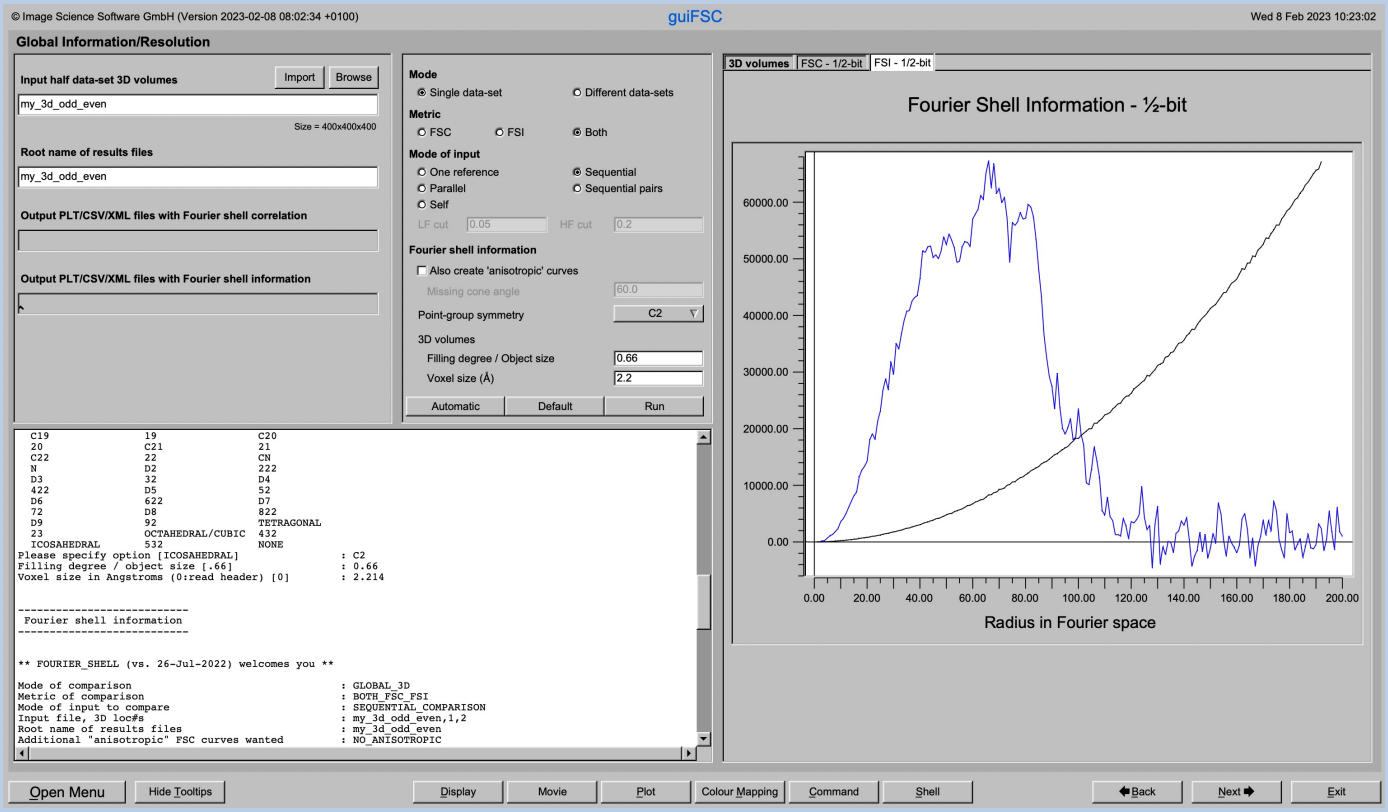
A Brief Introduction

Version 10-Oct-2023
www.ImageScience.de

The IMAGIC guiFSC program



The **guiFSC** program calculates the global and/or local resolution and/or information content within images/3D volumes/spectra.

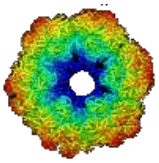
This is a brief hands-on on how to use IMAGIC GUI oriented programs and how to work with **guiFSC**:

CONTENT:

- IMAGIC GUI programs — How to use IMAGIC GUI programs
- guiFSC — How to calculate the global and the local resolution / Information content
- Error hints — How to send us feedback

FOURIER RING/SHELL INFORMATION, FOURIER RING/SHELL CORRELATION:

Refer to our internet pages https://www.ImageScience.de/metrics and https://www.ImageScience.de/fsc.



IMAGIC

GUI Programs

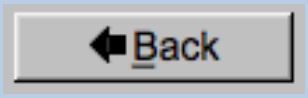
Workflow

The idea of **guiFSC** is to guide you through a typical procedure to get the global and local resolution/information content.

The workflow consists of several pages. Each page will perform a specific image processing step.

If the calculations are finished the results are shown and you can press the “Next” button to continue with the next page.

Of course, there is also a “Back” button. But be careful: when leaving a page the results shown on the page may get lost and when coming back you might have to do the calculations once more to get the results printed. The output files do not get lost, of course.



The Working Directory

If **guiFSC** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

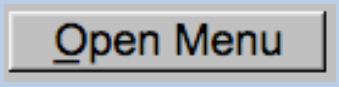
If **guiFSC** is called by an IMAGIC command in a terminal / command window

```
IMAGIC-COMMAND : guiFSC
```

the working directory will be the directory used in this window.

If you want to change this directory use the “Back” button(s)

or the “Open Menu” button



to navigate to the “Start” page where you can specify the working directory of **guiFSC**.

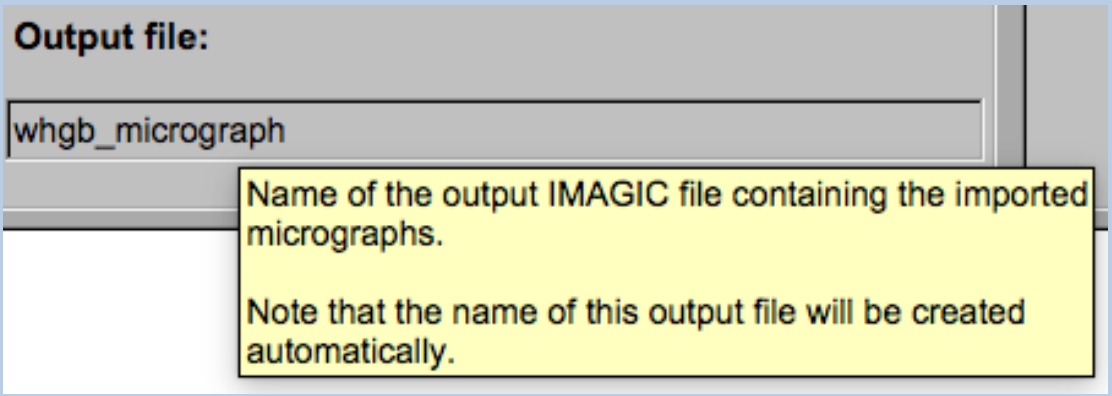
All output files will be stored in the working directory which you have specified on the start page.

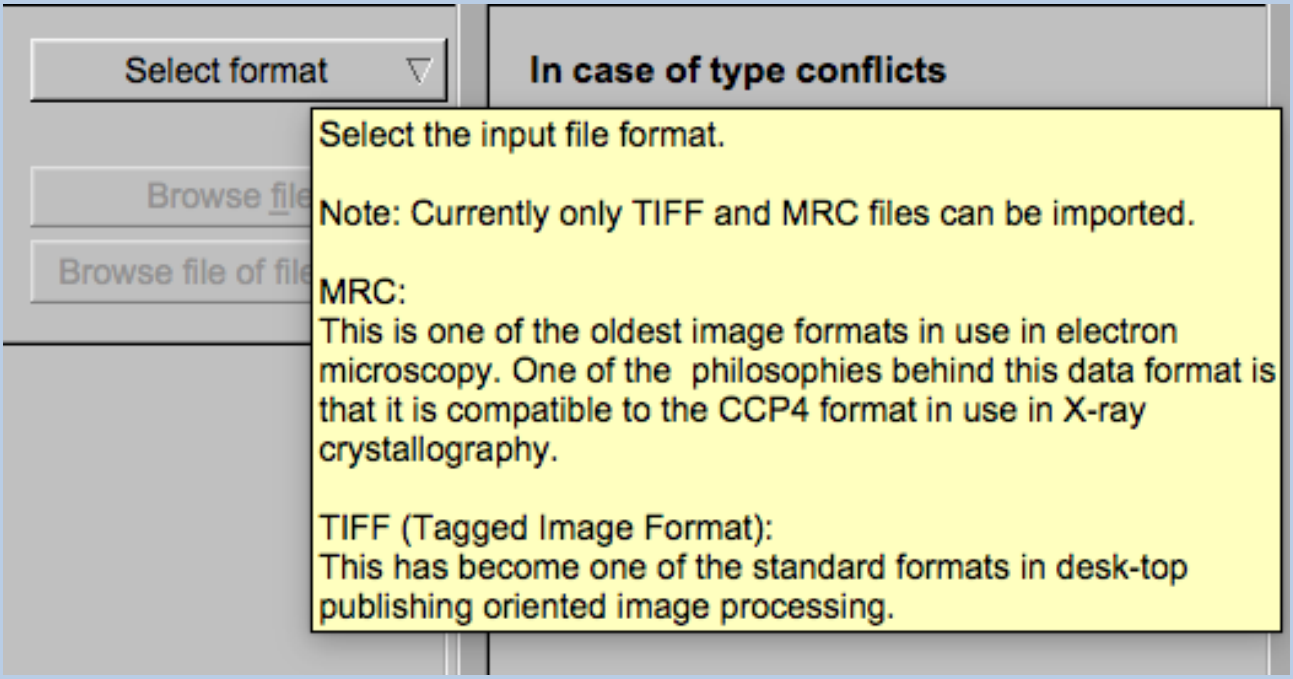
Input files can be chosen from other directories.

NOTE:
guiFSC will start with the “Global Information/Resolution” page. You need to prepare the data you can use the "Next” or "Menu button" to navigate to these these pages.

Help

Move the cursor on (nearly) any item (questions, radio buttons, display windows…) shown on the pages and you will get context sensitive help.



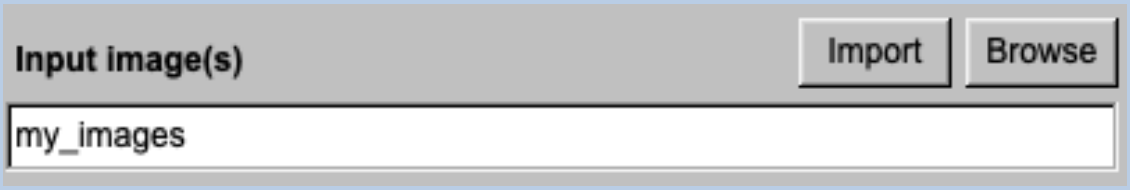


Input Files

Usually the input files on each page are output file(s) from the previous page(s) and are suggested automatically.

You can, of course, always use other input files names and even use other input directories.

To specify the input file you can enter the name of the file into the text field:



Or use the “Browse” button to browse for an IMAGIC image file (see net page):.

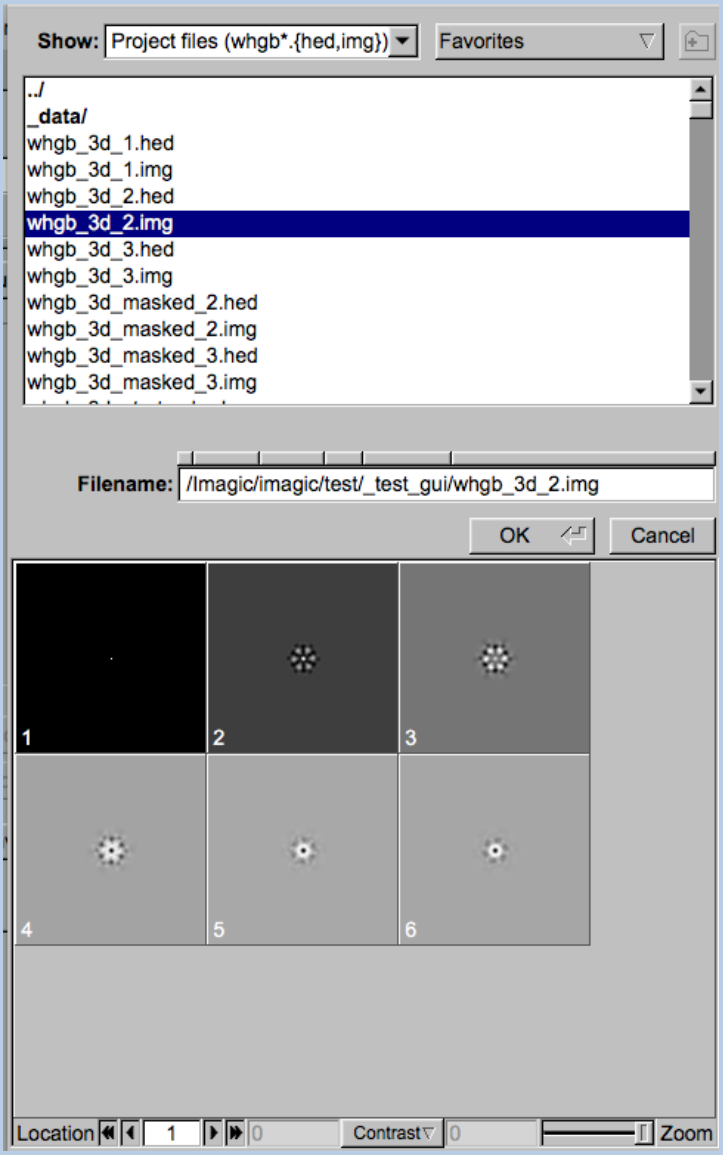
If the input file is in an IMAGIC but a 3DEM file use the “Import” button to create an IMAGIC image file. Note that a separate “EM2EM” window will open to calculate this conversion.

Refer to the **guiEM2EM** manual for further help.

Input File Chooser

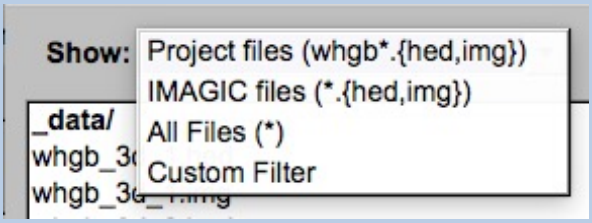
In most of the pages you are asked for input file(s) and you will find a “Browse file” button:

Pressing this button will open the IMAGIC file chooser:



Choose the wanted file by clicking its name

You can use a pre-selection of the files shown:



If the images are in IMAGIC format you can get a pre- view of the images.

Note that you can store your directory in “Favorites”.

