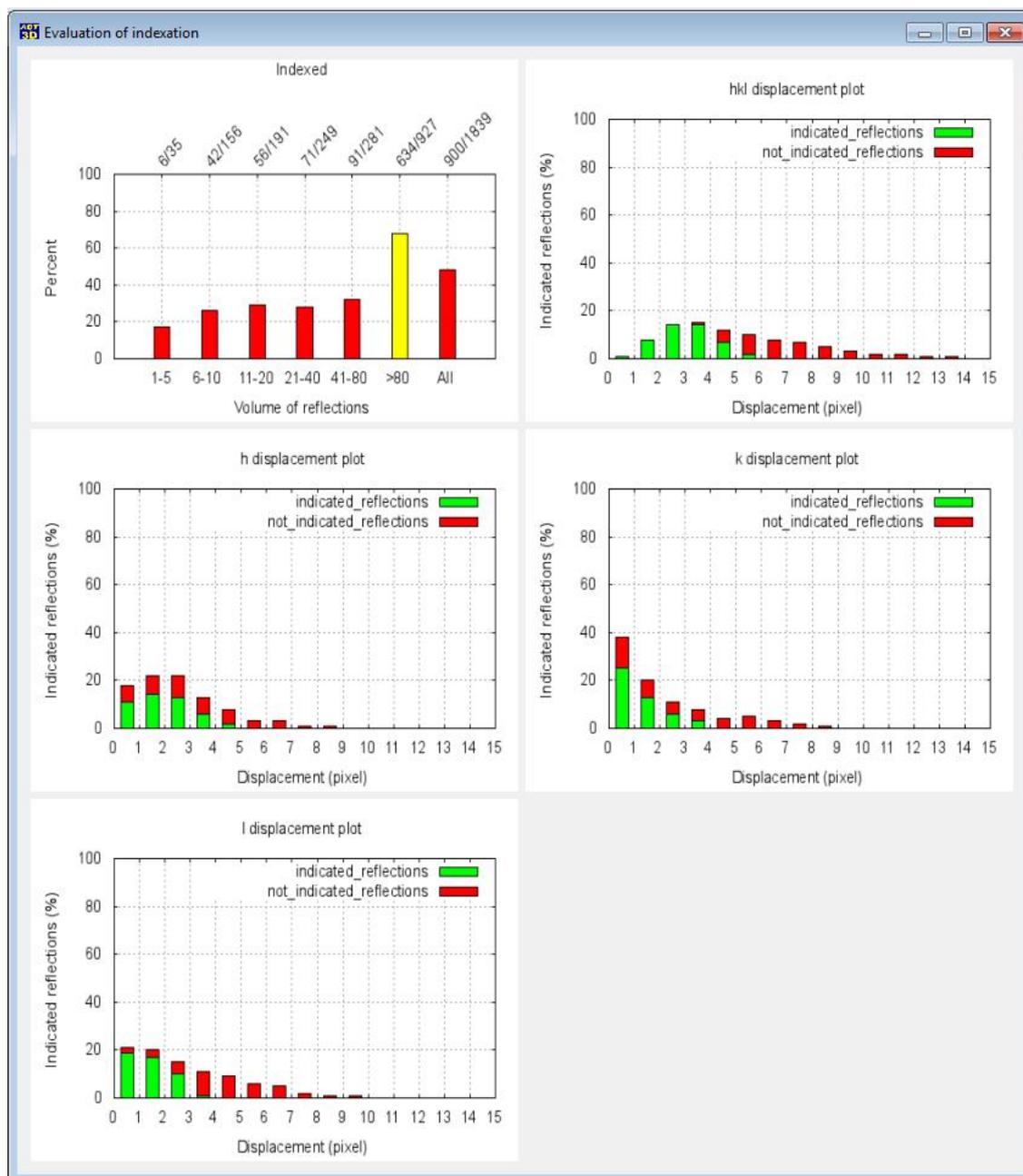
### 3.5 reflection indexation and intensity integration

Once the unit cell vectors are defined, the set of found reflections can be indexed and the relative intensities can be integrated. In *Options > Indexing Parameter* panel you can select the size of your indexation/integration boxes (*Positioning error*) (Figure 18). The suggestion is to take 40% of the shortest reciprocal vector (the longest cell parameter in direct space) and roughly equivalent lengths for the other two directions. If for example you have cell parameters  $a = 10 \text{ \AA}$ ,  $b = 5 \text{ \AA}$ ,  $c = 3 \text{ \AA}$ , the Positioning error should be 40% for a, 20% for b and around 12% for c.



**Figure 18.** Difference Vector Space Viewer with clusters and cell vectors.

The indexing and intensity integration routines are simultaneously called by pressing the *Peak Integration* icon of the *Main Menu*. Several evaluation charts appear as the reflections are indexed (Figure 18). The first plot shows percentage of the reflections indexed having a certain volume. The user should take care that most of large/strong reflections are indexed (yellow means  $> 60\%$  of reflections indexed and integrated, green  $> 80\%$ ). The other plots show displacement of reflection positions relative to the closest lattice node found. Here the user should observe a fading plot showing that there are only few reflections with a high displacement.



**Figure 18.** Evaluation charts related with the intensities extraction routine.

The intensity integration routine proposed in this tutorial is just a dummy version, though for working with the simplest structural cases proposed: BaSO<sub>4</sub> and Na<sub>2</sub>Ti<sub>6</sub>O<sub>13</sub>. You can extract the intensities for these two structures and use for next tutorials in *SIR* and *SHELX*.

