WCEN tutorial for beginners

In this exercise, you will open a file, modify its appearance for convenient viewing, determine the experimental parameters, apply correction factors, apply a transformation to display the data in reciprocal rather than detector space, and save the data as a reciprocal space file. In the process, you should become familiar with file input and output, color map adjustment, image repair, manual and automatic parameter determination, and data processing. *WCEN* and the test file *wcen_sample.smv* are at www.fiberdiffraction.org, under Software.

0. Open WCEN

1. Open the image

Open the sample NMV image (*File*/*Open*; select file in *Directories*, change *Filter* to …/*.smv if it does not already end that way, double click *wcen_sample.smv* or select it and click *OK*. [The *Options* button allows you to open files in other formats.] [The current version of *WCEN* does not always open successive files reliably. Close one image before opening another. If necessary, re-start *WCEN*.]

Rotate the image 90° (*Image*/*Rotate*/*90 degree*).

Open the coordinate window (*Windows*/*Coordinate*) and the full pattern window (*Windows*/*Full Pattern*) – these will help you navigate the image.

Use the scroll bars to look at the center of the image.

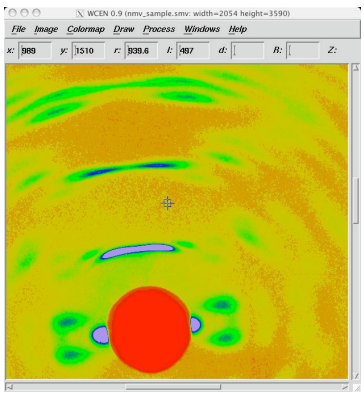
2. Adjusting the color

Open the *Colormap Control* window (Colormap/Customize).

The variable color map is particularly useful for looking at patterns that have a wide range of intensities, but include information in a narrow subset of that range. For example, fine structure within an intensity maximum can be visualized very effectively.

Drag the upper limit of the *histogram* to about 6,500 (lower right box). *Apply*.

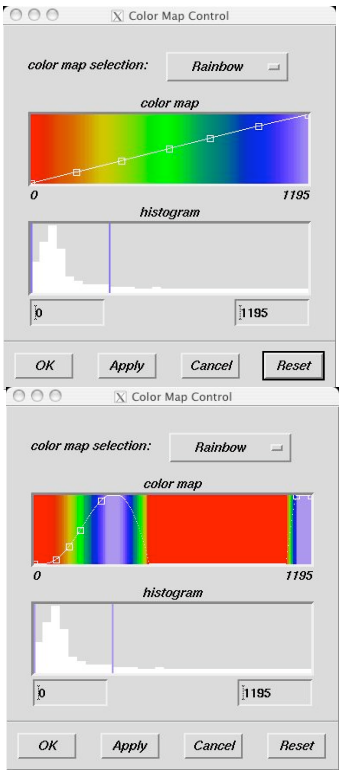
Drag the centre handle in the color map to change the map – observe the effects on the image. You can use *Reset* to restore the color map (*Reset* does not restore the histogram).



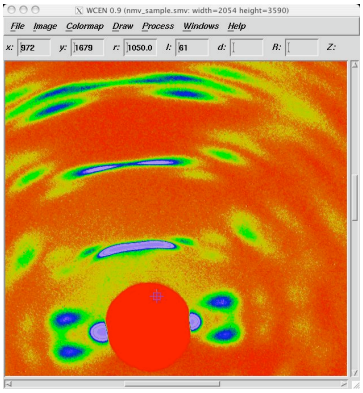
Use the scroll bars and the color map control to make the main window resemble the figure at left. Look at some of the intensities. Drag the cursor and watch "*x*:" and "*y*:" in the coordinate window to find the reflections at *x*=1020, *y*=1323 and *x*=1085, *y*=1690. There are several ways to make the intensities clearer – try them all.

(a) Select a different color map from the *color map selection* menu. Try *Keiichi*, *White-Black*, *Blue-Yellow* (just a colored version of *White-Black*), *Green-Purple* (more like *Keiichi*) and others. Drag the centre handle in the map to see the effect of changing the color map function. Click *Reset* and *Rainbow* color map before going on to part (b).

(b) The near-meridional layer lines in this pattern are very prominent. It is more difficult to see the other layer lines. Many reflections on the weak layer lines have intensities of about 600 (check this by moving the cursor and reading the value of "*I*:" in the coordinate window). You can feature the range around this value by making a more complex color map.



Add several new handles to the map (Mac: option click; Linux: middle click; on four points on the line – two on each side of the center handle).



Note that the intensities are the abscissa of the color map. Your goal is to make the color change very steep at around I=600. Drag the handles to do this.

The effects can be quite strange. You will learn by trial and error what works for each pattern. You may choose to add more handles. This technique is useful, for example, when a fine layer line structure is imposed upon a broader molecular transform (which is not the case in the sample pattern, but you can get the general idea from this exercise).

Determine experimental parameters

Open the *Reflection-Parameter* window (Wind*ows*/*Parameter*).