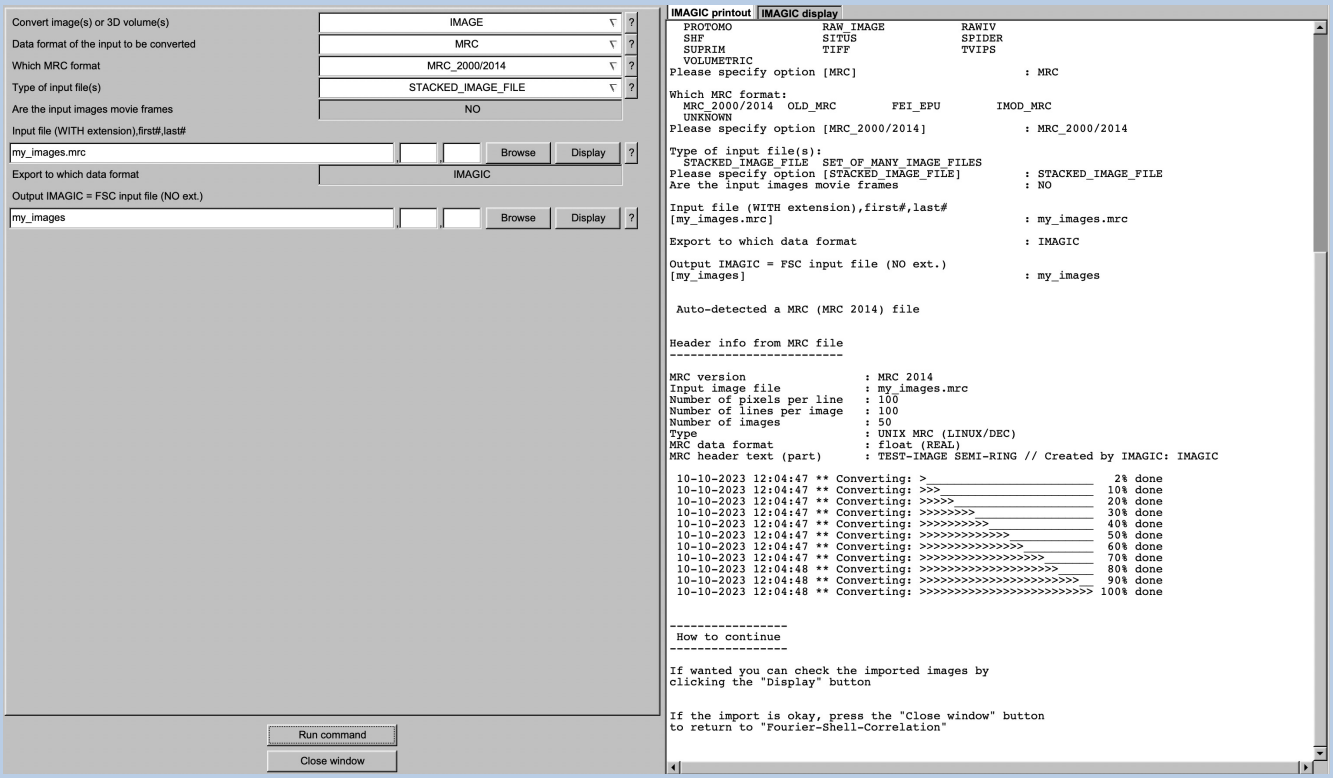
Output Files

Usually the names of the output files are suggested but it is your choice, of course. On each page you can specify these output file names on the left hand side. Enter the name of the file into the text field:

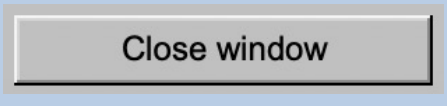
Import Buttons

You do not want to use the “Import page” you can use the “Import” button to import the input images/3D volumes from any 3DEM format. The “Import” button which is located above the text field specifying the name of the related file.

An additional “IMAGIC EM2EM” page will open. Specify all parameters needed and click the “Run command” button to import the images / 3D volumes:



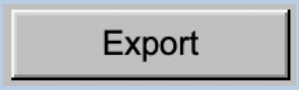
Click the “Close window” button to exit this additional window:



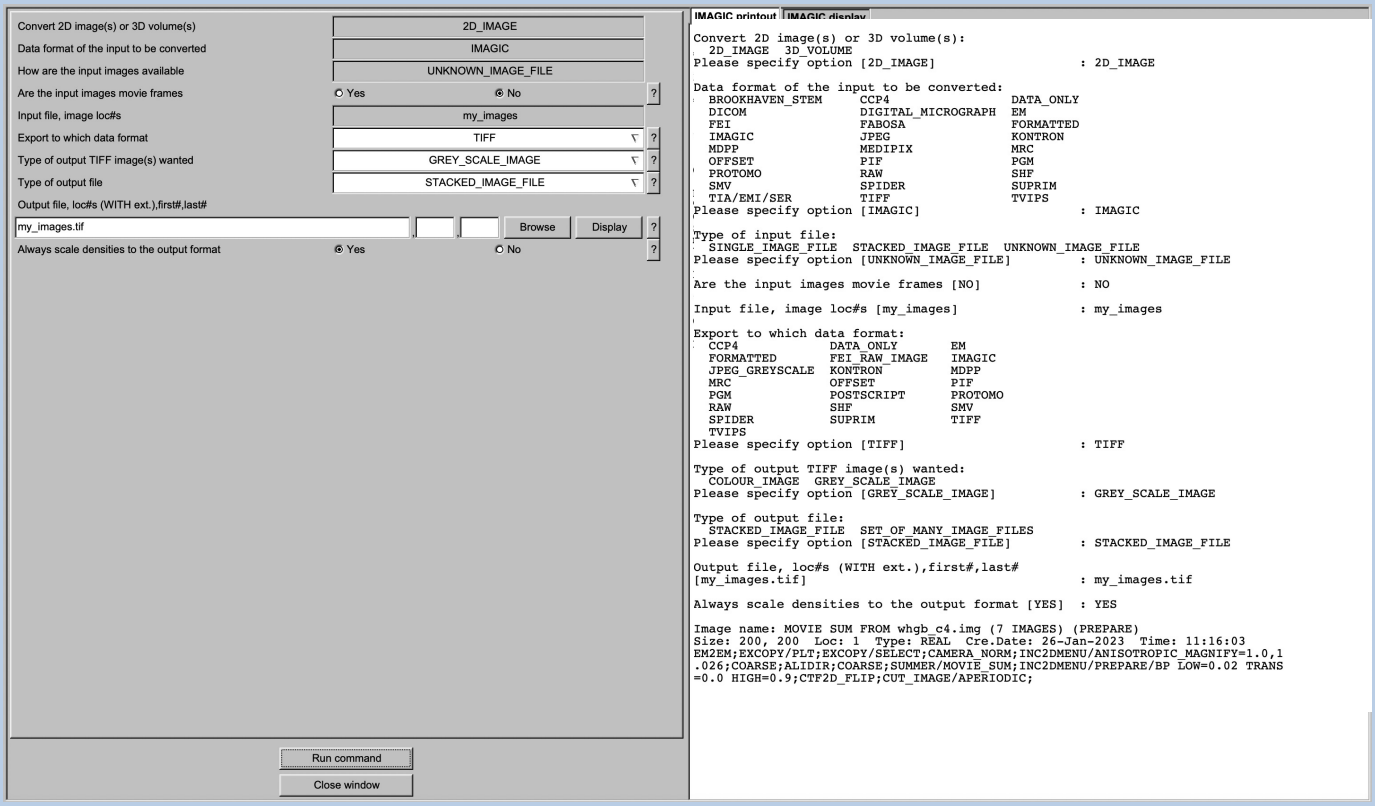
Refer to the **guiEM2EM** manual to get further help.

Export Buttons

You can export output images/3D volumes to any 3DEM format. Click the “Export” button which is located above the text field specifying the name of the related file.



An additional “IMAGIC EM2EM” page will open. Specify all parameters needed and click the “Run command” button to export the images / 3D volumes:



Click the “Close window” button to exit this additional window:

Refer to the **guiEM2EM** manual to get further help.

“Terminal Window”

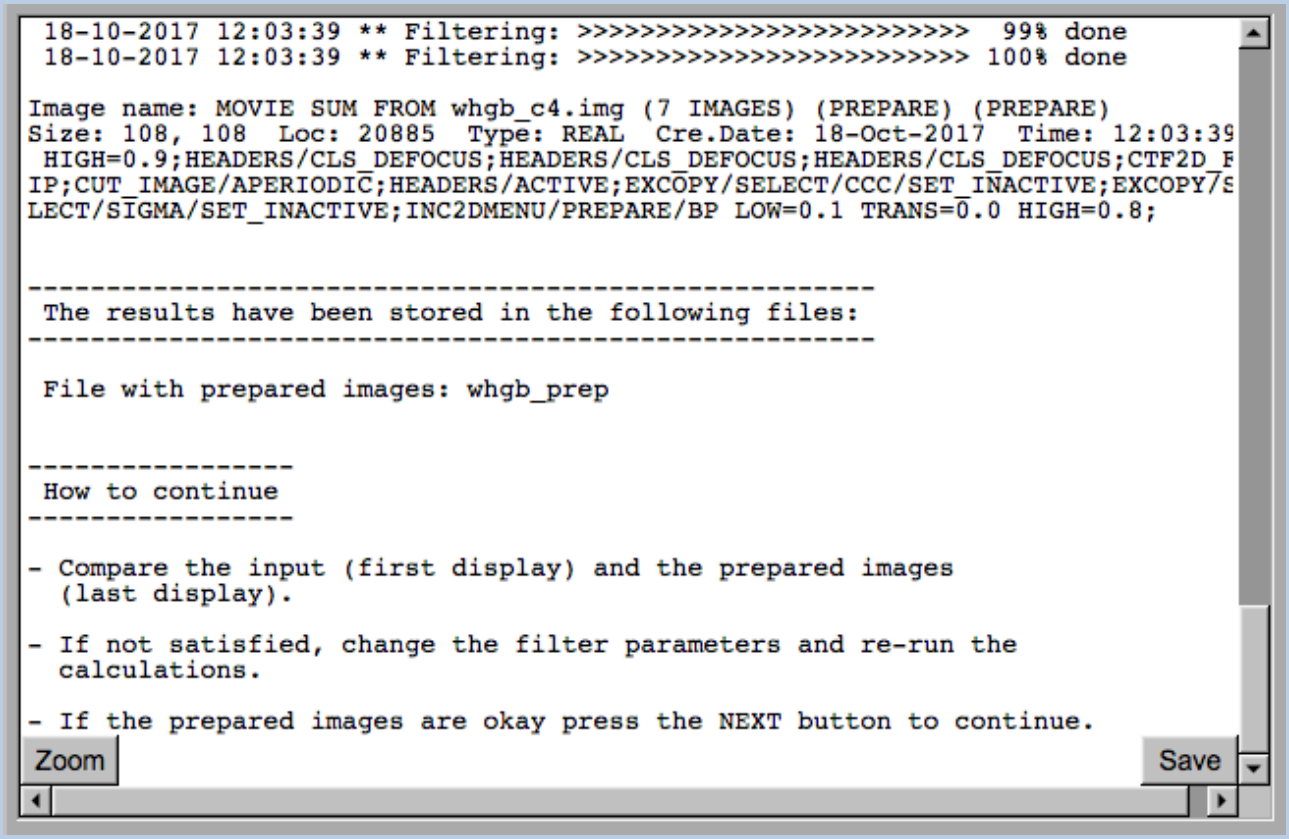
All print-out of an IMAGIC program started within the current **IMAGIC GUI program** page is shown in a kind of terminal window on the left hand side.

Most of the programs will end with a ”How to continue” giving some hints on how to check the results and on how to continue.

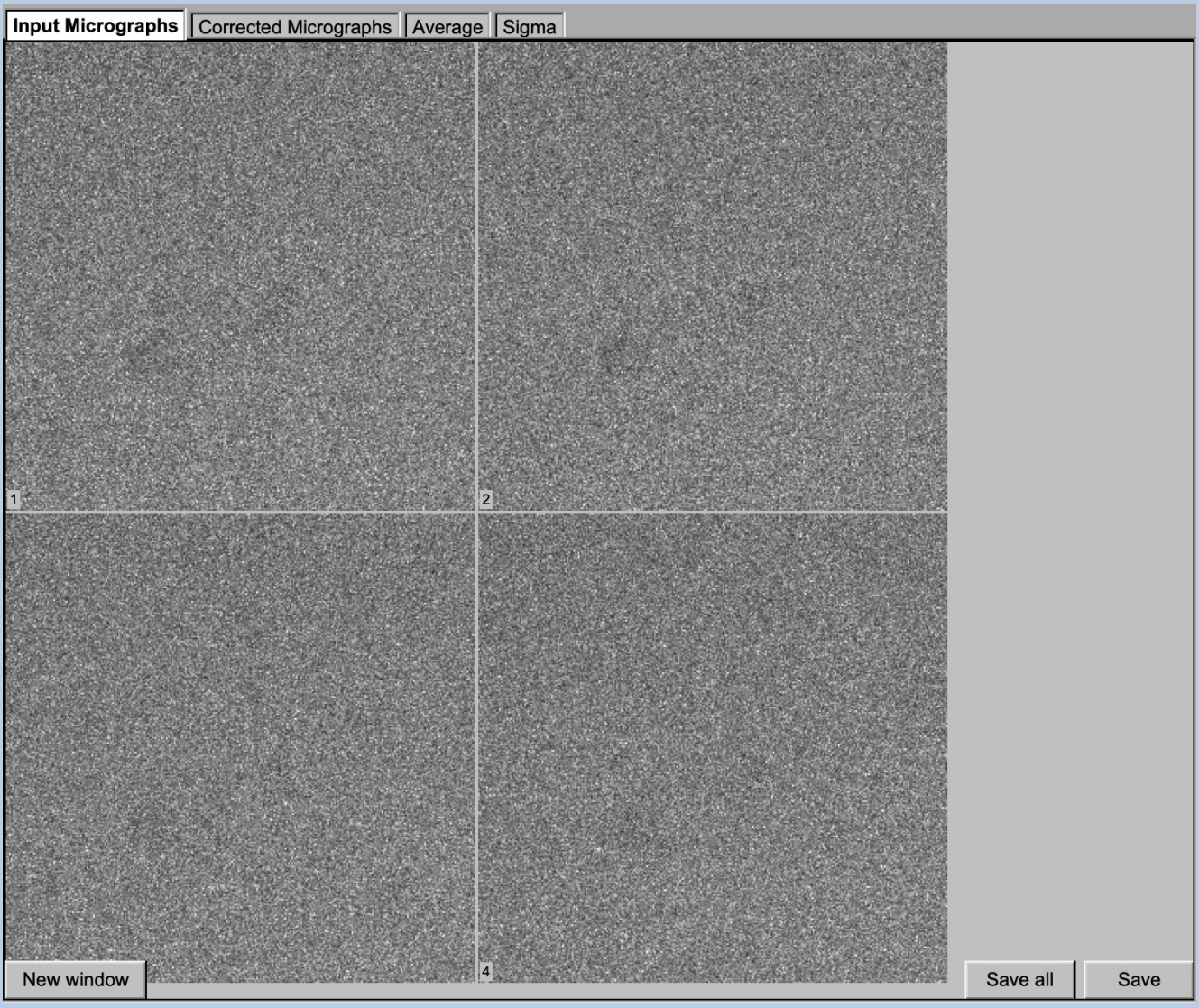
You can use the “Save” button to store the print-out in a text file.

