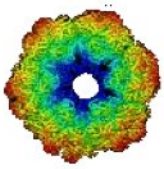


EM2EM

IMAGIC

Program to convert images from/to formats used in the "3DEM community"

Version 20-Aug-2019

1. Import Formats Supported

BROOKHAVEN STEM:

A format used to store STEM images. One data file actually contains two images: the large angle elastic scatter normalised signal, and the small angle elastic scatter signal (or bright field, if selected).

CCP4:

A format in use in X_ray crystallography to which the MRC format is largely but not completely compatible.

DATA_ONLY:

The input image file does not contain any header. The user has to specify image size and image format (corresponds to option OFFSET with offset = 0).

Same as RAW_IMAGE

DIGITAL MICROGRAPH:

GATAN's dmg, dm3 or dm4 image format.

EM:

Image data format used by the EM image processing system at the Max Planck Institute of Biochemistry (Martinsried, Germany) and by the TVIPS company (Tietz Image and Video Processing Systems, Gauting, Germany).

FABOSA:

Special image format used in the FABOSA project. Requires two files: a TIFF image and a text file with additional information (SDI).

FEI:

Image formats created by the FEI company: FEI_MRC (created by EPU), MRC_2000, MRC_2014, and FEI raw image format.

Import only.

FORMATTED:

Import ASCII formatted input files. Formatted ASCII files are rather inefficient because of storage needs and long conversion times required. YET, any computer and mailing system can handle ASCII so the format is universal. The user is requested to give the format of the formatted input image in Fortran style like: "20(I4)" etc.

IMAGIC:

Image processing in 2-D and 3-D. Developed by Marin van Heel and commercially distributed by: Image Science Software GmbH, Berlin, Germany. (www.ImageScience.de/formats)

JPEG:

JPEG is an acronym for the Joint Photographic Experts Group, which created this standard. For a long time this format has been the de facto file format for digital images/photography.

Should no be used in 3DEM image processing,

KONTRON:

Image format of the KONTRON ELEKTRONIK GmbH (Eching near Muenchen, Germany). This format is used in the IMCO/S system, for example.

MDPP:

Ross Smith's Micrograph Data Processing Program (N.Y.U. School of Medicine, NYU-MC).

MEDIPIX:

Raw MEDIPIX images (http://medipix.web.cern.ch/medipix/)

MRC:

This is one of the oldest image formats in use in electron microscopy. It is a relatively rich file format which allows for various data formats such as8-bits integers, (raw byte data), 16-bit integers, 32-bit REALs and complex formats. One of the philosophies behind this data format is that it is compatible to the CCP4 format in use in X-ray crystallography (not completely true).

OFFSET:

Do not read any input header values, i.e. skip a specified number of bytes and read image densities only. Of course, the user has to know the image size and format.

PIF:

Portable image format for EM data Purdue University (Tim Baker).

PGM:

Portable greymap format.

PROTOMO:

Protomo FFF format (vs. 1.8). Protomo is a software package used in electron tomography for marker-free alignment and 3D reconstruction of tilt series. The marker-free alignment is based on cross-correlation methods and projection matching. It also includes the refinement of geometric parameters of the tilt series. 3D reconstruction is carried out by weighted backprojection with general weighting functions that allow varying tilt angle increments. The software was originally developed for thin sections of insect flight muscle and paracrystalline protein arrays, but has since been successfully applied to various specimens in cryo-electron tomography. Author: Hanspeter Winkler.
www.electrontomography.org

RAW_IMAGE:

The input image file does not contain any header. The user has to specify image size and image format (corresponds to option OFFSET with offset = 0).

Same as DATA_ONLY

RAWIV:

The rawiv/volumetric data format is used to represent 3D volumetric data of scalar fields defined on a regular grid.

Same as VOLUMETRIC

SHF:

GATAN's simple header file format.

SITUS:

The 3D volumes format of Willy Wrigger's docking program Situs.

SPIDER:

The format(s) used by the SPIDER image processing system of J. Frank (Albany NY, USA).

SUPRIM:

Image format of the image processing system developed by J.P. Schroeter and P. Bretaudiere. The SUPRIM image format is also used in the PHOELIX image processing system written by B. Carragher, M. Whittaker and R. A. Milligan.

TIA (Emispec) / SER files:

TIA (Tecnai imaging and analysis) is the program used on FEI Tecnai and Titan microscopes for acquiring and displaying scanned images and spectra. It is based on ES Vision, originally created by the Emispec company, now taken over by FEI.

The format implemented in em2em is for .ser files (series file format) and relates to ES Vision 3.x, in later versions of TIA the format was updated to allow files greater than 2GB.