Center and twist

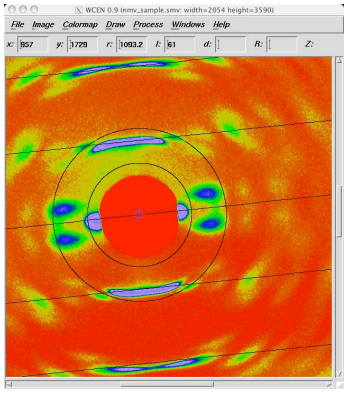
In the main window, click on the approximate center. In the *Reflection-Parameter* window, click *Parameter*/*Center from cursor*. The center coordinates (*x0* and *y0*) will change to the cursor coordinates.

Enter the specimen-detector distance (*distance (mm)*) as 114.7, the X-ray wavelength (*wavelength (Å)*) as 1.03, and the raster (*raster (µm)*) as 24. In this example, the distance was measured – we will not attempt to refine it. Raster size and wavelength are also not refined.

Enter the helical repeat (*repeat (Å)*) as 35. We will refine this value later. Click *Apply*. (*Apply* is not always required after changing parameters, but it is always safe to use it.)

Draw layerlines. In the main window, *Draw*/*Layerline*, click *Draw*. Leave the *Draw Layerlines* window open. In the main window, *Draw*/*Resolution Circle*. Enter *radius(Å)* 50. *Draw*. Enter *radius(Å)* 30. *Draw*. (50 and 30 are just convenient sizes for circles to guide your eye in later steps).

You now have an overlay of lines and circles well-suited to finding the pattern center. But the layer lines obviously have the wrong twist. Enter a trial value of twist (*twist (°)*) in the Reflection-Parameter window (try –5 to start with; -6 is better). In the Draw Layerlines window, *Draw*. (If you prefer, *Clear* before *Draw*.) By trial and error, you should be able to get layer lines whose twist (angular deviation



from the horizontal) matches the pattern quite well. Your pattern should now look something like the figure.

You can refine the center coordinates quite accurately by eye. In the main window, *Draw/Move Objects*. Click *enable move by arrow keys*. Enter the movement step size if you wish to change it (the default of 1 pixel is a good start). Either click the arrows in the *Move Objects* window, or click the main window and use the keyboard arrows. Move the overlay around until you are happy with the center. As you move it, *x0* and *y0* in the *Reflection-Parameter* window will change. As you refine the center, you may wish to refine the twist – simply change the twist in the *Reflection-Parameter* window and click *Draw* (or *Clear* followed by *Draw*) in the *Draw Layerlines* window.

Helical repeat

Refine the helical repeat by eye using the *Draw Layerlines* window. Simply change the repeat using the arrows and the step size box, and observe the fit of the layer lines to the pattern intensities.

A useful procedure when judging the fit of the lines and circles to the pattern is to vary the color map, watching the fit as the colors change. Use the color map pane in the *Color Map Control* window; this works best with only the original three handles present. If you have added handles, *Reset*, or if you have closed and re-opened the window since adding handles, command click (Mac) or right click (Linux) on the unwanted handles. Then move the middle handle, watching the fit of the changing colors to the lines and circles in the main window.

Tilt

Tilt can also be determined by eye, observing the fit of the high-order layer lines to the intensities. This procedure can be quite difficult, however, and if the sample is sufficiently well-oriented tilt can be better determined automatically.

Open the *Reflection* pane of the *Reflection-Parameter* window (*Windows/Reflection* in the main window, or *File/Open reflxn pane* in the *Reflection-Parameter* window).

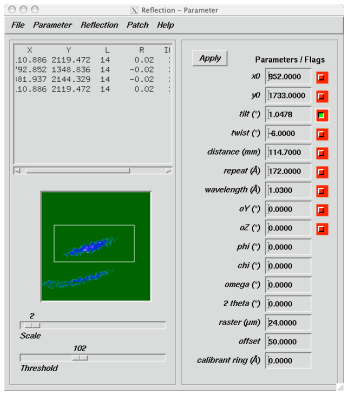
Select a set of equivalent reflections. To refine tilt, use well-defined reflections fairly close to the meridian. There is a good set at x=1020, y=1320 and its three equivalents. For each of these four reflections:

Click on the reflection in the main window. A magnified image appears in the image pane of the *Reflection-Parameter* window. You can adjust the scale: for these reflections, a scale of 2 is good. Drag the threshold to about 100 (102 works well) – the aim is to isolate a well-defined reflection. The threshold function temporarily sets all intensities below the threshold to zero. Drag a box around the reflection in the magnified image pane. Be sure that the box does not include any part of a neighboring reflection. In the *Reflection-Parameter* window, *Reflection/Box select*. The x and y coordinates of the center of gravity of the reflection appear in the reflection list. Repeat with the three equivalent reflections. They are at approximately (790, 1350), (880, 2145), and (1110, 2120). [(1) Ideally, the threshold should be selected for each reflection individually so that the number of points above the threshold, *nP* in the reflection list, is about the same. (2) There are ways to manipulate the image so

that adjacent reflections are temporarily removed (*Patch* menu), which makes box selection easier. These techniques are beyond the scope of this tutorial, but are discussed in the manual.]

