EMICOGRAD.DOC

(last update Sep 15, 1994)

CONTENTS

A. INTRODUCTION

EMICOGRAD is used to refine the orientations of up to 200 icosahedral particles against each other. This program was adapted from a version of SIMPLEX, written by Steve Fuller (Heidelberg) and based on the original MRC XCORRLN program (revised and renamed EMICOCOR at Purdue). Initial particle orientation refinement with EMICOGRAD usually follows EMICOFV and EMICOORG (or EMICOORG2), which, respectively give initial refinement of orientation and phase origin for individual particles. Subsequent cycles of EMICOGRAD generally follow re-refinement of origins, obtained directly with EMICOORG or EMICOORG2, or using EMCORORG with reprojected 3D reconstruction images (see [TSB.DOC]EMCORORG.DOC for more details about this procedure).

SIMPLEX is a program that can be run in either of two modes, i) a "gradient" mode in which the gradient of the change in residual for an individual image compared to all the other images is used to change the orientation of that image, and ii) a "simplex" mode in which changes are made in the orientations of all images at the same time by movements in the 4*(N_IMAGES) space of conformations. Both modes are iterative and allow the option of refining only some parameters and/or some images and holding the rest constant. Both methods work only on the orientation angles and a re-refinement of the origin is necessary.

EMICOGRAD only implements the gradient portion of SIMPLEX. In practice, this has proven to be the more useful of the two routines. The simplex mode appears to only work well at the final stage of refinement, where the orientations are fairly well determined (i.e. within a degree or less). The gradient method seems able to work well even for particle images in which the orientation is off by several degrees. Also, the simplex routine seems to require many more cycles to give effective refinement.

B. PROGRAM INPUT

```
1. N_IMAGES,IFLIP,ILIST (3I)
2. SCLMIN,SCLMAX,DELSCL (3F; DEFAULT=1.0,1.0,0.0)
3. ICO_IDIM,RES_MIN,RES_MAX (I,2F)
4. INCR,NSAMPL (2I)
5. DEL_THETA,DEL_PHI,DEL_OMEGA,NCYCLES,ERR_LIM,FMIN,FACTOR
   (3F,I,3F)
6. INPUT IMAGE FILENAME (A)
7. IMG_NUM,THETA,PHI,OMEGA,FFT_ORIGX,FFT_ORIGY,TRIGGER_DEL (I,6F)
```

```
1. N_IMAGES,IFLIP,ILIST (3I)
   -------------------------
```

N_IMAGES specifies the number of images to be correlated. The end of the data input (see 7.) contains relevant information about each of the individual images and how they are to be refined.

IFLIP enables/disables the evaluation of "hand" choices for the particle data. For IFLIP > 1 and N_IMAGES < 11, the program automatically tests all possible combinations of orientations (omega and omega+180) for the entire set of particle images to minimize the average phase differences between particles. Enabling this option considerably slows program execution (hence the limit of 10 for N_IMAGES) and therefore is only recommended during early stages of refinement and only if necessary.

Quite often, especially for particles with a skew icosahedral LATTICE symmetry (e.g. the T=13 rotavirus and reovirus or the T=7 papilloma viruses), the relative choice of hand for a given particle is readily apparent in the large phase residual difference between the particle in the "up" position (omega unchanged) verses the "down" position (omega+180 degrees). The relative orientations are much easier to identify when the number of particles compared in the program is small (N_IMAGES < 5). Once the relative orientations of a small number of well-correlated particles is established, it is much easier to add new particles and quickly determine the correct choice for the omega angle. Thus, for skew icosahedral lattice symmetries, it may be just as easy to sort out the relative hand choices directly. With non-skew symmetries (e.g. T=4 for the Nudaurelia capensis insect viruses or T=16 for the herpesviridae), the choice of hand may only manifest itself in higher resolution features (e.g. subunit twist) and the use of the IFLIP option may greatly facilitate the correct identification of the relative particle orientations.

If IFLIP = 1, then hand choices are ONLY EVALUATED. Particle omega angles WILL NOT BE FLIPPED during refinement.

ILIST controls the amount of output. Set ILIST = 0 for minimal output, and = 2 for maximum output.

