EMICO_SYS.DOC
(last update Jan 31, 1997)

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B. DEFINITION OF COMMON VARIABLES AND PARAMETERS USED IN EMICO PROGRAMS
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A. INTRODUCTION

The set of programs for computing the three-dimensional (3D) reconstruction of particles with icosahedral symmetry are based on the original core of programs developed at the MRC laboratory in Cambridge, England (circa 1970). Several modifications and additional routines were developed by Steve Fuller of the EMBL (Heidelberg) and Tim Baker of Purdue (West Lafayette, Indiana) which provide more quantitative and adaptable analysis of images of particles with icosahedral symmetry. This document just introduces the programs available. Specific instructions concerning the implementation of each of the programs appear in the documentation file for each program (e.g. BABE3:[TSB.DOC]EMICOLGFB.DOC).

------------------------------------------------------------------

The following is a list of available documentation for the programs required to compute 3D reconstructions of the icosahedral particles.

Abbreviations: C=complete, I=incomplete, N=not written

<table>
<thead>
<tr>
<th>DOCUMENTATION FILES:</th>
<th>STATUS</th>
<th>LAST UPDATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMICO_SYS.DOC</td>
<td>C</td>
<td>Jan 31, 1997</td>
</tr>
<tr>
<td>EMICO.DOC</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>EMICO3DR.DOC</td>
<td>C</td>
<td>Feb 10, 1997</td>
</tr>
<tr>
<td>EMICOFV.DOC</td>
<td>C</td>
<td>Oct 2, 1991</td>
</tr>
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<td>EMICOGRAD.DOC</td>
<td>C</td>
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</tr>
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<td>EMICOGRAD2.DOC</td>
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</tr>
<tr>
<td>EMICOPFTDSP.DOC</td>
<td>C</td>
<td>May 27, 1992</td>
</tr>
<tr>
<td>EMICOROT.DOC</td>
<td>C</td>
<td>Dec 10, 1990</td>
</tr>
<tr>
<td>EMICOSYM.DOC</td>
<td>C</td>
<td>Dec 10, 1990</td>
</tr>
<tr>
<td>EMCORORG.DOC</td>
<td>I</td>
<td>Sep 3, 1990</td>
</tr>
<tr>
<td>EMFFT.DOC</td>
<td>I</td>
<td>Mar 5, 1992</td>
</tr>
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The following is a list of documentation for programs no longer in use.

Abbreviations: C=complete, I=incomplete, N=not written

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<tr>
<th>OLD DOCUMENTATION FILES:</th>
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<tbody>
<tr>
<td>EMICOBG.DOC</td>
<td>C</td>
<td>Jan 5, 1991</td>
</tr>
<tr>
<td>EMICOCOR.DOC</td>
<td>I</td>
<td>Sep 16, 1987</td>
</tr>
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<td>EMICOFB.DOC</td>
<td>C</td>
<td>Sep 15, 1994</td>
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<tr>
<td>EMICOLG.DOC</td>
<td>C</td>
<td>Sep 15, 1994</td>
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<td>C</td>
<td>Jan 31, 1997</td>
</tr>
<tr>
<td>EMICOMAT.DOC</td>
<td>C</td>
<td>Dec 27, 1990</td>
</tr>
<tr>
<td>EMICOMATBG.DOC</td>
<td>C</td>
<td>Jan 31, 1997</td>
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<td>EMICOORG.DOC</td>
<td>C</td>
<td>Dec 17, 1990</td>
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<td>C</td>
<td>Dec 17, 1990</td>
</tr>
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<td>EMICOPFT.DOC</td>
<td>C</td>
<td>May 27, 1992</td>
</tr>
<tr>
<td>EMICOPFTCC.DOC</td>
<td>C</td>
<td>Feb 19, 1993</td>
</tr>
<tr>
<td>EMICOPRJ.DOC</td>
<td>C</td>
<td>Dec 11, 1990</td>
</tr>
<tr>
<td>EMPFT.DOC</td>
<td>C</td>
<td>Aug 24, 1992</td>
</tr>
<tr>
<td>EMPFTCC.DOC</td>
<td>C</td>
<td>Aug 24, 1993</td>
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<tr>
<td>SURFACE.DOC</td>
<td>C</td>
<td>Apr 4, 1990</td>
</tr>
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The following is a list of programs, subroutines and other files required to build a VAX/VMS version of the icosahedral particle 3D reconstruction system.

