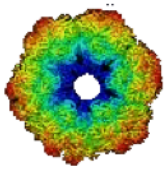

FSC

Fourier Shell Correlation

IMAGIC

Program to calculate the
Fourier Shell Correlation
of two 3-D volumes

Version 20-Aug-2019

PART 1: Introduction

PART 2: Use FSC -Fourier Shell Correlation

PART 3: Use EM2EM

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Content

1. INTRODUCTION

The three-dimensional Fourier Shell Correlation (FSC) was introduced by Harauz and van Heel in 1986. It measures the normalised cross correlation coefficient between two 3-D volumes over corresponding shells in Fourier space, i.e., as a function of the spatial frequency:

$$FSC_{1,2}(r) = \frac{\sum_{r_i \in r} F_1(r_i) \cdot F_2(r_i)^*}{\sqrt{\sum_{r_i \in r} |F_1(r_i)|^2 \cdot \sum_{r_i \in r} |F_2(r_i)|^2}}$$

r	*corresponding shell in Fourier space*
$F(r_i)$	*complex "structure factor" at r_i in Fourier space*
$\sum_{r_i \in r}$	*summation over all Fourier space voxels r_i in shell r*

The FSC is the straightforward three-dimensional generalisation of the earlier two-dimensional Fourier Ring Correlation (FRC) function (Saxton and Baumeister [1982], Van Heel et al. [1982]).

The (modified) **3-sigma criterion** indicates at which spatial frequency we are systematically gaining information significantly above the random noise level. Where we have to continue collecting information by adding more data of the same quality to the dataset we would certainly improve the dataset up to - and maybe even somewhat beyond - this point.

The **1/2-bit information** threshold criterium expresses where we have already collected a sufficient amount of data in the final 3D reconstruction to allow a direct structural interpretation at that resolution level. The 1/2-bit curve is calibrated to approximately yield resolution values comparable to resolution values in use in X-ray crystallography (FOM).

We suggest using the 1/2-bit threshold curve as a general-purpose indicator of interpretable resolution in FSC curves.

PLEASE NOTE:

Under-sampling remains one of the worst sins one can commit in this field. You should never claim any resolution level beyond **2/3rd of the Nyquist frequency**.

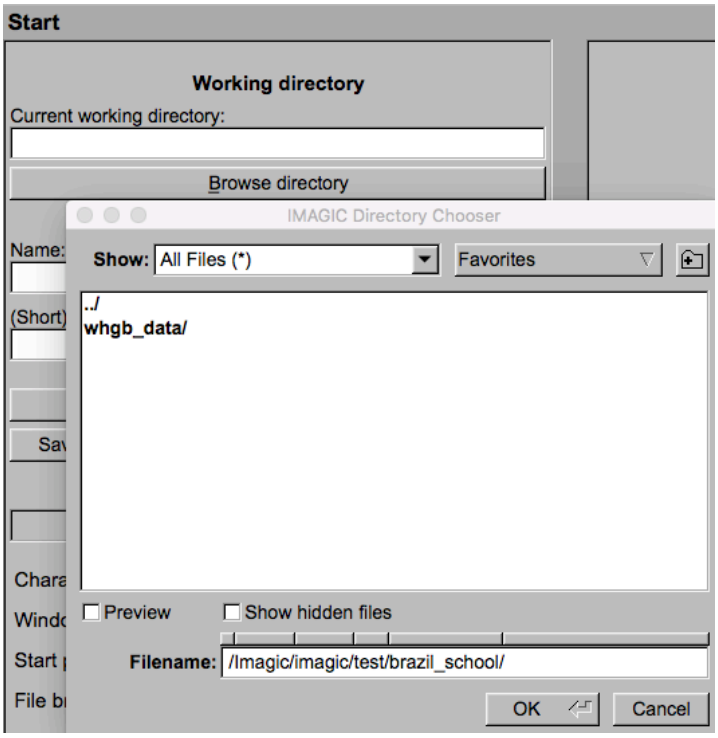
The FSC is a measure to compare the similarity of two 3D data-sets. If it is used to estimate the resolution of a 3D reconstruction you have to make sure that the two 3D subsets do not contain artificial similarities.

2. USE FSC - FOURIER SHELL CORRELATION

2.1. The FSC Start Page

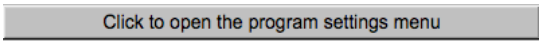
Before doing any calculations, you have to give some information on the "Start" page:

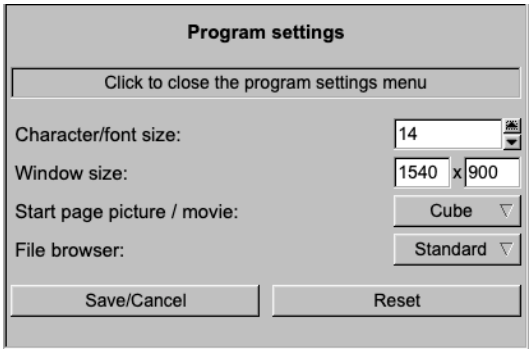
1. Specify your working directory. You can type the name into the text box or use the "Browse directory" button.