2. SCLMIN,SCLMAX,DELSCL (3F; DEFAULT=1.0,1.0,0.0)

SCLMIN,SCLMAX define the range of radial scale factors and DELSCL the radial step size. These parameters are designed to allow variation of the radial sampling of each image transform independently. To have no variation use: 1.0,1.0,0.0 (the DEFAULT).

NOTE: The option to adjust radial scaling is currently inactive, and thus the values for SCLMIN,SCLMAX, and DELSCL have no effect whatsoever on the refinement calculations.

3. ICO_IDIM,RES_MIN,RES_MAX (I,2F)

ICO_IDIM defines the size of the FFT calculated for each image. ICO_IDIM must = 128, 256, 512, or 1024 and it must be greater than the largest image dimension (all particle images typically have identical NCOL,NROW dimensions).

RES_MIN and RES_MAX specify the lower and upper resolution limits of the data to be used in the refinement calculations. These values are expressed in transform pixel units (TPU). As an example, for a particle image of bovine papilloma virus (BPV) recorded at 36,000X, scanned at 25 microns, and Fourier transformed in a 128 by 128 pixel array, the following relationships can be established:

```
    Each pixel = scan raster in nm / magnification
               = (25000 nm/36000)
               = 0.694 nm

     Each TPU  = 1.0 / (pixel size * ICO_IDIM)
               = magnification / (scan raster in nm * ICO_IDIM)
               = (36000/(25000 nm*128))
               = 0.0113 nm-1
               = 1/88.9 nm
```

Judicious choices of RES_MIN and RES_MAX significantly improve the success of the EMICOGRAD refinement.

RES_MAX is typically estimated from inspection of a few representative particle Fourier transforms (displayed with EMFFTDSP). Refinement proceeds more effectively if, initially, the value of RES_MAX is set slightly lower than the expected maximum resolution of the data. Initial refinement often results in changes in particle orientations by several degrees. Use of high resolution (noisy) information (RES_MAX too high) tends to reduce the effectiveness of the refinement of these large scale orientation adjustments (> 1 degree). Towards the end of refinement, with a large number of particle images, and where small orientation adjustments are made (< 1 degree), RES_MAX can be increased to match the maximum expected resolution of the data. In the BPV example above, the maximum resolution was determined to be 36.0 TPU (= 3.5 pixel resolution = 2.5 nm resolution). For initial refinement, RES_MAX was set to 26 TPU (4.9 pixel resolution; 3.4 nm resolution). As refinement progressed, RES_MAX was increased to 31.0 TPU and then to 36.0 TPU.

NOTE: RES_MAX must be < ICO_IDIM/2 since this is the limit of the Fourier transform data. Since micrographs typically should be scanned at a pixel resolution AT LEAST TWICE AS FINE as the expected resolution, RES_MAX should never exceed ICO_IDIM/2. If, for example, the data are digitized at a raster step size that is three times finer than the expected resolution, RES_MAX should not be set any higher than ICO_IDIM/3.

The choice of RES_MIN also has a significant effect on refinement success. Setting RES_MIN too low has the effect of overemphasizing very low resolution features which generally carry little if any useful information concerning the relative orientations of the icosahedral symmetry axes of the particle. RES_MIN can be estimated by noting where the circularly symmetric features in the particle transform begin to diminish. In the BPV example, RES_MIN was determined to be 6.0 TPU (21.3 pixel resolution; 14.8 nm resolution) which is a resolution corresponding to about one fourth the particle diameter. Thus, for BPV, the spherically symmetric part of the transform extends to about 14.8 nm resolution and this part of the transform carries no useful information about the orientation of the icosahedral symmetry axes. In some instances it may be useful to increase RES_MIN towards the end of refinement as a way of reducing possible over-emphasis of lower resolution features which may incorrectly bias the orientation parameters.

4. INCR,NSAMPL (2I)

   INCR and NSAMPL determine how the transform is divided up into

annular bands. INCR is the increment between bands (= width of each band) in INTEGER TPU and NSAMPL specifies the number of samples (annuli) to take in each band. The number of bands (NBAND) is determined in the following way:

```
NBAND = IFIX((RES_MAX-RES_MIN)/INCR)
```

NOTE: The program imposes certain restrictions to make sure no attempt is made to access non-existent data (outside the limits of the transform).