All information is taken from a web page of Dr Chris Boothroyd (Juelich Research Centre, Germany): www.er-c.org/cbb/info/TIAformat/

TIFF:

Tagged Image Format: this has become one of the standard formats in desk-top publishing oriented image processing. Desk-top flat-bed scanners, for example, can all generate this image format.

Note: A coloured TIFF image can be converted to either a grey scale image or three images (red, green, blue).

TVIPS:

Image formats used by the TVIPS (Tietz Video and Image Processing Systems) company, Germany. The two formats used are EM and TIFF with a specific tag.

VOLUMETRIC:

The rawiv/volumetric data format is used to represent 3D volumetric data of scalar fields defined on a regular grid.

Same as RAWIW.

2. Export Formats Supported

CCP4:

A format in use in X_ray crystallography to which the MRC format is largely but not completely compatible.

DATA_ONLY:

The input image file does not contain any header. The user has to specify image size and image format (corresponds to option OFFSET with offset = 0).

Same as RAW_IMAGE

EM:

Image data format used by the EM image processing system at the Max Planck Institute of Biochemistry (Martinsried, Germany) and by the TVIPS company (Tietz Image and Video Processing Systems, Gauting, Germany).

FABOSA:

Special image format used in the FABOSA project. Requires two files: a TIFF image and a text file with additional information (SDI).

FEI_RAW_IMAGE:

FEI raw image format.

FORMATTED:

Import ASCII formatted input files. Formatted ASCII files are rather inefficient because of storage needs and long conversion times required. YET, any computer and mailing system can handle ASCII so the format is universal. The user is requested to give the format of the formatted input image in Fortran style like: "20(I4)" etc.

IMAGIC:

Image processing in 2-D and 3-D. Developed by Marin van Heel and commercially distributed by: Image Science Software GmbH, Berlin, Germany. (www.ImageScience.de/formats)

JPEG (greyscale):

JPEG is an acronym for the Joint Photographic Experts Group, which created this standard. For a long time this format has been the de facto file format for digital images/photography.

NOTE:
JPEG creates an 8-bit output file. The imageis compressed and scaled to 256 densities values. Not recommended as input for serious image processing. It is more intended as output of results.,

KONTRON:

Image format of the KONTRON ELEKTRONIK GmbH (Eching near Muenchen, Germany). This format is used in the IMCO/S system, for example.

MDPP:

Ross Smith's Micrograph Data Processing Program (N.Y.U. School of Medicine, NYU-MC).

MRC:

This is one of the oldest image formats in use in electron microscopy. It is a relatively rich file format which allows for various data formats such as8-bits integers, (raw byte data), 16-bit integers, 32-bit REALs and complex formats. One of the philosophies behind this data format is that it is compatible to the CCP4 format in use in X-ray crystallography (not completely true).

PIF:

Portable image format for EM data Purdue University (Tim Baker).

PGM:

Portable greymap format.

POSTSCRIPT:

PostScript is a page description language designed by Adobe Systems Incorporated. It provides a device independent standard for electronic printing.

PROTOMO:

Protomo FFF format (vs. 1.8). Protomo is a software package used in electron tomography for marker-free alignment and 3D reconstruction of tilt series. The marker-free alignment is based on cross-correlation methods and projection

matching. It also includes the refinement of geometric parameters of the tilt series. 3D reconstruction is carried out by weighted backprojection with general weighting functions that allow varying tilt angle increments. The software was originally developed for thin sections of insect flight muscle and paracrystalline protein arrays, but has since been successfully applied to various specimens in cryo-electron tomography. Author: Hanspeter Winkler.
www.electrontomography.org

RAW_IMAGE:

The input image file does not contain any header. The user has to specify image size and image format (corresponds to option OFFSET with offset = 0).

Same as DATA_ONLY

RAWIV:

The rawiv/volumetric data format is used to represent 3D volumetric data of scalar fields defined on a regular grid.

Same as VOLUMETRIC

SHF:

GATAN's simple header file format.

SITUS:

The 3D volumes format of Willy Wrigger's docking program Situs.

SPIDER:

The format(s) used by the SPIDER image processing system of J. Frank (Albany NY, USA).

SUPRIM:

Image format of the image processing system developed by J.P. Schroeter and P. Bretaudiere. The SUPRIM image format is also used in the PHOELIX image processing system written by B. Carragher, M. Whittaker and R. A. Milligan.

TIFF:

Tagged Image Format: this has become one of the standard formats in desk-top publishing oriented image processing.