With the “Zoom” button you can open a separate larger window showing the print-out.

Note that the “Save” and “Zoom” buttons are only visible when the cursor is moved into the terminal window.



Display



In the right part of a typical **IMAGIC GUI program** page you will find displayed images - usually the input and the output images.

You can press the tabs to toggle between the various displays.

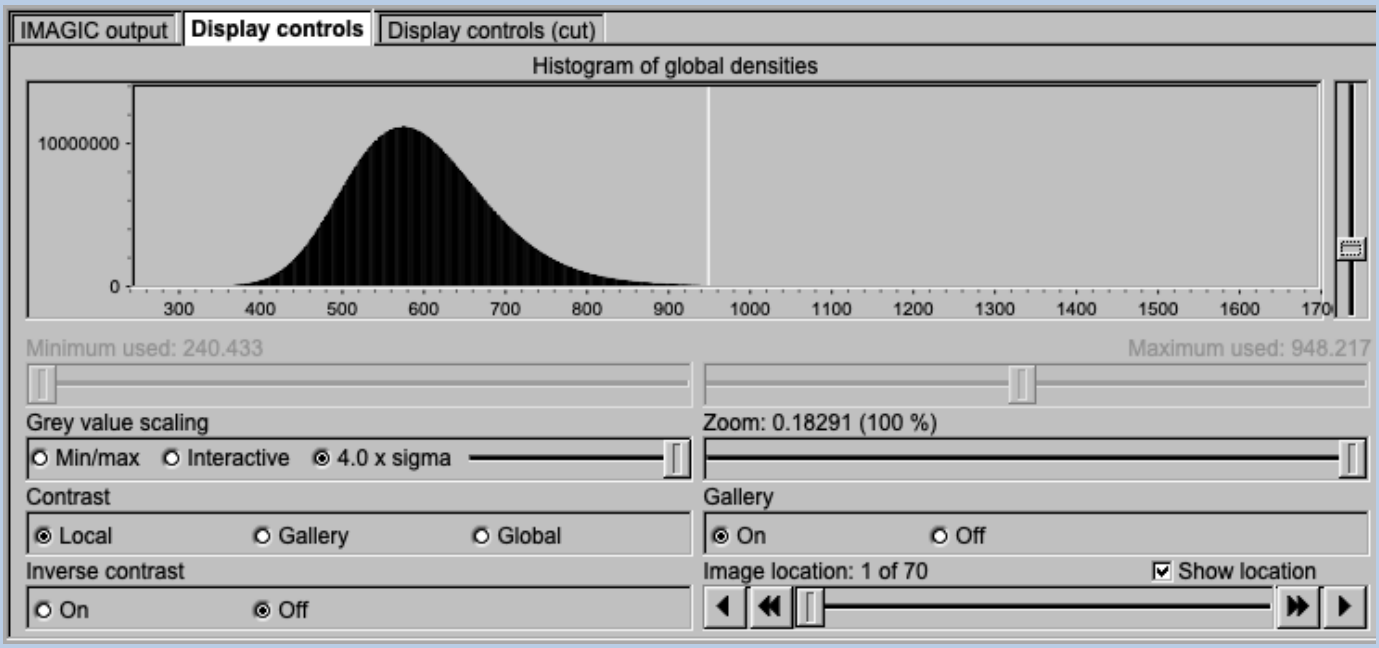
Double click into the wanted images or use the “New Window” button to get an enlarged display window. Use “Save” to store the display (JPG).

To adjust the display settings use the related display control tab on the left hand side of the page. Refer to **guiDISPLAY**.

“Display Control” Tabs

The visualisation settings of images is shown on the right-hand side of each **IMAGIC GUI program** page can be adjusted in its own related “Display control” tab on the bottom left part of each page. Also refer to **guiDISPLAY**.

Grey value scaling:	Adjust the contrast	
	Min/Max:	Scale the grey-values to minimum/maximum
	Interactive:	Set the limits by giving numbers
	Sigma:	Use an amount of sigma to set the limits
Contrast	How to calculate the grey value scaling	
	Local:	Calculated in each image separately
	Global:	Calculated using all image densities (as displayed in the histogram)
	Gallery:	Calculated in the currently displayed images
Inverse contrast:	Use one of the radio buttons	
Zoom	Enlarge the displayed images	
Gallery	On	Display the images in a gallery (may be you need another zoom to see more than one image)
	Off	Show only one image
Image Locations.	Use the slider or the arrows to select image locations	



“Plot Control” Tabs

The visualisation settings of curves/spectra is shown on the right-hand side of an **IMAGIC GUI program** page can be adjusted in its own related “Plot control” tab on the bottom left part of each page. Also refer to **guiPLOT**.

Style, Colour, Grid: Adjust the curve line style, the colour and add a grid if wanted

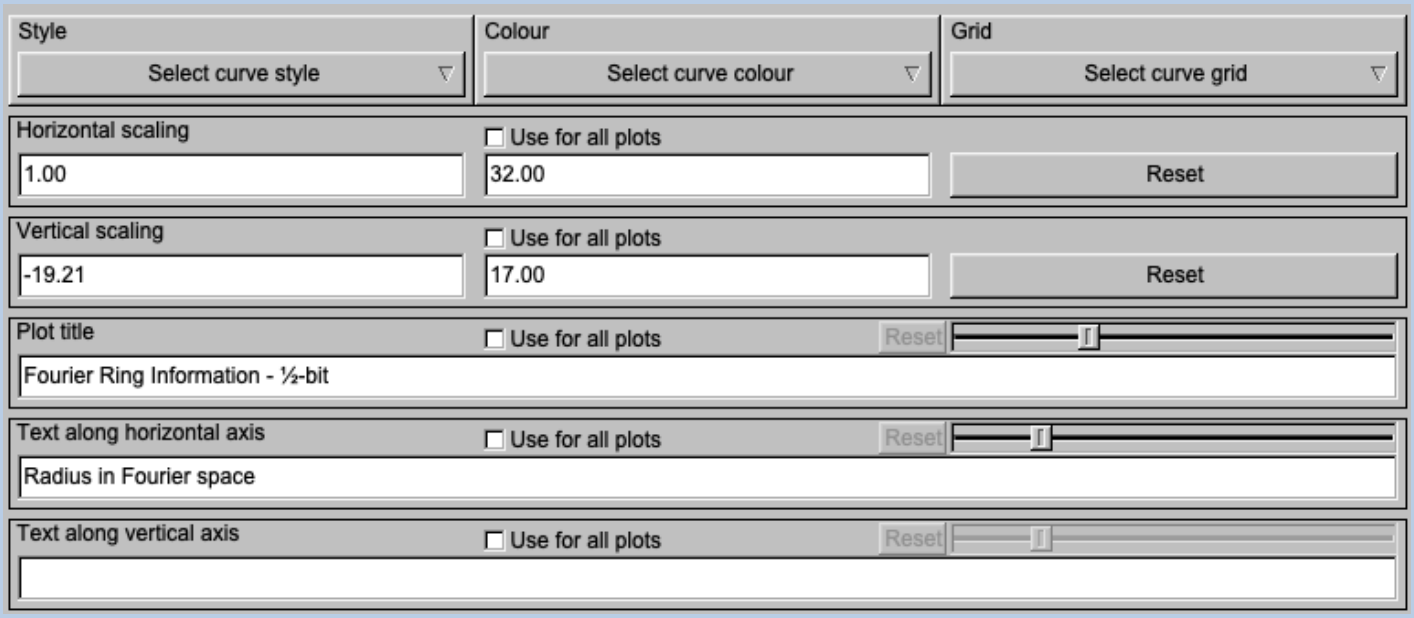
Horizontal, vertical scaling: Set minimal and maximal horizontal or vertical limits

Plot title Set the text of the plot title

Text along ... Set the text along the given axis

Use for all plots: Use the setting for all plots in a file independent of of what is input in the PLT file

Reset:. Reset to the automatic values

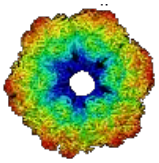


The Toolbar

There is a toolbar at the bottom of each **guiFSC** page.

The toolbar buttons:

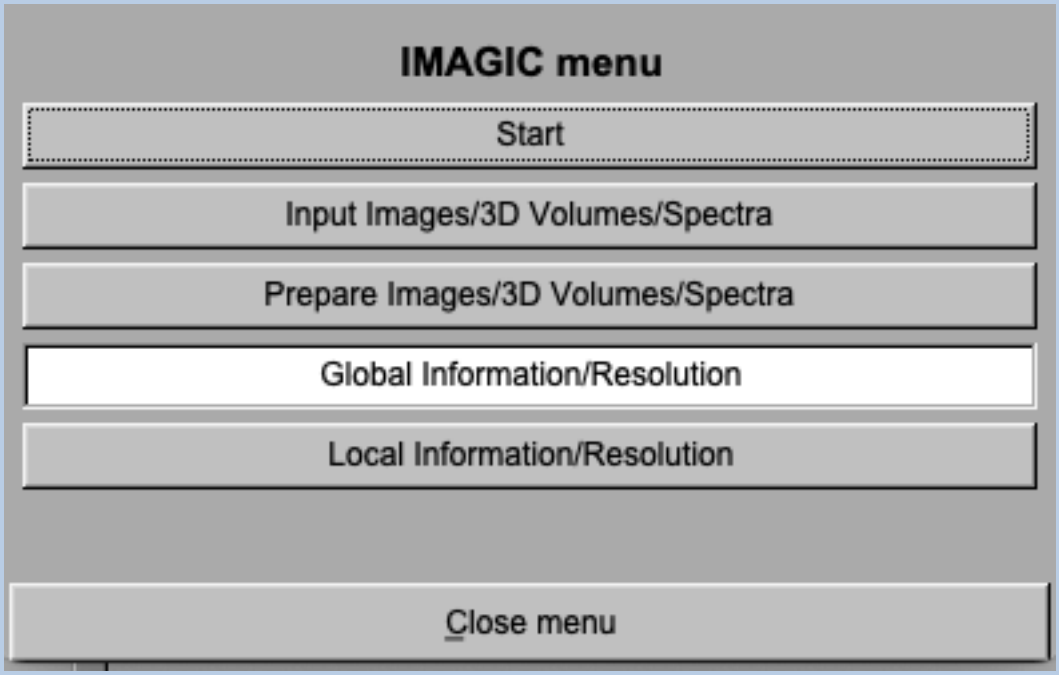
Open Menu	Open the MENU to navigate to each page wanted
Hide Tooltips / Show Tooltips	Show or hide the context sensitive tooltips (the help text may sometimes disturb)
Display	Open a DISPLAY page to visualize IMAGIC images. Refer to **guiDISPLAY**.
Movie	Open a MOVIE page (display in an endless loop). Refer to **guiDISPLAY**
Plot	Open a PLOT page to show IMAGIC curves. Refer to **guiPLOT**
Colour Mapping	Open a DISPLAY page to visualize IMAGIC images using a colour map stored in another input.
Command	Open a list to run any IMAGIC command. Refer to **guiIMAGIC**.
Shell	Run a shell / terminal page. command
Back	Go to the previous page
Next	Continue with the next page
Exit	Exit **guiFSC**



IMAGIC

guiFSC

The guiFSC Menu



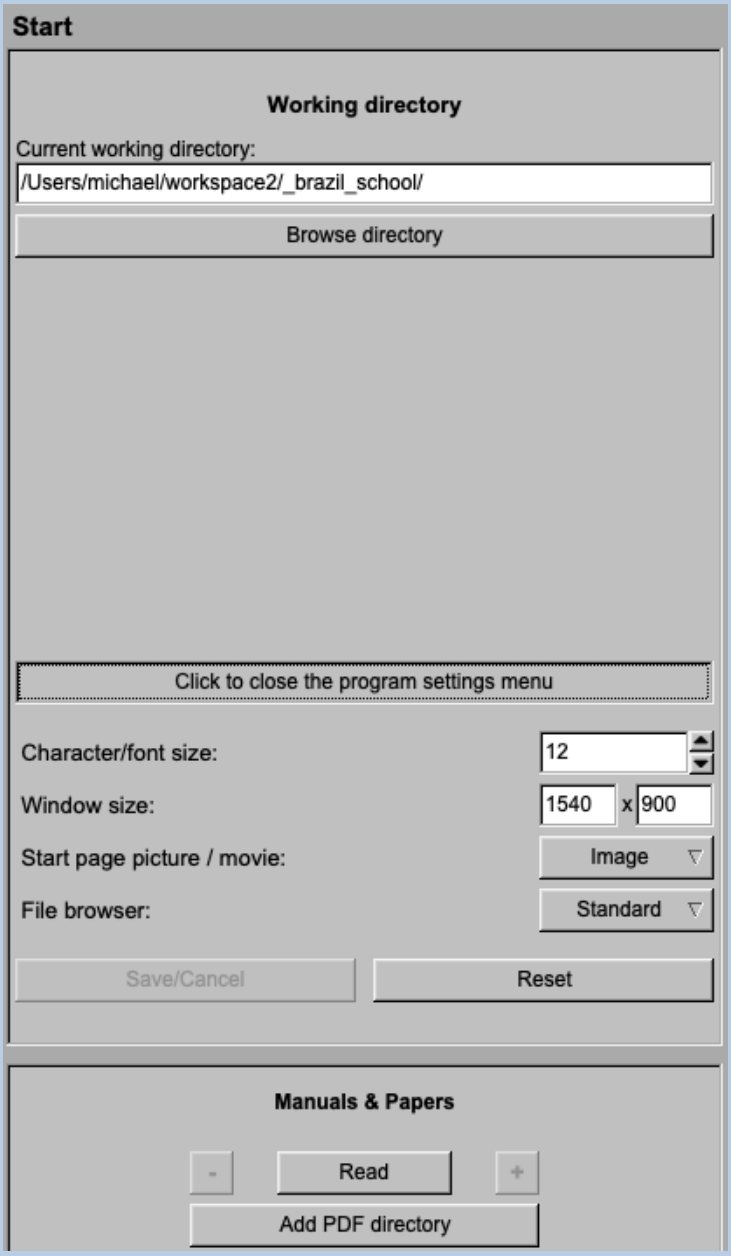
PAGES:

Start	Page to adjust **guiFSC** program parameters
Import Images...	Import or specify the input. Cut out a part, if wanted.
Prepare Images...	Pre-treatment: Mask, filter, normalise variance, resize, summing ...
Global information/Resolution	Calculate the global information content / resolution of the input data
Local information/Resolution	Calculate the local information / resolution maps of the input data

NOTE:
guiFSC will start with the “Global Information/Resolution” page. If you need to prepare the data you can use the IMAGIC menu buttons to navigate to the wanted pages.

