

EM-BFACTOR: A package for sharpening high resolution information in electron cryomicroscopy

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The program EMBFACTOR sharpens high resolution information in 3D maps obtained by electron cryomicroscopy according to the approach by Rosenthal and Henderson (JMB 333:721–745, 2003). This program allows automatic determination of the Bfactor for maps at a resolution higher than 10 Å. The program also allows application of the Bfactor found, or a Bfactor value provided by the user, to sharpen the map and compensate the decay of the amplitudes. Before sharpening, the map is weighted in Fourier space by C_{ref} , a function computed from the FSC that represent the correlation between the map and a perfect reference, in order to avoid noise amplification. Furthermore, the program also places the structure factor amplitudes of the map on an absolute scale. The program accepts 3D maps in any format common in EM (e.g. MRC, Spider, Xmipp, PIF, EM, etc) and also accepts FSC curves in the format of most common packages for single particles cryoEM (Frealign, Spider, EMAN, Xmipp, Bsoft). As output, the program produces the sharpened map and a Guinier plot that allows assessment of the sharpening.

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1 Installation

- Uncompress the file `embfactor.zip`.
- You will find these directories:
 - `bin`, where the executable binary `embfactor` is.
 - `doc`, where this documentation is.
- Set up your `PATH` environment variable to have direct access to the program.

Tested machines

So far, the package has been built and made available for the following platforms:

x86 (64 bits) under linux
Intel-based (64 bits) Macs under OSX

In case of problems in these or requests for other platforms, contact me and I'll try my best to sort them out.

2 Description

The method for determination of the Bfactor and its application to sharpen 3D maps and compensate the decay of the amplitudes was presented by Rosenthal and Henderson as described in their article JMB 333:721–745, 2003.

The method for Bfactor determination relies on the fact that, beyond 10 Å, the average scattering amplitude is essentially constant in a Guinier plot (which show the natural logarithm of the spherically average structure factor as a function of the square resolution (Å⁻²)). According to Wilson statistics, the average scattering amplitude in that range is approximately $\sqrt{N_{\text{atoms}}}$. N_{atoms} can be approximated by the number of equivalent atoms of identical scattering factor and that can be easily computed from the molecular mass.

For Bfactor determination, the program proceeds as follows:

- Computes the spherically averaging structure factor amplitudes.
- Applies a noise-weighting function (see C_{ref} below) to better represent the average signal in the map.
- Computes the natural logarithm of the average amplitude as a function of resolution (Å⁻²).
- Determines the line that best fits the data by least squares. For the fitting, a range of resolution is used, typically [10-15Å, R_{max}], where R_{max} is the maximum resolution in the map as assessed by means of the FSC.
- Computes the Bfactor affecting the data from the slope of the line fitted. The Bfactor for sharpening (B_{restore}) is thus set as the negative of the Bfactor affecting the data.

Sharpening is carried out by applying the Bfactor in the form $e^{-(B_{\text{restore}}/4d^2)}$, where d is spatial frequency. In order to avoid noise amplification at high frequency, down-weighting of resolution shells containing noise is carried out. The noise-downweighting function (C_{ref}) is computed from the FSC (Fourier Shell Correlation) as $\sqrt{2\text{FSC}/(1 + \text{FSC})}$. So, in the end, the map is sharpened by means of the function:

$$C_{\text{ref}} e^{-(B_{\text{restore}}/4d^2)} = \sqrt{2\text{FSC}/(1 + \text{FSC})} e^{-(B_{\text{restore}}/4d^2)}$$

For Bfactor application, the program proceeds as follows:

- Reads the FSC curve from an input file.
- Slightly smooths the FSC by a moving-average procedure.
- Computes the C_{ref} function from the smooth FSC.
- Sharpen the input map with the function $C_{\text{ref}} e^{-(B_{\text{restore}}/4d^2)}$.

Finally, the structure factor amplitudes of the sharpened map are placed on an absolute scale by setting the zero angle scattering to $0.28 \times N_{\text{atoms}}$ and the average scattering amplitude in the Wilson regime equal to $\sqrt{N_{\text{atoms}}}$.

The program generates a Guinier plot with the average structure factor amplitudes of the original map, the noise-weighted map, the sharpened map. The zero angle scattering and Wilson statistics used for scaling the map are also indicated. This plot can be used for assessment of the sharpening. This plot is generated in Postscript file and in text format.