In the BPV example, with RES_MIN,RES_MAX = 6,31, and INCR,NSAMPL = 5,5 the transform is divided up into 6 annular bands, the first five of which will be 5 TPU wide (6-10, 11-15, 16-20, 21-25, 26-30) and the last of which will be 1 TPU wide (31-31).

5. DEL_THETA,DEL_PHI,DEL_OMEGA,NCYCLES,ERR_LIM,FMIN,FACTOR
   (3F,I,3F)

DEL_THETA,DEL_PHI,DEL_OMEGA are the initial step sizes for the three orientation angles. A value of 0.0 fixes the angle so it cannot change during refinement of other parameters.

NCYCLES specifies the number of cycles of refinement to carry out. In the gradient method each step size is tried for each image until there is no improvement in residual or until N_SUB_CYCLES has been passed and then the step sizes are decreased by a factor = FACTOR. This is considered as one cycle. When one image continues to improve but the others not at a given step size, the others are held fixed while that one is refined. If NCYCLES = 0, then no refinement is performed, but the error matrix for the image comparisons is calculated.

ERR_LIM is used to help keep bad images from throwing off the entire refinement. Images with phase errors against another image greater than this value are not included in the refinement calculation. However, NOTE that they ARE INCLUDED IN THE FINAL ERROR MATRIX CALCULATION, which is one reason that the two values of residual may disagree slightly. In the simplex method this control parameter should be set high (180deg) because the fit rapidly discovers that one way to improve the residual over all images is to move the worst ones to such bad orientations that they exceed ERR_LIM and so make no contribution. In the gradient method ERR_LIM allows the better images to be refined without interference from the worst. Typically, ERR_LIM is set high (90 degrees or higher) in the initial refinement runs. As the refinement progresses, and poorer data are identified and removed from the data set, ERR_LIM should be reduced (often to a value 5-10 degrees above the average cross-phase residual).

FMIN is a control parameter used exactly as in EMICOFV. It establishes a cut off threshold for transform values included in the calculations. Typically FMIN = 1.0. If FMIN is set too high, too few data may be used in the calculations and the results may be biased by data at one particular (often low) resolution. If FMIN is set too low, the results are likely to be strongly influenced by noise in the data.

FACTOR is the factor by which the step sizes are reduced after each cycle (DEFAULT = 0.8). The smaller FACTOR is, the faster the step size decreases with each cycle. For example, with FACTOR = 0.8 (the DEFAULT) and step sizes (DEL_THETA,DEL_PHI,DEL_OMEGA) = 1.0, after the first cycle of refinement, the step sizes will be :0.8,0.64,0.51,.41,etc.. For slower refinement, set FACTOR higher (e.g. 0.9, which would give step sizes of 1.0,0.9,.81,.73,.66,etc.), or, for faster refinement, set FACTOR lower (e.g. 0.5, which would give step sizes of 1.0,0.5, 0.25,0.12,.06,.03,etc.).

6. INPUT IMAGE FILENAME (A)

Input to EMICOGRAD is in the form of IMAGE data, either as individual images stored in separate files, or as a group of images stored in a single file, or both. Read [TSB.DOC]EMPROGS.DOC for descriptions of the two different IMAGE file formats (INTEGER*2 or BYTE) used at Purdue. The easiest way to input data is from the byte-packed data files, however, it is possible to mix data types as is shown below.

7. IMG_NUM,THETA,PHI,OMEGA,FFT_ORIGX,FFT_ORIGY,TRIGGER_DEL (I,6F)

IMG_NUM refers to the storage position of the IMAGE data for byte-packed format data. For INTEGER*2 format IMAGE data, IMG_NUM is a dummy variable that can have any value except -999. When IMG_NUM =-999, this signals that a new data file must be opened to read in the next IMAGE. NOTE: This signal is ONLY needed when a BYTE-format data file is closed in order to open another IMAGE file (BYTE or INTEGER*2 format). Thus, the -999 flag SHOULD NOT BE USED after input of data from an INTEGER*2 format file (see examples below).

THETA,PHI,OMEGA are the current values of the three orientation angles for each particle image (obtained from previous runs of EMICOGRAD or EMICOFV).