The “Start” Page

This page is not part of the **guiFSC** workflow and can only be reached using the “Back” or the “Open Menu” button(s).



On this page you can set some program parameters:

a) the working directory
b) the size of the **guiFSC** program windows and/or text (a re-start is needed)
c) the type of file browser

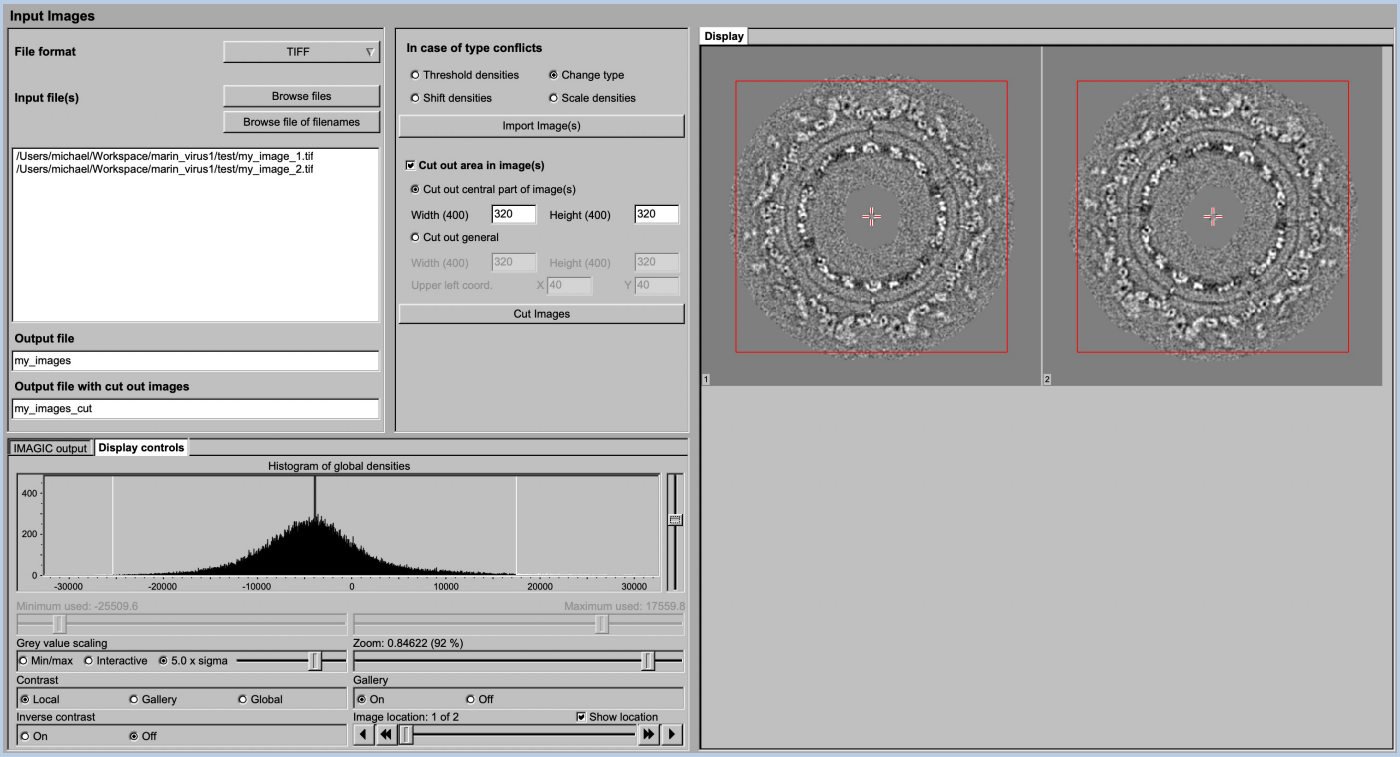
Start Working

guiFSC starts with the “Global Information/Resolution” page.

The workflow using the “Next” button will guide you through all Global Information/Resolution pages.

If you need to prepare the data you can use the “Back”, “Next” or “Open Menu” buttons to navigate to these preparation pages.

The “Import” Page



DESCRIPTION:

Convert import image/3D volume/spectra files using any 3D-EM format (or TIFF) into a single (stacked) IMAGIC image file.

The page can be skipped if your input images/3D volumes/spectra are already stored in IMAGIC format.

If wanted you can cut-out parts of the input.

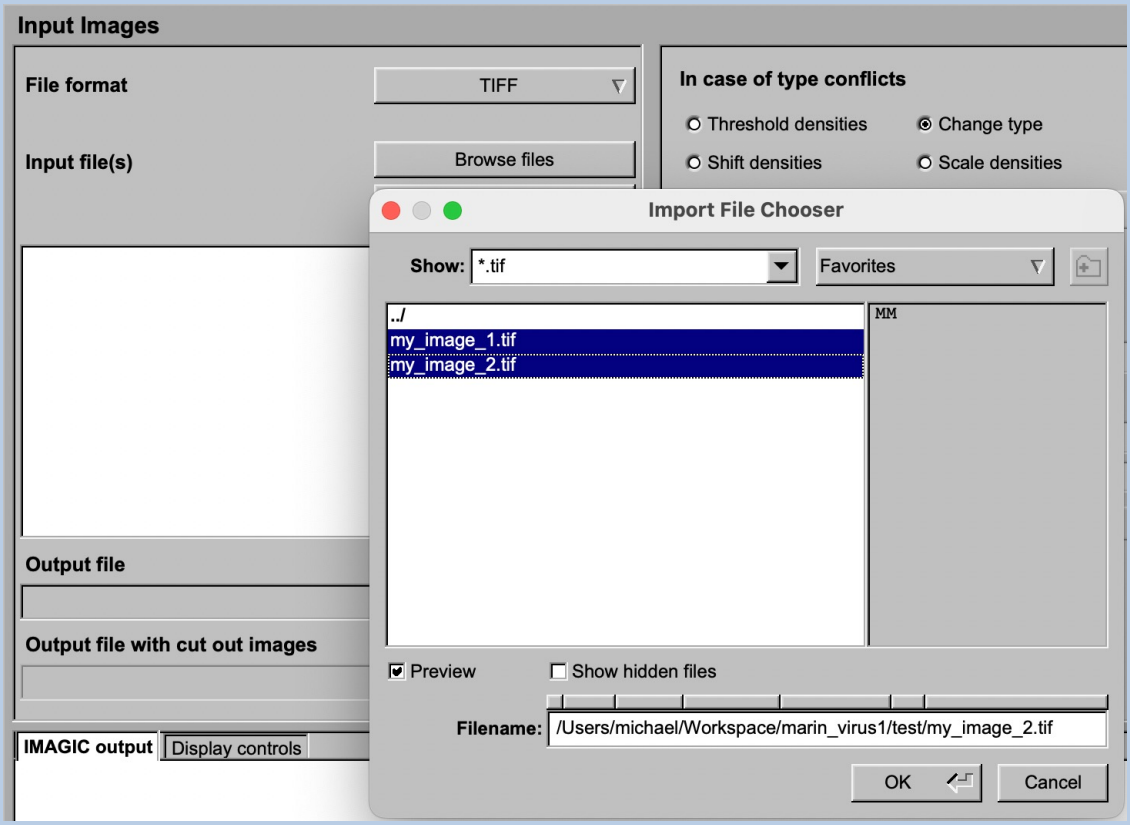
Also refer to program **guiIMPORT**.

IMPORT :

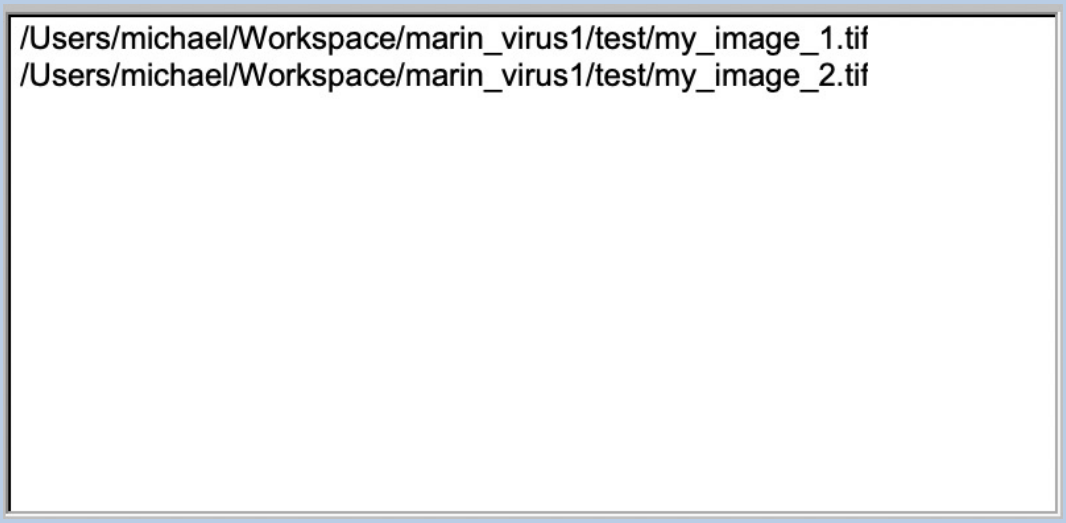
Specify the file format in which your input images/3D volumes/spectra are stored. Click the “Select format” button

and choose one of the formats in the listing.

Now you can specify the input files or a “File of filenames” text file (containing the names of the wanted input files) with the “Browse” button. Refer to chapter “Input Files” and ”Input. File Chooser” for help.



If wanted you can edit the list of files. But be careful there is no automatic control of file names in this list.



Next, you need to specify the name of the output file which is the IMAGIC image file which will contain the imported images/3D volumes/spectra.

Depending on the format of the input you have to specify a number of parameters or options.

Format TIFF, for example:

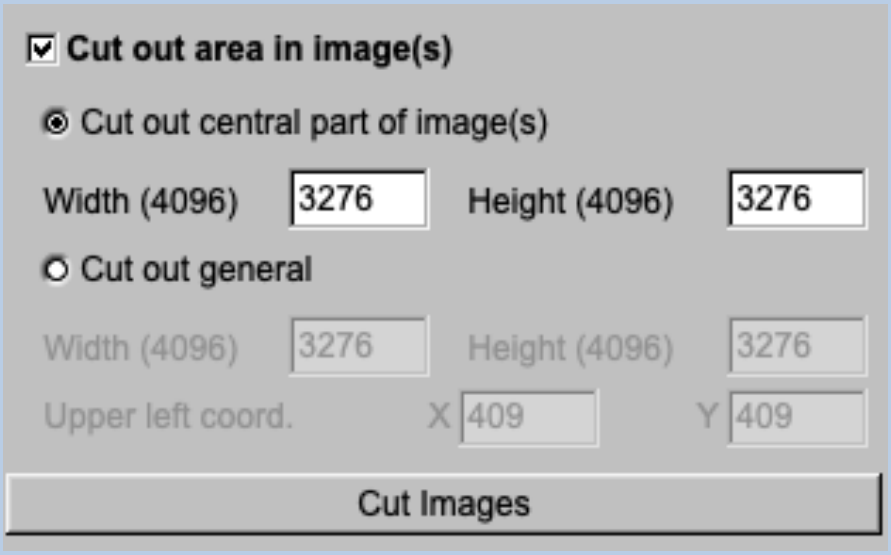
Having specified every information needed click the “Import” button to start the import of the images/3D volumes/spectra.

The imported images/3D volumes/spectra are shown in the display/plot tab on the right-hand side. See chapters “A Typical Page - Display control tabs” or “A Typical Page - plot control tabs”.

CUT:

Having imported the input images/3D volumes/spectra, you may want to not use the full size but only a part of them.

Clicking the “Cut out area” option you can cut-out parts of the imported images/3D volumes/spectra :

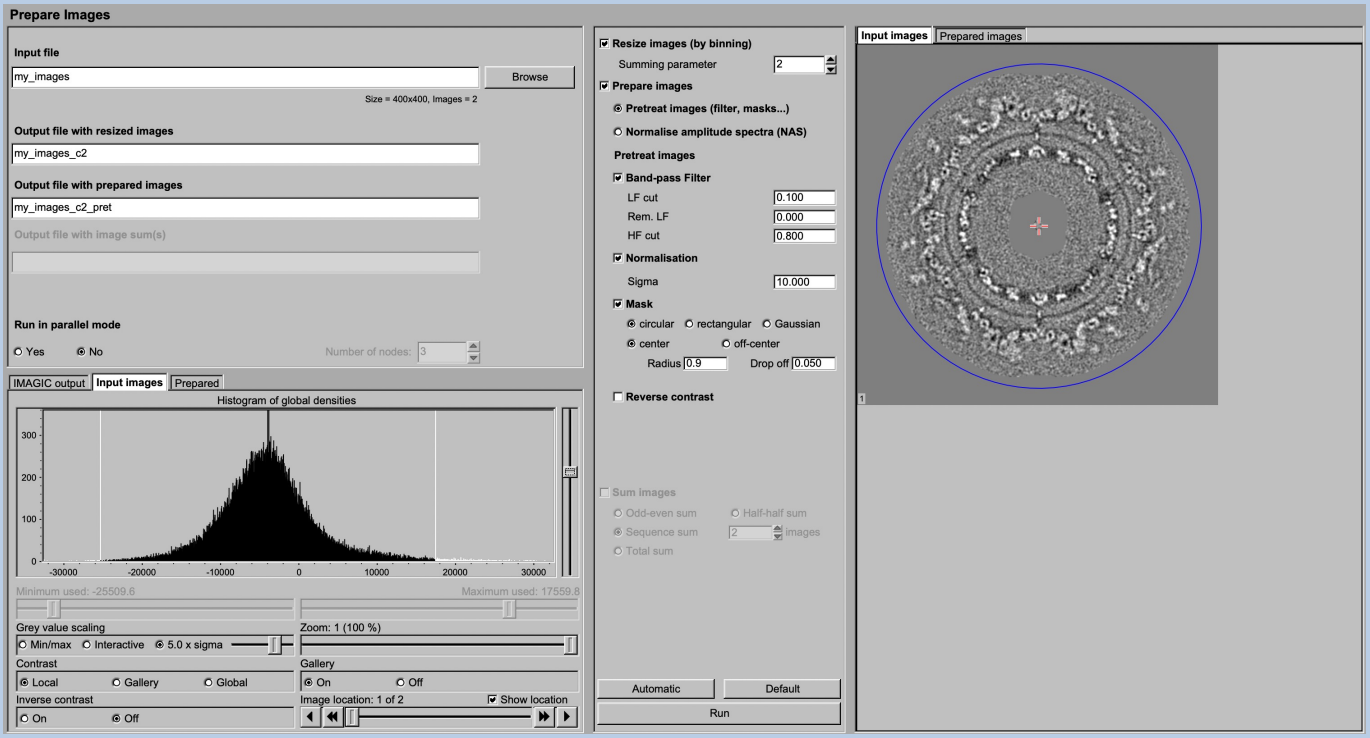


The chosen part is shown in the display window. You can cut-out a central part or any part wanted. The cut-out part is the same in all images/3D volumes/spectra, of course.

