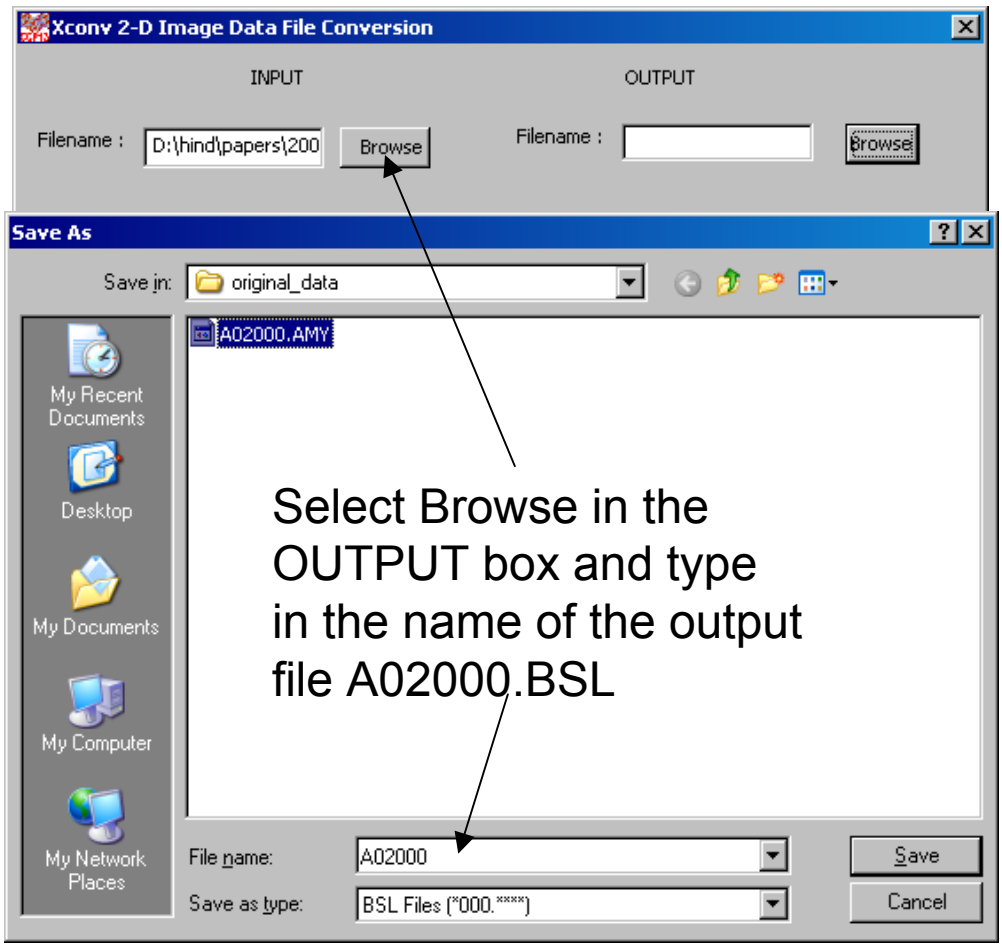
FibreFix Tutorial 3:
Analysing an amyloid diffraction pattern

Introduction.

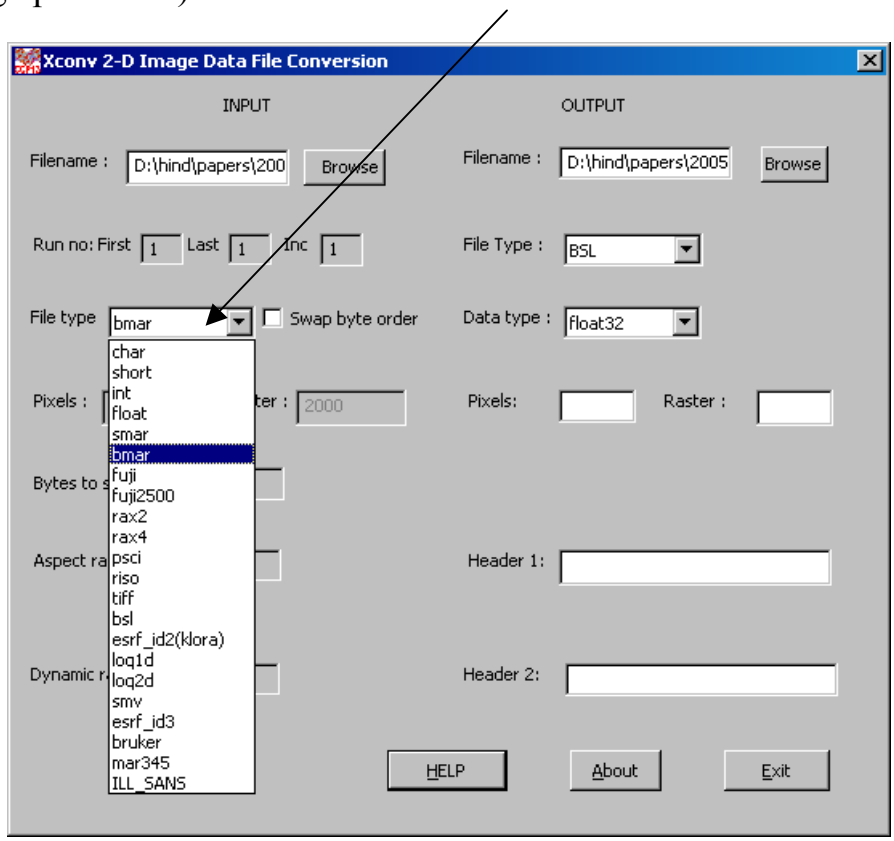
Amyloids are an interesting assortment of mostly β-structures associated with plaque formation in various diseases such as Alzheimers. The β-structures often form long fibrils which can be oriented with varying degrees of success to yield fibres which give quite rich diffraction patterns. However, there is usually a great deal of disorientation evident in the diffraction patterns. Here FibreFix has been used to analyse a quite well oriented amyloid pattern kindly provided by Dr. Louise Serpell (Sussex University).

Application of XCONV for file format conversion

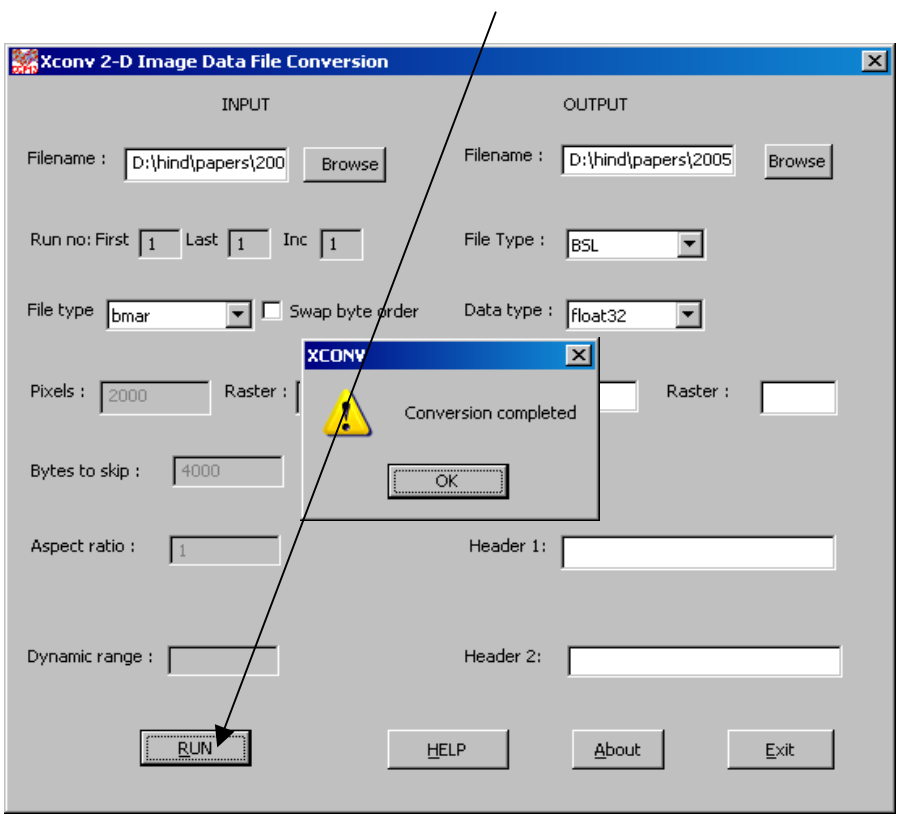
With most images one can go straight to FibreFix and open the file. However, it is also possible to convert the image directly to BSL format using XCONV as below. Click Browse and select the input file name – in this case AMYLOID_S_1_015.IMAGE



Select the file type of the input file, in this case it is bmar (big mar image plate data)