A detailed description of the procedure implemented in the program is described in the following articles:

- [1] Optimal Determination of Particle Orientation, Absolute Hand, and Contrast Loss in Single-particle Electron Cryomicroscopy
PB Rosenthal and R Henderson
Journal of Molecular Biology 333:721-745, 2003.
<http://dx.doi.org/10.1016/j.jmb.2003.07.013>

- [2] EM-BFACTOR: Sharpening high resolution information in single particle electron cryomicroscopy.
JJ Fernandez, D Luque, JR Caston, JL Carrascosa.
Journal of Structural Biology 164:170-175, 2008.
<http://dx.doi.org/10.1016/j.jsb.2008.05.010>

Please, cite these articles if you use EM-BFACTOR in your works.

3 Usage

The program uses the routine library from the well-known EM package Bsoft (<http://bsoft.ws>) and also follows the conventions therein. The command line user interface adheres to typical Unix-style practices and the syntax is:

```
embfactor [options] inputmap.img outputmap.img
```

The options are tags or tag-value pairs, each tag starting with a hyphen followed by a word or acronym. There must be a space between the option tag and the value. Every option is specified in the usage printed when only the program name is used. This usage list indicates whether an option takes one or more comma-separated values. The user can use any truncated version of a tag as long as it is unique:

```
-tag val1,val2,...
```

To get the list of options, just type the program name: embfactor.

```
Usage: embfactor [options] inputmap.img outputmap.img
-----
Determines and applies B-factor on images, according
to Rosenthal & Henderson's approach
(J.Mol.Biol. 333:721-745, 2003).

Actions:
-Bfactor -500      Bfactor (Å2). Multiplied in reciprocal space by
                  exp(-Bfactor/(4*s2)).
                  (default: automatic determination of Bfactor.)

Parameters:
-verbose 1         Verbosity of output.
-sampling 1.5      Sampling (Å/pixel; default from input file).
-molweight 120000  Molecular weight (Daltons)
-resolution 6,10   Resolution limits (Å) for B-factor determination
                  (default: Nyquist,15).
-maxresol 6[,7]    High resolution limit (Angstroms) for B-factor
                  application (default: max.resol. for
                  determination). Two values define the resolution
                  limits for a raised-cosine lowpass filter.
-offset 100        Offset to add to the B-factor before sharpening
                  (default: 0).
-noscale           Flag to disable that the map is put in an absolute
                  scale. Available only for backward compatibility.

Input:
-FSC file          FSC curve.

Output:
-guinier file.txt  Text file containing a Guinier plot, to be
                  displayed with gnuplot.
                  A postscript version is also generated on
                  'file.txt.ps'
```

The option tags can be truncated as long as they remain unique, e.g., "-v 7" will set the verbosity level to 7. The same happens with the other tags. For "-molweight" the truncated version is "-mo" and for "-maxresol" is "-ma".

Input and Output image formats

As far as image format is concerned, embfactor can deal with in any format common in EM (e.g. MRC, Spider, Xmipp, PIF, EM, etc.). This flexibility is thanks to the use of the Bsoft library. In addition, support for Xmipp format has also been included in this program. Data file formats are recognized by the file extension following the last period (".") in the file name. The image format extensions supported are in the following table.

Image format extension supported

BioRad (pic), Brix (brx), CCP4 (map), Digital Instruments (di), EM (em), Goodford (pot), GRD (grd), Imagic (img), JPEG, (jpg), MFF (mff), Image Magick (miff), MRC (mrc), PIC (bp), PIF (pif), Spider (spi), Suprim (spm), TIFF (tif), RAW (raw), Xmipp (xmp).

Examples:

1. embfactor [options] inputmap.mrc outputmap.spi

The inputmap is assumed to have MRC format, and the resulting sharpened map is written in Spider format.

2. embfactor [options] inputmap.xmp outputmap.mrc

The inputmap is assumed to have Xmipp format, and the resulting sharpened map is written in MRC format.

3. embfactor [options] inputmap.mrc outputmap.mrc

The inputmap is assumed to have MRC format, and the resulting sharpened map is also written in MRC format.

Additionally, note that a file can be read using a particular format by specifying this format's extension after a colon (":").