The name of the output file containing the cut-out images/3D volumes/ spectra is suggested on the left-hand side. As usual you can change this name, of course.

Having specified everything click the “Cut” button to run the calculations.

The “Prepare” Page

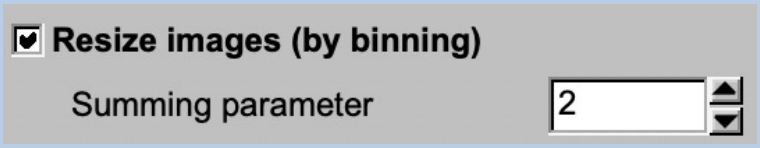


DESCRIPTION:

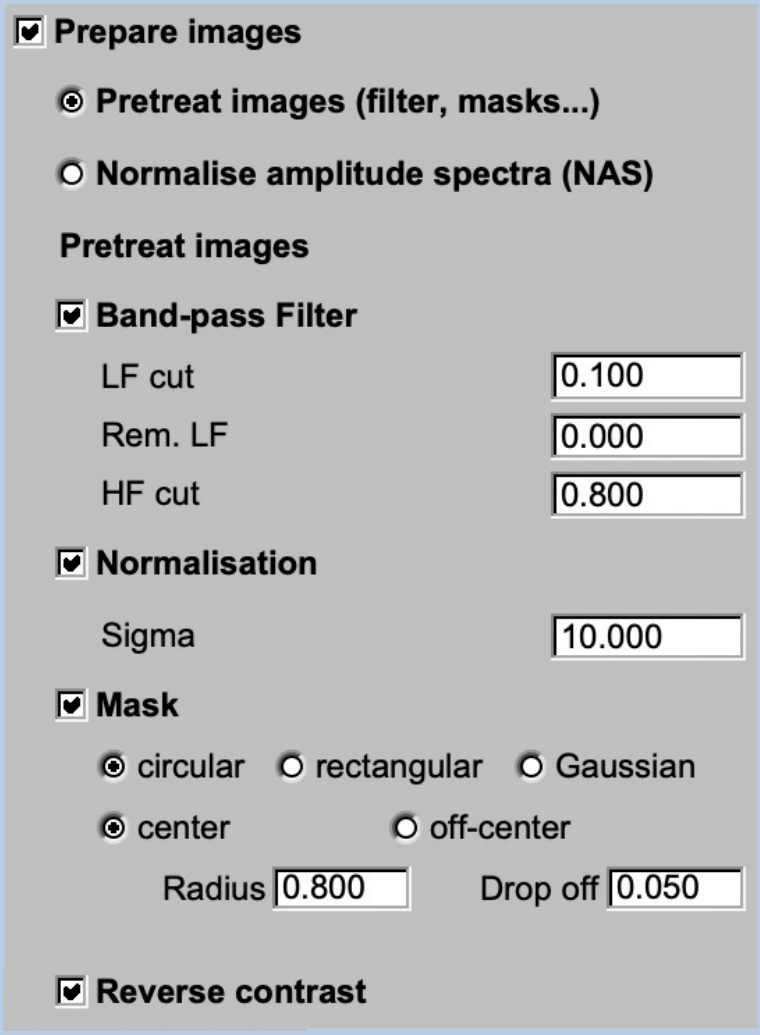
It can be helpful to pre-treat the input import image/3D volume/spectra by imposing a band-pass filter, normalise the variance, impose a mask and...

NOTE:
Of course, you can skip this page if no such treatment is wanted/needed.

You can resize the images by binning

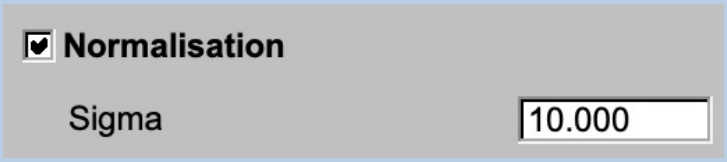


You can pre-treat the images. Options are

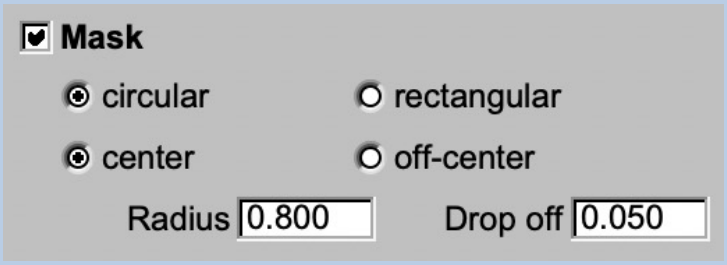


Options are band-pass filtering

normalise the variance in each image

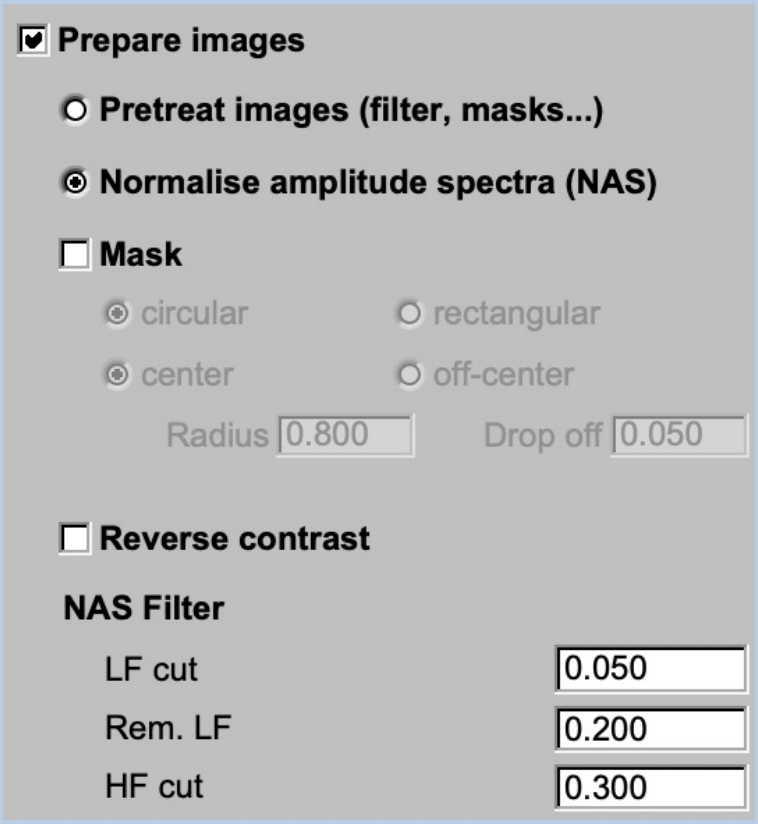


imposing a mask



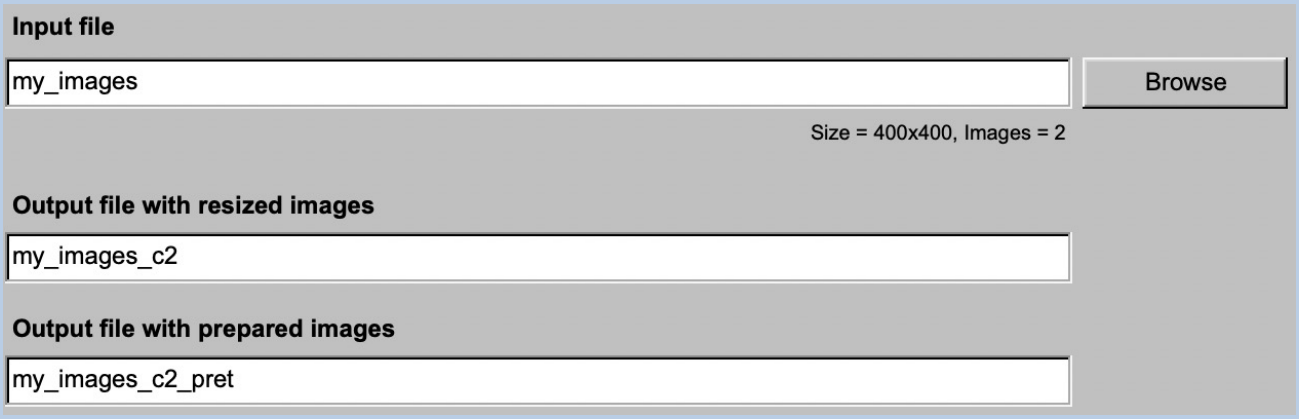
if wanted you can also reverse the contrast.

Instead of using these pre-treatment options you can apply a NAS filter which means that the amplitude spectra of the images are normalised:

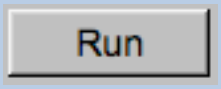


Imposing a mask and reversing the contrast are also options here.

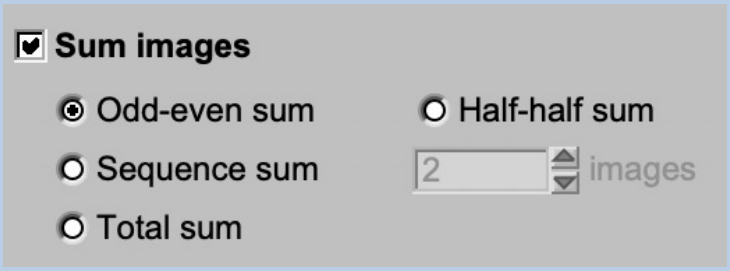
As usual, specify the names of the input and the output files:

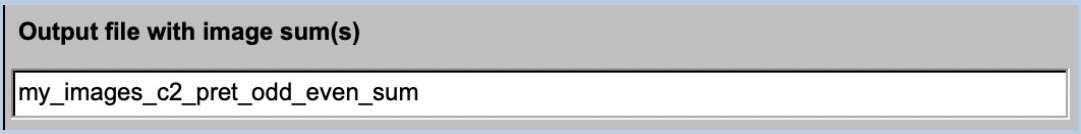


Start the calculations by clicking the “Run” button:



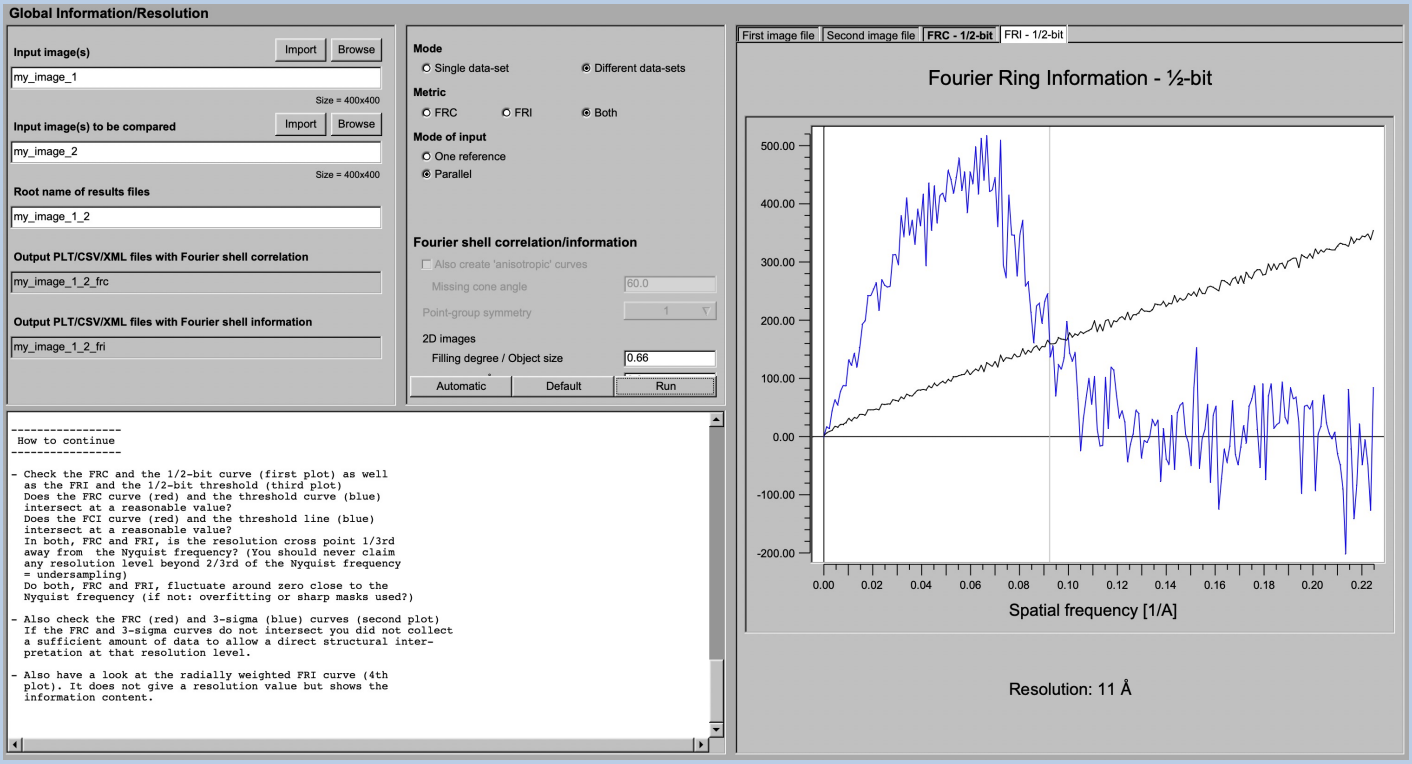
You can also create various image sums which you may need for the subsequent calculations of the global and local information content in your images.





Note:
The preparation was described for images. But input can also be curves/spectra/1D image or 3D volume(s) files. In this case the pages are modified for the other dimension but the content and the use of the pages are the same or similar.