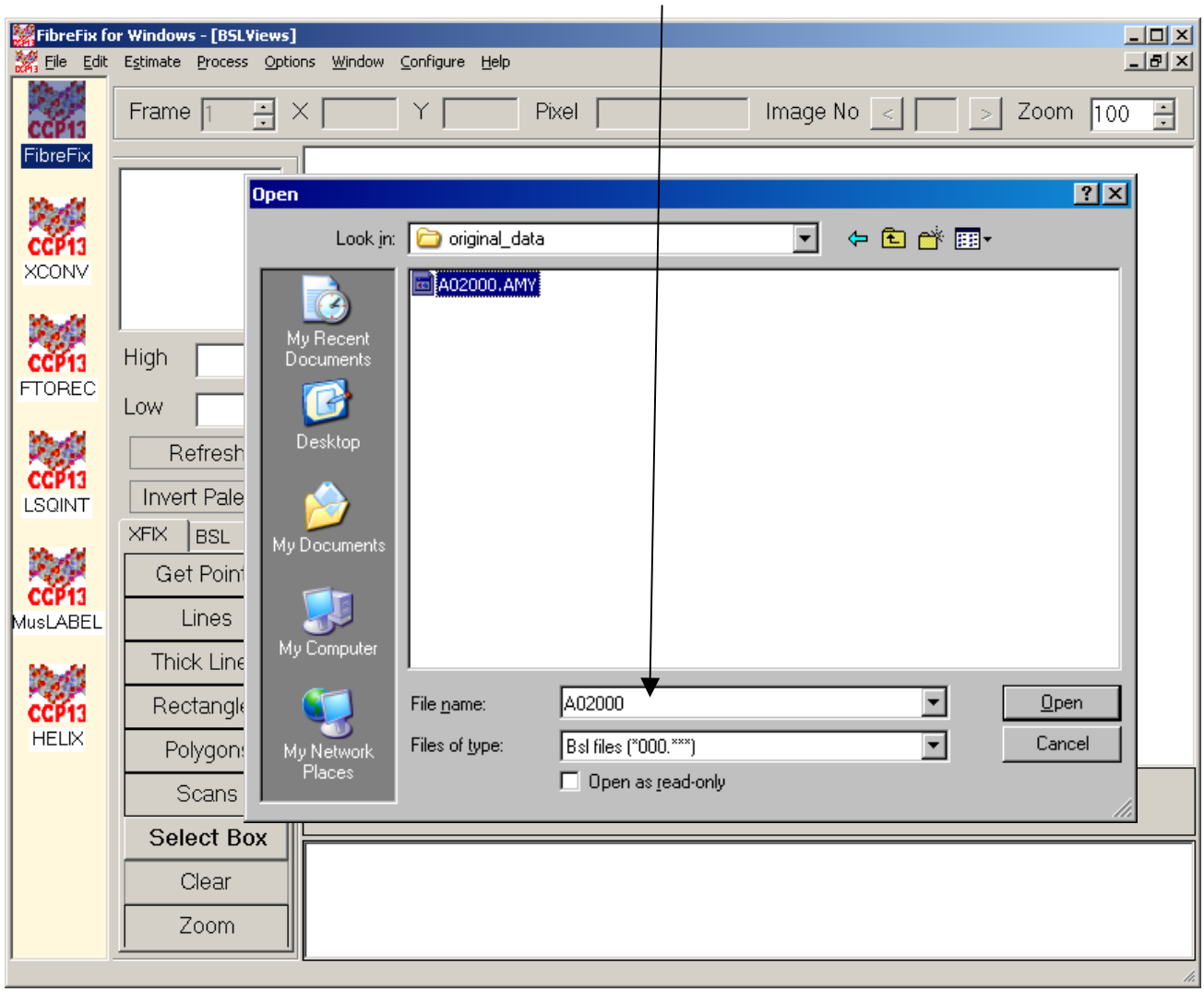


Click run and an icon will show up when the conversion to BSL is completed



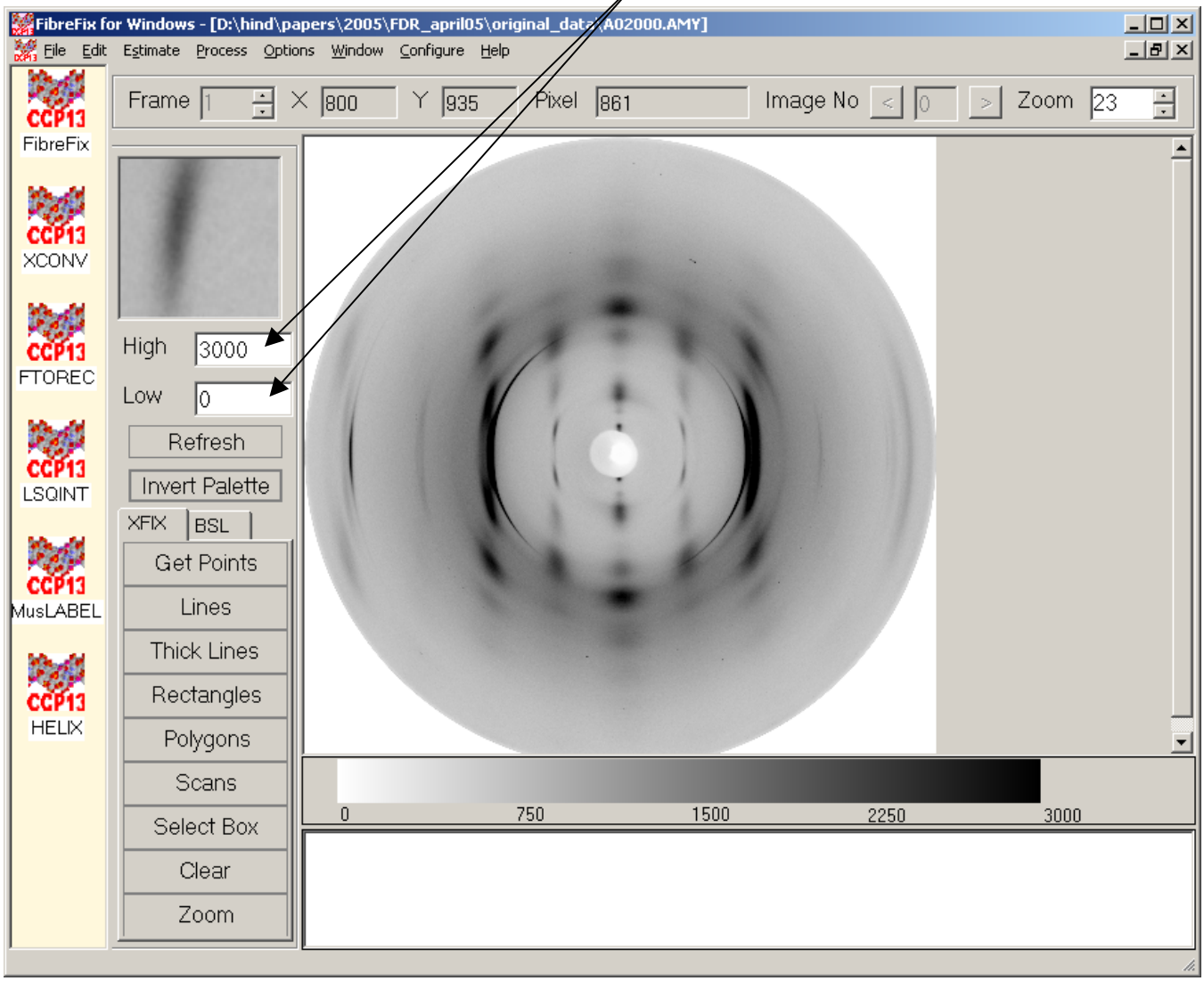
Application of FibreFix for pre-processing the pattern

Open FibreFix, select File, then open and select the name of the input BSL file



Alternatively, if the filename is in an explorer window, it can be picked up and dropped into the image window which will have the same effect