Using the command **Main Menu** > *File* > *Save standard HKL file*, intensities can be saved in a standard *hkl* file where each line corresponds to a certain reflection. For each reflection the following values are reported: *h*, *k*, *l*, *Intensity* and *estimated standard deviation* (e.s.d. – simply defined as square root of intensity). Pay attention that in this *hkl* file you have intensities values and not  $F_{hkl}$ ! You have to take care of this when you want to use this file for *SIR* or *SHELX*.

## 4. ADT3D keyword shortcuts and mouse keys

<b>General</b>	
Ctrl + O	Open an <i>mrc</i> file
Ctrl + S	Save active <i>mrc</i> file
Ctrl + Q	Quit <i>ADT3D</i> without saving / prompt
Ctrl + A	Show <i>ADT3D</i> About dialog
Ctrl + B	Show Qt about dialog
Ctrl + W	Close the current dataset
Shift + F4	Close current window
Ctrl + M	Show <i>mrc</i> header info in the right properties window
Ctrl + B	Rebinning the active image stack
Ctrl + R	Downsample the active image stack using a Gaussian filter
Ctrl + 2	Open the 2D viewer for the active dataset

<b>3D Viewer</b>	
Esc	Toggle between move mode and select mode
Space	Toggle between four window and single window view
A	rotate scene to view along cell vector 1 (red)
B	rotate scene to view along cell vector 2 (green)
C	rotate scene to view along cell vector 3 (blue)
Alt + A	save the actual marked reflex as cell vector 1 (red)
Alt + B	save the actual marked reflex as cell vector 2 (green)
Alt + C	save the actual marked reflex as cell vector 3 (blue)
Shift + A	store actual view direction as cell vector 1 (red)
Shift + B	store actual view direction as cell vector 2 (green)
Shift + C	store actual view direction as cell vector 3 (blue)

<b>Difference Vector Space Viewer (Visualization Mode)</b>	
middle mouse button	move the scene
left mouse button	rotate the scene
mouse wheel	zoom in / out
<b>Difference Vector Space Viewer (Editing Mode)</b>	
left mouse button	select a reflex

## 5. Proposed examples

In this tutorial we propose 4 exemplary ADT data sets from relatively easy crystal structures. For all of them you have an *mrc* file containing a 2D diffraction patterns stack collected with ADT method. All the acquisition are performed coupling ADT with precession of the beam.<sup>[3,6]</sup> All the three data sets are already binned for saving memory (see § 2.4). If you still have problem with the memory of your computer, bin them again. The tilt axis azimuth is between 40° and 50° for all the data sets except Hemimorphite\_BeamStop, where the tilt axis is between -40° and -30°. The *cif* files for the first three data sets are provided and you can use them for comparing cell parameters.

### 5.1 BaSO<sub>4</sub>

The first example is BaSO<sub>4</sub>. This structure is relatively easy as the cell is orthorhombic primitive and contains only 5 independent data atoms. You can load the 2D diffraction patterns stack and go through the whole procedure exploring all the options. After reconstructing the 3D reciprocal space, you can easily determine the cell parameters with the automatic procedure. A *Threshold* of 250 or more is suggested for the *Find Reflection* parameters. Then you can try to refine (or spoil) your result changing manually the cell parameters (pay attention to the determinant of the cell vector matrix!). Once the cell is satisfactory, you can rotate the volume looking for the two glide plane extinction families and extract the intensities. Pay attention to your cell setting and compare it with the values reported in the *cif* file. If the setting is different are different (i.e. a, b and c are exchanged), change the cell accordingly... or take care in the space group selection when you run *SIR* or *SHELX*.

### 5.2 Na<sub>2</sub>Ti<sub>6</sub>O<sub>13</sub>

The second example is Na<sub>2</sub>Ti<sub>6</sub>O<sub>13</sub>. You don't need to go through all the options of *ADT3D* and you can run directly all the steps until the cell determination (the cell parameters have a global scale factor due to the missing fine calibration in the present test version, but angles and length ratios should be correct). For the *Find Reflection* parameters, use *Min. volume* 5, *Max. volume* 5000 and *Threshold* 250. These values are important later for a correct intensity extraction. On the contrary, for the *Cell Determination* parameters you are free to try until you get a good result. Suggestion, take care of the *cut off center* and use the full difference vector space. Also in this case you can extract an *hkl* file with the intensities that you can use later for *SIR* or *SHELX* tutorials.

### 5.3 Natrolite

The third example is natrolite – Na<sub>2</sub>Al<sub>2</sub>Si<sub>3</sub>O<sub>10</sub>·2H<sub>2</sub>O. Natrolite is a more complicate case, having an orthorhombic F-centered (pseudo-tetragonal) cell and a more complicate structure. For natrolite you can consider a good result just finding the correct cell. Can you distinguish between the orthorhombic and the tetragonal setting? Which margin of error do you have?

### 5.4 Hemimorphite\_BeamStop

The forth examples comes from another inorganic sample. Unlike the other data seta, this data set was acquired using a beam stopper in order to shield the transmitted beam. The presence of the beam stopper prevents to perform an automatic pattern centering so you have to set the

center by the use of a Friedel pair in any one of the slides where a pair is clearly recognizable. You have also to select *use beam stopper mode* in the *Centering routine* panel.

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## Credits

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