

The following is an outline of the procedure for obtaining three-dimensional maps of diffuse intensity:

1. IMAGE ORIENTATION:

- (a) Use DENZO and SCALEPACK to find the crystallographic parameters from the oscillations.
- (b) Use DENZO to output the crystallographic orientation matrix for each of the stills.

2. IMAGE PROCESSING:

- (a) Threshold the stills to mark overflow pixels (`thrshim, proc.mode`).
- (b) Window the stills to define image borders (`windim, proc.mode`).
- (c) Apply a mode filter to get rid of Bragg peaks (`modeim, proc.mode`).
- (d) Find the polarization of the beam by analyzing a typical diffraction image. For example, use TV6 to obtain an azimuthal intensity distribution in a thin annulus about the beam spot, and use `gnuplot` to fit the polarization.
- (e) Correct for the polarization effect using appropriate parameters (`polarim, proc.polarim`).
- (f) Correct for solid-angle normalization (`normim, proc.normim`).
- (g) Calculate average properties of images (`avgrim, subrfim, avsgrim, proc.avgim`).

3. LATTICE GENERATION:

- (a) Calculate image scale factors (`avgrf, proc.makeref, proc.scale`).
- (b) Generate a `genlat` input file called, for example, `genlat.input` (`proc.makeline, proc.genlat.input`).
- (c) Generate the lattice (`genlat, genlat.input, proc.genlat`).
- (d) Transform the lattice, if necessary, to correct for DENZO orientation errors (`xflt`).

4. VISUALIZATION

- (a) **EXPLORER** can be used to view the lattice immediately after it is generated. Run EXPLORER from the `./visualization` directory, and open the `mapview.map` map, which uses `mw.float` as a template for reading lattices. Enter the filename of the lattice in the appropriate box, and select a threshold for the isosurface rendering routine. The view can be manipulated in the Render window using the mouse.
- (b) **Shell images** can be generated using `shimlt` (reduced image) or `shim4lt` (whole image). They can be viewed using the `xseesh` scripts. The script `proc.shimlt` can be used to generate a sequence of shell images in increasing resolution shells. Shell images are easiest viewed from lattices which have had their spherically-averaged component subtracted, using `avgrlt` and `subrflt`. Otherwise, viewing thresholds have to be set by hand to bracket the average value in the shell.

## 5. ANALYSIS

- (a) The **internal symmetry** of the lattice can be characterized by calculating the difference between a lattice and its symmetry-averaged counterpart. The only symmetry-averaging currently implemented in `symlt` is  $P4_1$ . The file `script.sym` contains a transcript of a sample session from the unix shell.
- (b) The techniques to calculate the best-fit **scale factor** between two lattices are used to determine the **reproducibility** of diffuse maps, and to measure the *difference* between the crystals of nuclease both with and without substrate analog. A transcript of a session where the best scale factor was found between two lattices is in `script.scale`.
- (c) Results are **displayed** using `gnuplot`. The file `script.plot` shows a transcript of a session with `gnuplot` to display results.