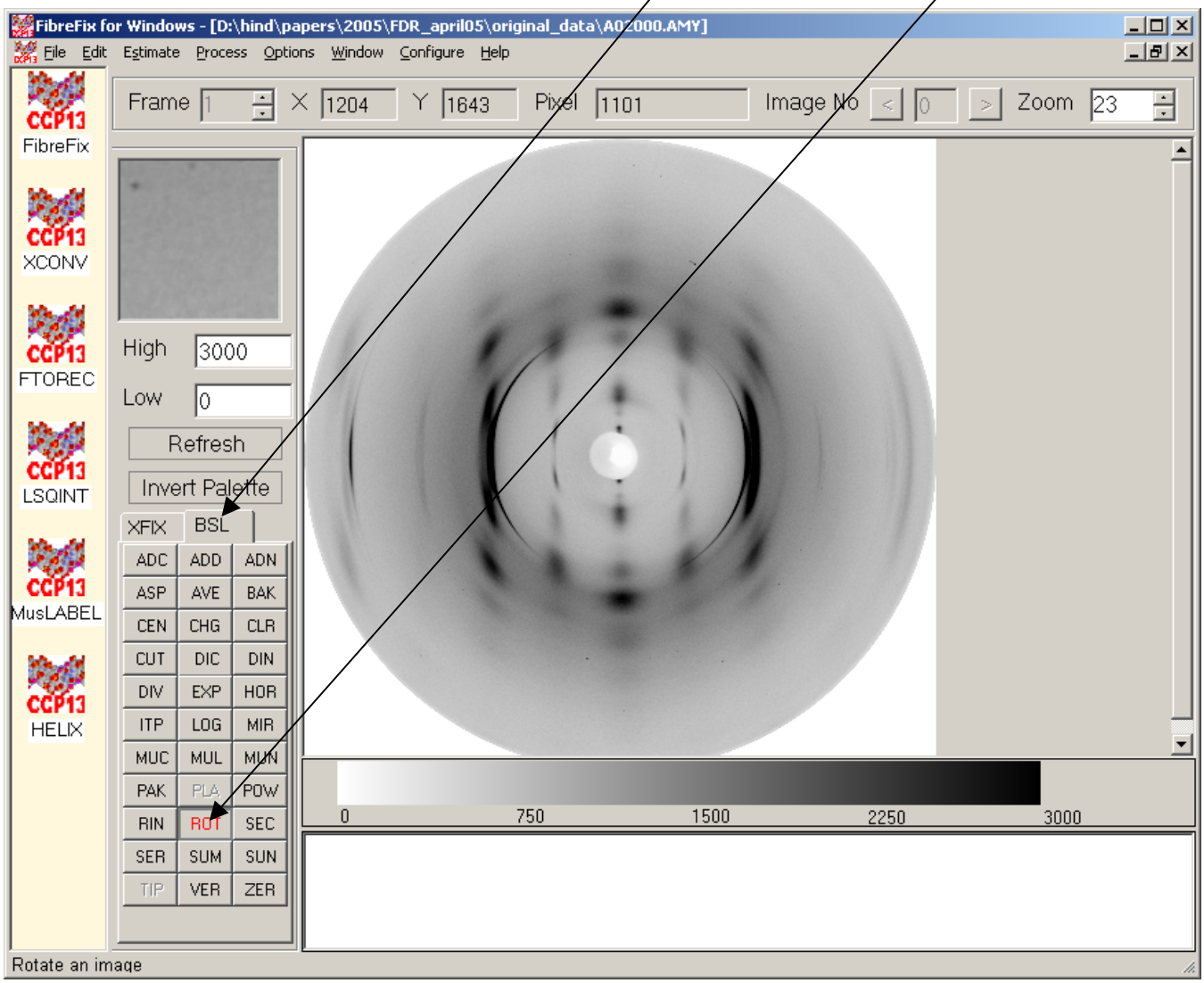
The diffraction pattern is then displayed. The threshold settings for optimum display can either be set by manually inserting High and Low values ……..



….. OR the same effect can be achieved by right-clicking on the mouse and moving the mouse in the image area either up and down or side to side.

The counts corresponding to a particular grey value in the image can be seen on the scale bar below the image.

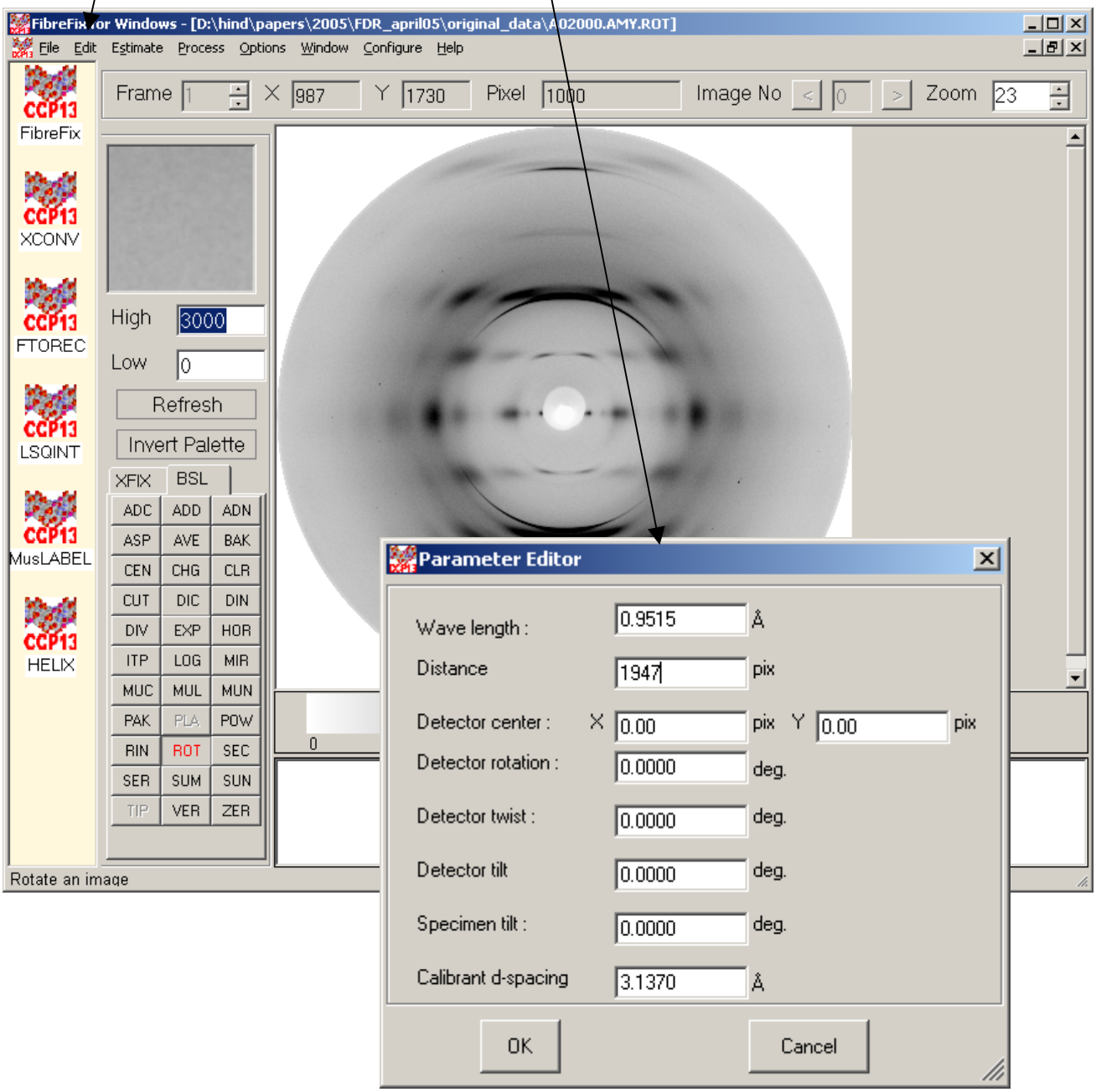
Since the meridian of this pattern is in the horizontal direction and the equator is in the vertical direction, we need to rotate the pattern. Click on BSL and then select ROT. Then type in the rotation angle (90).



The image can now be seen with the meridian vertically oriented and it is now ready for preprocessing with FibreFix

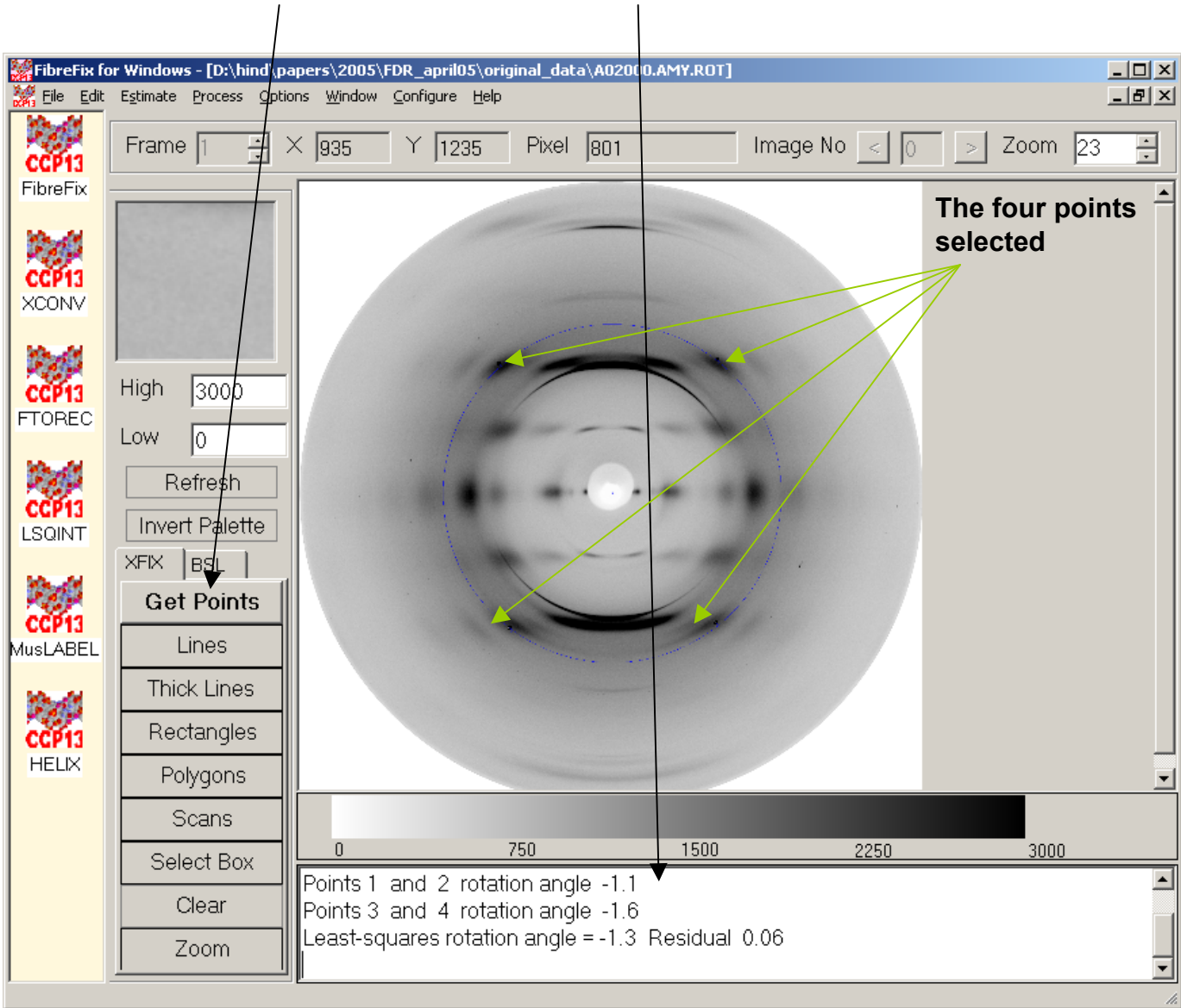
Application of FibreFix for calculating various parameters for the pattern e.g. centre, rotation of the pattern and the specimen tilt

Select Edit, then Parameters. The parameter Editor will show up. Type in the values for the various known parameters (e.g. wavelength) in the units shown on the right side of each.