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

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





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

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

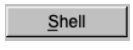
Save the settings with the 

button. Note that **FSC** 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)



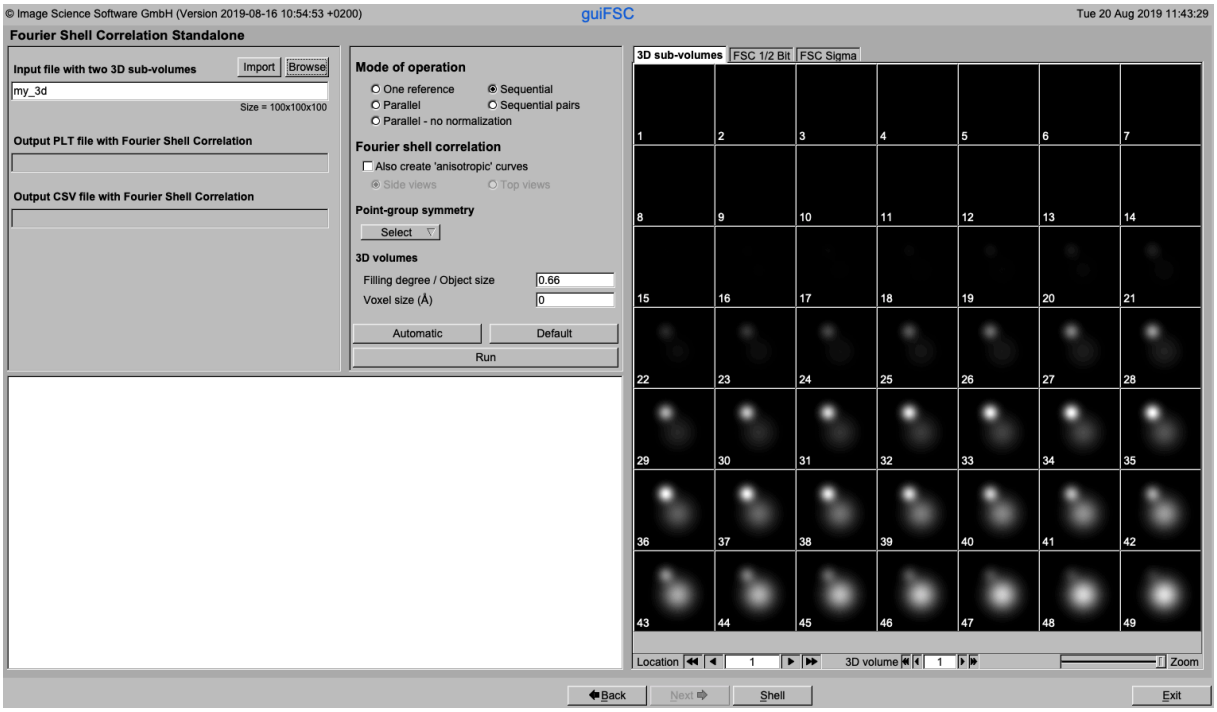
Run a shell command (depending on your operating system)

Exit the **FSC** program

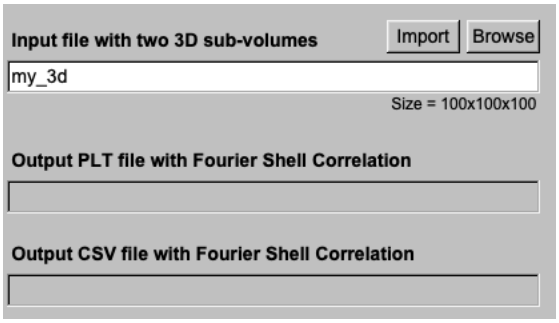
4. Click the 

button to start converting images.