The “Global Information/Resolution” Page

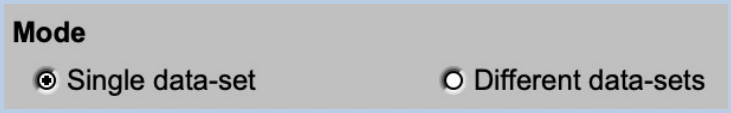


DESCRIPTION:

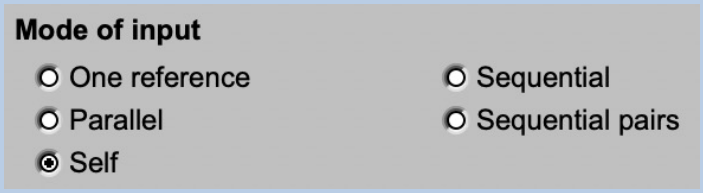
Calculate the global information content (using the Fourier ring or shell information metric (FRI/FSI) and/or the global resolution (using the Fourier ring or shell correlation metric (FRC/FSC).

The comparison(s) can be calculated between different images / 3D volumes or by comparing each image / 3D volume with itself.

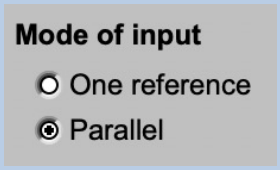
Specify if input are two different or a single data-set. Note that these two different option are not relevant for the calculations but to give better help and tool tips.



In “Single data-set” you have to choose which of the following input modes is to be used



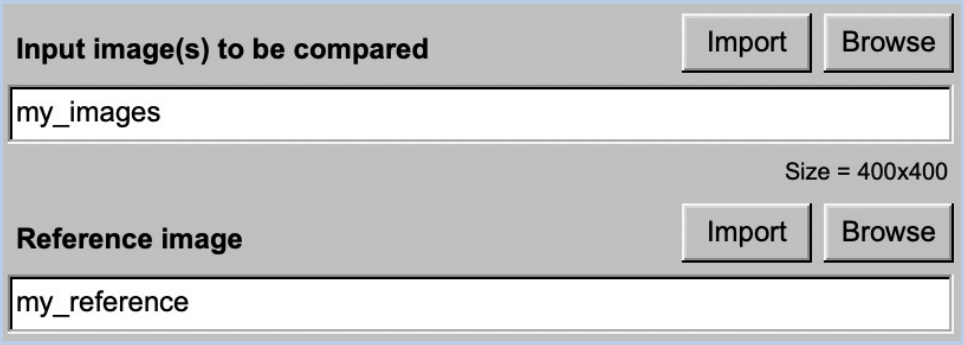
in “Different data-set” the input mode can be one of the following:



Modes of input are:

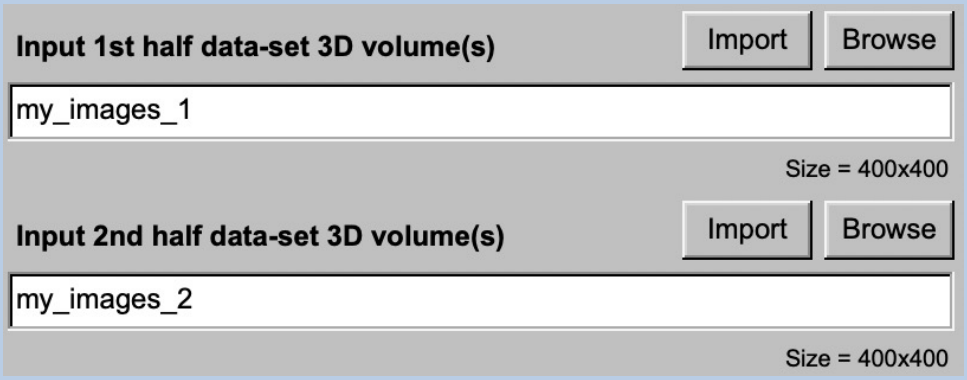
- One reference:

  The input images / 3D volumes are compared with a single reference



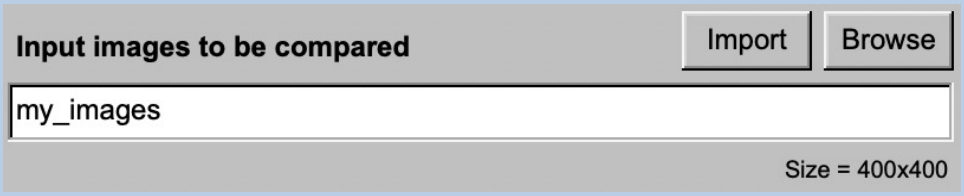
- Parallel:

  The images / 3D volumes in the first input file 1 are compared one-by-one with the images / 3D volumes in the second input file. Both input files are expected to contain the same number of images / 3D volumes.



- Sequential:

  The images / 3D volumes to be compared are stored in a single input file. The comparison is in sequential order which means The image / 3D volume #1 is compared with #2, #2 with #3 etc.

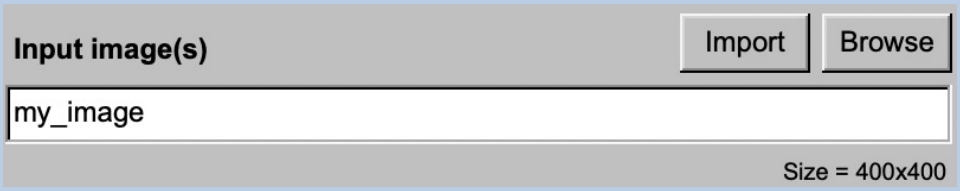


- Sequential pairs

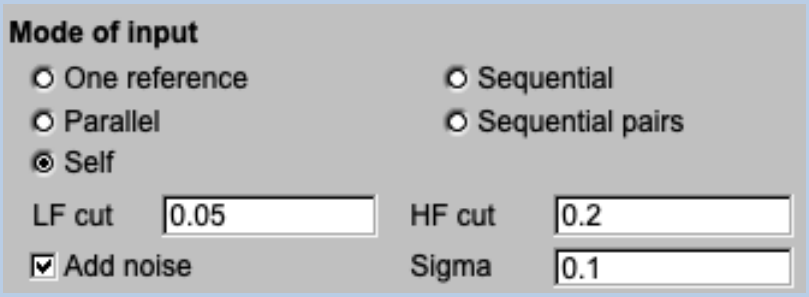
  Same as sequential but the comparison is done pair-wise: The image / 3D volume #1 is compared with #2, #3 with #4, #5 with #6 etc.

- Self

  In case of a “Single dataset” there is an additional mode of input option “Self” which allow to calculate the information content/resolution using single images / 3D volumes which are only compared with itself but NOT with any other images / 3D volumes.



Note that in case of option “Self” you have to specify the parameters of a band-pass filter and you can add noise to remove high frequency information introduced by this very specific “self compare” technique:



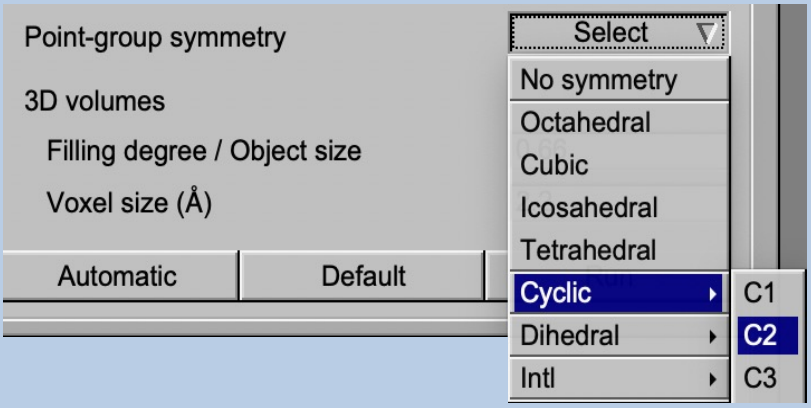
Next you are expected to choose the wanted metric:

Options are:

Images	FRC	Global resolution using the Fourier Ring Correlation
	FRI	Global information using the Fourier Ring Information
	Both	Calculate both, FRC and FRI
3D volumes	FSC	Global resolution using the Fourier Shell Correlation
	FSI	Global information using the Fourier Shell Information
	Both	Calculate both, FSC and FSI

In case of input images you have to specify (or check) the filling degree (move the cursor to the input field to get help) and the pixel size:

In case of input 3D volumes you also have to specify the symmetry:

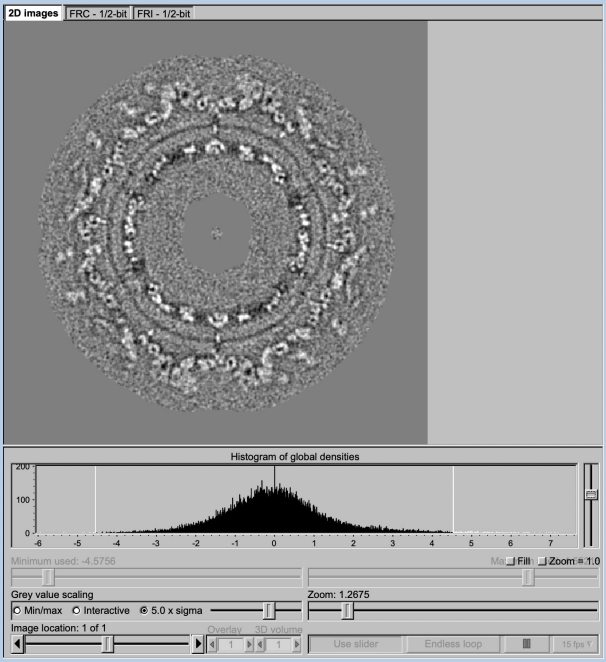


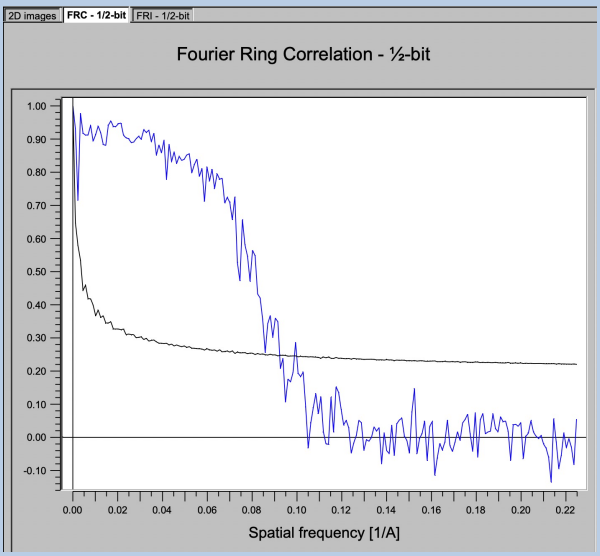
Move the cursor to an input fields to get context sensitive help.

As usual you can reset all parameters to the last values you have used by clicking the “Default” button, clicking the “Automatic” button will reset all parameters to the values suggested by **guiFSC**.

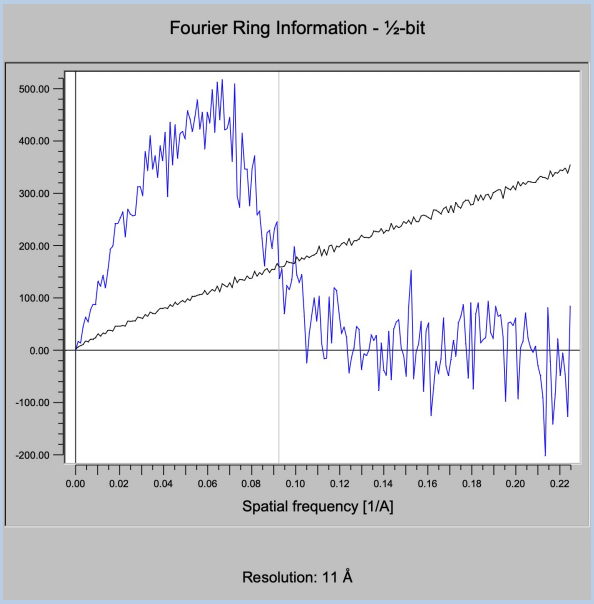
Clicking the “Run” button will start the calculations.

As usual the input images and the resulting information and/or resolution curves are displayed in the display/plot tabs on the right-hand side.



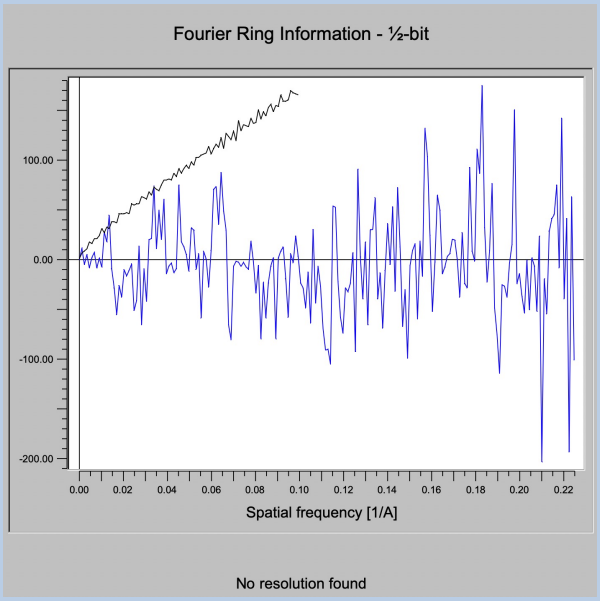


In contrast to the FRC/FSC curve the FRI/FSI plots also show the estimated resolution value in case FRI/FSI curve and the ½-bit threshold curve intersect:



The ½-bit information curve indicates where a sufficient amount of data is collected to allow a direct interpretation at that resolution level. The overall resolution achieved is estimated by the intersection of the FRI/FSI curve (blue) and the 1/2 Bit curve (black).

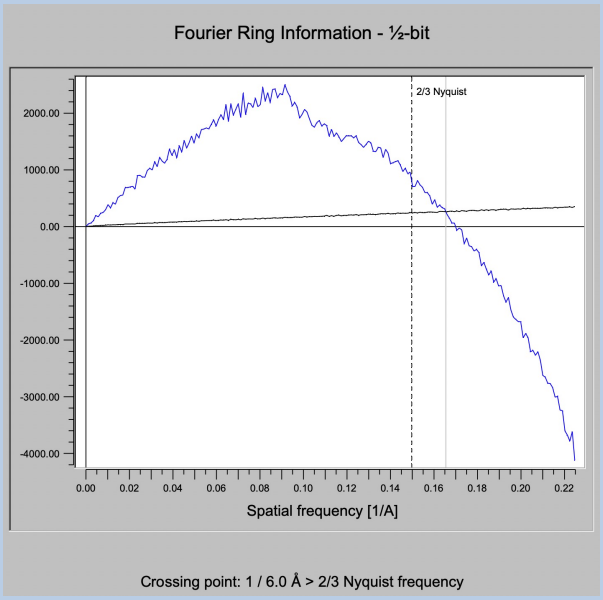
No resolution is printed if the estimated resolution value is too small :



No resolution but a warning is printed if the estimated resolution value is too small or too close to the Nyquist frequency.