FFT_ORIGX,FFT_ORIGY are the pixel coordinates of the particle center (the point 0.0,0.0 corresponding to the lower left corner of the boxed particle image). These values are estimated or refined by EMCORORG, EMICOORG, or EMICOORG2.

TRIGGER_DEL is a value that is compared to the sum of delta's for the parameters, and refinement of an image only occurs when it is higher than the sum of deltas. This provides a means of allowing only some images to refine at the coarser stages of refinement while holding the rest fixed and then refining the rest at finer values of delta. If an image is always to be held fixed, this value should be set to -1.0. If <CR> or 0.0, TRIGGER_DEL will be set = 9999.0 so the image will always be refined.

Three examples below show different acceptable ways to enter data. In each of the examples, the method for specifying the data filename is also illustrated.

```
EXAMPLE 1: 3 images, all from one BYTE-format packed file.
----------------------------------------------------------
USER5:[TSB.HPV]10810_SUB.PCK
1,  83.962,   -21.034,   151.002, 49.254,    50.740, 0.0
3,  80.010,     7.917,   316.013, 50.779,    48.940, 0.0
4,  78.250,     4.452,    82.712, 49.294,    50.295, 0.0
```

```
EXAMPLE 2: 4 images from 4 separate INTEGER*2 format files.
------------------------------------------------------------
USER5:[TSB.HPV]1.IMG
0,  83.962,   -21.034,   151.002, 49.254,    50.740, 0.0
USER5:[TSB.HPV]3.IMG
0,  80.010,     7.917,   316.013, 50.779,    48.940, 0.0
USER5:[TSB.HPV]4.IMG
0,  78.250,     4.452,    82.712, 49.294,    50.295, 0.0
USER5:[TSB.HPV]5.IMG
0,  80.899,   -12.501,   147.055, 51.218,    47.827, 0.0
```

```
EXAMPLE 3: 5 images from 3 different IMAGE data files.
------------------------------------------------------
USER5:[TSB.HPV]10810_SUB.PCK
1,  83.962,   -21.034,   151.002, 49.254,    50.740, 0.0
3,  80.010,     7.917,   316.013, 50.779,    48.940, 0.0
     -999,
USER5:[TSB.HPV]4.IMG
0,  78.250,     4.452,    82.712, 49.294,    50.295, 0.0
USER5:[TSB.HPV]10810_SUB.PCK
5,  80.899,   -12.501,   147.055, 51.218,    47.827
7,  89.387,    30.500,   183.001, 49.051,    49.086
```

After refinement, the program outputs the refined positions in the file EMICO.DAT in the proper format for another EMICOGRAD run. The particle phase origins should always be re-refined after running EMICOGRAD (with EMICOORG, EMICOORG2, or EMCORORG).

C. RUNNING EMICOGRAD IN BATCH MODE

The following is an example of a BATCH command file which runs EMICOGRAD and refines 5 human wart virus particles. In this particular example the data has already passed through several cycles of orientation (EMICOGRAD) and origin (EMICOORG or EMICOORG2 or EMCORORG) refinement. See [TSB.FOR]EMICOGRAD.BCH for a sample BATCH command file.

```
$ SET DEFAULT USER5:[TSB.HPV]
$ RUN USER5:[TSB.EXE]EMICOGRAD.EXE
5, 1, 2
1.0, 1.0, 0.0
128, 6., 35.
5, 5
.5,.5,.5, 3, 90.,     1.000
USER5:[TSB.HPV]10810_SUB.PCK
 1, 83.962,   -21.034,   151.002, 49.254,    50.740
 3, 80.010,     7.917,   316.013, 50.779,    48.940
 4, 78.250,     4.452,    82.712, 49.294,    50.295
 5, 80.899,   -12.501,   147.055, 51.218,    47.827
 7, 89.387,    30.500,   183.001, 49.051,    49.086
$EXIT
```