2.2. The Fourier Shell Correlation Page



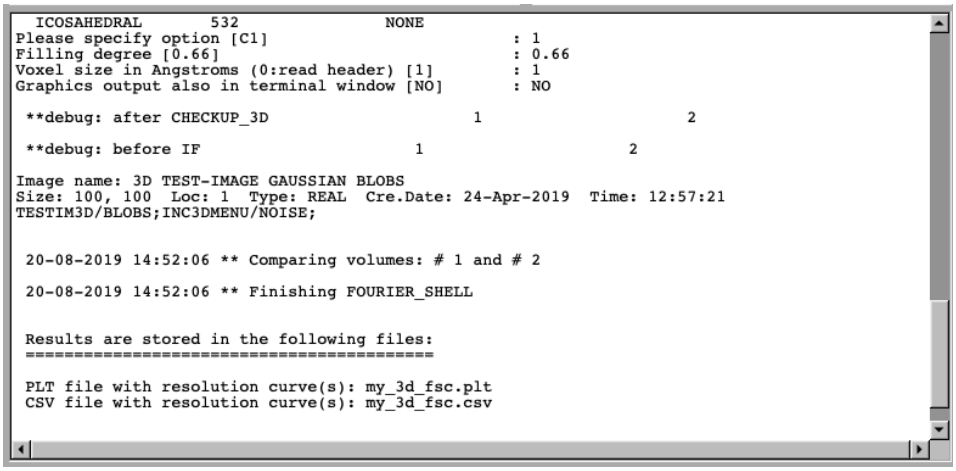
1. On the left-hand side you have to specify the input file name(s). The names of the resulting output files are also listed.



2. In the second block is a list of all parameters which can be adjusted before running the **FSC** calculations. Your last answers given are shown. When pressing the Automatic button, the answers suggested by the **FSC** program are listed whereas clicking into the Default button will re-load your last values used.

   You will also find the. . Run button to start the **FSC** calculations.

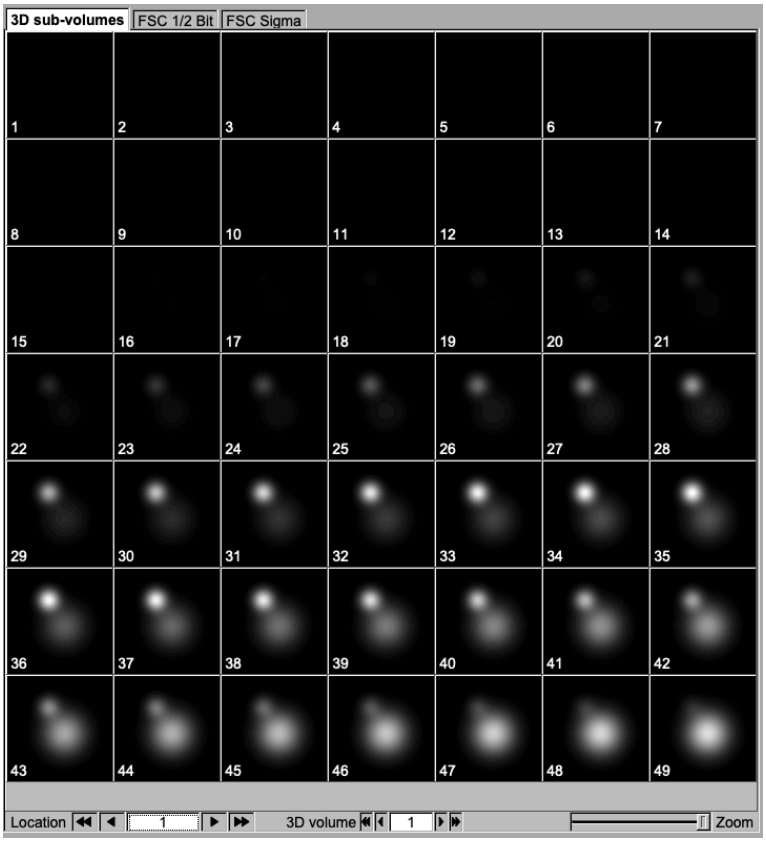
3. On the left bottom part of the page you can find the print-out of the program while calculating the Fourier shell correlation.



To enlarge the print-out window move the cursor into the print-out window and click the Zoom button.

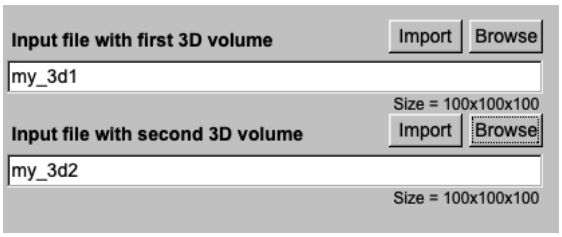
The Save button will store the print-out in a text file.

4. On the right-hand side you find the display windows (input 3-D volumes) and the results windows of the FSC calculations. The various tabs can be used to activate the wanted window.



2.2.1. Specify the input file(s)

1. **FSC** needs to know the input file(s) containing the 3-D volumes for which the Fourier shell correlation is to be calculated.



(for example)

2. Import: If the input format is not **IMAGIC** use the Import buttom to open the **EM2EM** page to convert your input file to an **IMAGIC** file. Refer to chapter 3 to learn on how to import volumes stored in non- **IMAGIC** files.

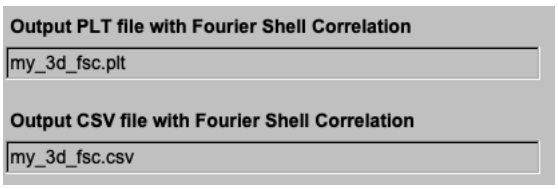
3. Write: If the input format is **IMAGIC** you can type the file name into the text box.