FORTRAN PROGRAM DRIVERS

-----------------------
| EMICO.FOR
| EMICO3DR.FOR
| EMICOCOR.FOR
| EMICOFV.FOR
| EMICograd.FOR
| EMICOORG.FOR
| EMICOORG2.FOR
| EMICOPFT.FOR
| EMICOPFTCC.FOR
| EMICOPFTDSP.FOR
EMICOPRJ.FOR
EMICOROT.FOR
EMICOSYM.FOR

EMCORORG.FOR
EMFFT.FOR
EMIMG.FOR
EMIMGFFT.FOR
EMMAP.FOR
EMPFT.FOR
EMPFTCC.FOR
EMPFTREF.FOR
SURFACE.FOR

SUBROUTINE OBJECT LIBRARIES
---------------------------
JUSTEM$DKA0:[TSB.FOR]TSBLIB.OLB
BABE3:[TSB.LEX]LEXI.OLB
BABE3:[TSB.NEWFV]SDFLIB.OLB

SUBROUTINE LIBRARY FILES (all on disk BABE3:)
------------------------
EMICOLIB.SUBS
EMFFT.SUBS
EMIMG.SUBS
EMMAP.SUBS
FFTLIB1.SUBS
FFTLIB2.SUBS
IMGLIB.SUBS
IMG_PACK.SUBS
MAPLIB1.SUBS
MAPLIB2.SUBS
MISCLIB.SUBS
PFTLIB.SUBS

INCLUDE FILES (contain many COMMON block declarations):
------------------------------------------------------
EM.CMM
EMICO.CMM
EMICORAD.INC
EMICOSYM.INC
LEXI.CMM

Brief descriptions of the icosahedral and related programs (in alphabetical order):

<table>
<thead>
<tr>
<th>PROGRAM</th>
<th>DESCRIPTION</th>
</tr>
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</table>


EMCORORG  Determine particle origin(s) by cross-correlation methods.
EMFFT     Multi-purpose FFT data manipulations.
EMICO      Multi-purpose icosahedral data processing.
EMICO3DR   Compute 3D-reconstruction of icosahedral particle
EMICOCOR   Cross-correlate particle images for scaling (rarely used).
EMICODIF   Difference MAP between raw and reprojected data.
EMICOFV    Determine particle view orientation (theta,phi,omega).
EMICOGRAD  Multiple particle, cross-common lines orientation refinement.
EMICOORG   Refine particle origin.
EMICOORG2  BATCH mode common-lines origin refinement.
EMICOPFT   Compute polar Fourier transforms of icosahedral projections.
EMICOPFTCC Cross-correlate raw image data with model PRJs and PFTs.
EMICOPFTDSP Display icosahedral PRJs or PFTs.
EMICOPRJ   Project 3D icosahedral map in evenly spaced views for one half of the icosahedral asymmetric unit (also EMMAP "X").
EMICOROT   Rotate 3D 2-fold MAP to equatorial (theta=90) orientation.
EMIMGBOX   Window out individual particles from the scanned micrograph.
EMIMG      Multi-purpose IMAGE data manipulations.
EMIMGFFT   Compute 2D Fourier transform of particle IMAGE.
EMMAP      Multi-purpose 2D/3D MAP data manipulations.
EMMAPDSP   Display 2D/3D MAP with contours/grey-levels (also EMMAP "D").
EMMAPPRJ   Project 2D/3D MAP from any view direction (also EMMAP "X").
EMMAP3DT   3D FFT of 3D MAP: produce 3D SFs.
EMPFT      Compute projections and polar Fourier transforms of 3D data.
EMPFTCC    Cross-correlate raw image data with model PRJs and PFTs.
EMPFTREF   Combines work of EMPFT and EMPFTCC for refinements.
EMSF       General purpose SF manipulation program
EMSF3DBT   Inverse 3D FFT of 3D SF data; produce 3D MAP file
SIMPLEX    Multiple particle, cross-common lines orientation refinement.
SURFACE    Compute 3D MAP depth-cue representation (also EMMAP "B").
<table>
<thead>
<tr>
<th>OLD PROGRAM</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMICOBG</td>
<td>Combine icosahedral data and solve for G's.</td>
</tr>
<tr>
<td>EMICOFB</td>
<td>Fourier-Bessel synthesis of 3D MAP (&quot;standard&quot; 2-fold view).</td>
</tr>
<tr>
<td>EMICOLG</td>
<td>Compute g's from G's.</td>
</tr>
<tr>
<td>EMICOMAT</td>
<td>Sets up normal matrices for each particle.</td>
</tr>
</tbody>
</table>
A former (Circa 1989-1992), "typical" protocol for processing icosahedral particles involved running programs in the following order:

1. EMIMG DISPLAY raw digitized IMAGE data
2. EMIMGBOX BOX out individual particles
3. [EMIMG] Normalize data/remove gradients/etc.
4. [EMFFT] FOURIER TRANSFORM IMAGE data
   (estimate RES_MIN, RES_MAX)
5. EMCORORG Initial particle ORIGIN estimate
6. EMICOFV Initial particle ORIENTATION estimate
7. EMICOORG Single particle ORIGIN refinement
8. EMICOGRAD<-- Interparticle ORIENTATION refinement
   [SIMPLEX] Interparticle ORIENTATION refinement
   > [EMICOORG2] Multiple particle ORIGIN refinement
9. EMICO3DR Set up normal MATRICES for particles, compute
   Gn's the gn's, and finally a 3D MAP with the
   FOURIER BESSEL procedure
10. [EMICOSYM] Enforce full 532 symmetry on 3D MAP.
11. EMMAP ("X") Reproject 3D MAP in refined view orientations
12. EMCORORG Refine particle ORIGINs by CC with projections
    go back to 8 Add/delete particles, increase resolution,
    etc.
    OR
13. EMPFT Model-based PFT refinement
    go back to 10 Add/delete particles, increase resolution,
    etc.

NEED TO ADD NEW PROTOCOL (EMPFT, EMPFTCC, EMPFTREF)
Additional programs for analysis of icosahedral particle data:

EMICODIF, EMICOPFT, EMICOPFTDSP, EMICOPFTCC, EMICOPRJ, EMICOROT
EMMAP, EMMAPDSP, EMMAP3DT
EMPFT, EMPFTCC, EMPFTREF
EMSF, EMSF3DBT
SURFACE

Older routines:

EMICOMAT, EMICOBG, EMICOLG, EMICOFB, EMICOMATBG, EMICOLGFB

B. DEFINITION OF COMMON VARIABLES AND PARAMETERS USED IN EMICO PROGRAMS

<table>
<thead>
<tr>
<th>NAME</th>
<th>DEFINITION</th>
</tr>
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<tbody>
<tr>
<td>FFT_ORIGX,</td>
<td>pixel coordinates of the particle center (the point</td>
</tr>
<tr>
<td>FFT_ORIGY</td>
<td>0.0,0.0 corresponding to the lower left corner of</td>
</tr>
<tr>
<td>FFT_STEPSIZE</td>
<td>width of each transform annulus, given by:</td>
</tr>
<tr>
<td></td>
<td>(ICO_IDIM*RSCALE)</td>
</tr>
<tr>
<td></td>
<td>------------------ TPU</td>
</tr>
<tr>
<td></td>
<td>(ICO_DIAM*ICO_NSAMP)</td>
</tr>
<tr>
<td>FMIN</td>
<td>the fractional minimum amplitude (relative to the mean)</td>
</tr>
<tr>
<td></td>
<td>of data used in the refinement. This sets a threshold</td>
</tr>
<tr>
<td></td>
<td>so that data points with smaller amplitudes are</td>
</tr>
<tr>
<td></td>
<td>ignored. See EMICOFV.DOC for more details.</td>
</tr>
<tr>
<td>ICO_DIAM</td>
<td>the diameter of the original boxed particle (in pixels).</td>
</tr>
<tr>
<td>ICO_IDIM</td>
<td>transform dimension (same for X or Y directions: must</td>
</tr>
<tr>
<td></td>
<td>be 128, 256, 512, or 1024).</td>
</tr>
<tr>
<td>ICO_NSAMP</td>
<td>the number of annuli per interval of 1/ICO_DIAM (= 1</td>
</tr>
<tr>
<td></td>
<td>for single or 2 for double sampling of the FFT data.</td>
</tr>
<tr>
<td></td>
<td>(see EMICOMAT.DOC).</td>
</tr>
<tr>
<td>INCR</td>
<td>width of each band in number of transform annuli.</td>
</tr>
<tr>
<td></td>
<td>NBAND*INCR must be &lt; ICO_IDIM/2.</td>
</tr>
<tr>
<td>MINR,MAXR</td>
<td>the inner and outer radii (in INTEGER TPU steps) of</td>
</tr>
<tr>
<td></td>
<td>the band of data thought to be correlated icosahedrally.</td>
</tr>
<tr>
<td></td>
<td>These parameters should be carefully chosen as outlined</td>
</tr>
<tr>
<td></td>
<td>in EMICOFV.DOC.</td>
</tr>
</tbody>
</table>
NANNULI  number of annuli into which the transform is divided (EMICOMAT.DOC,EMICOBG.DOC,EMICOLG.DOC).