Examples:

1. embfactor [options] mymap.orig:spi mymap.sharp:mrc

The inputmap "mymap.orig" is read as having Spider format, and the resulting sharpened map is called "mymap.sharp" and written in MRC format.

2. embfactor [options] mymap.vol:xmp outputmap.mrc

The inputmap "mymap.vol" is read as having Xmipp format, and the resulting sharpened map is written in MRC format.

3. embfactor [options] inputmap.dat:mrc outputmap.mrc

The inputmap "inputmap.dat" is read as having MRC format, and the resulting sharpened map is written in MRC format.

Recommendation: Although the program can deal with maps in any mode (unsigned char, short, integer, float), it is recommended that the data type in the input format be float (e.g. Spider, Xmipp, MRC Mode 2, etc.).

4 Options and Parameters

-Bfactor <value>

The program `embfactor`, by default, automatically determines the Bfactor to be applied by means of Rosenthal and Henderson's approach. However, if the user indicates a specific value with this option, this is the Bfactor value that will be applied. Negative B-factor values are expected. Positive B-factor values will further smooth the map. A Bfactor of 0.0 may be used just to obtain the curve of the original map on a Guinier plot.

-FSC <value>

This option allows the user to enter the name of the file containing the FSC curve. If this option is not given, the program does not use Noise-weighting with the function C_{ref} , hence applying the Bfactor as usual with other standard programs.

The FSC stored in the file can follow the convention for the FSC in the following packages: `Frealign`, `Spider`, `EMAN`, `Xmipp`, `Bsoft`. Also, the FSC curve can be input in raw format (a text file, with the FSC values in a single column). The program tries to guess the format by itself: First, it checks the extension: `Frealign` (`mrc`), `spider` (`spi`), `EMAN` (`eman`), `Xmipp` (`xmp`, `fsc` or `frc`), `Bsoft` (`bsoft`), raw format (`raw`). If it is not possible to guess the file type from the extension, the program tries to guess it from the first line of the file and the structure of its contents.

The appendix shows examples of the FSC files coming from those packages and that are interpreted by `embfactor`. The raw format accepted by the program is described as well.

-guinier <value>

This option is used to enter the name of the file to store the Guinier plot in text format. Additionally, a colour Postscript version of the Guinier plot is generated, provided that the well-known program "`gnuplot`" is available in the system. The postscript Guinier plot is stored in a file with the name given by the user here, and the ".ps" extension added.

The Guinier plot shows the zero angle scattering and the Wilson regime when the molecular mass is specified in command line. If not, these values are shown in an approximate way as estimated from the experimental data.

-maxresol <value>[,<value2>]

This option is used to enter the maximum resolution (value) at which Bfactor is applied. All the components at frequencies higher than that value are cancelled out. If this option is not given, by default Bfactor restoration is applied up to the maximum resolution used for Bfactor determination (see option "`-resolution`").

This option also allows the user to specify a raised-cosine lowpass filter in order to smoothly stop the Bfactor correction. In this case, the user has to indicate two values: the pass-band (value) and the cut-off frequency (value2). All the components at frequencies higher than the cut-off are cancelled out. All the components between the pass-band and the cut-off are applied a raised-cosine falloff over the Bfactor correction curve.

-molweight <value>

This option is used to enter the molecular mass (Da) of the structure in the input map. If this option is used, the map is placed on an absolute scale. Otherwise, there is no scaling of the map.

-noscale

This option allows the user to request that the map should not be placed on an absolute scale, even though the molecular weight had been given. This option is provided for backward compatibility.

-offset <value>

This option allows the user to enter an additional value for the Bfactor before sharpening the map. The entered value is added to the Bfactor found by the automatic Bfactor determination procedure or to the entered value with the option “-Bfactor”. This input value can be positive (to reduce the strength of the sharpening) or negative (to increase the strength of the sharpening further).

-resolution <hi,lo>

The resolution range (Å) to be used in the least squares procedure for Bfactor determination is entered by means of this option. If this option is not given, by default the resolution range used is from 15 Å to the Nyquist frequency.

-sampling <value>

This option is used to enter the pixel size (Å). If this option is not given, the pixel size is assumed to be 1 Å, or is taken from the header of the input size, if available.

-verbose <value>

The program works in silent mode by default. With this option, the user can request different levels of verbosity, from 1 (minimal output) to 7 (maximal output). Verbosity level of 1 is recommended for Bfactor determination, as it shows the details of the least squares fitting.