There are two suboptions:

> GREYSCALE_TIFF: Output is a grey scale TIFF image

> COLOUR_TIFF: Three input images are expected to be the red, the green and the blue part of coloured image will be converted to a coloured TIFF output image.

This is only an option for 2D images.

TVIPS:

Image formats used by the TVIPS (Tietz Video and Image Processing Systems) company, Germany. The two formats used are EM and TIFF with a specific tag.

VOLUMETRIC:

The rawiv/volumetric data format is used to represent 3D volumetric data of scalar fields defined on a regular grid.

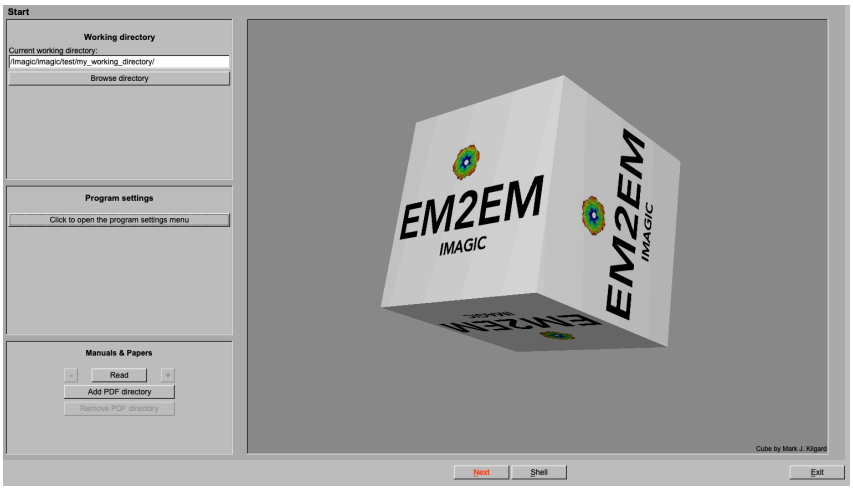
Same as RAWIW.

X-PLOR:

A system for X-ray crystallography and NMR.

Only 3D volumes can be exported.

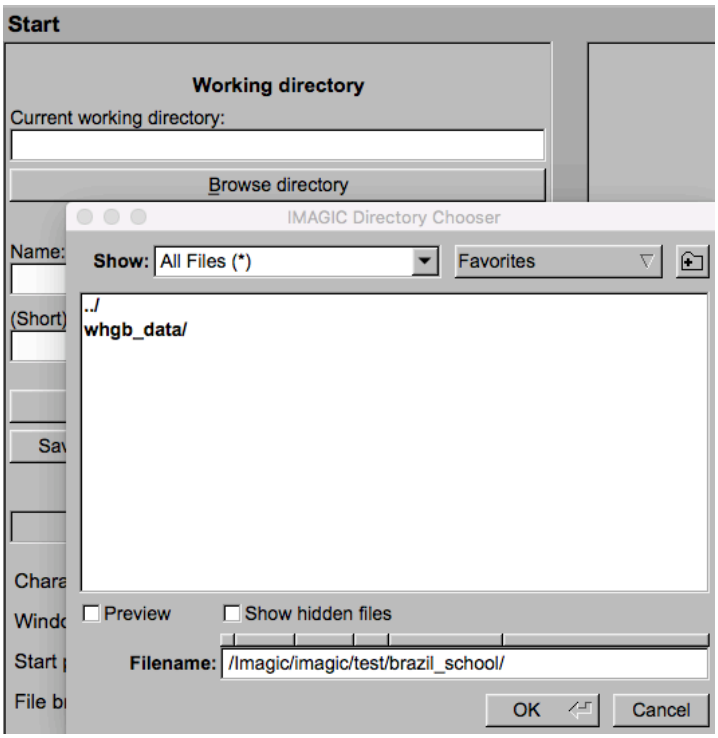
3. USE EM2EM



3.1. The EM2EM Start Page

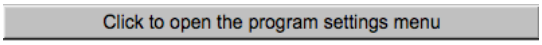
Before doing any calculations, you have to give some information on the "Start" page:

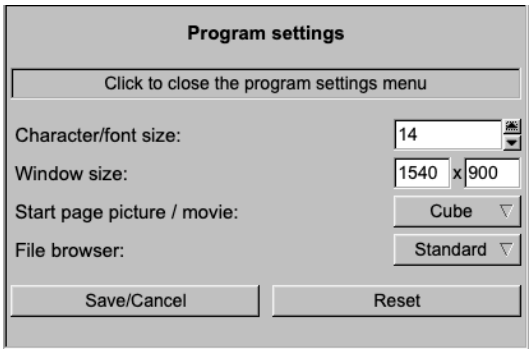
1. Specify your working directory. You can type the name into the text box or use the "Browse directory" button.