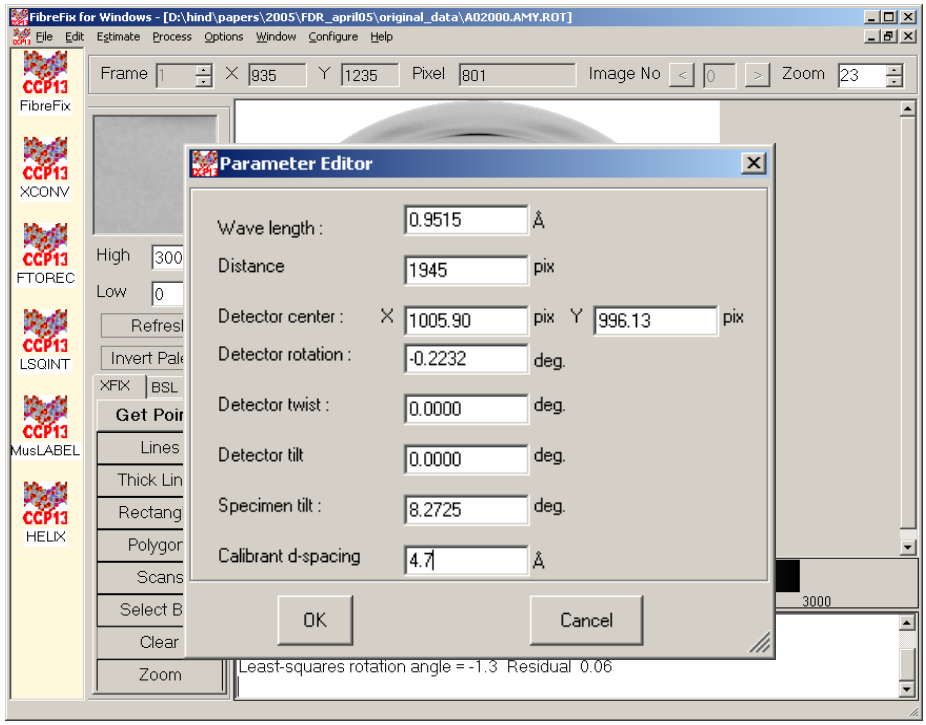


Select Get Points, then select and left click on four points that lie on a circle centred on the centre of the pattern. The enlarged image in the small top left window will aid selection of the peak positions. Doing a right click will then bring up a box with various options. Select Estimate Centre, followed by Estimate Rotation.

This will then calculate, based on the four points selected, both the pixel values of the centre of the pattern and the rotation angle of the pattern needed to make the meridian exactly vertical and equator horizontal. A little screen will show up giving these two parameters as well as these being reproduced in the log file in the bottom window.

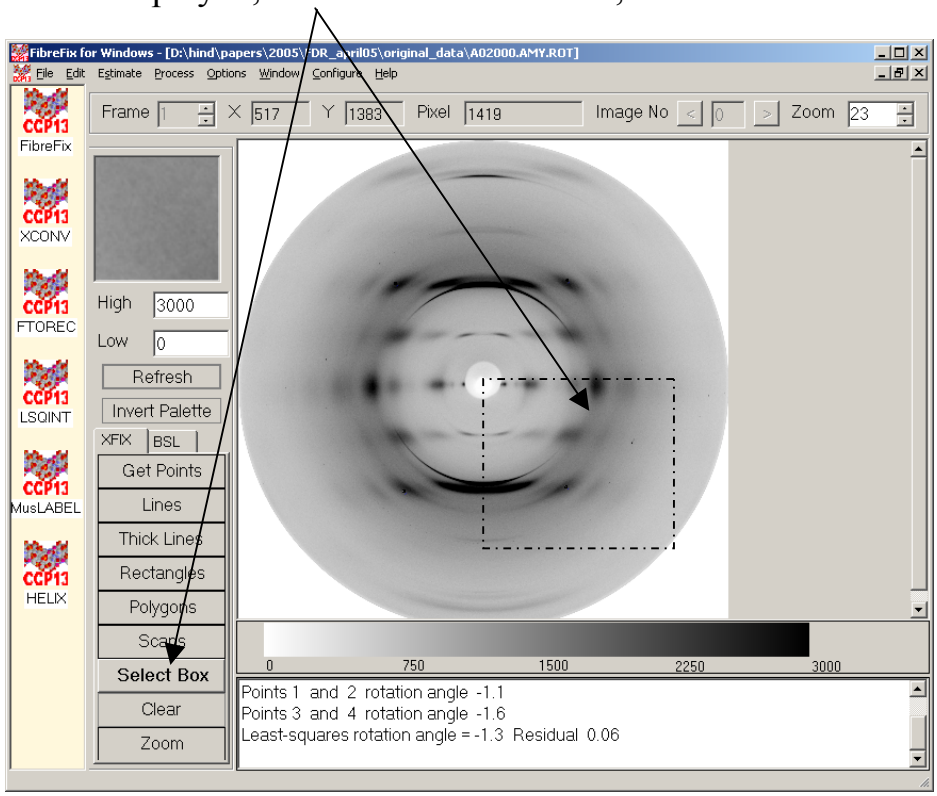


Select Edit, then Parameters. The parameter Editor will show up giving the values of the parameters that FibreFix has calculated.

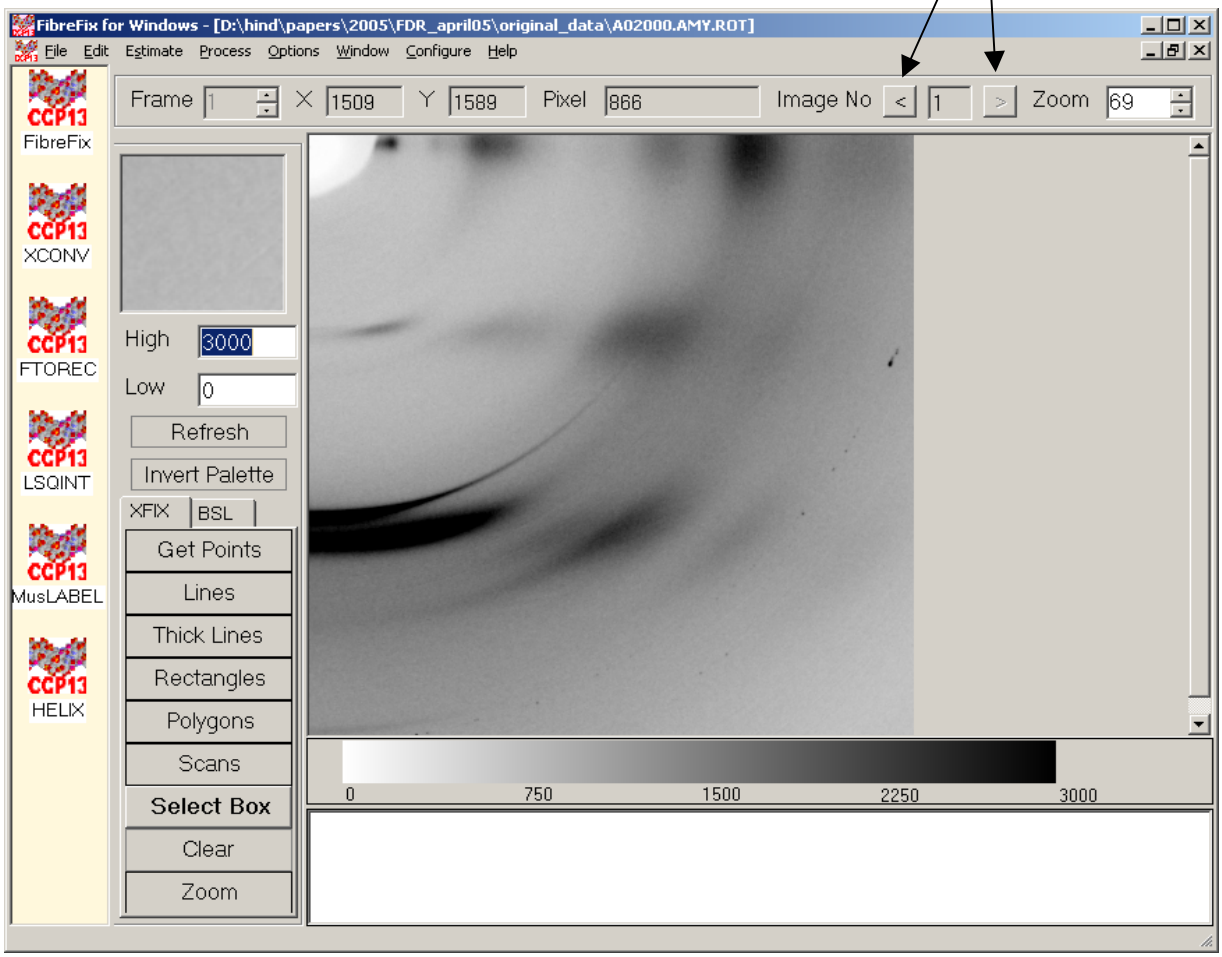


Refinement of the parameters values within FibreFix

Click on 'Select Box' under FibreFix, then select an area e.g. bottom right quadrant. Another image will be displayed, shown in the next slide, of the selected area.