The most important options for Bfactor determination and application are: -sampling, -resolution, -FSC, -molweight, -maxresol, -guinier, -Bfactor.

5 Examples

5.1 Examples of commands

1. Automatic Bfactor determination, using the resolution range [6,10]. The input map is in Spider format. The input FSC curve is in Spider format. The pixel size is 2.18 Å. A molecular mass of 0.8 MDa is specified for placing the map amplitudes on an absolute scale. As output, the program produces the sharpened map in MRC format, and the Guinier plot in text format in stored on the file “guinierplot” and also –as long as “gnuplot” is available– in Postscript format in the file “guinierplot.ps”. The verbosity level is set to 1.

```
embfactor -v 1 -molweight 800000 -FSC myFSCcurve.spi -sampling 2.18
-guinier guinierplot -resol 10,6 inputmap.spi outputmap.mrc
```

2. Automatic Bfactor determination, using the resolution range [7,10]. The input map is in MRC format. The input FSC curve has been extracted from the Frealign logfile. The pixel size is 2.18 Å. Bfactor application is done only up to 8 Å resolution. No scaling of the map is carried out as no molecular mass is specified. As output, the program produces the sharpened map in Spider format. No Guinier plot is wanted. The verbosity level is set to 1.

```
embfactor -v 1 -FSC myFSCcurve.mrc -sampling 2.18
-resol 10,7 -maxresol 8 inputmap.mrc outputmap.spi
```

3. Application of a specific Bfactor of -500 Å². The input map is in Xmipp format. The input FSC curve follows a raw format. The pixel size is 2.18 Å. The maximum resolution to apply sharpening is up to 8 Å resolution. A molecular mass of 0.8 MDa is specified for placing the map amplitudes on an absolute scale. As output, the program produces the sharpened map in MRC format, and the Guinier plot in text format in stored on the file “guinierplot” and also –as long as “gnuplot” is available– in Postscript format in the file “guinierplot.ps”. The program works in silent mode.

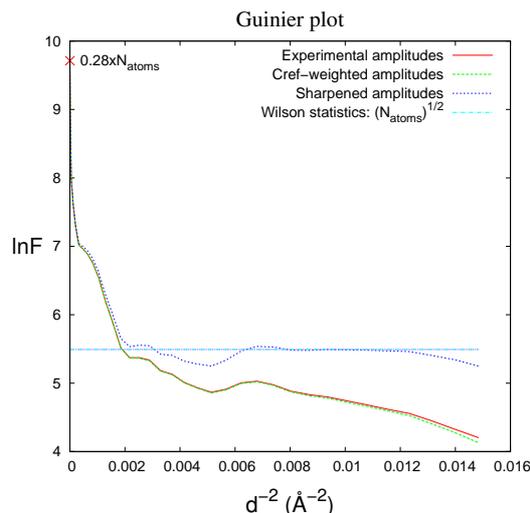
```
embfactor -Bfactor -500 -molweight 800000 -FSC myFSCcurve.raw
-sampling 2.18 -guinier guinierplot -maxresol 8
inputmap.xmp outputmap.mrc
```

4. Automatic Bfactor determination, using the resolution range [7,10]. The input and output maps are in Xmipp format. The input FSC curve has been computed with Xmipp. The pixel size is 2.18 Å. Bfactor application is done only up to 8 Å resolution, but with a smooth falloff between 8 and 7 Å. No scaling of the map is carried out as no molecular mass is specified. The Guinier plot in text format in stored on the file “guinierplot” and also –as long as “gnuplot” is available– in Postscript format in the file “guinierplot.ps”.

```
embfactor -v 1 -FSC myFSCcurve.xmp -sampling 2.18 -resol 10,7
-maxresol 8,7 -guinier guinierplot inputmap.xmp outputmap.xmp
```

5.2 Example of Postscript Guinier plot provided by the program

In the following figure, an example of Guinier plot provided by the program is shown. A Guinier plot shows the natural logarithm of the spherically average structure factor amplitude as a function of the resolution (\AA^{-2}). The plot has Guinier curves corresponding to the original map, the noise-weighted map, and the sharpened map. Additionally, the plot also contains information used for placing the map on an absolute scale: zero angle scattering ($0.28 \times N_{\text{atoms}}$) and the average scattering amplitude in the Wilson regime ($\sqrt{N_{\text{atoms}}}$).



