**PROGRAM: soft\_rec**

**PROGRAM: soft\_het**

**PROGRAM: auto\_mask**

Auto\_mask is a program for doing solvent flattening of an input spider volume.

**Usage:**

**./auto\_mask** *vol1*=<invol.spi> *amsklp=*<mask low-pass limit (in Å)> *box=<*box size (in pixels)> *smpd=*<sampling distance (in Å)> *voltyp*=<spider|simple> [msk=<mask radius (in pixels)>] [debug=<yes|no>]

**Comments:**

The algorithm for background removal is based on low-pass filtering. First, the volume is low-pass filtered to *amsklp* before clustering the voxels into two groups using the k-means algorithm and assigning them background (=0) and foreground (=1) values. Low-pass filtering to *amsklp/2* before multiplication with the input volume softens the sharp-edged envelope. The mask and the masked volume are written to files. *voltyp* refers to which program has been used to generate the spider volume (the byte order may differ).

**PROGRAM: emc**

Emc is a program for Expansion Maximization Compression style ab initio 3D reconstruction for single-particle EM. This reconstruction algorithm is based on Bayesian maximum likelihood estimation and it was originally developed with a Poisson noise model for reconstructing X-ray free electron laser data (REFS). In SIMPLE it is implemented without relying on any noise model. Instead the variance of the low-pass limited correlation is used as a parameter for doing probabilistic orientation assignment. The algorithm is unpublished and more work is required to understand the effects of the input parameters and their automated assignment. No heterogeneity analysis has been implemented. Emc will replace the common lines based routines for doing ab initio reconstruction because of its increased robustness towards noise, its insensitivity to the distribution of orientations in the single-particle population and its automated symmetry weighting due to the probabilistic orientation assignment. The emc algorithm can deal with single-particle populations with orientations distributed in single-axis tilt geometries. Common lines-based reconstruction algorithms generally fail if not all orientations are present.

**Usage:**

**./emc** *fstk*=<fprojs.fim> *maxits=*<maximum nr of iterations (typically 15)> *lp=<*low-pass limit (in Å)> *amsklp=*<mask low-pass limit (in Å)> *epsilon*=<correlation variance (typically 0.01)> *nbest*=<nr of best solution to grid with probabilistic weight (typically 10)> [msk=<mask radius (in pixels)>] [hp=*<*high-pass limit (in Å)>] [*nthr*=<nr of openMP threads>] [debug=<yes|no>]

**Comments:**

If the particle is symmetric, *nbest* should have the minimum value of the number of equivalence positions in the point group. One may need to play around with *epsilon* and *nbest* for optimal performance. Input reference volumes and heterogeneity analysis will be implemented.