EMROTFLT.DOC

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A. INTRODUCTION

   EMROTFLT is used to calculate and plot the power spectrum of images with axial symmetry and compute rotationally symmetrized averages. It is a highly revised version of the original MRC program RFILTIM. The program finds the best position for the postulated rotational symmetry axis (in the Fourier transform of the IMAGE) and then decomposes the transform into cylindrical harmonics about this origin. Those harmonics consistent with the postulated symmetry of the IMAGE are then combined to produce a rotationally filtered image. The best (and original) literature reference to this procedure is, Crowther, R. A. & Amos, L. A. (1971) "Harmonic Analysis of Electron Microscope Images with Rotational Symmetry" J. Mol. Biol. 60:123-130.

   Another program, EMIMG (option "J"), which operates in REAL SPACE, is an alternative to EMROTFLT for applying a rotational symmetry to an IMAGE. The advantage of EMROTFLT is that it includes routines for refining the origin of the symmetry axis, calculating and plotting a power spectrum, and producing a file (EMROTFLT.LG) which contains the little g's. Also, note that EMROTFLT produces output in MAP format, whereas option "J" of EMIMG produces output in IMG format.

   EMROTFLT contains a variety of interactive options to provide maximum flexibility. Most options have DEFAULT values given at the user prompt. **WARNING:** In many instances the DEFAULT values (e.g. NROT = 1) are not the best choice.

B. PROGRAM INPUT

1. IMAGE FILENAME (A)
2. NROT,NANNULI,FFT_STEP,PHI0,GZERO (2I,2F,I)
3. NLR,DEL_LR,ASCAL,BSCAL,ELIPX,ELIPY (I,5F)
4. IOPT1,IOPT2,IOPT3,IOPT4 (4I)

```
5. [IF IOPT1=0]            5A. FFT_ORIGX,FFT_ORIGY (2F)
   [IF IOPT1=1]            5A. DXMIN,DXMAX,DELDX (3F)
                           5B. DYMIN,DYMAX,DELDY (3F)
                           5C. NB_STEP (I)
                           5D. NB_SKIP (I)
6. [IF IOPT2=0]            6A. POW_SCALE (F)
7. [IF IOPT4=0]            7A. BES1,BES2 (2I)
                           7B. MAP_FILENAME (A)
                           7C. MAP_FILE_HEADER (18A4)
                           7D. IOUT (I)
```

1. FILE NAME (A)

   Name of file that contains the IMAGE to be analyzed.

2. NROT,NANNULI,FFT_STEP,PHI0,GZERO (2I,2F,I)

   NROT is the rotational symmetry order that will be searched to
refine the symmetry axis phase origin. It is also the symmetry
that is enforced on the data about the symmetry origin (DEFAULT
NROT = 1).

   FFT_STEP is the width of each annulus in the transform (DEFAULT
= 1.0 TPU).

   NANNULI specifies the number of annuli into which the FFT of
the IMAGE is divided (limit = (IDIM/2)-1, where IDIM is the
dimension of the FFT). With most data and FFT_STEP = 1.0, NANNULI
should normally be set much lower than the DEFAULT (often
DEFAULT/2), otherwise the high frequency noise will bias the
origin refinement.

   PHI0 is the angle by which the reconstruction is rotated with
respect to the original (input) image. A positive angle rotates
the reconstruction counter-clockwise.

   GZERO determines whether G-zero terms are included in the
Fourier-Bessel calculation of the reconstruction. The DEFAULT
(=0) is to include G-zero in the reconstruction. Otherwise, enter
"1" to compute the reconstruction with only the azimuthal terms.

3. NLR,DEL_LR,ASCAL,BSCAL,ELIPX,ELIPY (I,5F)

   NLR sets the number of radial steps to be computed in the
reconstruction (DEFAULT = radius in pixels in boxed image =
FFT_ORIGX). Set NLR higher than the DEFAULT (and DEL_LR smaller
than 1.0) to obtain finer sampling of the reconstruction.

DEL_LR is the size of the radial steps (in pixels) in the reconstruction (DEFAULT = 1.0 when the DEFAULT for NLR is chosen). If a value other than the DEFAULT is used for NLR and nothing is entered for DEL_LR, then DEL_LR is automatically set equal to FFT_ORIGX/NLR to keep the NCOL,NROW size of the reconstruction equal to that of the input IMAGE. If DEL_LR is set > 1.0 with NLR set equal to or less than its DEFAULT value, the outer portions of the particle will be missing in the rotationally averaged reconstruction.