4. Browse: If the input format is **IMAGIC** you use the Browse button to browse for the file wanted.

2.2.2. Output files

1. The resulting Fourier shell correlation curves are stored in an **IMAGIC** PLT file and in a CSV file.

2. Note that the output file names are created automatically:

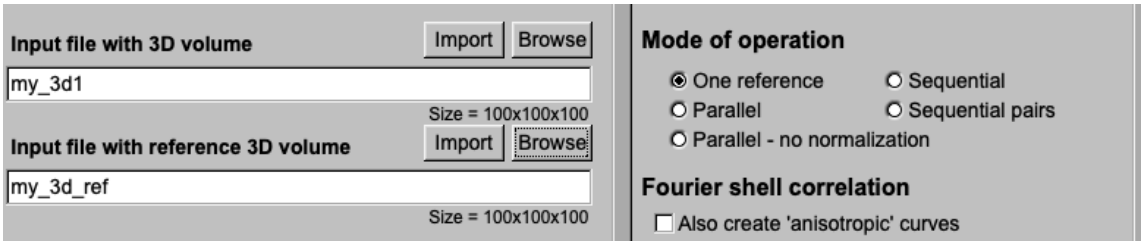


2.2.3. The input parameters

1. The parameters to be used to calculate the Fourier shell correlation are listed in the middle of the **FSC** page.

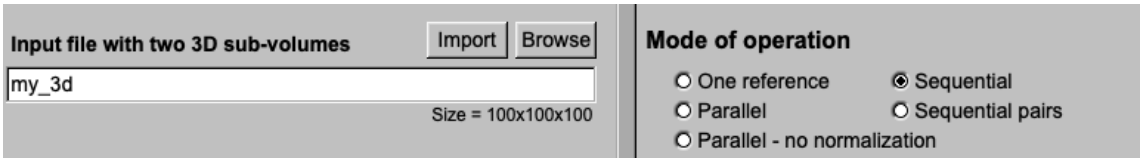
2. You can choose a number of options how the input 3D volumes are available and which comparison is wanted:

ONE REFERENCE:



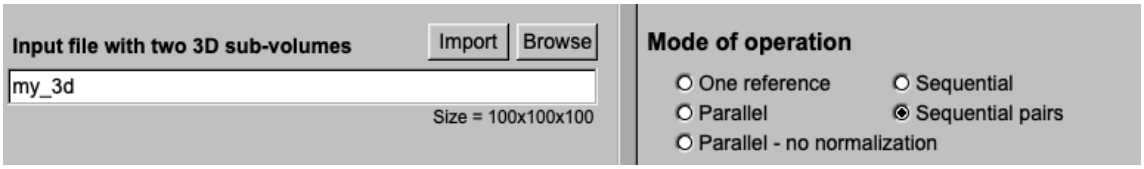
A (set of) 3-D volume(s) will be compared to one reference stored in a second input file.

SEQUENTIAL:



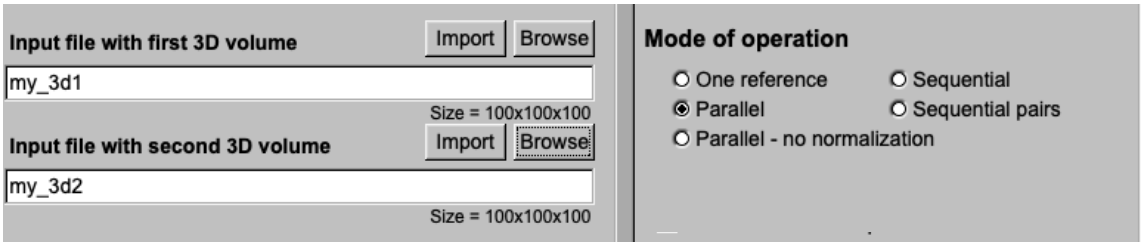
The input 3D volumes are stored in one file and will be compared in a sequential way: each odd location to its even neighbour, i.e. loc#1 to loc#2, loc#2 to loc#3, etc.

SEQUENTIAL PAIRS:



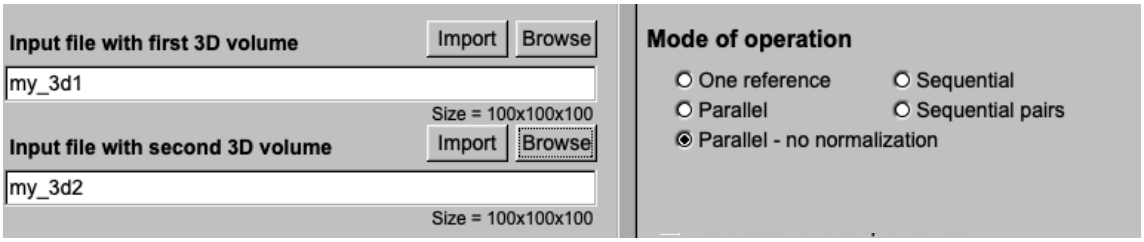
The input 3D volumes are stored in one file and will be compared sequentially. Each odd location is compared to its next neighbour, i.e. loc#1 to loc#2, loc#3 to loc#4, etc. This option typically used after the FSC option of 3-D reconstruction of multiple 3-D volumes.