NOTE: You can store your directory in "Favorites".

2. If wanted you can also change some **EM2EM** program settings:





May be, your computer window/monitor is too small and you want to reduce the **EM2EM** window:

In this case you normally also have to adjust the font size:

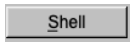
Save the settings with the 

button. Note that **EM2EM** will re-start.

3. There are a number of additional buttons on this start page, as well as on the subsequent import/export page:

Go to the next page (import/export images)

Go back to the starting page (after having converted images)



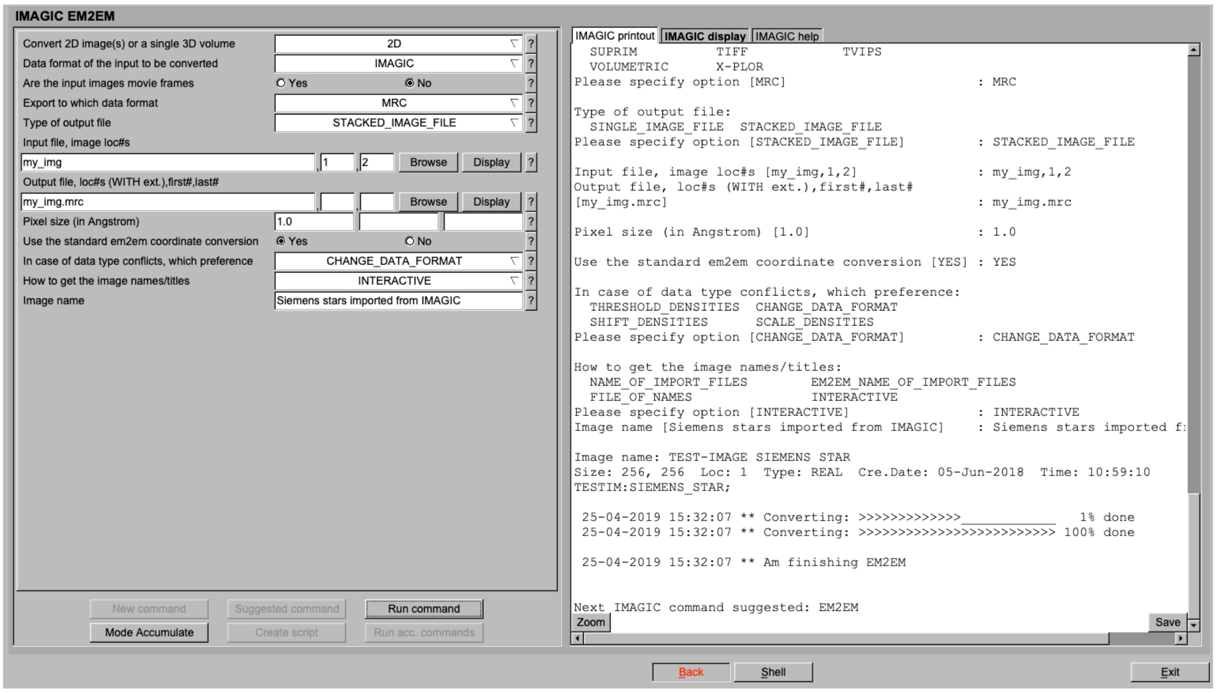
Run a shell command (depending on your operating system)

Exit the **EM2EM** program

4. Click the 

button to start converting images.

3.2. The EM2EM Image Conversion Page



1. On the left-hand side is a list of questions (the "user interaction block") asking for all parameters needed to run the conversion.

   You will also find the Run command button to start the conversion.

2. On the right-hand side you find the print-out / display page. The various tabs are used to show the print-outs, plotted curves or displayed images.