ASCAL ands BSCAL are radial weighting factors which may be used to artificially increase the strengths of the high frequencies (DEFAULT = 1.0,0.0).   USE ONLY WITH CAUTION!! Annulus number IR is scaled by the factor (ASCAL + IR*BSCAL).

ELIPX and ELIPY are scale factors used to remove or enforce an elliptical distortion on the data (DEFAULT = 1.0,1.0, for no distortion). For example, if ELIPX > 1.0, then the input IMAGE is expanded in the horizontal direction before processing. DO NOT use ELIPX and ELIPY to magnify or demagnify the reconstruction (with ELIPX = ELIPY); instead, use NLR and DEL_LR.

4. IOPT1,IOPT2,IOPT3,IOPT4 (4I)

   The following program control options are used in the following way (DEFAULT = 0,0,0,0):

   IOPT1 = 0   do not refine symmetry axis origin.
         = 1   refine the symmetry axis origin.

   IOPT2 = 0   compute the power spectrum.
         = 1   do not compute the power spectrum.

   IOPT3 = 0   no graphics or file output of the power spectrum.
         = 1   plot power spectrum on graphics.
         = 2   save power spectrum results in EMROTFLT.TMP.
         = 3   plot power spectrum and save results in
               EMROTFLT.TMP.

   IOPT4 = 0   compute the rotationally symmetrized average.
         = 1   do not compute the rotationally symmetrized
               average.
         = 2   compute the rotationally symmetrized average and
               save the little g's in EMROTFLT.LG.

5. ORIGIN REFINEMENT OPTIONS:

[IF IOPT1=0: DO NOT REFINE SYMMETRY AXIS POSITION]

    5A. ORIGX,ORIGY (2F)

        Enter the position of the symmetry axis (DEFAULT =
    FFT_ORIGX, FFT_ORIGY which are usually the coordinates of
    the center of the boxed IMAGE).

    [IF IOPT1=1: REFINE SYMMETRY AXIS POSITION]

    5A. DXMIN,DXMAX,DELDX (3F)

        DXMIN, DXMAX, and DELDX specify the range of search in
    the X direction relative to ORIGX, and the X step interval,
    measured in pixels. (DEFAULTS=-2.5,2.5,0.25)

    5B. DYMIN,DYMAX,DELDY (3F)

        DYMIN, DYMAX, and DELDY specify the range of search in
    the Y direction relative to ORIGY, and the Y step interval,
    measured in pixels. (DEFAULTS=-2.5,2.5,0.25)

    5C. NB_STEP (I)

        NB_STEP specifies the number of transform annuli to lump
    together in bands when computing residuals for the search
    procedure. (DEFAULT = 5: Limits = 1 to NANNULI).

    5D. NB_SKIP (I)

        NB_SKIP specifies the number of bands nearest the origin
    of the transform to omit from the search procedure
    (DEFAULT=0; Limits: 0 -(NANNULI/NB_STEP)-1. NOTE: The
    higher the rotational symmetry, the further the
    corresponding harmonic terms will be from the origin of the
    transform. Usually NB_SKIP should be set = 1 or higher.

        The best ORIGX,ORIGY found by the origin refinement is
    used for the power spectrum and reconstruction calculations.

6. POWER SPECTRUM OPTION [IF IOPT2=0]

    6A. POW_SCALE (F)

        POW_SCALE is a scale factor used to normalize the power
    spectrum (DEFAULT = 100.0). All terms are normalized with

G-zero equal to POW_SCALE. Set POW_SCALE negative to get
out unscaled values of the power spectrum.

7. RECONSTRUCTION OPTIONS

[IF IOPT4=0 or 2]

7A. BES1,BES2 (2I)

These specify the range of Bessel orders to be included
in the reconstruction. DEFAULT values have a lower limit
of 0 (to include the G-zero term) and an upper limit
determined by several factors such as the value chosen for
NROT. The user is prompted with the appropriate upper
limit as a DEFAULT. The upper limit (BES2) should be
reduced to eliminate higher order terms that merely add
noise to the reconstruction. BES2 can be estimated by
direct inspection of the computed power spectrum. The BES2
limit is calculated according to the following
relationship:

$$BES2 = (INT(2.0*PI*NLR*DEL\_LR*FFT\_STEP*NANNULI/IDIM) + 2.0))$$

Using reovirus ISVP images (127x127 pixels) as an example,
and NLR = 63, DEL_LR = 1.0, FFT_STEP = 1.0, NANNULI = 31,
IDIM = 128 (FFT dimension), BES2 = 98. Thus, for ISVP
images in which most particles are oriented along the
characteristic five-fold view (NROT=5), the maximum value
allowed for BES2 would be 95.