PARALLEL:



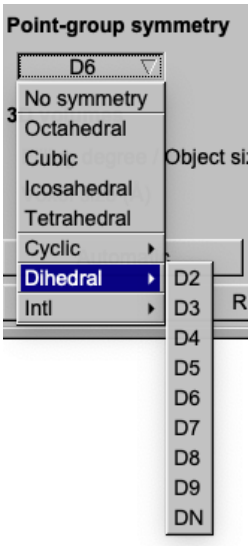
The input 3D volumes are stored in two files and will be compared location by location.

PARALLEL - NO NORMALISATION:



Special option for a measurement of the FSC theoretical behaviour (signal versus noise). This is a special version of the PARALLEL option. No normalisation by the rotationally-averaged amplitude spectra is applied.

3. The threshold to estimate the resolution strongly depends on the point-group symmetry of the input particles. So, you have to specify this symmetry. Select the point-group symmetry using the pull-down menu. You can either use the 'international' or the 'Schoenflies' notation.

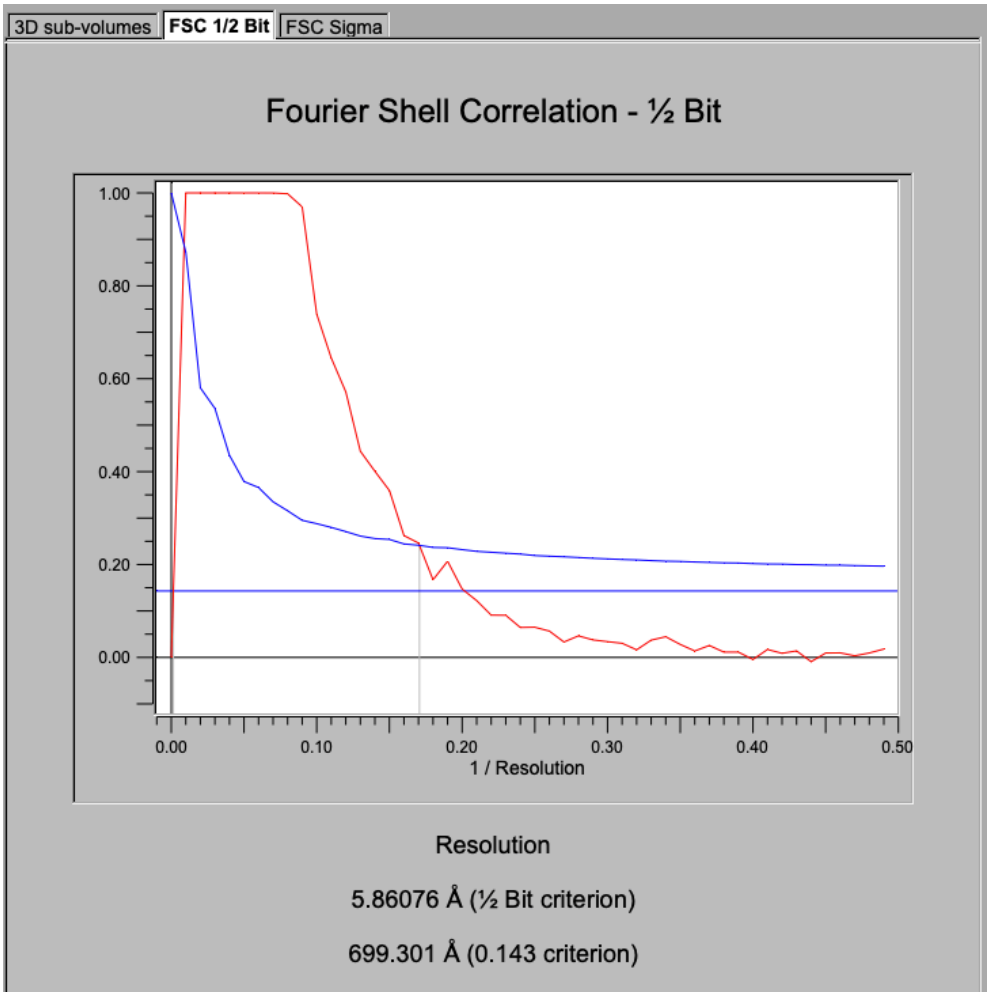


4. Also, the size of the 3D object within the 3-D reconstruction volume influences the theoretically expected FSC levels. The smaller the 3-D

structure within the reconstruction volume, the larger the corresponding 'convolution' sphere in 3D Fourier space in which the complex structure factors are highly correlated. Please give the filling degree D/L, i.e. the ratio of the object size D (height/width) in voxels and L, the linear size of the 3-D volume (X, Y or Z). A typical value would be 0.66, indicating that 2/3 of the linear width of the 3D volume is filled by the object.