3. Move the cursor into the print-out window and click the Zoom button to enlarge the print-out window.

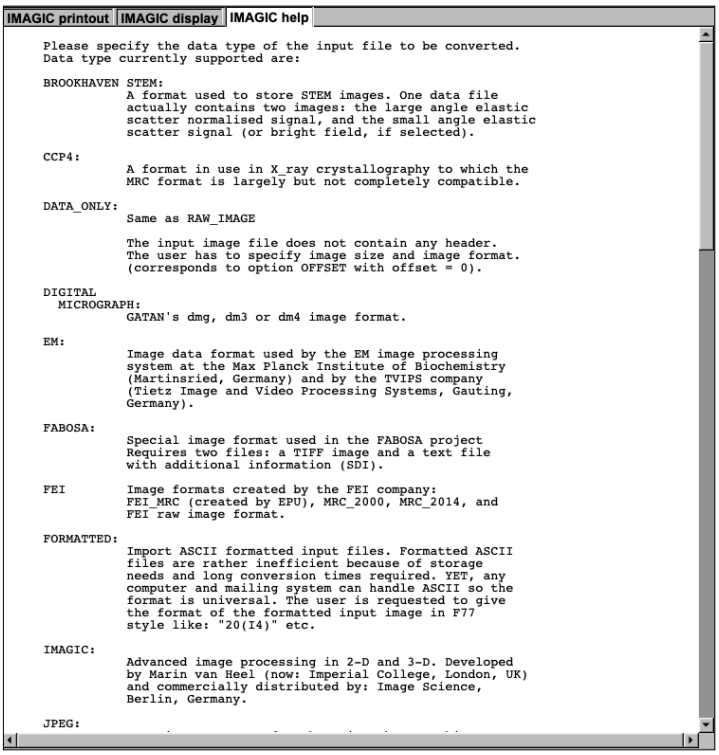
   The Save button will store the print-out in a text file.

3.2.1. The User Interaction Block (UIB)

1. **EM2EM** first asks for all file names and parameters needed before any calculation can be started.

2. **EM2EM** questions will often have default values which appear in the text/value boxes. Of course, values and file names are only suggested. You are free to choose whatever you wish

3. **EM2EM** questions always have an associated help, which can be accessed by clicking the related ? button



Move the cursor into the print-out window and click the Zoom button to enlarge the print-out window.

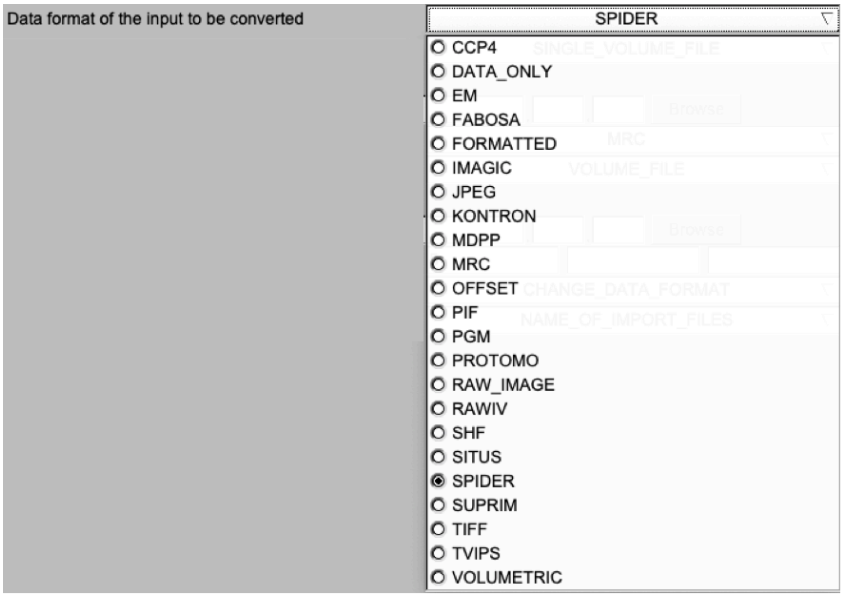
The Save button will store the print-out in a text file.

3.2.2. Typical UIB Questions

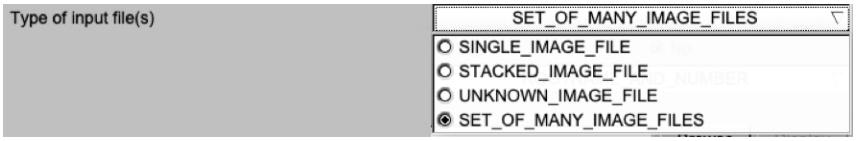
Typical **EM2EM** questions are:

- Do the input file(s) contain 2-D image(s) or 3-D volumes:

- The format of the input images:



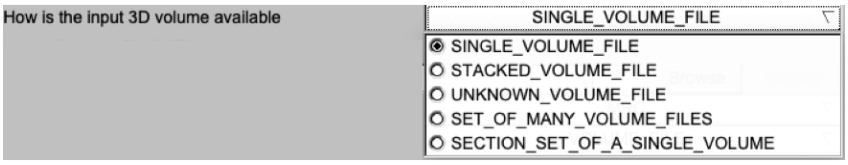
- The way the input images are stored:



The options depend of the input format given. Possible options are:

Single image file	Input is a single file containing a single 2-D image.
Stacked image file:	Input is a single file containing a stack of 2-D images.
Unknown image file:	Input is a single file either containing a single 2-D image or a stack of 2-D images.
Set of many image files:	Input is a set of 2-D image files.