D. PROGRAM OUTPUT FROM PREVIOUS EXAMPLE BATCH JOB

```
Particle     Filename         Theta    Phi   Omega  Orig_X  Orig_Y
1         10810_SUB.PCK       83.96  -21.03  151.00  49.25   50.74
2         10810_SUB.PCK   80.01    7.92  316.01   50.78   48.94
3         10810_SUB.PCK   78.25    4.45   82.71   49.29   50.29
4         10810_SUB.PCK   80.90  -12.50  147.05   51.22   47.83
5         10810_SUB.PCK   89.39   30.50  183.00   49.05   49.09

       1     2     3     4     5
  1 56.8  58.2  49.9  55.0  54.2
    56.8  89.6  91.4  77.9  87.8
  2 58.2  72.3  60.6  54.7  48.8
    89.6  72.3  80.1  83.3 110.0
  3 49.9  60.6  70.2  61.6  64.4
```

```
    91.4  80.1  70.2  83.9 100.5
  4 55.0  54.7  61.6  64.6  62.6
    77.9  83.3  83.9  64.6  98.3
  5 54.2  48.8  64.4  62.6  62.6
    87.8 110.0 100.5  98.3  62.6
----------------------------
Up  54.8  58.9  61.3  59.7  58.5
Dwn 80.7  87.1  85.2  81.6  91.9
Bst 54.8  58.9  61.3  59.7  58.5

Image orientations after refinement
-----------------------------------
Particle     Filename         Theta    Phi   Omega  Orig_X  Orig_Y
1         10810_SUB.PCK       83.95  -20.33  151.77  49.25   50.74
2         10810_SUB.PCK       80.04    7.90  315.98  50.78   48.94
3         10810_SUB.PCK       77.50    3.70   82.71  49.29   50.29
4         10810_SUB.PCK       80.90  -12.02  147.80  51.22   47.83
5         10810_SUB.PCK       89.39   30.45  183.00  49.05   49.09

Orientation changes relative to original images changes in hand
are indicated by ***
-----------------------------------------------
Particle    Filename          Theta    Phi   Omega  Orig_X  Orig_Y
1        10810_SUB.PCK        -0.01    0.70   0.77    0.00    0.00
2        10810_SUB.PCK         0.03   -0.02  -0.04    0.00    0.00
3        10810_SUB.PCK        -0.75   -0.75   0.00    0.00    0.00
4        10810_SUB.PCK         0.00    0.48   0.75    0.00    0.00
5        10810_SUB.PCK         0.00   -0.05   0.00    0.00    0.00

       1     2     3     4     5
  1 56.3  56.2  47.9  56.7  48.7
    56.3  91.0  89.8  77.1  84.3
  2 56.2  71.7  61.6  53.0  48.8
    91.0  71.7  85.8  84.1 110.0
  3 47.9  61.6  68.8  56.0  61.7
    89.8  85.8  68.8  77.9 103.9
  4 56.7  53.0  56.0  63.1  56.8
    77.1  84.1  77.9  63.1 102.7
  5 48.7  48.8  61.7  56.8  62.8
    84.3 110.0 103.9 102.7  62.8
    ----------------------------
Up  53.2  58.3  59.2  57.1  55.8
Dwn 79.7  88.5  85.3  81.0  92.7
Bst 53.2  58.3  59.2  57.1  55.8
```

The following shows the improvement in the residuals for the 5 particles refined.

```
Bef 54.8  58.9  61.3  59.7  58.5
Aft 53.2  58.3  59.2  57.1  55.8
```

```
Hand residuals
Mean of 16 residuals = 1776.88
#     Residuals
-----------------------
                 12345
 1    1417.74    |||||
 2    1656.11    |||~|
 3    1678.14    ||~||
 4    1683.07    |~~~~
 5    1720.36    |~|||
 6    1775.80    |~|~~
 7    1787.59    ||||~
 8    1828.97    ||~~|
 9    1834.39    |~|~|
10    1840.06    |~~|~
11    1842.47    |||~~
12    1845.25    |~||~
13    1846.58    ||~~~
14    1879.24    ||~|~
15    1883.92    |~~||
16    1910.41    |~~~|

Where | indicates the given orientation and ~ indicates the
opposite orientation

Error as a function of resolution vs image
------------------------------------------
Image 1 vs image
 Resln      1     2     3     4     5  Avg
 6- 10   33.8  35.7  34.0  25.6  30.6  32.7
           54    61    52    32    42     241
11- 15   53.8  60.3  60.0  52.0  55.8  56.6
           25    13    22    10     9      79
16- 20   64.4  50.3  38.0  41.5  59.3  51.9
           38    45    27    27    33     170
21- 25   77.7 103.5  68.7  79.4  40.2  69.7
           38    26    31    33    50     178
26- 30   73.3  79.1  56.8  78.3  72.9  71.9
           38    19    29    33    39     158
31- 35   68.2  77.9  61.4  96.5  65.7  75.8
```