The Guinier plot is also stored onto a text file, with 5 columns that correspond to the following 5 fields: (1) resolution (\AA^{-2}), (2) natural logarithm of the spherically average structure factor amplitude from the original map, (3) from the noise-weighted map, (4) from the sharpened map and (5) the average scattering amplitude in the Wilson regime ($\sqrt{N_{\text{atoms}}}$). A piece of a text file containing this plot is shown in the following. This text file allows the user to prepare the plot with his/her favourite graphical plotting program.

```

0.000000  9.712327  9.712327  9.712327  5.492646
0.000013  8.934300  8.934164  8.935593  5.492646
0.000051  7.974760  7.974257  7.978405  5.492646
0.000116  7.622321  7.621428  7.630568  5.492646
0.000205  7.295247  7.294247  7.310562  5.492646
...
0.013986  4.325380  4.277796  5.340898  5.492646
0.014847  4.202241  4.129067  5.249811  5.492646

```

5.3 Example of the output report

On the execution of the following command, with verbosity level 1,

```
embfactor -v 1 -molweight 708000 -FSC myFSCcurve -sampling 2.18  
-guinier guinplot -resol 12,8 -maxresol 8,7 inmap.xmp outmap.mrc
```

the following output report is shown on console:

```
Reading FSC file: myFSCcurve  
  FSC file: Spider Format  
  
Computing the spherical average of the structure factor amplitudes  
Wilson Regime: 242.90      Zero Angle Scattering: 16520.00  
Applying Cref weighting  
Automatic determination of the B-factor affecting the data:  
  Fitting y=a+b*x to Cref-weighted spherically averaged structure factor curve:  
  Resolution range for fitting: [12.00,8.00]  
  Fitting parameters: a: 0.027726      b: -85.745102  
  B-factor found: 342.98  
  
Restoring amplitudes by applying B-factor: -342.98  
  Max. resolution range for B-factor application: 8.00 Angs  
  (Raised-cosine filter 8.00-7.00 Angs)  
  
Computing the spherical average of the structure factor amplitudes  
Placing the structure factor amplitudes on an absolute scale.  
Writing output map.  
  
Writing Guinier plot.
```

This report shows the parameters for the least squares fitting and the Bfactor that was finally found out. The report shows on console the progress of the program. In this particular example, note that the FSC file specified in the command did not have a specific extension. So, the program has had to get into the file and analyze the structure of the contents to guess its format.

6 Advices and comments

- Whenever possible, enter the FSC curve. The FSC allows more accurate Bfactor determination because of the noise-weighting function.
- Whenever possible, enter the molecular mass of the structure under study. This allows placement of the map on an absolute scale.
- For Bfactor determination, the default low resolution limit is 15 Å. It may be advisable to play around with values in the range [20 Å, 10 Å] for a better least squares fitting.
- For Bfactor determination, the default high resolution limit is the Nyquist frequency. It is strongly recommended that this limit is set up to the resolution attained by the input map, as assessed by the researcher by means of the FSC and one of the criteria currently used in the field (0.5-FSC, 0.143-FSC).
- The maximum resolution for sharpening is, by default, the high resolution limit used for determination. If the latter is not set, then it is strongly recommended that a value is entered, even though the program already uses noise-weighting in the sharpening. Typically the resolution attained by the map, as assessed by the 0.143-FSC, is a potential useful value for this parameter.
- For Bfactor correction, it is recommended that the raised-cosine lowpass filter is used in order to make a smooth transition to zero. Use a transition range of 1 or 2 angstroms (e.g. -maxresol 8,7). It is always advisable to use these smooth transitions to avoid abrupt cut-offs in Fourier space, as they would generate ripples in real space.
- It is advisable to generate the Guinier plot in order to assess the effect of the sharpening.
- For Bfactor determination, it is advisable to use verbosity level 1 in order to check out the least squares procedure and take note of the Bfactor finally found out.
- Although the program can deal with maps in any mode (unsigned char, short, integer, float), it is recommended that the data type in the input format be float (e.g. Spider, Xmipp, MRC Mode 2, etc.). This data type guarantees that the scale of the output map can be properly represented.

