EMICO_SYS.DOC

(last update Jan 31, 1997)

CONTENTS

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

DOCUMENTATION FILES:	STATUS	LAST UPDATE
EMICO_SYS.DOC	C	Jan 31, 1997
EMICO.DOC	N	
EMICO3DR.DOC	C	Feb 10, 1997
EMICOFV.DOC	C	Oct 2, 1991
EMICOGRAD.DOC	C	Sep 15, 1994
EMICOGRAD2.DOC	N	
EMICOPFTDSP.DOC	C	May 27, 1992
EMICOROT.DOC	C	Dec 10, 1990
EMICOSYM.DOC	C	Dec 10, 1990
EMCORORG.DOC	I	Sep 3, 1990
EMFFT.DOC	I	Mar 5, 1992

EMIMG.DOC	I	Feb 25, 1992
EMIMGFFT.DOC	C	Nov 27, 1989
EMMAP.DOC	I	Apr 4, 1990
EMPFTREF.DOC	C	Aug 24, 1993
EMPROGS.DOC	C	Mar 12, 1992
EMSYSTEM.DOC	C	Sep 8, 1989
LEXI.DOC	I	Sep 2, 1987

The following is a list of documentation for programs no longer in use.

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

OLD DOCUMENTATION FILES:	STATUS	LAST UPDATE
EMICOBG.DOC	C	Jan 5, 1991
EMICOCOR.DOC	I	Sep 16, 1987
EMICOFB.DOC	C	Sep 15, 1994
EMICOLG.DOC	C	Sep 15, 1994
EMICOLGFB.DOC	C	Jan 31, 1997
EMICOMAT.DOC	C	Dec 27, 1990
EMICOMATBG.DOC	C	Jan 31, 1997
EMICOORG.DOC	C	Dec 17, 1990
EMICOORG2.DOC	C	Dec 17, 1990
EMICOPFT.DOC	C	May 27, 1992
EMICOPFTCC.DOC	C	Feb 19, 1993
EMICOPRJ.DOC	C	Dec 11, 1990
EMPFT.DOC	C	Aug 24, 1992
EMPFTCC.DOC	C	Aug 24, 1993
SURFACE.DOC	C	Apr 4, 1990

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
EMICOGRAD.INC
EMICOSYM.INC
LEXI.CMM

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

PROGRAM   DESCRIPTION
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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").

OLD PROGRAM	DESCRIPTION
EMICOBG	Combine icosahedral data and solve for G's.
EMICOFB	Fourier-Bessel synthesis of 3D MAP ("standard" 2-fold view).
EMICOLG	Compute g's from G's.
EMICOMAT	Sets up normal matrices for each particle.

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

```
NAME      DEFINITON
------------------------------------------------------------------
FFT_ORIGX,     pixel coordinates of the particle center (the point
FFT_ORIGY      0.0,0.0 corresponding to the lower left corner of
               the boxed particle image).

FFT_STEPSIZE   width of each transform annulus, given by:
                 (ICO_IDIM*RSCALE)
               -------------------- TPU
               (ICO_DIAM*ICO_NSAMP)

FMIN      the fractional minimum amplitude (relative to the mean)
          of data used in the refinement.  This sets a threshold
          so that data points with smaller amplitudes are
          ignored.  See EMICOFV.DOC for more details.

ICO_DIAM  the diameter of the original boxed particle (in pixels).

ICO_IDIM  transform dimension (same for X or Y directions: must
          be 128, 256, 512, or 1024).

ICO_NSAMP the number of annuli per interval of 1/ICO_DIAM (= 1
          for single or 2 for double sampling of the FFT data.
          (see EMICOMAT.DOC).

INCR      width of each band in number of transform annuli.
          NBAND*INCR must be < ICO_IDIM/2.

MINR,MAXR the inner and outer radii (in INTEGER TPU steps) of
          the band of data thought to be correlated icosahedrally.
          These parameters should be carefully chosen as outlined
          in EMICOFV.DOC.
```

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 (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 (THETA/PHI/OMEGA). THETA is measured in degrees positive from the Z-axis towards the X-axis; PHI is measured in the XY plane in degrees positive from X towards Y; 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.)

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RESULTS - 3D OF NEGATIVELY-STAINED ICOSAHEDRAL VIRUSES

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Jack, A., Harrison, S. C. & Crowther, R. A. (1975) "Structure of Tomato Bushy Stunt Virus II. Comparison of Results Obtained by Electron Microscopy and X-ray Diffraction" J. Mol. Biol. 97:163-172.

Kam, Z. & Gafni, I. (1985) "Three-dimensional Reconstruction of the Shape of Human Wart Virus Using Spatial Correlations" Ultramicrosc. 17:251-262.

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NOTE: This list is horribly incomplete!

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