5. The voxel size is needed to estimate the resolution. The voxel size is expected in Angstrom. If the voxel size is already specified in the headers of the input images the value is shown in the box. In this case do NOT change the value.

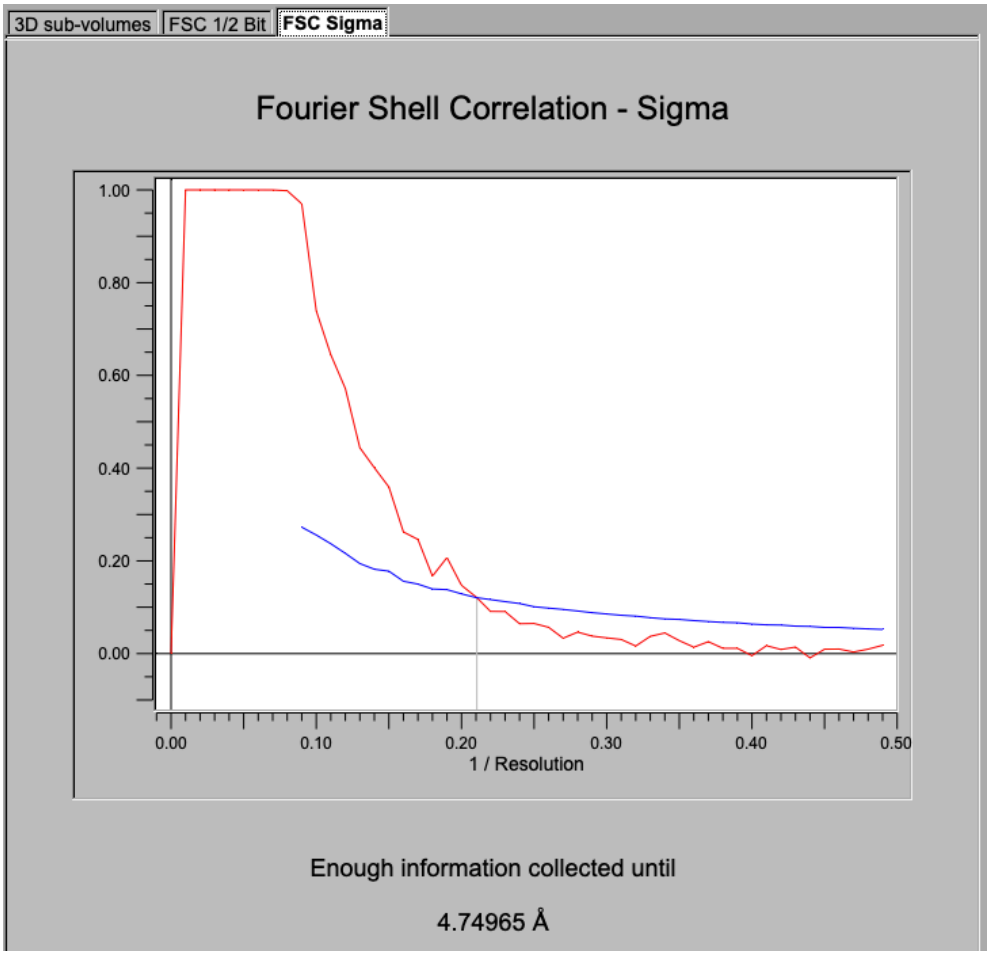
2.2.4. The Results Page - ½ Bit



1. The 1/2 Bit information curve indicates where you have already collected a sufficient amount of data in the final 3-D reconstruction to allow a direct structural interpretation at that resolution level. The overall resolution achieved in the 3-D volume is estimated by the intersection of the FSC curve (red) and the 1/2 Bit curve (blue).

