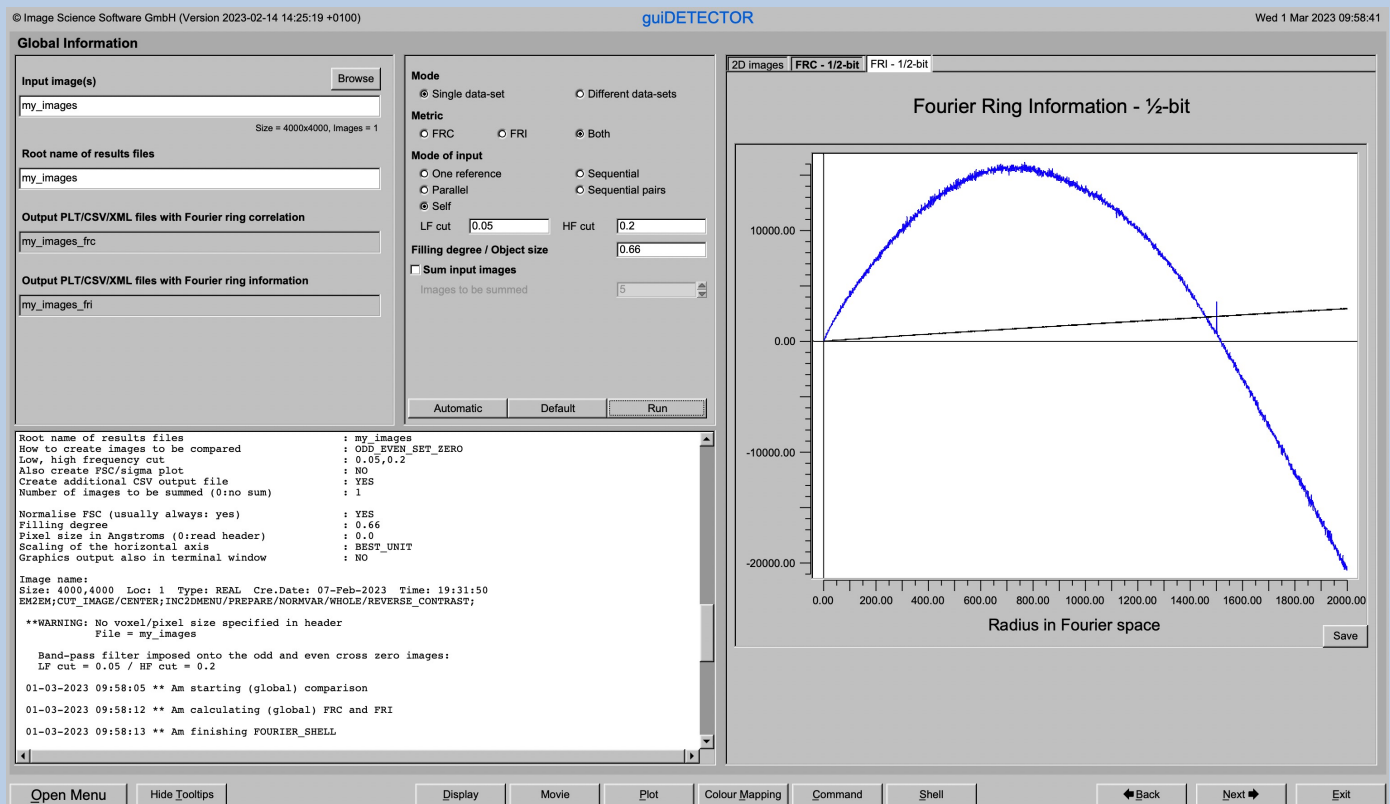




A Brief Introduction

Version 10-Oct-2023
www.ImageScience.de
© Michael Schatz (Image Science)

The IMAGIC guiSPECTRA program



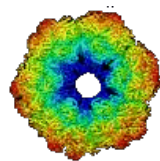
The **guiSPECTRA** program offers a number of options to estimate the global and local information content of in a data-set of curves/spectra/1D images.

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

CONTENT:

- IMAGIC GUI programs
 - **guiSPECTRA**
 - Import Spectra
 - Prepare Spectra
 - MSA and Classification.
 - Global Information
 - Local Information
 - Error hints
- How to use IMAGIC GUI programs
 - How to use **guiSPECTRA**
 - How to import spectra
 - How to pre-treat spectra
 - How to find the main variances and classes
 - How to estimate the global information content
 - How to estimate the local information content
 - How to send us feedback





IMAGIC

GUI Programs

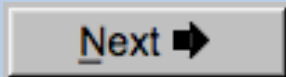


Workflow

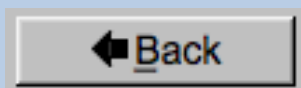
The idea of **guiSPECTRA** is to guide you through a typical camera/detector correction measurement or camera .

