

EMROTAVG.DOC

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Documentation unfinished

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A. INTRODUCTION

EMROTAVG is used to align and average a set of particle images in which the particles are all nearly aligned with respect to the view direction (same THETA,PHI) but are more or less randomly oriented with respect to the rotation angle (OMEGA) about the view direction.

The program allows certain control in weeding out 'bad' particle images so that better average images are computed.

The program works by starting with a REFERENCE IMAGE (REF_IMG) and estimates the location of its center by use of a cross-correlation procedure. A MAP (called PS_MAP) of the square-root of the power spectrum of REF_IMG is computed and converted to polar form. Note that PS_MAP is a translation-invariant function since the location of the origin of the power spectrum is always known. A PS_MAP is then computed for each individual image and rotationally cross-correlated with the PS_MAP calculated from REF_IMG.

B. PROGRAM INPUT

1. FILENAME (A)
2. REF_ID,PHANTOM (2I)
3. RES_MIN,RES_MAX,NANNULI,NROT (2F,2I)
4. ANG1,DEL_ANG,NANG (2F,I)
5. DX,DY,DPHASE (3F)
6. IMG_NUM1,IMG_NUM2,IMG_NUM_STEP (3I)
7. NCYCLE (I)
8. CHANGE HEADER FOR EMROTAVG.AVG FILE NO<CR> YES(1) (I)

1. FILE NAME (A)

Name of file that contains the IMAGE data set.

2. REF_ID,PHANTOM (2I)

REF_ID is the number of the IMAGE in the data set to be selected as the REFERENCE. Set PHANTOM = 1 to produce intermediate graphics output (mainly useful for debugging purposes).

3. RES_MIN,RES_MAX,NANNULI,NROT (2F,2I)

RES_MIN and RES_MAX are the inner and outer radii (in floating point TPU steps) of the region of the Fourier transform thought to contain data that ought to correlate between different images viewed in the same orientation. These constants should be carefully chosen as outlined below.

RES_MIN and RES_MAX specify the lower and upper resolution limits of the data to be used in the alignment calculations. These values are expressed in REAL*4 transform pixel units (TPU). The DEFAULTS for these parameters are 1.0 and (IDIM1/4).

Judicious choices of RES_MIN and RES_MAX significantly improve the success of the alignment process.

RES_MIN and RES_MAX are typically estimated by inspection of a few representative particle Fourier transforms (displayed with EMFFTDSP) as is done for programs like EMICOFV (see [TSB.DOC]EMICOFV.DOC). Typically, the value for RES_MAX is set slightly lower than the value corresponding to the maximum expected resolution of the data. This helps reduce uncertainties introduced by the presence of high resolution noise.

NOTE: RES_MAX must be $< (IDIM1/2)-1$, the limit of the Fourier transform data. Since micrographs typically should be scanned at a raster step size (pixel resolution) AT LEAST TWICE AS FINE as the expected resolution, RES_MAX should never exceed $(IDIM1/2)-1$. If, for example, the data are digitized at a raster step size three times finer than the expected resolution, RES_MAX should not exceed $IDIM1/3$.

The choice of RES_MIN also has a significant effect on the success of the alignment. Setting RES_MIN too low has the effect of overemphasizing very low resolution features, which often do not convey useful information concerning the relative alignment and rotation of images. The minimum value for RES_MIN is estimated by noting where the particle transform is no longer

circularly symmetric. (see [TSB.DOC]EMICOFV.DOC for further discussion concerning the choice of RES_MIN).

NANNULI determines how many annuli there are between RES_MIN and RES_MAX in the PS_MAP.

(DEFAULTS=NINT(RES_MAX)+1,1)

4. ANGL,DEL_ANG,NANG (2F,I) (DEFAULTS=-90.0,1.0,180)

5. DX,DY,DPHASE (3F) (DEFAULTS=3.0,3.0,90.0)

6. IMG_NUM1,IMG_NUM2,IMG_NUM_STEP (3I) (DEFAULTS=1,BIMG_NUM_LIM,1)

IMG_NUM1 and IMG_NUM2 identify the first and last IMAGES to be tested against the REFERENCE. By setting IMG_NUM_STEP > 1 (DEFAULT=1), IMAGES in the data file will be ignored. For example, if IMG_NUM1,IMG_NUM2,IMG_NUM_STEP = 1,20,3, then only IMAGES # 1, 4, 7, 10, 13,16, and 19 in the BYTE-packed file will be compared to the REFERENCE.