If the input contains 3-D volume(s):



The options depend of the input format given. Possible options are:

Single volume file:	Input is a single file containing a single 3-D volume.
Stacked volume file:	Input is a single file containing a stack of 2-D volumes.
Unknown volume file:	Input is a single file either containing a single or a stack of 3-D volumes.
Set of many volume files:	Input is a set of 3-D volume files.
Section set of single 3-D:	Input is a set of 2-D image files each file containing a single section of a single 3-D volume.

- The name of the input images:

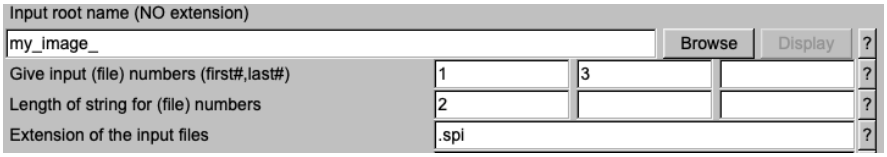
Usually you can type the file name into the text box or browse the file.

If the input is a set of files you can specify the input file names in two ways:

The options are:

Root name and number:	The input file names have a common root name followed by a number.
File of file names:	The input file names will be read from a text file, which we have to provide.

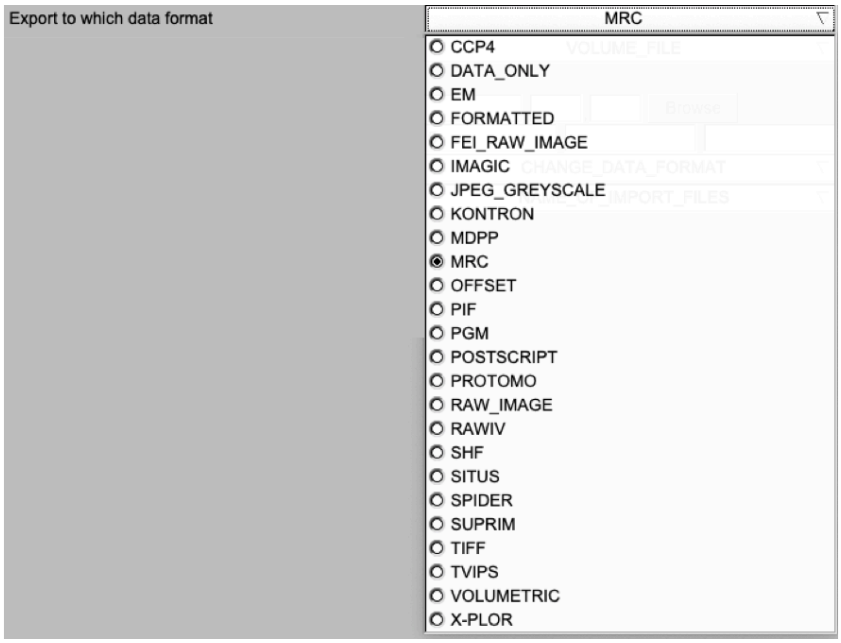
Choosing root-name and numbers you have to answer these questions:



Press the ? button to get additional help.

- Do the input files contain movies (2-D mode only):

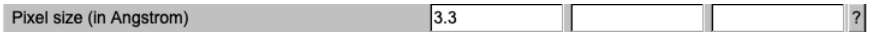
- The wanted format of the output images:



- The way the output images are to be stored (options depend on the input and output files and formats used):

- The name of the output images:

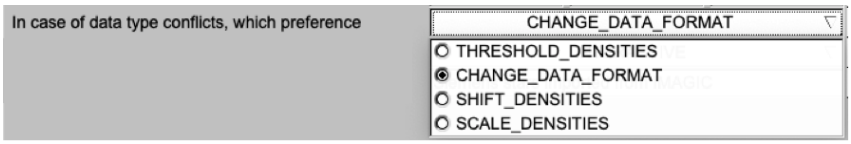
- Specify the pixel size to be stored in the header(s) of the output file(s):



- How to handle different coordinate systems:

Sometimes the import format and the export format have different coordinate systems. Usually **EM2EM** does a related conversion. By choosing NO, you will obtain mirrored or flipped 2D images/volumes or 3-D volumes with the wrong handedness. This option was the special request of specific users.