2. The horizontal blue line correlates to the 0.143 criterion (not suggested to use).

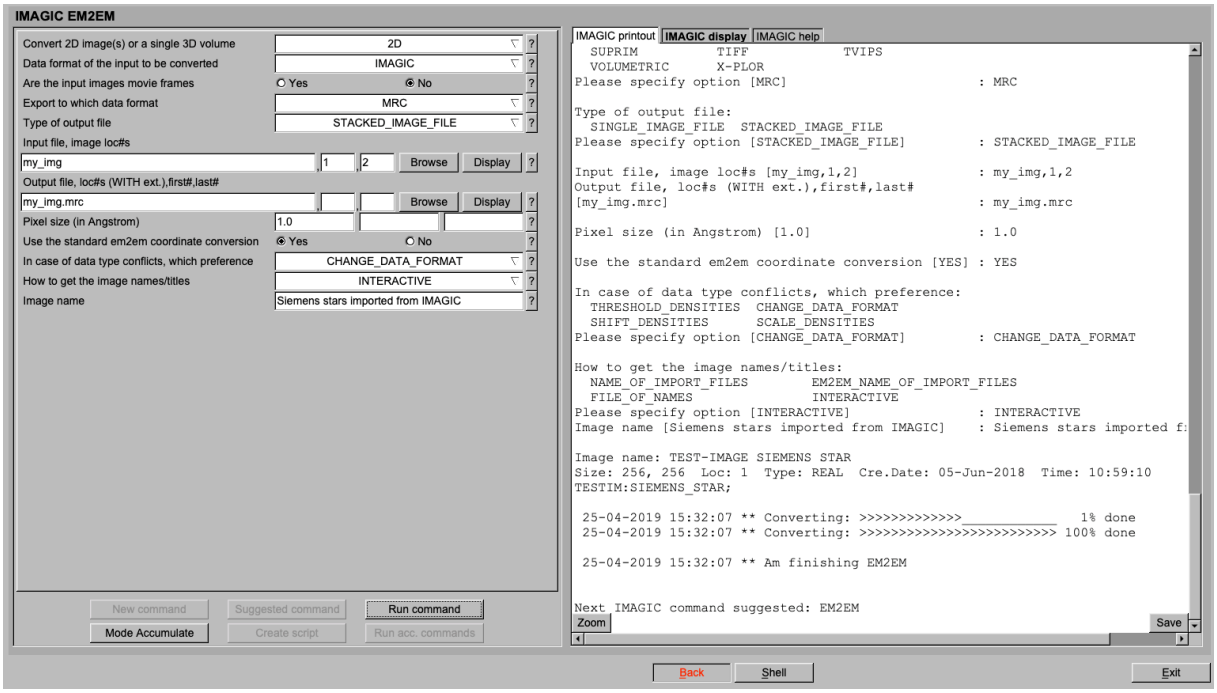
2.2.5. The Results Page - 3 Sigma



The Fourier shell correlation curve is shown in red, the 3 Sigma curve in blue. The 3 Sigma criterion indicates at which spatial frequency we are systematically gaining information significantly above the random noise level. Where you have to continue collecting information by adding more data of the same quality to the dataset we would certainly improve the dataset up to - and maybe even somewhat beyond - this point.

3. USE EM2EM

3.1. The EM2EM Image Conversion Page



5. 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.

6. 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.

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

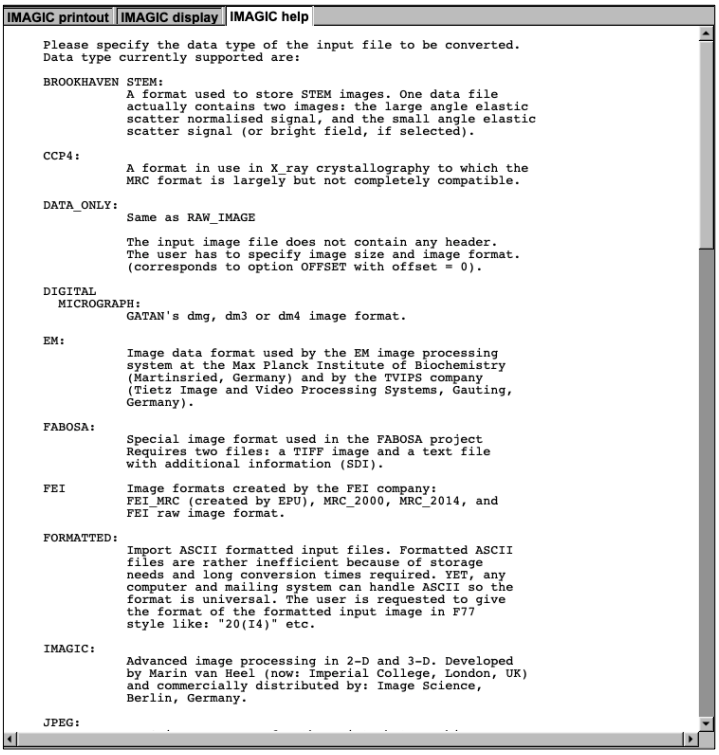
   The Save button will store the print-out in a text file.

2.2.6. The User Interaction Block (UIB)

1. **EM2EM** 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



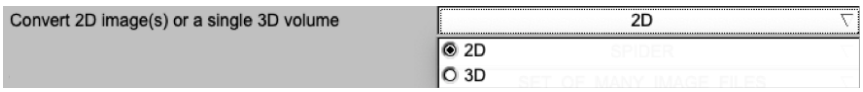
Move the cursor into the print-out window and click the Zoom button to enlarge the print-out window.