You can toggle between the original image, shown in the previous slide, and the selected area image by changing the image number using the arrows. Then select Process and Refine and select Centre, rotation and specimen tilt



Finding the camera length

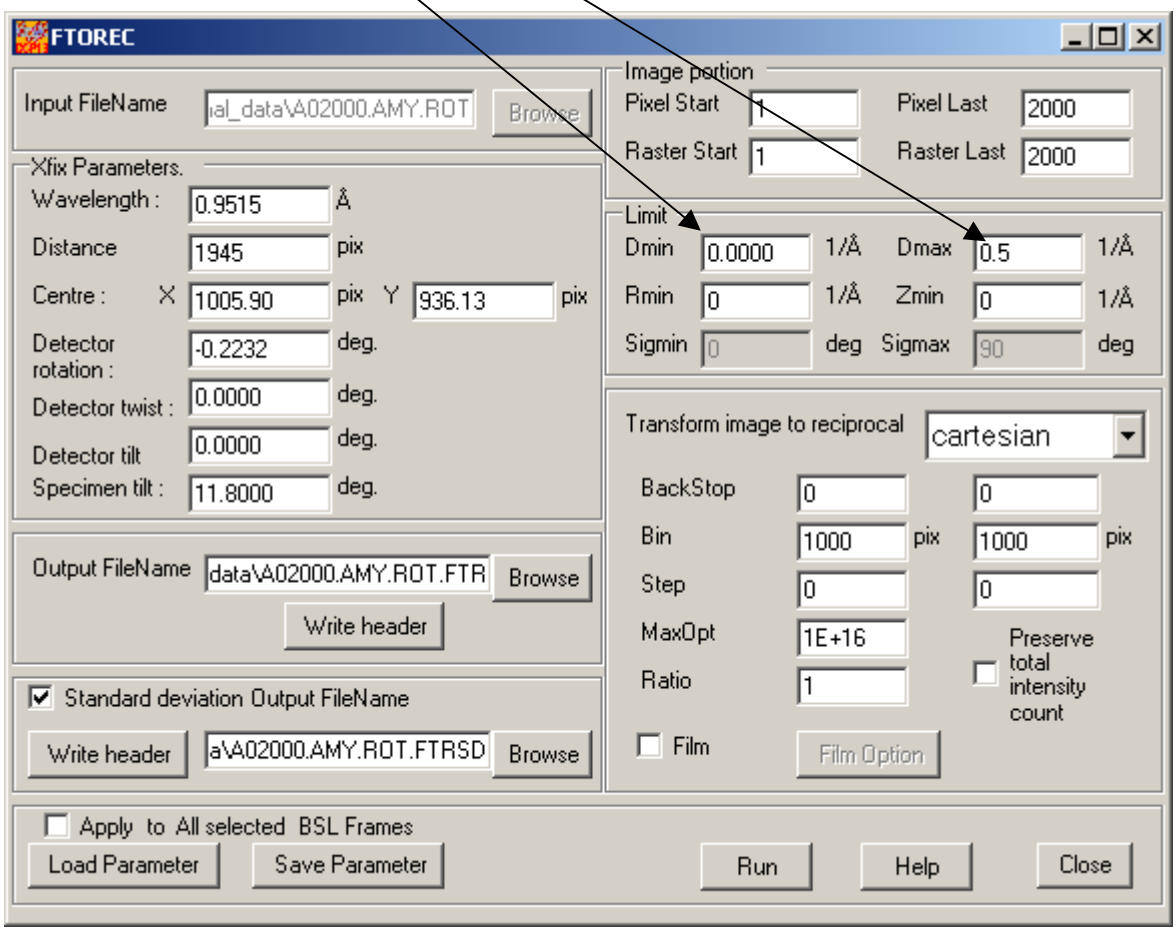
Finding the specimen tilt and converting the image to reciprocal space requires knowledge of the camera length. This value needs to be inserted **in pixels** in the Parameter box which can be selected from the Edit drop down menu. If the camera length is not known by direct measurement and conversion into pixels using the detector pixel size, but there is a calibration peak or ring on the pattern, then the d-spacing corresponding to this peak or ring needs to be inserted into the Parameter Box. Then one should click on Get Points in the FibreFix box and left click on one or more points of the same spacing on the pattern (at least one point, but ideally several points around a calibration ring). A right click will then ask Estimate Distance. Left click on this and the camera length will be determined and its value inserted into the Parameter Box.

Estimating the Specimen Tilt.

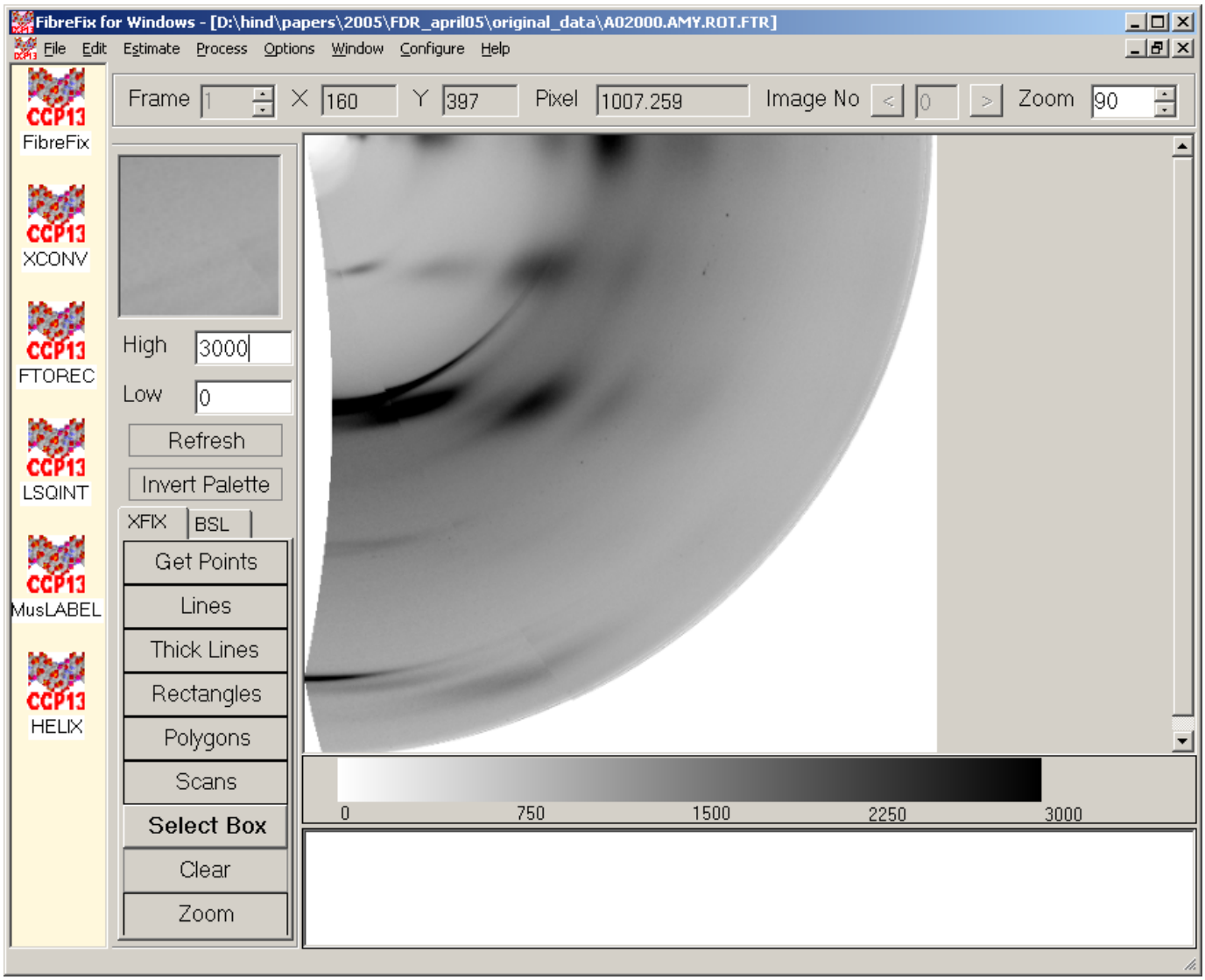
Once the camera length has been defined, clicking on Get Points and selecting (left clicking) four equivalent reflections in the four quadrants of the diffraction pattern followed by a right click will bring up the box where the option Estimate Tilt can be selected.