25	25	13	25	19	107

```
Image 2 vs image
 Resln     1     2     3     4     5  Avg
 6- 10   35.7  54.0  33.0  48.1  34.3  39.4
           61    36    44    35    64     240
11- 15   60.3  66.9  83.1  68.4  49.9  64.0
           13    20    12    18    23      86
16- 20   50.3  79.5  62.3  39.4  42.2  54.2
           45    42    43    36    58     224
21- 25  103.5  86.0  68.5  57.1  56.9  71.3
           26    32    32    41    44     175
26- 30   79.1  75.7  88.1  50.0  85.6  76.2
           19    48    40    34    40     181
31- 35   77.9  99.8  78.7  95.5 100.1  88.9
           25    22    21    23     9     100

Image   3 vs image
 Resln     1     2     3     4     5  Avg
 6- 10   34.0  33.0  31.4  30.9  35.1  33.0
           52    44    44    47    54     241
11- 15   60.0  83.1  68.4  59.7  67.8  66.4
           22    12    19    19    24      96
16- 20   38.0  62.3  82.6  50.5  66.2  61.1
           27    43    39    49    50     208
21- 25   68.7  68.5  85.4  74.8  79.7  76.3
           31    32    42    40    48     193
26- 30   56.8  88.1  90.5  90.1  95.0  84.0
           29    40    29    36    20     154
31- 35   61.4  78.7  89.1  70.2  83.7  77.6
           13    21    17    18    20      89

Image   4 vs image
 Resln     1     2     3     4     5  Avg
 6- 10   25.6  48.1  30.9  34.8  48.9  38.2
           32    35    47    36    50     200
11- 15   52.0  68.4  59.7  75.1  57.6  64.6
           10    18    19    28    20      95
16- 20   41.5  39.4  50.5  59.5  35.6  46.8
           27    36    49    32    16     160
21- 25   79.4  57.1  74.8  80.9  77.0  73.9
           33    41    40    42    58     214
26- 30   78.3  50.0  90.1  92.2  55.6  74.1
           33    34    36    31    25     159
31- 35   96.5  95.5  70.2  64.9  55.0  78.0
           25    23    18    38    10     114
```

```
Image   5 vs image
 Resln     1     2     3     4     5  Avg
 6- 10   30.6  34.3  35.1  48.9  64.0  40.6
           42    64    54    50    30     240
11- 15   55.8  49.9  67.8  57.6  89.4  60.2
            9    23    24    20     5      81
16- 20   59.3  42.2  66.2  35.6  68.4  55.8
           33    58    50    16    38     195
21- 25   40.2  56.9  79.7  77.0  47.8  60.6
           50    44    48    58    53     253
26- 30   72.9  85.6  95.0  55.6  78.9  77.4
           39    40    20    25    21     145
31- 35   65.7 100.1  83.7  55.0  78.1  76.0
           19     9    20    10    15      73

Resolution - Average self and cross error for all images
6- 10     41.6    35.7
           200     962
11- 15    67.3    61.3
            97     340
16- 20    71.5    50.2
           189     768
21- 25    73.5    68.9
           207     806
26- 30    81.2    75.4
           167     630
31- 35    77.4    80.0
           117     366

Image orientations in proper format for another run
USER5:[TSB.HPV]10810_SUB.PCK
1,  83.953, -20.331, 151.767,  49.254,  50.740,  10.0
3,  80.036,   7.895, 315.977,  50.779,  48.940,  10.0
4,  77.500,   3.702,  82.712,  49.294,  50.295,  10.0
5,  80.899, -12.021, 147.805,  51.218,  47.827,  10.0
7,  89.385,  30.450, 183.004,  49.051,  49.086,  10.0
```