PLEASE NOTE:

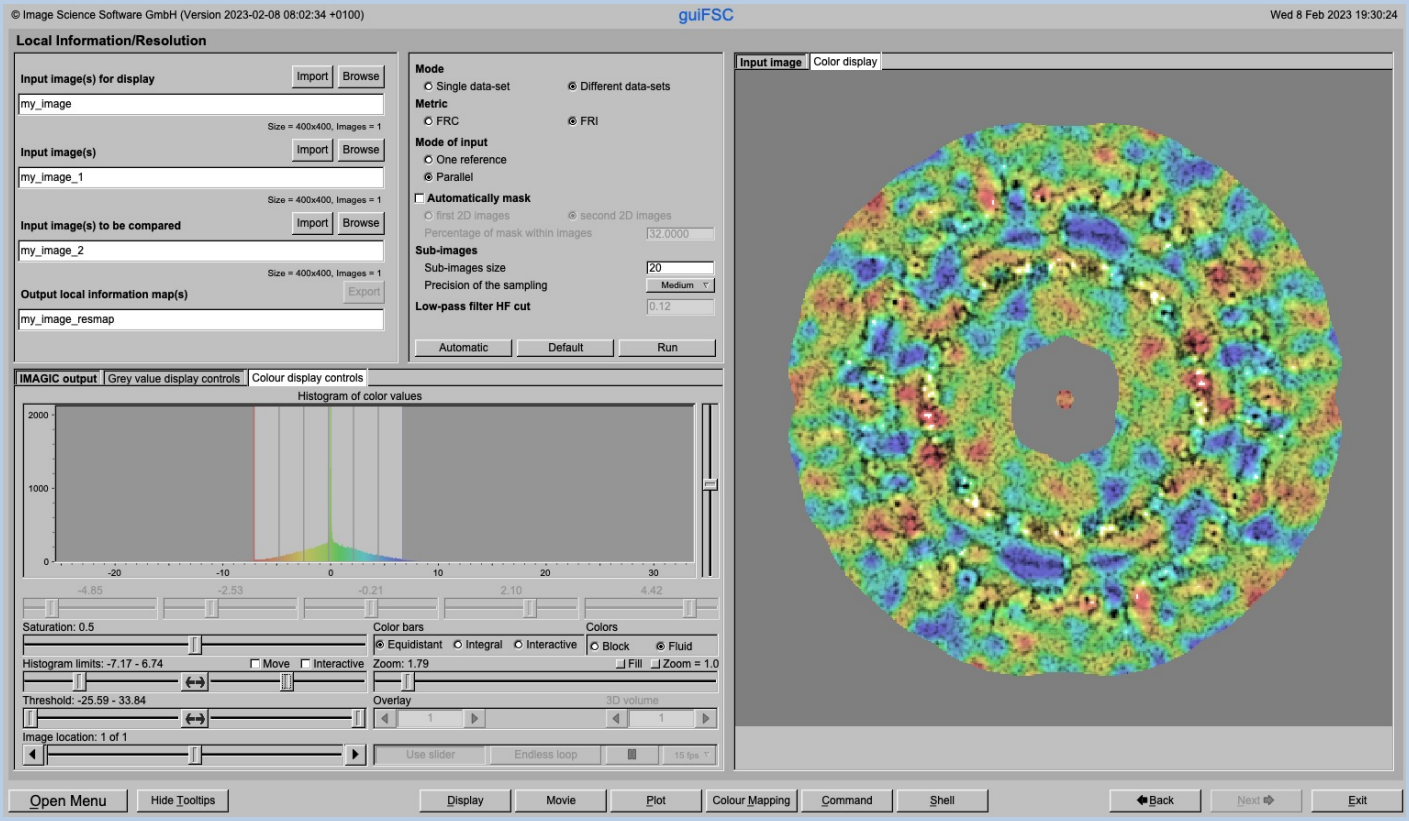
Under-sampling remains one of the worst sins one can commit in estimating the resolution / information content in images / 3D volumes. You should never claim any resolution level beyond 2/3rd of the Nyquist frequency.



As usual you can click the “Next” button to navigate to the next page which is the “Local Information / Resolution” page.

Note:
Global information/resolution was described for images. But input can also be curves/spectra/1D image or 3D volume(s) files. In this case the pages are modified for the other dimension but the content and the use of the pages are the same or similar.

The “Local Information/Resolution” Page

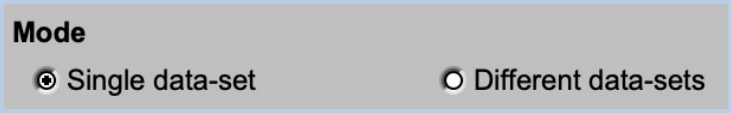


DESCRIPTION:

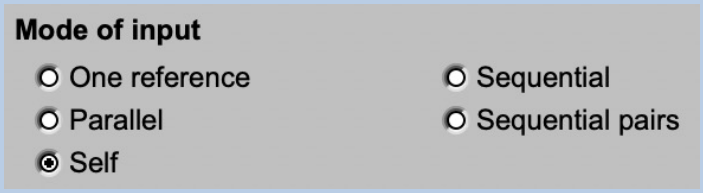
Calculate the local information content (using the Fourier Ring or Shell Information metric (FRI/FSI) and/or the local resolution (using the Fourier Ring or Shell Correlation metric (FRC/FSC).

The comparison(s) can be calculated between different images / 3D volumes or by comparing each image / 3D volume with itself.

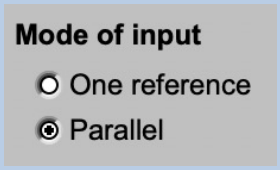
Specify if input are two different or a single data-set. Note that these two different option are not relevant for the calculations but to give better help and tool tips.



In “Single data-set” you have to choose which of the following input modes is to be used



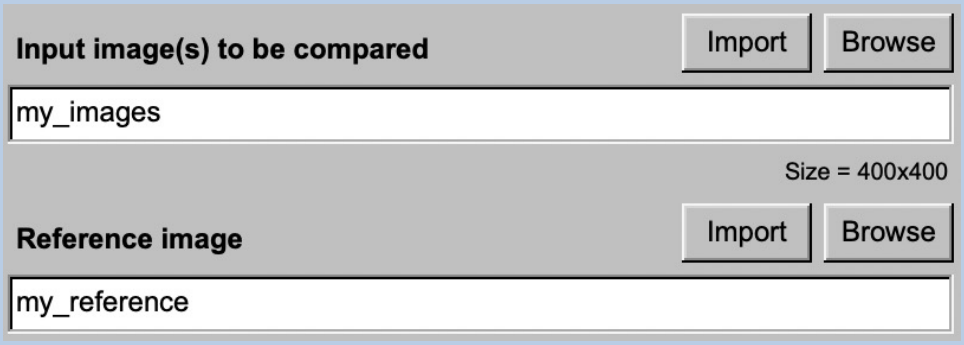
in “Different data-set” the input mode can be one of the following:



Modes of input are:

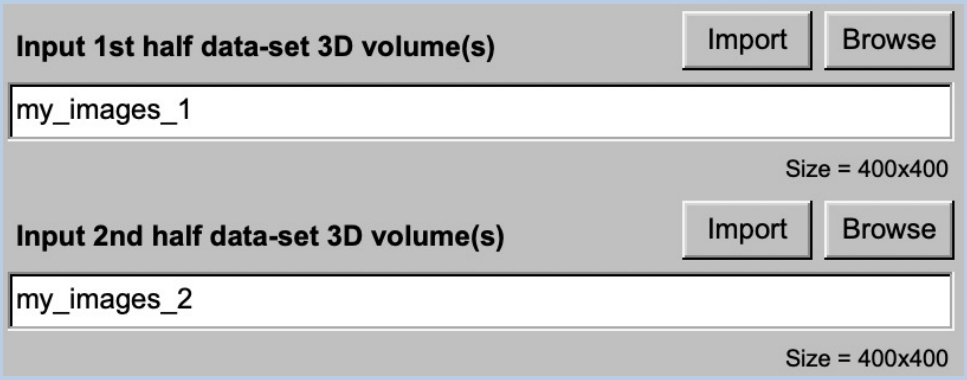
- One reference:

  The input images / 3D volumes are compared with a single reference



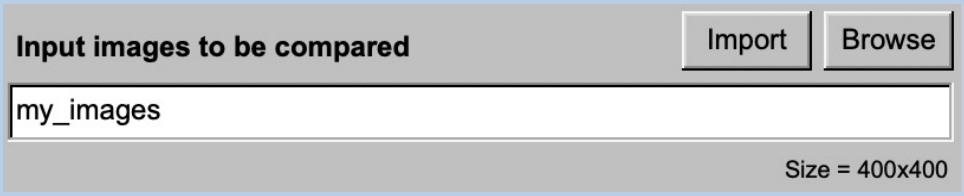
- Parallel:

  The images / 3D volumes in the first input file 1 are compared one-by-one with the images / 3D volumes in the second input file. Both input files are expected to contain the same number of images / 3D volumes.



- Sequential:

  The images / 3D volumes to be compared are stored in a single input file. The comparison is in sequential order which means The image / 3D volume #1 is compared with #2, #2 with #3 etc.

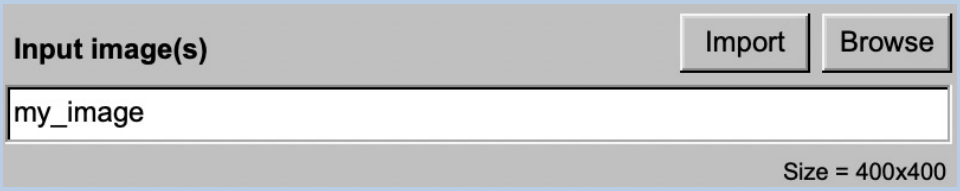


- Sequential pairs

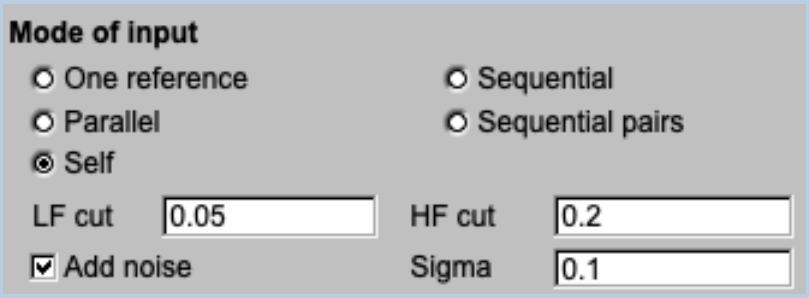
  Same as sequential but the comparison is done pair-wise: The image / 3D volume #1 is compared with #2, #3 with #4, #5 with #6 etc.

- Self

  In case of a “Single dataset” there is an additional mode of input option “Self” which allow to calculate the information content/resolution using single images / 3D volumes which are only compared with itself but NOT with any other images / 3D volumes.

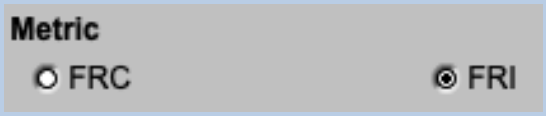


Note that in case of option “Self” you have to specify the parameters of a band-pass filter and you can add noise to to remove high frequency information introduced by this very specific “self compare” technique:



The first input file which name you have to specify is asked for all option. It is NOT needed for the information/resolution calculations. It is only needed in the COLOURISED DISPLAY where it is used as grey-image which is colourised by the calculated information/resolution map. So this image / 3D volume usually is a sum of the input images compared are the full resolution 3D volume in case an odd and even 3D reconstruction is used. When using the “Self” comparison mode this input file is usually the same as the input file to be used for comparison.

Next you are expected to choose the wanted metric:

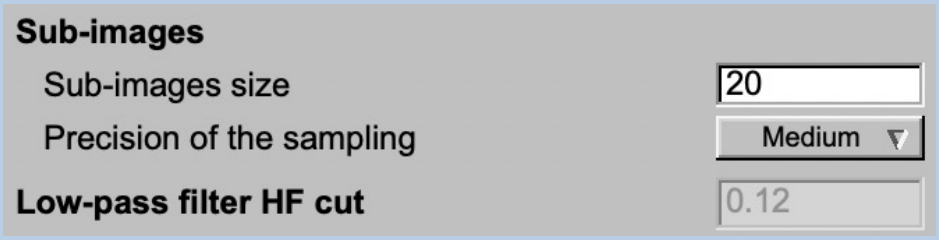


Options are:

Images	FRC	Global resolution using the Fourier Ring Correlation
	FRI	Global information using the Fourier Ring Information

3D volumes	FSC	Global resolution using the Fourier Shell Correlation
	FSI	Global information using the Fourier Shell Information

The input images / 3D volumes are windowed to calculate the information content / resolution locally. You can change the size of this window area as well as the precision of the related sampling. The hight frequency cut of the low-pass filter I calculated automatically:

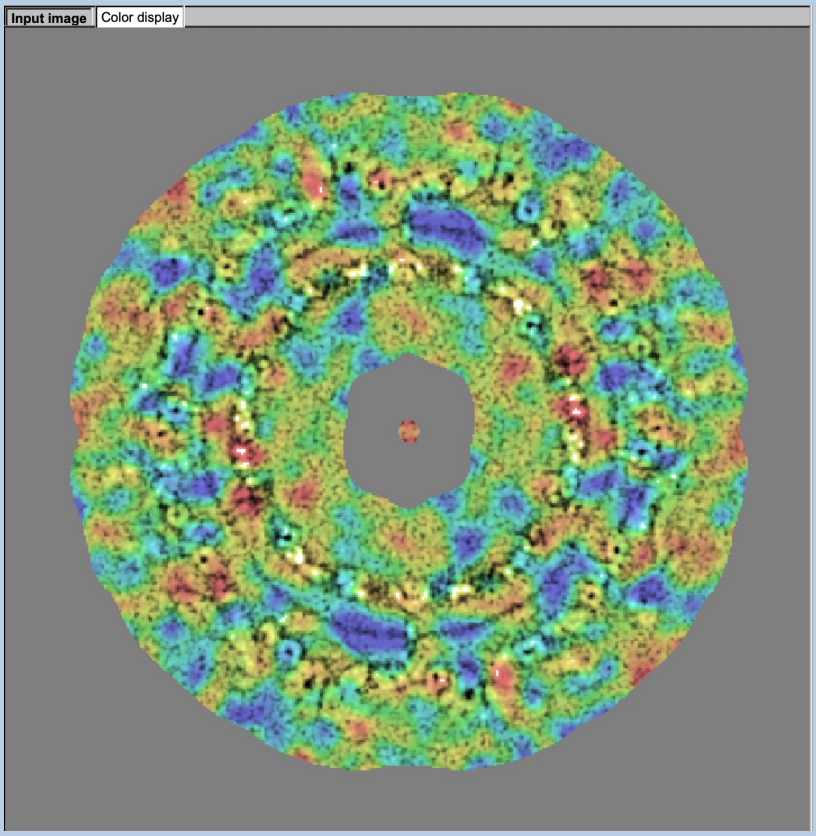


Before starting the calculations adjust the display settings of the grey scale image displayed on the right-hand side. Refer to the “guiDISPLAY manual” if you need help.