Conversion to reciprocal space - Application of FTOREC

Once the image has been centred, aligned and the camera length and specimen tilt have been determined click on Process and then FTOREC. The FTOREC window will then appear and will already contain many of the parameters already determined. Other parameters such as the range of the pattern to process (Dmin and Dmax) can be inserted as required.



Output from FTOREC

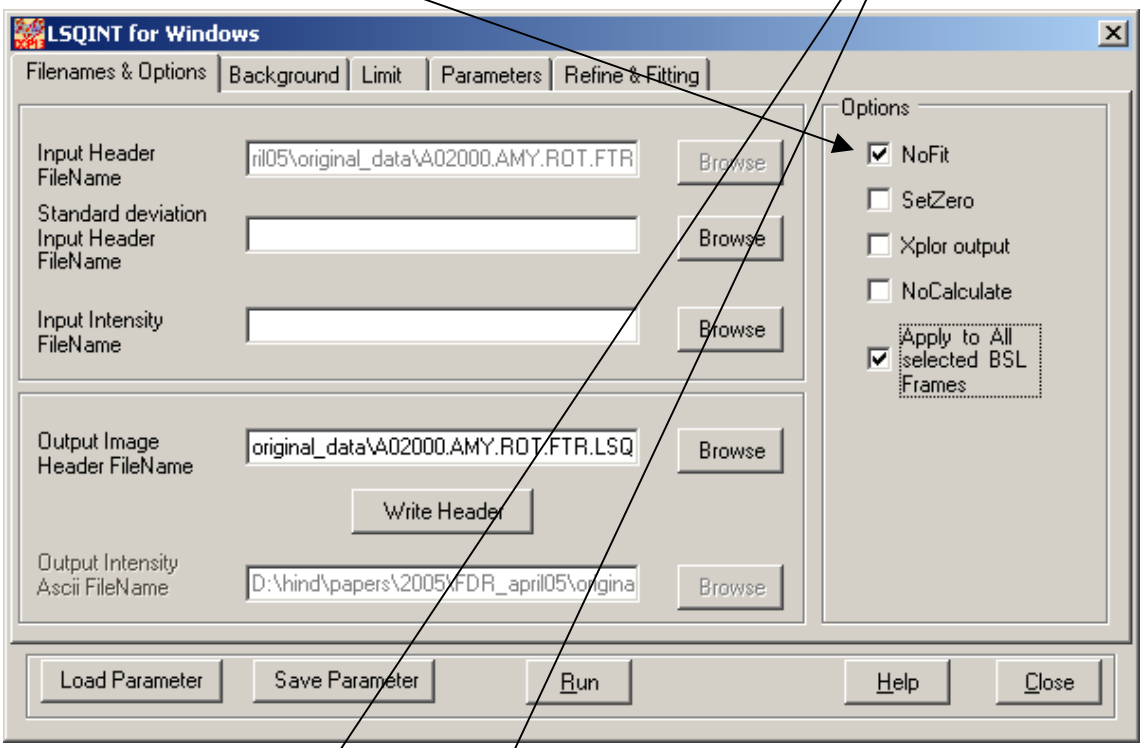


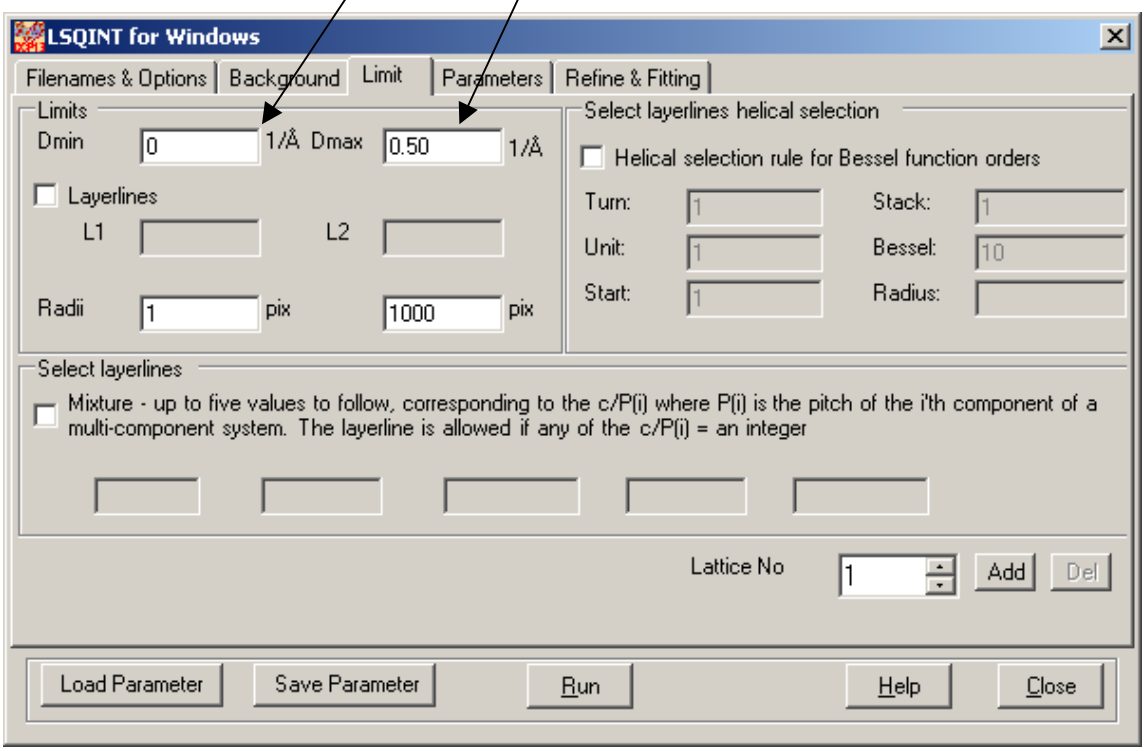
If the parameters used are reasonable then the FTOREC output image should show reflections on horizontal straight layer-lines. Most of the meridian will be missing. The only intensity which appears on the meridian is the region corresponding to the tilt of the fibre.

Note that FTOREC also produces a second image which is the standard deviation between the four quadrants of the original pattern. It gives a visual (and numerical if required) estimate of the efficacy of the FTOREC conversion.

Fitting of the FTOREC pattern using LSQINT:

LSQINT attempts to fit the observed peaks both in terms of peak shape (including disorientation etc) and intensity. Under the Filenames and Options window select NoFit. Then select the Limit window and input the range in reciprocal space to be fitted. The NoFit operation attempts to fit the peak shapes without trying to fit the relative intensities of the peaks.