E. FLOW CHART FOR EMICOGRAD PROGRAM

```
*******************
*      MAIN       *
* (EMICOGRAD.FOR) *
*******************
      *
      *-- GRAD_INPUT -- GRAD_READ -- <below>
      *
      *-- GRAD_ORIENTATIONS
      *
      *-- CALC_FINAL_RESIDUAL_ALL_IMAGES -- CALC_DIFF_I_ALL --
<below>
      *
      *-- PRINT_ERROR_MATRIX
      *
      *-- CHECK_FOR_ACTIVE
      *                     |-- SET_BEST_HAND -- <below>
      *                     |-- CALC_GRAD_I_ALL -- <below>
      *-- GRADIENT_REFINE --|-- RETURN_TO_UNIT_TRIANGLE -- <below>
      *                     |-- CALC_DIFF_I_ALL -- <below>
      *
      *-- GRAD_ORIENTATION_CHANGES
      *
      *-- CALC_FINAL_RESIDUAL_ALL_IMAGES
      *
      *-- PRINT_ERROR_MATRIX
      *
      *-- CALCULATE_HAND_SET
      *
      *-- PRINT_HAND_SET
      *
      *-- CALC_ERROR_VS_RESLN
      *
      *-- PRINT_ERROR_VS_RESLN
      *
      *-- CALC_PRINT_AVG_ERROR_ALL_IMAGES
      *
      *-- GRAD_OUTPUT
      *                    |-- PIRADDEG        |-- STRING_UPPER
      *                    |-- IMG_OPEN -------|--
IMG_PACK_BIMGCOM_CLEAR
      *                    |                   |-- FILE_CHECK
      *                    |
      *                    |-- IMG_PACK_FIND -- IMG_PACK_NRECS
      *                    |-- IMG_PACK_FILL -- IMG_PACK_NRECS
      *-- GRAD_READ -------|-- IMG_FILL
      *                    |-- IMG_FFT_FILL --- FFT_CLEAR
      *                    |-- FFT_2D --------- FOURT -- L6TOL9
      *                    |-- FFT_TO_ATBT ---- PIRADDEG
      *                    |-- ZMINC ---------- LINFIT

      *                     |-- CALC_COM_LINES -- <below>
      *-- CALC_DIFF_I_ALL --|-- FILL_COM_LINES -- ICO_INTERP_SHIFT
      *                     |-- CALC_DIFF_WGT -- ICO_INTERP_SHIFT

      *                     |-- RETURN_TO_UNIT_TRIANGLE -- <below>
      *                     |-- CALC_COM_LINES -- <below>
      *-- CALC_GRAD_I_ALL --|-- FILL_COM_LINES -- ICO_INTERP_SHIFT
      *                     |-- CALC_DIFF_WGT -- ICO_INTERP_SHIFT
      *                     |-- NORM_GRAD

      *                    |-- ICO_ROTMAT
      *-- CALC_COM_LINES --|-- ICO_COMLIN
      *                    |-- ICO_TRMEET -- PIRADDEG
      *                    |-- ICO_TRMEET_EX -- PIRADDEG

      *                             |-- POLAR_TO_COSINES
      *-- RETURN_TO_UNIT_TRIANGLE --|-- VECMUL
      *                             |-- GOOD - GEOMETRY_INIT -
GENROT - DMATMUL
      *                             |-- EQUIVALENT_VIEW -- <below>

      *                     |-- PRINT_MAT ---------|--
ROTMAT_TO_AXIS
      *                     |-- POLAR_TO_COSINES   |--
COSINES_TO_POLAR
      *                     |-- VECMUL
      *-- EQUIVALENT_VIEW --|-- COSINES_TO_POLAR   |-- NORM
      *                     |-- ANGLE_TO_PMAT -----|-- DOT
      *                     |-- POLAR_TO_ROT_MAT   |-- CROSS
      *                     |-- TRANSPOSE3         |--
COSINES_TO_POLAR
      *                     |-- MATMUL             |--
POLAR_TO_ROTMAT
      *                                            |-- VECMUL
      *-- SET_BEST_HAND --|-- CALCULATE_HAND_SET --|--
ADD_TRUE_TO_HAND
      *                   |-- PRINT_HAND_SET       |--
HAND_RESIDUAL
```