Acknowledgements

The routine library developed for Bsoft (Heymann and Belnap, JSB 157:3–18, 2007) has been used in this program to take advantage of the ability to deal with EM maps in any common format. The routines for least squares fitting were taken from Numerical Recipes in C (Press et al, Cambridge University Press, 1992). The development of this program was initiated at the MRC Laboratory of Molecular Biology, Cambridge, UK. Discussions with A. Ionel and B. Zuber have been of great help. This work has been possible thanks to grants from the Spanish Ministry of Science, the MRC, EMBO and EU through the network of excellence 3DEM-NoE.

7 History

V. June 2008

- Original package as described in J.Struct.Biol. 164:170-175, 2008.

V. December 2009

- The problems with the scaling of the amplitudes when no molecular mass was given through the command line has been sorted out. In the previous version, these problems were solved by using the option “-noscale”. Now, this version places the map on an absolute scale as long as the molecular mass is given. Otherwise, scaling is not done.
- The problems with versions of gnuplot older than 4.2 are solved.
- The FSC files from Xmipp 2.3 are now supported.
- A raised-cosine lowpass filter is used to make a smooth transition to zero.

V. April 2013

- FSC curves in the formats followed by Xmipp 2.x and Xmipp 3.x are now supported.
- The program interprets FSC curves in the format followed by Bsoft 1.8.x and beyond. Previous versions of Bsoft are also supported.
- The program now has abilities to accept FSC curves in raw format (a text file with the FSC values in a single column). This raw format makes embfactor more independent of the program used to compute the FSC.
- A bug while reading the FSC curve in Eman format has been fixed. This bug produced a slight under-estimation of the Bfactor.
- The program is provided as static binaries for linux and OSX.

V. May 2014

- The OSX version of embfactor is built for 64 bits so that it can now sharpen large maps. There are no changes in the source code of the program. The Linux version remains the same (V. April 2013).

8 References

- of special interest.

References describing the methods implemented in EMBFACTOR

- Optimal Determination of Particle Orientation, Absolute Hand, and Contrast Loss in Single-particle Electron Cryomicroscopy.
P.B. Rosenthal and R. Henderson.
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Appendix: Formats for FSC curves

The program embfactor accepts FSC curves that follow the convention for the FSC in the following packages: Frealign, Spider, EMAN, Xmipp, Bsoft. In addition, the program also accepts FSC curves in raw format. The program tries to guess the format by itself: First, it checks the extension: Frealign (mrc), spider (spi), EMAN (eman), Xmipp (xmp), Bsoft (bsoft), raw format (raw). If it is not possible to guess the file type from the extension, the program tries to guess it from the first line of the file and the structure of its contents. In the following, the structure of these formats are described and examples are shown.

1. FSC in Frealign format

Frealign shows the FSC in the output log file. The FSC can be extracted from the log and stored into a file with the following structure.

- All the lines in the file must start with the character 'C'.
- The first line is a title line.
- There must be 14 columns, apart from the 'C' column. The normalized frequency (i.e., in the range [0, 0.5]) is stored in the third column. The FSC in the fifth column.

Example:

C	NO.	RESOL	RING RAD	FSPR	FSC	QFACT	QRAN+SIG	SSNR	RFACT	PFREE	PNOISE	NONZERO	TOTVOX
C	2	279.0	0.0078	1.72	0.998	0.154	0.004	61.06	0.037	0.00	0.00	17	17
C	3	139.5	0.0156	1.66	0.990	0.058	0.005	21.40	0.156	0.00	0.00	41	41
.
.
C	46	6.2	0.3516	90.28	0.004	0.014	0.020	0.36	0.585	0.00	0.00	13157	13157
C	47	6.1	0.3594	88.48	0.018	0.031	0.043	0.42	0.585	0.00	0.00	13553	13553

2. FSC in Spider format

The Spider command 'RF 3' computes the FSC between two volumes and generates a DOC file with the resulting curve. The structure of this file follows this convention:

- The first line is a title line, starting with ' ;'
- There must be 7 columns. The normalized frequency (i.e., in the range [0, 0.5]) is stored in the third column. The FSC in the fifth column.