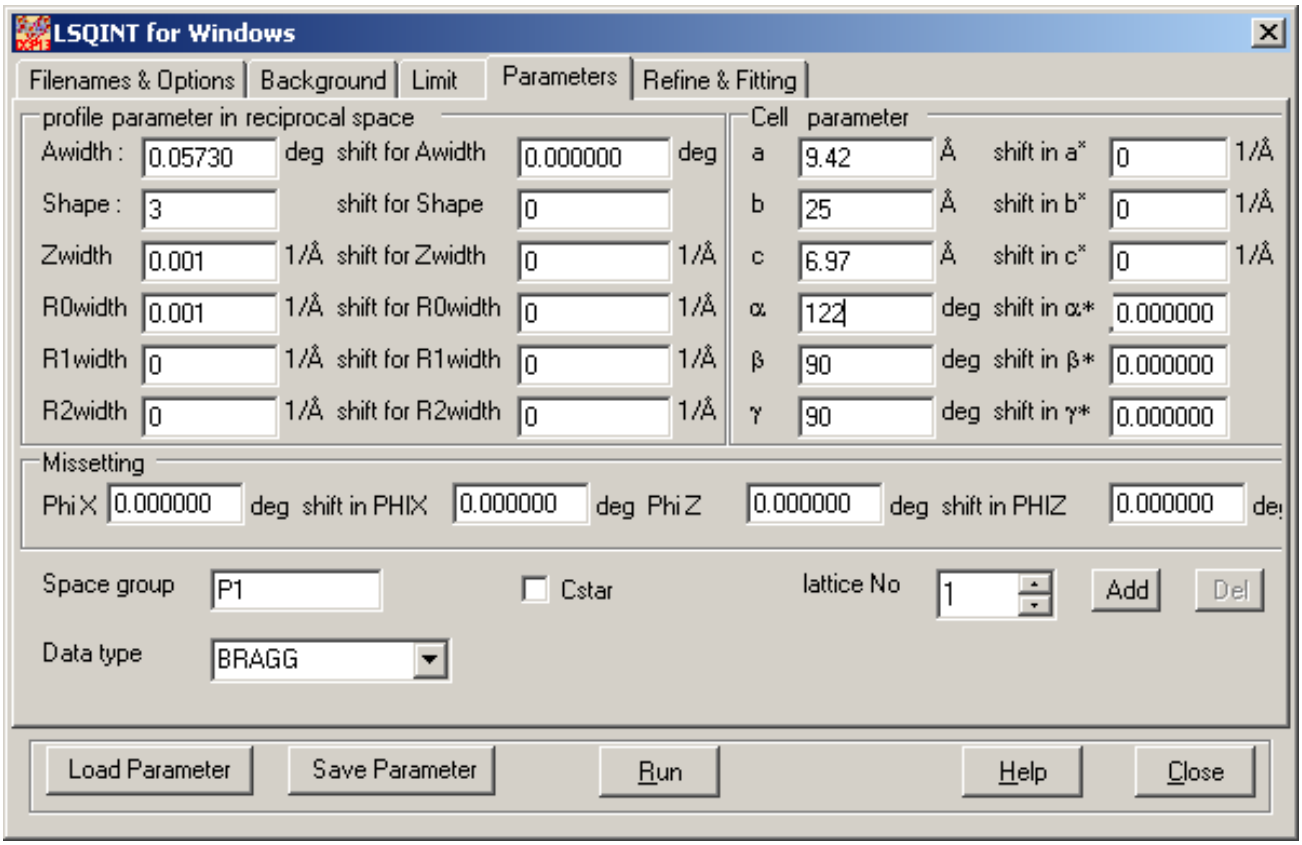




Selecting NoFit Parameters

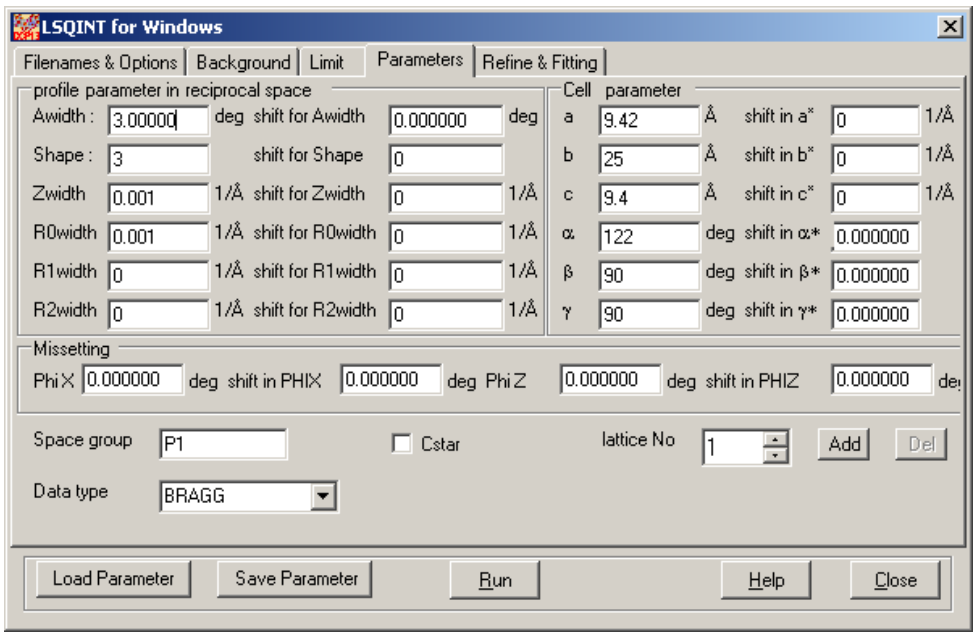
The fibre disorientation and peak shape will depend very much on the particular specimen being studied and the nature of the X-ray beam and camera. Appropriate parameters can be determined by trial and error, but it is often useful, if similar patterns have been analysed before, to use previous estimates as starting values.

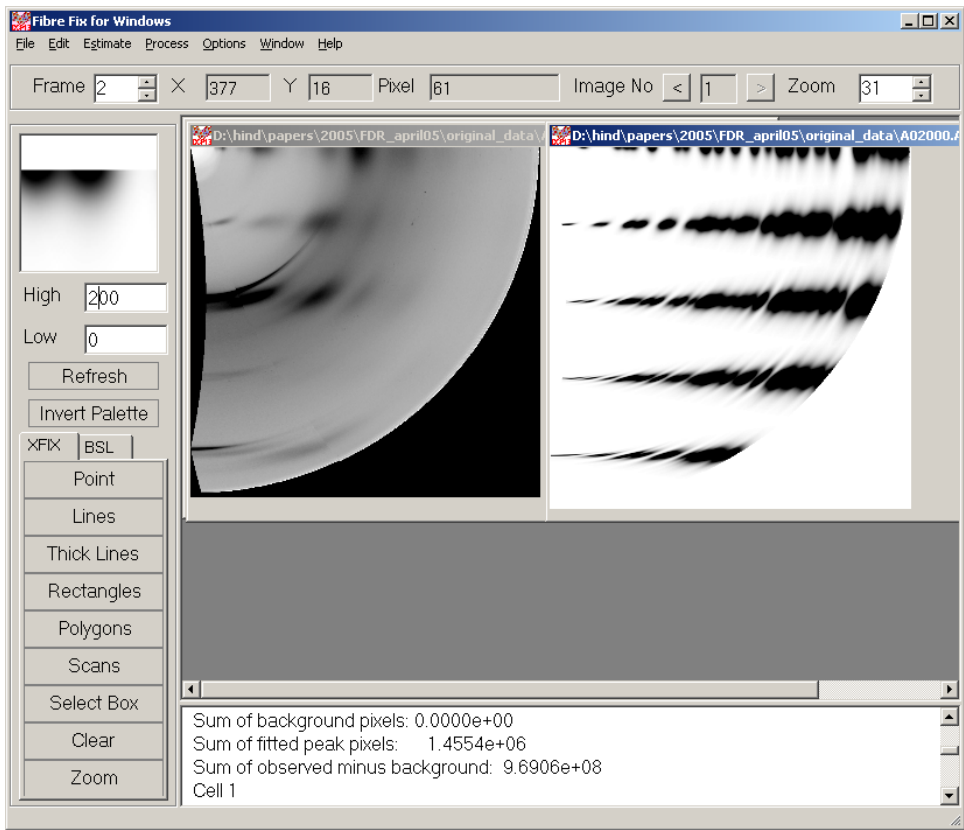
Select the Parameters window and put in estimates of the Profile and Cell parameters. Here, the cell parameters of the published proposed model were used for the starting values.



If you go to a particular parameter name, like Awidth, and hold the cursor there, a flag comes up saying what the parameter is. For example Awidth is the width of the orientation distribution function and Zwidth is 1/ particle length. If the pattern is clearly sampled then select BRAGG in the Data type window.

Clicking on Run in the Parameters window will generate a NoFit pattern. Visual inspection will then indicate whether any parameters need changing. The Awidth, Zwidth, R0width etc parameters can be manually changed and the process Run again until a close simulation of the observed peak shapes is observed.



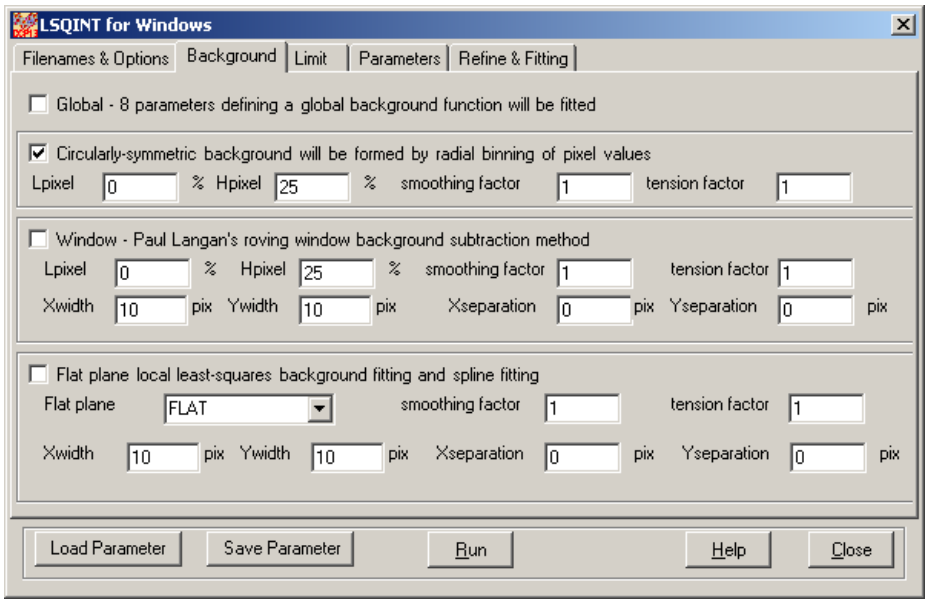


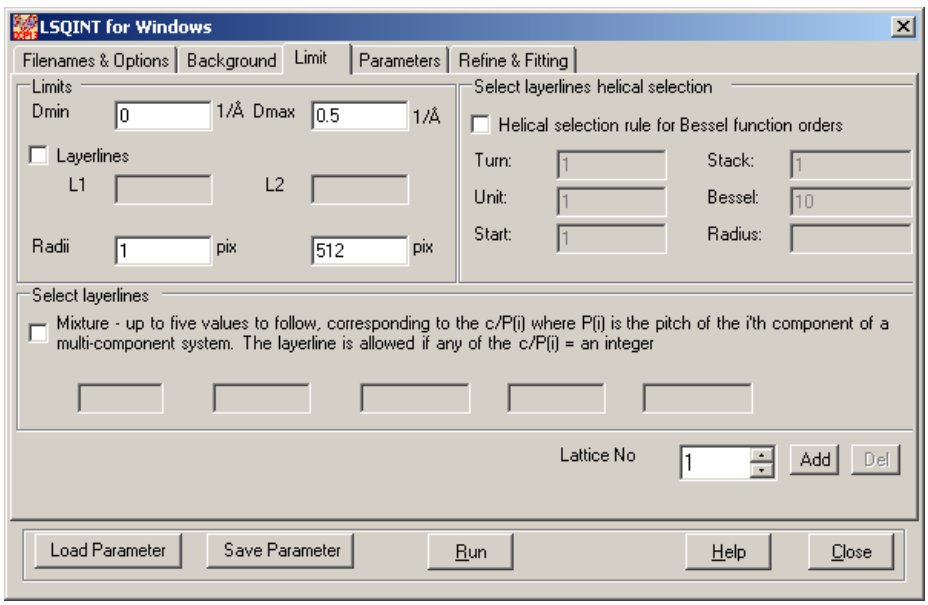
Application of LSQINT for fitting background and peak intensities, as well as to refine the cell and profile parameters

To estimate the peak intensities once the peak shapes have been defined, go to the Filenames and Options window and **un**select NoFit.