7B. MAP_FILENAME (A)

Name of the file for storing the rotationally
symmetrized output MAP.

7C. HEADER FOR MAP FILE (18A4)

Type any header information you wish up to 72
characters long.

7C. IOUT (I)

This is used to signal whether MAP output format is
INTEGER*2 (DEFAULT IOUT=0) or REAL*4 (IOUT=1).

If IOPT4 = 2, the values of the little gs are saved in

an output file called EMROTFLT.LG.

C. RUNNING THE PROGRAM

The program is normally run interactively but may be run in BATCH mode if desired (see FINAL NOTES below).

After the big G's are computed for all annuli in the Fourier transform, the power spectrum is listed at the terminal (and in the file EMROTFLT.TMP if IOPT3=2 or 3). The values of the power spectrum are normalized to make G-zero = POW_SCALE for positive values of POW_SCALE (DEFAULT = 100.0) or are left unscaled if POW_SCALE is entered as a negative value. The % of the total azimuthal power in the transform contributed by the chosen rotational symmetry provides a measure of the strength of that symmetry relative to others.

The power spectrum may be displayed on the raster graphics screen with regular or logarithmic scaling. The absolute scale of the power of the n-fold rotational component (Pn: ordinate axis) is plotted as a function of harmonic frequency (abscissa axis). Follow prompts to achieve optimum scaling of the graphics plot.

The resulting filtered data are saved as a MAP file which may be further manipulated and displayed on the graphics screen using EMMAP and EMMAPDSP.

D. FINAL NOTES

1. See [TSB.FOR]EMROTFLT.BCH for an example BATCH command procedure such as that shown below. Realize that EMROTFLT can be run in any of a number of different modes as determined by the values of IOPT1-IOPT4.

```
$ SET DEFAULT BERNAL2:[TSB.VIRUSES.REO]
$ RUN JUSTEM$DKA0:[TSB.EXE]EMROTFLT.EXE
BERNAL2:[TSB.VIRUSES.REO]EMROTAVG.AVG
5, 31, 1.0, 0.0, 0
63, 1.0, 1.0, 0.0, 1.0, 1.0
0, 0, 0, 0
63., 63.
100.0
0, 95
[TSB.REO]EMROTAVG.ROT
This is a rotational average of an already averaged REO series.
0
$ EXIT
```

E. FLOW CHART FOR EMROTFLT PROGRAM

```
*******************
*      MAIN       *
*  (EMROTFLT.FOR) *
*******************
        *                                        |-- STRING_UPPER
        *-- IMG_OPEN ----------------------|--
IMG_PACK_BIMGCOM_CLEAR
        *                                        |-- FILE_CHECK
        *-- IMG_PACK_FIND - IMG_PACK_NRECS
        *
        *-- IMG_PACK_FILL
        *
        *-- IMG_FILL
        *
        *-- FFT_SETDIM_DEF_SAME
        *
        *-- IMG_FFT
        *                  |-- PIRADDEG  |-- STRING_UPPER
        *-- ROTFLT_INFO--|-- FFT_OPEN--|-- FILE_CHECK
        *
        *-- ROTFLT_MAXBES
        *
        *-- FFT_TO_ATBT -- PIRADDEG
        *
        *-- ROTFLT_ORIGIN -- PIRADDEG
        *
        *-- ROTFLT_BG -- PIRADDEG
        *                          |-- PIRADDEG
        *-- ROTFLT_POWSPEC --------|-- ROTFLT_POWPLT -- [GRAPHICS]
        *                          |-- HITCR
        *
        *              |-- PIRADDEG
        *-- ROTFLT_LG --|-- ROTFLT_FILBES
        *
        *-- ROTFLT_LGOUT
        *
        *-- ROTFLT_FB -- MAP_WRITE -- STRING_UPPER
```