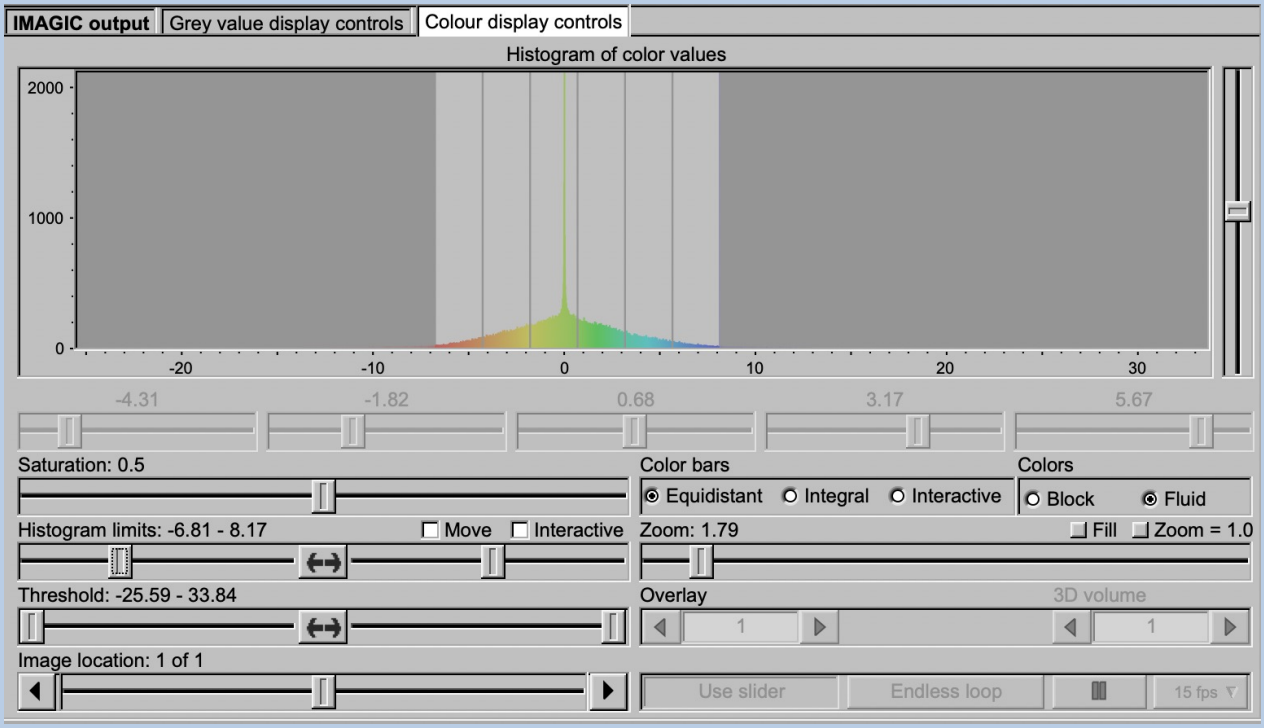
As usual you can reset all parameters to the last values you have used by clicking the “Default” button, clicking the “Automatic” button will reset all parameters to the values suggested by **guiFSC**.

Clicking the “Run” button will start the calculations.

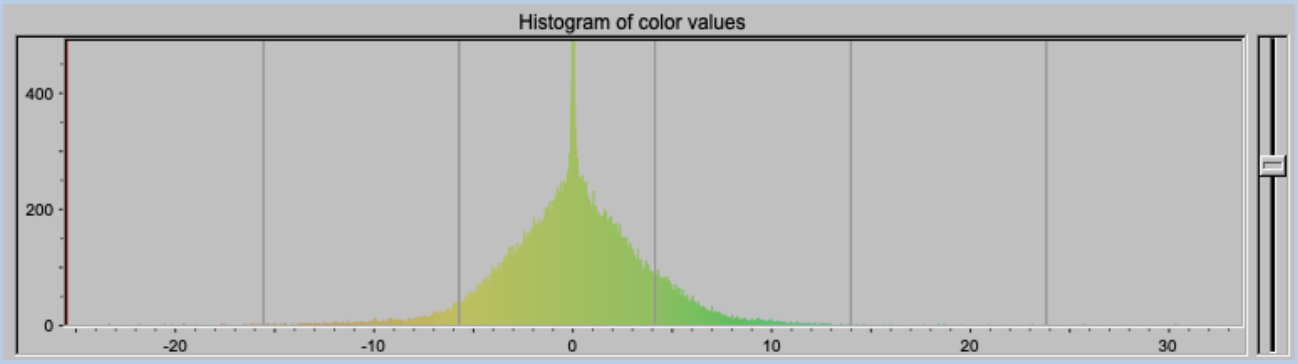
The output file will contains the local information / resolution map(s) but the result showing the local information / resolution is the colourised display on the right hand-side. The local information content/ resolution is colour coded in a palette from red (low) to blue (high).



Use the “Colour display controls” to adjust the colourised display on the right-hand side.



The histogram shown in the “Colour display controls” is the histogram of colour values used.



Use the slider to adjust the vertical scaling of the histogram.

The colour mapping can be adjusted by a number of parameters and options. Refer to the ”**guiColourMapping** manual” for details.

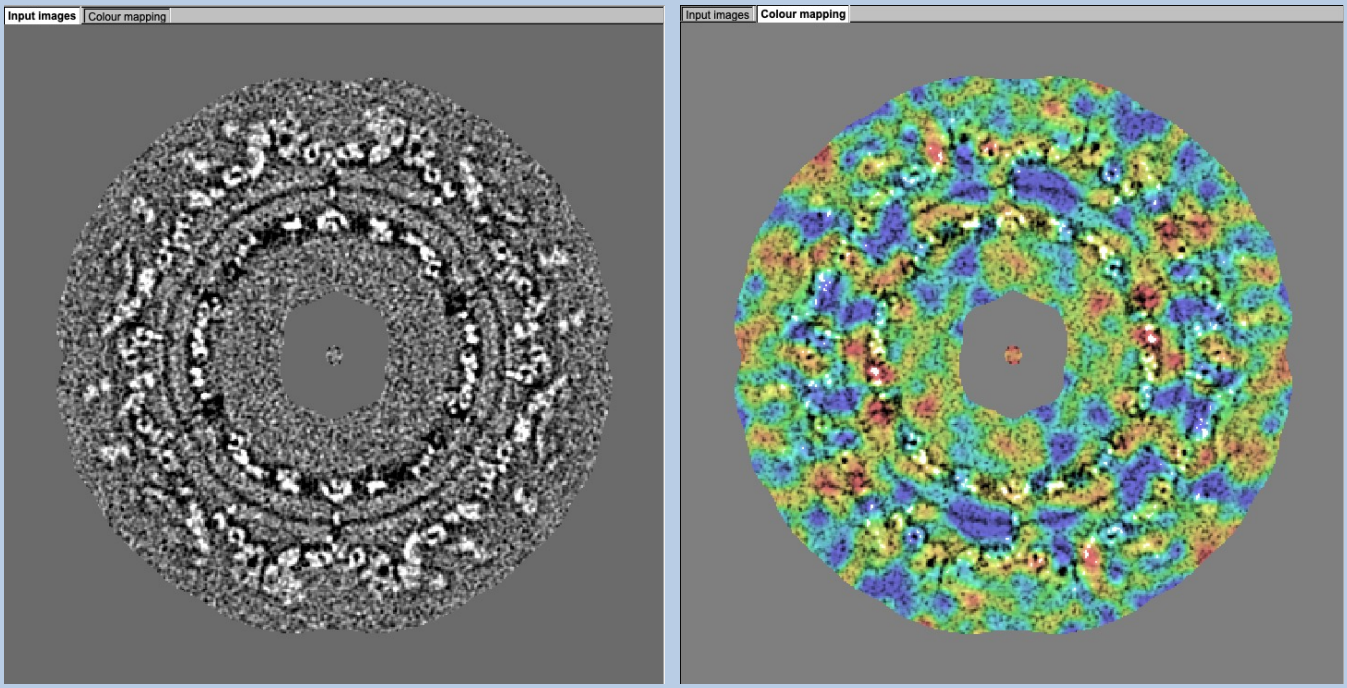
Saturation:	Adjust the saturation
Histogram limits:	Use the two sliders to adjust between which values the colour palette is used.
	Interactive: Set the limits by giving numbers
	Move: Move the chosen limits through the palette
Colour bars	How to use the colour palette
	Equidistant: The colour palette is used linearly
	Integral: The colour palette is squeezed according to the number of histogram values
	Interactive: Use the histogram sliders to set the colour bars
Colours:	Block: Fixed colour between two colour bars
	Fluid: The colours are changing continuously
Zoom	Enlarge the displayed image using the slider
	Fill: Fit image size to window size
	Zoom = 1.0. Display image 1:1
Threshold:	Adjust the threshold limits with the two sliders. Colours below the threshold are displayed in grey, colours above the threshold are displayed white
Image Locations.	Use the slider or the arrows to select image locations

COMPARE TWO DIFFERENT
LOCAL INFORMATION / RESOLUTION MAPS:

Note that the value of each colour is an absolute value. If you want to compare different information / resolution maps make sure that the same “Histogram limits” are used. Activate the ”Interactive” option and specify the same limits in both colourised displays for a correct comparison.

Display Windows

The image(s) are displayed in the display windows at the right hand side. Click the related tab to get the wanted display window.



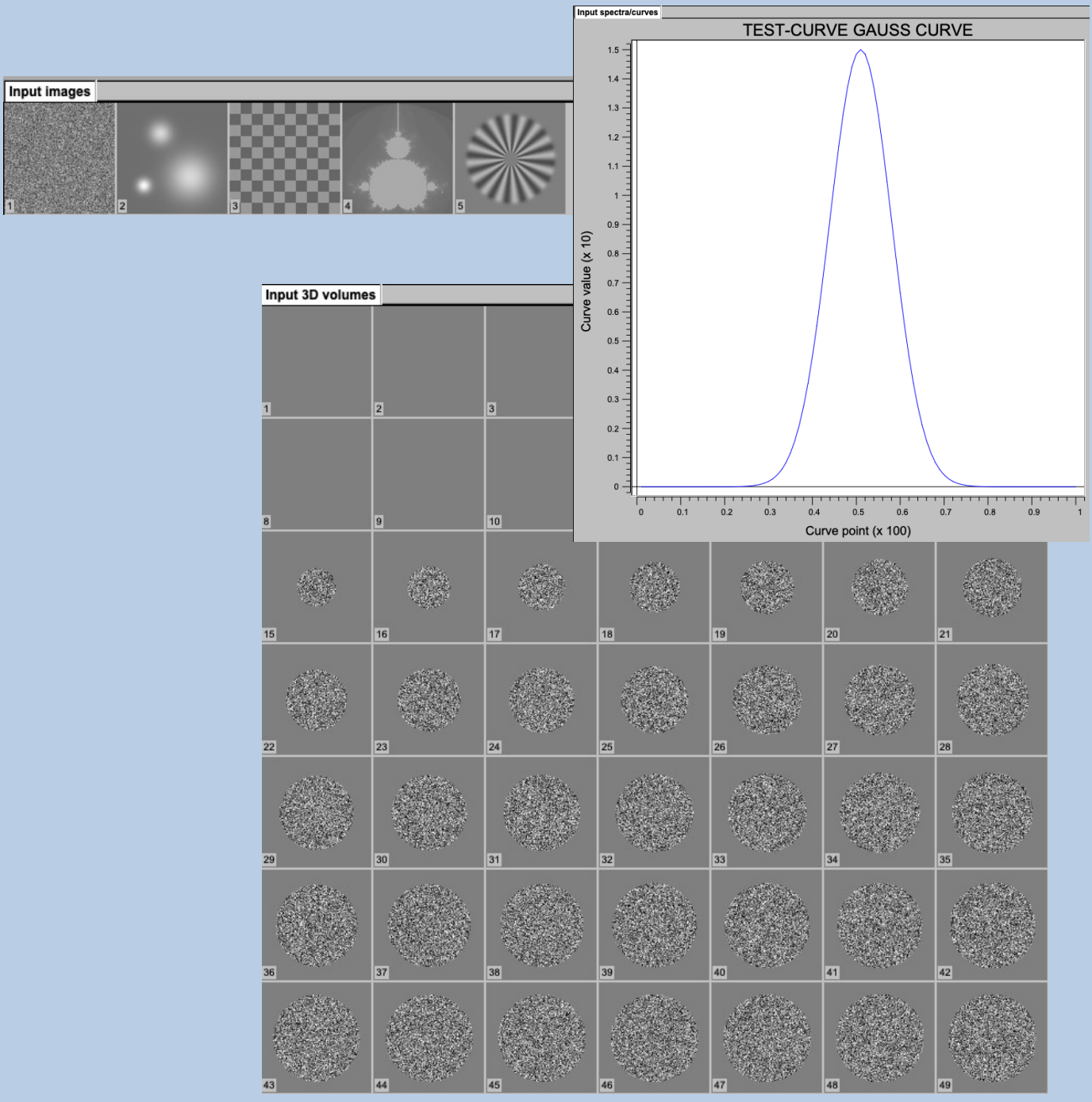
Moving the cursor into the display window there are a few options:

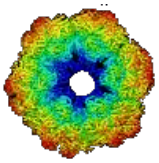
New window	Open the display window a larger separate display window
Save	Save the current displayed image in a JPG image
Save all	Save the whole window in a JPG image

Dimensions

In the previous pages **guiFSC** was described for images.

But input can also be curves/spectra/1D image or 3D volume(s) files for global information/resolution and 3D volumes for local information/ resolution. In these cases the pages are modified for the other dimension but the content and the use of the pages are the same or similar.





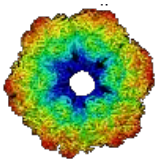
IMAGIC

guiFSC

Not (yet) possible

The following options are not (yet) possible:

- Run in batch mode.
- Store output files and results of different pages in different sub-directories of the working directory.



IMAGIC

guiFSC

Feedback / Error hints

We intensively tested the **guiFSC** program and tried to find all possible errors and inconsistencies. But the program is very complex and still in progress. So you may still find some problems.

We are happy to get feed-back. Please send your comments, error hints etc. to

imagic@ImageScience.de

THANK YOU VERY MUCH.

Image Science

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