7. NCYCLE (I) (DEFAULT=1)

This specifies the number of cycles of refinement. If NCYCLE is set >2, then DEL_ANG is reduced by half and NANG is doubled in size with each cycle beyond the second one. ANGL is then automatically adjusted to allow the orientation search to encompass angles centered about the angle of best correlation from the previous cycle.

NSAMPL is the number of radial sample steps to be taken within the band. NSAMPL is almost always set equal to the DEFAULT value (MAXR-MINR+1). At this value the sample step size equals one TPU.

FMIN is the fractional minimum amplitude (relative to the mean) of data used in the refinement. Data points with smaller amplitudes are ignored. For FMIN > 0.0 the average value of F in each radial zone is calculated and these values are fit, via a Wilson plot, to a straight line to establish the expected average value of F which is then used as a cutoff. For FMIN < 0.0 the average value of F for the entire required area is calculated and this cutoff is applied to all the F's. The DEFAULT for FMIN is 1.0, a value that is useful in most instances. Use FMIN > 1.0 or < 1.0 WITH CAUTION.

C. RUNNING THE PROGRAM

The program is normally run in BATCH mode and output in the *.LOG and EMICOFV.TMP files should be examined very carefully to determine the most probable THETA,PHI,OMEGA orientation angle.

NOTE: Output from this program requires careful analysis, since the correct orientation angle QUITE OFTEN turns out NOT to be the orientation at which the BEST residual value is found due to the low signal-to-noise in most EM data (especially of frozen-hydrated specimens). EMICOFV gives the most trustworthy results ONLY if the particle phase origins (FFT_ORIGX,FFT_ORIGY) are fairly accurately known. This is not always easy to do, especially for particle image data that is noisy (the case for most images of frozen-hydrated specimens). In addition, the results of EMICOFV are highly sensitive to the values chosen for MINR, MAXR, and FMIN (see 3.).

D. FINAL NOTES

1. See JUSTEM\$DKA0:[TSB.FOR]EMROTAVG.BCH for an example BATCH command procedure.

E. FLOW CHART FOR EMROTAVG PROGRAM

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*****
*          MAIN          *          FLOW CHART FOR EMROTAVG PROGRAM
* (EMROTAVG.FOR) *
*****
*
*-- IMG_OPEN ----- | -- STRING_UPPER
*                   | -- IMG_PACK_BIMGCOM_CLEAR
*                   | -- FILE_CHECK
*-- IMG_PACK_GETINFO
*
*-- LX_INIT
*
*-- IMG_PACK_FIND - IMG_PACK_NRECS
*
*-- IMG_PACK_FILL
*
*-- LX_PUTIMG_STRETCH | -- IMG_FFT
*                   | -- COPY_R4
*                   | -- FFT_FLIP
*-- FFT_SETDIM_DEF_SAME | -- FFT_PSHIFT
*                   | -- CCF_FFT
*-- ROTAVG_REF ----- | -- ROTAVG_PEAK
*                   | -- FFT_PSHIFT
*-- IMG_PACK_GETRANGE | -- PS_SQRT_FFT
*                   | -- COR_CENTER
*-- IMG_FFT           | -- MAP_POLAR
*                   | -- LX_PUTMAP_STRETCH
*-- PS_SQRT_FFT
*
*-- COR_CENTER
*
*-- LX_PUTMAP_STRETCH
*
*-- MAP_POLAR
*
*-- RCF
*
*-- CCF_FFT           | -- MAP_PEAKSEARCH
*                   | -- PEAK_SORT_HI
*-- ROTAVG_PEAK ----- | -- PEAK_CHK
*                   | -- PEAK_SORT_HI
*-- IMG_SHIFT         | -- TRUS_POLYS
*
*-- ROTAVG_BESTANG -- IMG_ROT
*
*-- IMG_ROT
*

```

```
*-- IMG_SYM
*
*-- IMG_BKGND
*
*-- IMG_FFT
*
*-- FFT_COMPARE -- PIRADDEG
*
*-- IMG_ADDIMG
*
*-- COPY_I2
*
*-- IMG_CLEAR
*
*-- IMG_STATS
*
*-- IMG_BKGND
*
*-- IMG_SCALE
*
*-- IMG_WRITE
```