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

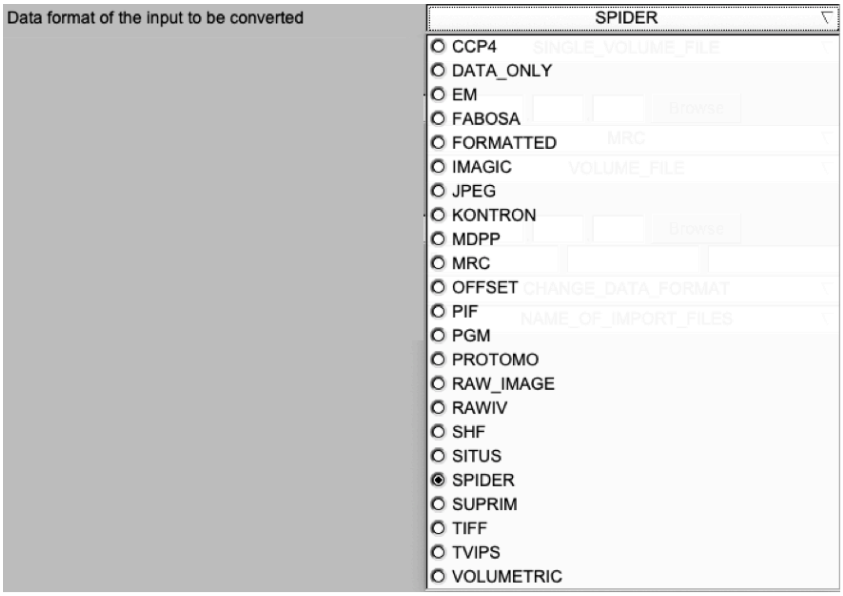
2.2.7. Typical UIB Questions

Typical **EM2EM** questions are:

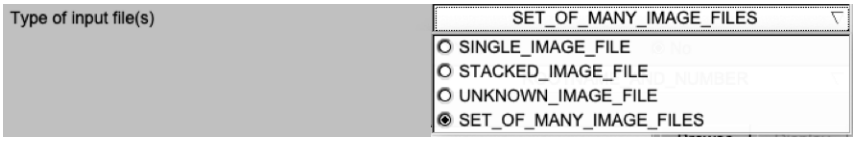
- Do the input file(s) contain 2-D image(s) or 3-D volumes:



- The format of the input images:



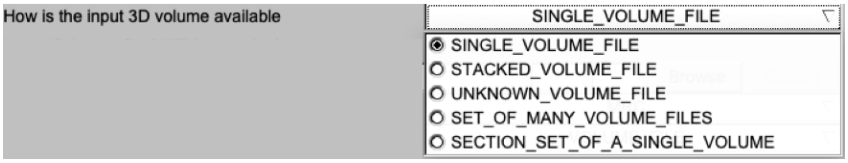
- The way the input images are stored:



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:

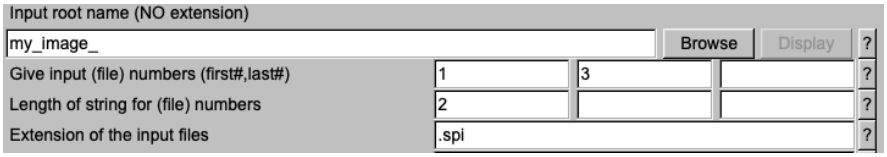
Usually you can type the file name into the text box or browse the file.

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.

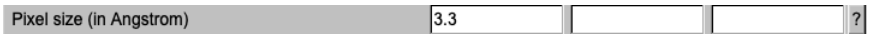
Choosing root-name and numbers you have to answer these questions:



Press the ? button to get additional help.

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

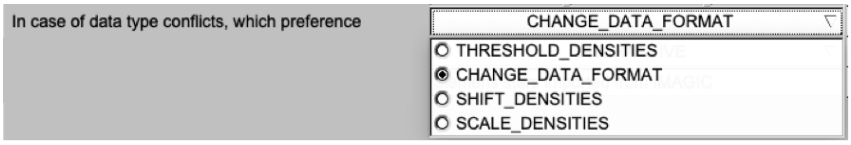
- Specify the pixel size to be stored in the header(s) of the output file(s):



- 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:

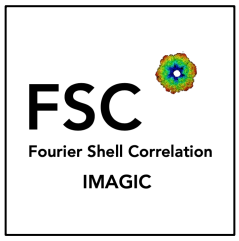
In case of an import/export data type conflict scale the image densities so that the minimum/maximum values of the scaled image fit to the minimum/maximum values allowed by the export data type.

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.



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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.

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