- What to do in case of data type conflicts:



If import and export format do not support the same data type (REAL, LONG, INTG, PACK) and/or do not have the same signed/unsigned data properties the image density values cannot be simply taken over.

Here you have to specify what to do if such a conflict occurs:

THRESHOLD_DENSITIES:

In case of an import/export data type conflict threshold the too large and/or the too small density values.

NOTE: The import/export data type will be the same as well as the file size. But due to possible thresholding some (too high and/or too low) density values can be changed.

EXAMPLE: MRC images created by FEI are stored in the non-sstandard unsigned INTG format. But since FEI never uses densities larger than the maximal value allowed by INTG one can use option THRESHOLD_DENSITIES to keep the data type INTG (and hence can also keep the file size).

CHANGE_DATA_FORMAT:

In case of an import/export data type conflict change the export data type (for example from PACK/byte to INTG/int).

NOTE: The density values remain the same but the file size will increase.

EXAMPLE: MRC PACK/byte images are stored as non-standard signed bytes. To keep all density values unchanged one can use option CHANGE_DATA_FORMAT to store the export image in INTG/int format.

SHIFT_DENSITIES:

In case of an import/export data type conflict shift all image densities accordingly.

NOTE: The density values change but the data type (and hence the file size) remain unchanged.

EXAMPLE: MRC PACK/byte images are stored as non-standard signed bytes. Using the option SHIFT_DENSITIES the non-standard image density values (-128 to 127) are shifted to 0 - 256 so that they can be stored as standard PACK/byte images.

NOTE: Sometimes the import/export data type conflict cannot be solved by shifting all image densities. In this case the image densities MUST be scaled to the export data type.

SCALE_DENSITIES:

In case of an import/export data type conflict scale the image densities so that the minimum/maximum values of the scaled image fit to the minimum/maximum values allowed by the export data type.

EXAMPLE: A REAL/float image should be exported to a JPEG image. JPEG only allows PACK format. Because the range of REAL values is much larger than the range of PACK values the REAL image densities will be scaled to PACK format (0 - 256).

NOTE: If there is no import/export data type conflict the image densities and the data type will remain the same.

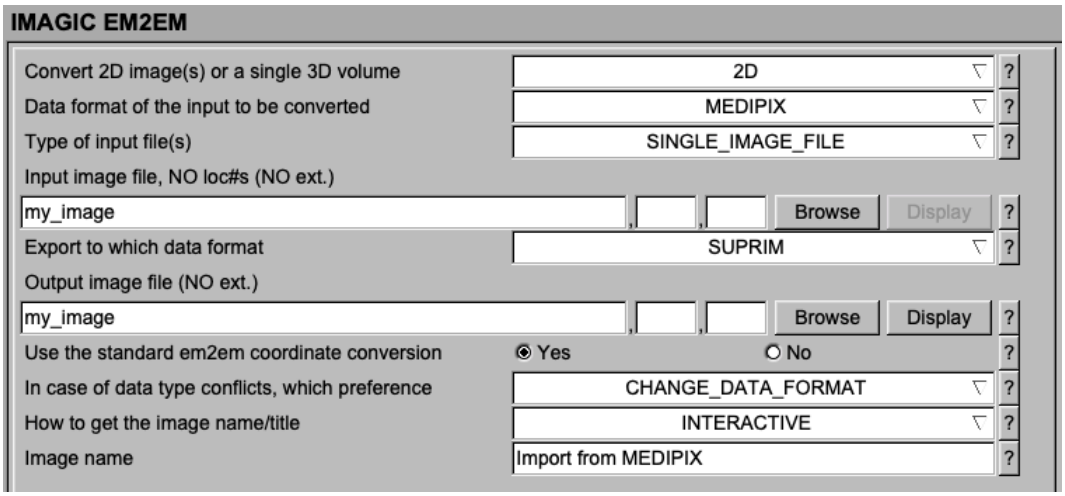
- Text in header file(s):

Some formats allow to store a text/comment in the headers of the images.