Example:

;	spi/dat	10-MAR-2004	AT	14:31:39	mydata.dat	
3	5	8.86525E-03	2.3633	0.99897	1.0000	5.0000
4	5	1.24113E-02	1.7200	0.99951	0.86603	12.000
...
...
140	5	0.49468	97.077	0.10921	2.42821E-02	15264.
141	5	0.49823	102.96	9.95071E-04	3.49310E-02	7376.0

3. FSC in EMAN format

The EMAN program 'eotest' computes the FSC between two volumes and generates a text file with the following structure:

- There is no title line.
- There must be 2 columns. The first is the index of the ring, and the second column is the FSC. It is assumed that the FSC curve covers the whole Fourier range, up to the Nyquist frequency.

Example:

1	0.999964
2	0.999643
...	...
...	...
31	0.070059
32	0.053562

4. FSC in Xmipp format

Embfactor is able to interpret FSC curves generated by Xmipp (Version 2.x and 3.x). The Xmipp program 'xmipp_resolution_fsc' computes the FSC between two volumes and generates a text file with the FSC curve.

In Xmipp 2.x, this file has the following structure:

- The first line is a title line, starting with '#'
- There must be 4 columns. The resolution (\AA^{-1}) is stored in the first column and the FSC in the second one.

Example:

#	Resol. [1/Ang]	FRC	FRC_random_noise	Resol. [Ang]
0.004067		0.99897	1.0000	245.88
0.005693		0.99951	0.86603	175.65
...	
...	
0.226917		0.10921	2.42821E-02	4.41
0.228546		9.95071E-04	3.49310E-02	4.38

In Xmipp 3.x, however, the structure of the file is:

- Several lines with titles (starting with '#'), metadata labels (containing '_'), etc.
- There must be 5 columns. The resolution (\AA^{-1}) is stored in the first column and the FSC in the second one.

Example:

#	XMIPP_STAR_1 *			
#				
data_noname				
loop_				
_resolutionFreqFourier				
_resolutionFRC				
_resolutionErrorL2				
_resolutionFRCRandomNoise				
_resolutionFreqReal				
0.000203	0.999922	69952.892439	1.154701	4919.999886
0.000406	0.999521	59838.569238	1.000000	2459.999943
...
...
0.030285	0.003878	12125.848819	0.024063	33.020133
0.030488	0.026332	11995.467567	0.034826	32.799999

5. FSC in Bsoft format

Embfactor is able to interpret FSC curves generated by Bsoft (any version, before or later 1.8). The Bsoft program 'bresolve' computes the FSC between two volumes and shows the FSC in the output log. To produce such a log on console (or with re-direction to a file), the option '-v 7' should be used. The FSC curve can be extracted from the log and stored onto a text file, to be used as an input for embfactor.

In Bsoft (versions up to 1.7.x), the structure of the FSC curve is:

- The first line is a title line, starting with 'Radius'
- There must be 8 columns. The resolution (\AA^{-1}) is stored in the second column and the FSC in the fifth one.

Example:

Radius	s(1/A)	Res(A)	Sigma2	FSC	DPR	SSNR	RA+B
0.00	0.00000	9999.00	1.4142	1.0000	0.00	0.000	0.000
1.00	0.00222	450.00	0.4312	0.8902	42.41	3.503	0.302
...
...
45.00	0.10000	10.00	0.0126	0.0000	104.98	0.000	0.915
46.00	0.10222	9.78	0.0180	0.0000	106.39	0.000	0.923

Bsoft 1.8.x and beyond uses the following structure:

- The first line is a title line, starting with 's(1/A)'
- There must be 6 columns. The resolution (\AA^{-1}) is stored in the first column and the FSC in the third one.

Example:

s(1/A)	Res(A)	FSC	DPR	SSNR	RA+B
0.00000	9999.00	1.0000	0.00	0.000	0.000
0.00222	450.00	0.8902	42.41	3.503	0.302
...
...
0.10000	10.00	0.0000	104.98	0.000	0.915
0.10222	9.78	0.0000	106.39	0.000	0.923

6. FSC in Raw format

This version of embfactor is able to deal with FSC curves saved in so-called raw format. Basically, this should be a text file with the FSC values in a single column. This format essentially makes embfactor independent of the program used to calculate the FSC: just extract the FSC values and places them in a file like this. The structure of the file should then be:

- There is no title line.
- The FSC values must be placed in a single column. As in the eman case, it is assumed that the FSC curve covers the whole Fourier range, up to the Nyquist frequency.

Example:

0.999964
0.999643
..
..
0.070059
0.053562