The reflections must be indexed in order to refine parameters. The reflections chosen for this example are not on the near-meridional layer lines, so they will have to be indexed on a long helical repeat. It turns out that for this pattern, a repeat of five times the near-meridional reflection repeat works well. Change the repeat in the *Parameter* pane to five times the value you determined earlier (it will be about 172). You might want to check the fit by re-drawing the layer lines (*Draw Layerlines* window, click *Draw*). Select all four reflections in the reflection list (hold down shift to select multiple reflections). *Reflection/Autoindex*). The layer line numbers (14 in this case), reciprocal space radii, and a label for the set (1 in this case) will appear in the list for each reflection.

You may now refine tilt. If you wished, you could have added more sets of reflections to make the refinement more accurate. But we will work with one set. Click the flag box for tilt (the box beside *tilt* in the parameter pane). It should turn green. *Parameter/Refine selected*. The tilt should change (probably to a value between 0° and 1°).



If there are enough well-defined reflections (for example, in a TMV pattern, a filamentous phage pattern, or in many crystalline fiber diffraction patterns), all the parameters can be determined automatically in this way rather than by eye. In the case of the pattern used here, the combination of manual and automatic determination seems to work better. The detector mis-setting angles (*oY* and *oZ*) can only be refined automatically, and only for very well-ordered patterns.

You now have a full set of parameters. Your R-P window should look something like the figure. You can save your parameters in a parameter file (*Parameter/Save into file*).

Repair

This step could be completed earlier (and sometimes should be!). For most patterns, it will not be necessary. It will probably be convenient for you to clear the layer lines at this point (*Draw Layerlines* window, *Clear*).

In this pattern, there are two rows of defective pixels. Their intensity values can be replaced by values determined by interpolation from the adjacent rows. In the main window, click close to the left extremity of the defective rows (around *y*=1180). Open the *Pixel Intensities* window (main window, *Windows/Pixel Intensities*). You will easily be able to recognize that the defective rows are 1182 and 1183. If you wish to examine other parts of the image, remember that you must click *Update* in the *Pixel Intensities* window to look at the new main window cursor position.

In the main window, *Image/Repair*. Enter the top left and the bottom right points in a rectangle. Intensity values in this rectangle will be replaced by interpolated values. In this case, you can see from the *Pixel Intensities* window that the top left pixel in the defective area is (1, 1182), and the bottom right pixel is (2054, 1183). Move the cursor in the main window, then *Update* in the *Pixel Intensities* window to see the right end of the row in the *Pixel Intensities* window. In the *Image Repair* window, click *Repair*. Observe the effect on the image. Close the *Image Repair* window.

Correction Factors

You should now correct the intensities for polarization (needs some user input) and geometric effects.

In the main window, *Process/Correction*. The *Data Correction* window opens.

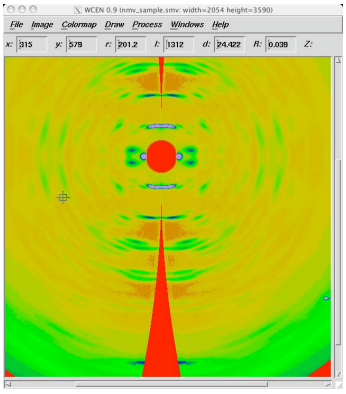
You do not need the absorption correction (this is for film absorption; this image is a CCD image). The incident beam is from a synchrotron, so you do not need to change the beam type. For this beamline, τ' (see the manual) is 0.995; ρ' (the angle between the plane of polarization and the detector axis) is 0°. Detector Fog is subtracted from all intensities before correction; for CCD data, it is usually a number that was added to all intensities in order to avoid negative values. In this case, it has been read from the input file header (it is 50 after a program scaling step), and you do not need to change it.

Click *Apply*. You may need to change the color map to see the pattern well.

Transformation

Convert the data from detector space into reciprocal space, removing the experimental distortion.

Click *Process/Transform*. The *Data transformation* window opens. We will choose to express the data in Cartesian coordinates (*reciprocal*). [You may want to try polar coordinates as well. Note that correction and transformation are irreversible, so if you want to experiment with other transformation options, you will need to re-read the original file. You will not have to re-determine the parameters if you saved a parameter file.] We will average the four quadrants (flag *4Q*). For this data set, a bin size of 0.0005 Å-1 in both *x* and *y* (*Bin Size: x-rad*; *Bin Size: y-rad*) is useful. Choose a convenient resolution range: *lower* 1000 Å to upper 4 Å works well for this data set. *Apply*.



Use the Color map window to make the reciprocal space image look pretty. You may notice some discontinuities in the highest layer lines. These will appear if you did not get the parameters quite right; they appear at the edge of the blind region of the quadrants with the larger blind region. With practice, you will learn to avoid them.

You can save this image if you wish (main window, *File/Save as*), You have now completed the tutorial.