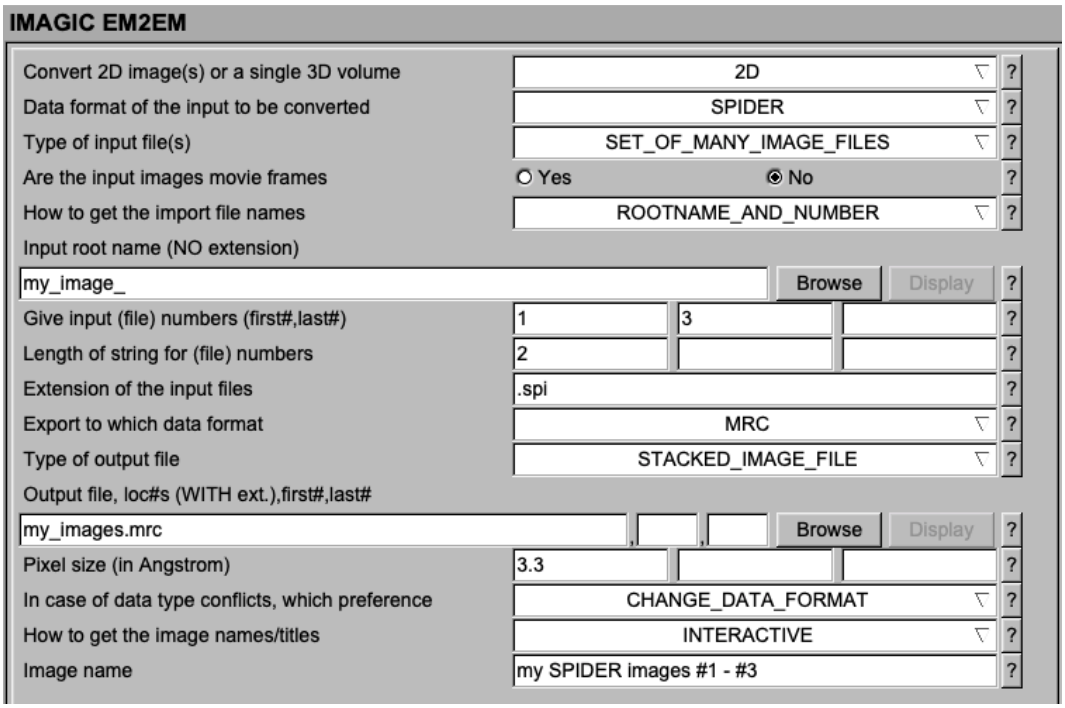
3.2.3. Examples

Some example on how to convert images using **EM2EM**:

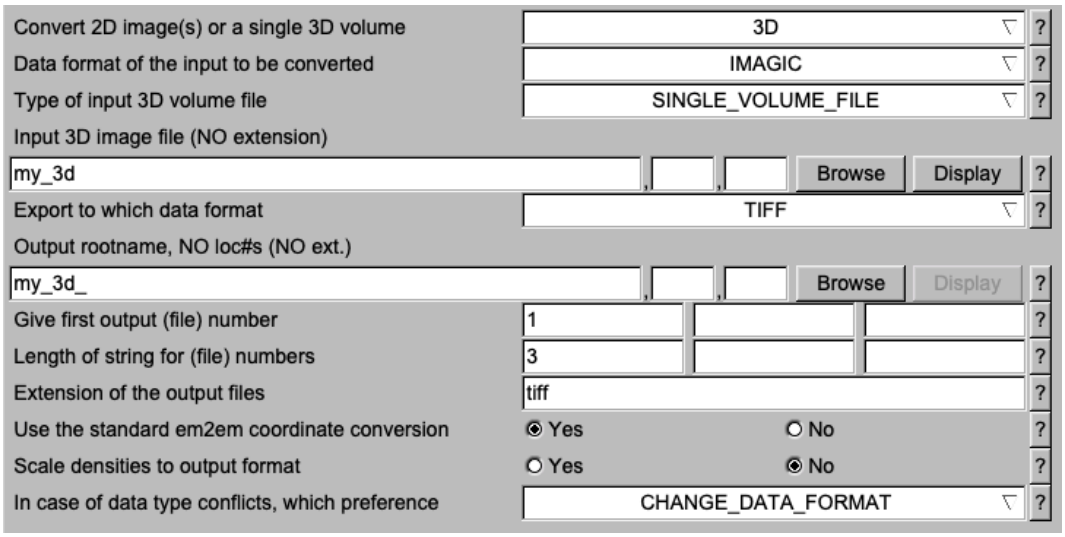
- Convert a single MEDIPIX image into a SUPRIM image:



- Convert SPIDER image files named my_image_01.spi, my_image_02.spi and my_image_03.spi into MRC format and store the image in a single MRC file:



- Convert a 200x200x200 3-D volume in IMAGIC to TIFF files:



Note that the output will be a series of 200 TIFF 2-D image files named my_3d_001.tiff, my_3d_002.tiff … my_3d_200.tiff.

ERROR HINTS

We tried to find and correct all errors. If you still find some mistakes please send your error hints to michael@ImageScience.de so that we can improve this tutorial. Thank you very much.

CONTENT