NBAND  number of bands into which the Fourier transform is subdivided for scaling purposes in EMICOMAT and EMICOBG.

NSAMPL  the number of radial sample steps to be taken within each annular band of the transform.

RADIUS  number of radial steps in real space, i.e. the outer radius of the reconstruction in pixels (EMICOLG.DOC).

RADMIN  the distance in reciprocal space beyond which data points are considered to be independent (usually equal to the reciprocal of twice the particle diameter (1/2*diameter) expressed in REAL*4 TPU. See EMICOFV.DOC for more details.

RES_MIN, RES_MAX  define the lower and upper radial limits of data thought to be correlated icosahedrally. See EMICOFV.DOC for a complete description of how to estimate these limits (MINR,MAXR).

RScale  radial scale factor (normally = 1.0 for images of frozen-hydrated particles boxed from a single micrograph.

STEP_SIZE  size of each radial step in pixels used in EMICOLG (a ratio relative to the pixel size in the original scanned image).

THETA, PHI, OMEGA  three Euler angles that define the particle view orientation (Klug/Finch convention given in J. Mol. Biol. (1968) 31:1-12).

TPU  transform pixel unit.

C. DEFINITION OF "STANDARD" TWO-FOLD PARTICLE ORIENTATION

3D reconstructions of icosahedral particle are computed in the "standard" 2-fold orientation. In this view the 3D MAP contains the entire icosahedral particle viewed along a 2-fold axis such that three mutually perpendicular two-fold particle axes are aligned with an XYZ Cartesian MAP coordinate system (NCOL columns in the X direction; NROW rows in the Y direction; NSEC sections in the Z direction: see JUSTEM$DKA0:[TSB.FOR]EMPROGS.DOC for further details about the storage of MAP data).
The original MRC program produced a 3D MAP oriented with a two-fold axis parallel to the Z direction and a five-fold axis parallel to the Y direction.

The standard orientation is useful since:

1. Any equatorial view (\(\text{THETA}=90\)) can easily be computed from the 2-fold MAP.

2. The 2-fold MAP conforms to the Klug & Finch convention as described in J. Mol. Biol. 31:1-12 (1968) for the particle orientation (\(\text{THETA}/\text{PHI}/\text{OMEGA}\)). \(\text{THETA}\) is measured in degrees positive from the Z-axis towards the X-axis; \(\text{PHI}\) is measured in the XY plane in degrees positive from X towards Y; \(\text{OMEGA}\) is measured in degrees positive, counterclockwise about the viewing direction.

3. EMMAP and EMICOPRJ can be used to obtain 2-D projected views, and EMICOROT to compute a 3-D MAP with the Z-axis of the MAP coincident with an equatorial view.

D. REFERENCE LIST

The following lists provide a guide to the literature that deals with icosahedral virus structure and three-dimensional reconstruction methods.

GENERAL (METHODS/REVIEWS/ETC.)


RESULTS - 3D OF NEGATIVELY-STAINED ICOSAHEDRAL VIRUSES


RESULTS – 3D OF UNSTAINED, FROZEN-HYDRATED ICOSAHEDRAL VIRUSES

NOTE: This list is horribly incomplete!