LSQINT can fit the background and refine the profiles and cell parameters, as well as fitting intensities as BRAGG peaks.

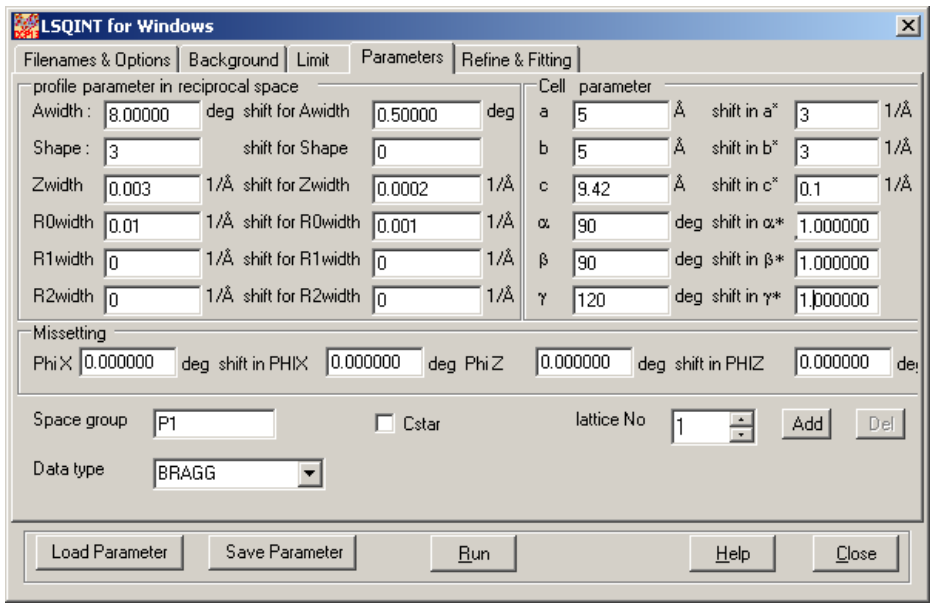
Under the Background window, select the method of background fitting, here the circularly-symmetric method was chosen. Other parameters such as the range of the pattern to process (Dmin and Dmax) can be inserted as required in the Limit window.

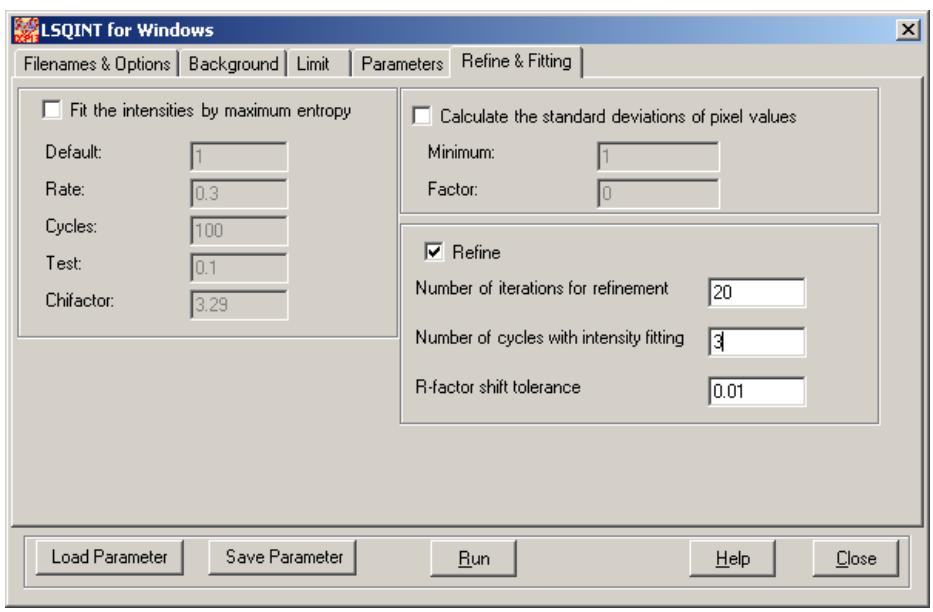




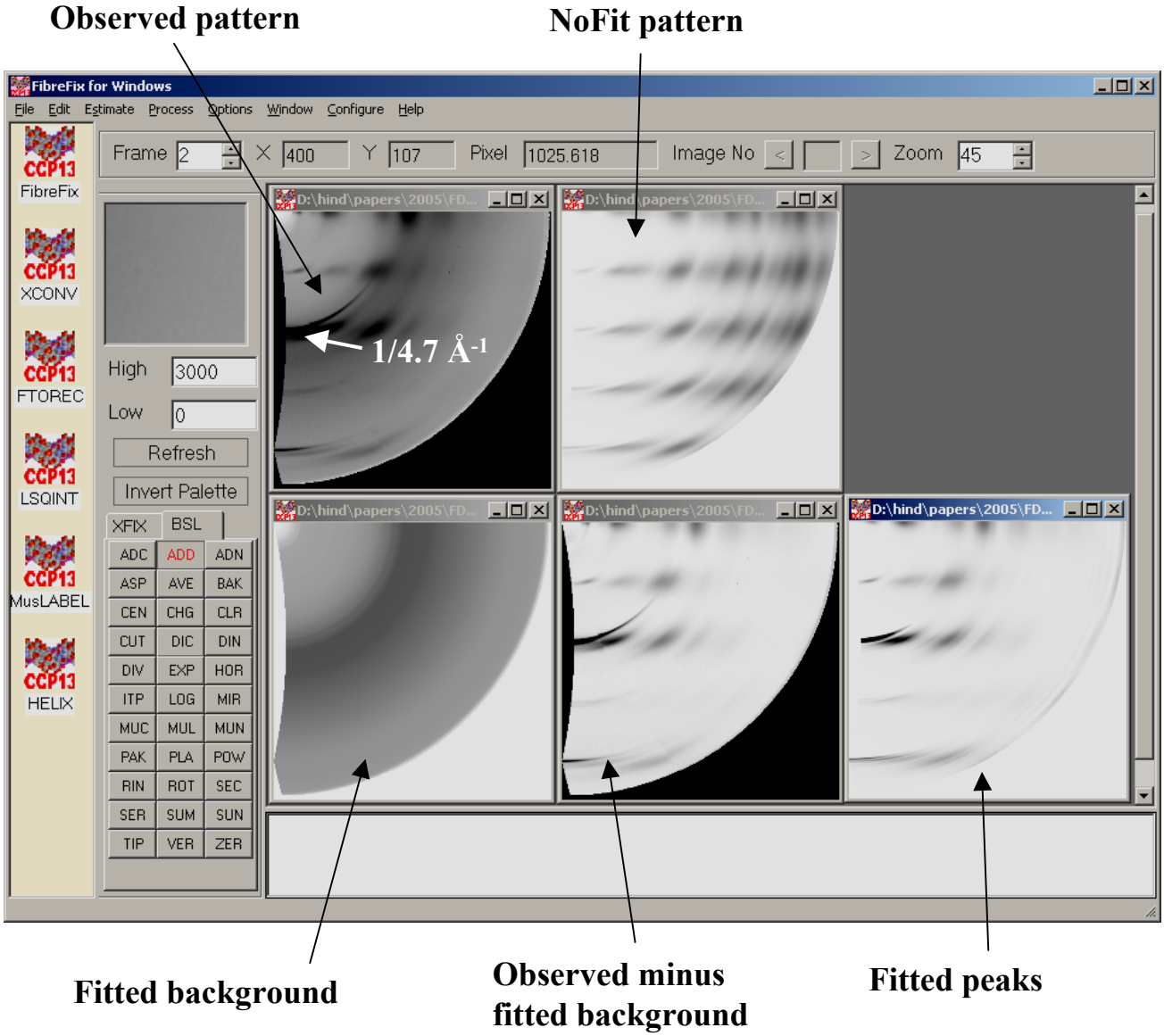
Under the Refine and Fitting window select Refine. Here, for example, the number of cycles could be 3. Try using different starting values for the unit cell parameters ‘a’ and ‘b’ in the Parameters window. For example, in this case 5 Å was used for each, but this was often changed, as well as changing the starting values and the shifts for the profile parameters.

When there is a complete set of parameter starting values, click on Run in the Refine and Fitting window to generate an LSQINT image.





The best fit obtained with LSQINT for the Amyloid pattern



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