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

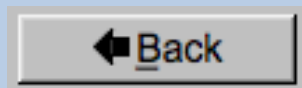
If **guiSPECTRA** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

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

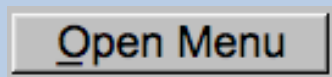
```
IMAGIC-COMMAND : gui-SPECTRA
```

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 **guiSPECTRA**.

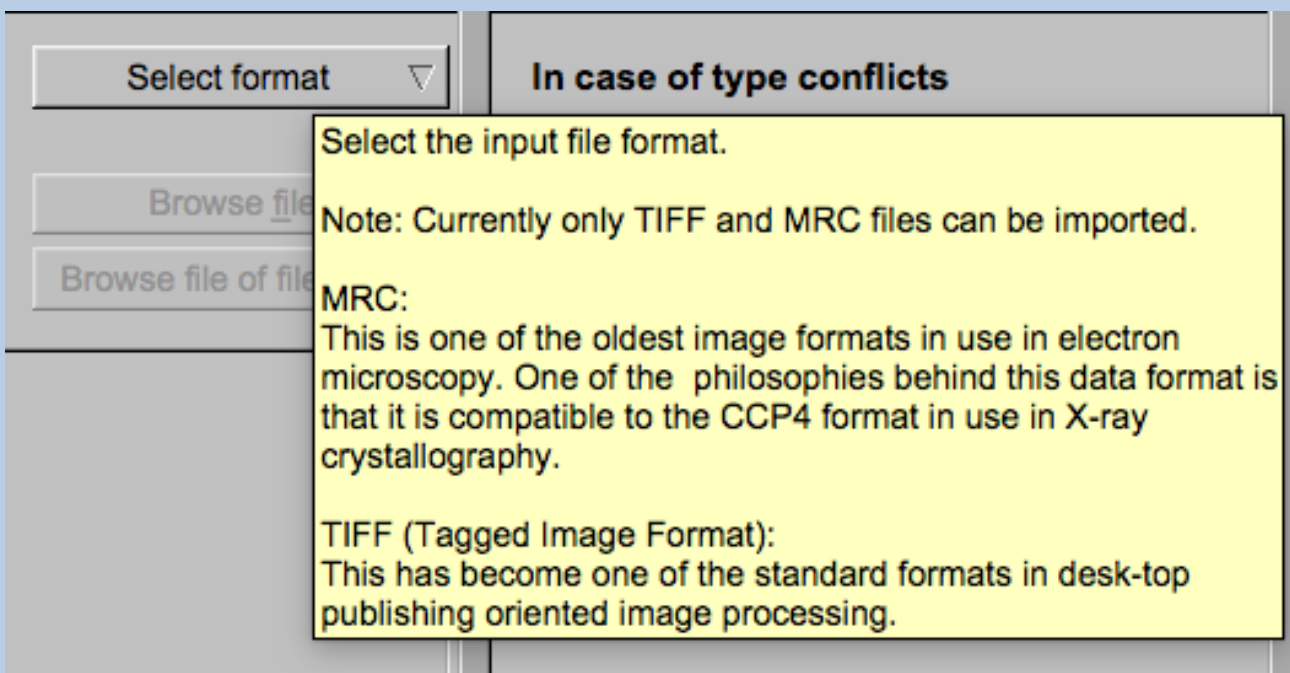
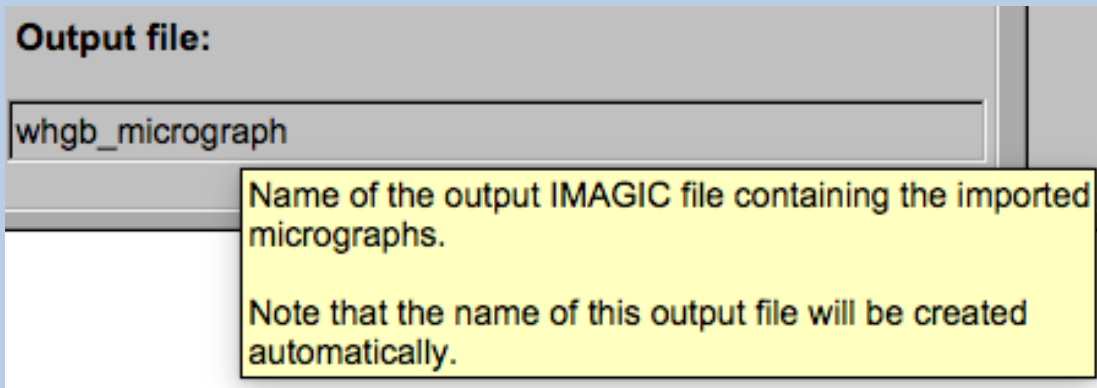
All output files will be stored in the working directory which you have specified on the start page.

Input files can be chosen from other directories.



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.

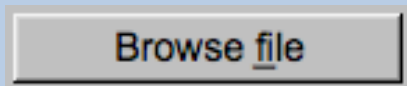
You can, of course, always use other input files names and even use other input directories.

Input file	
<input type="text" value="my_spectra"/>	<input type="button" value="Browse"/>

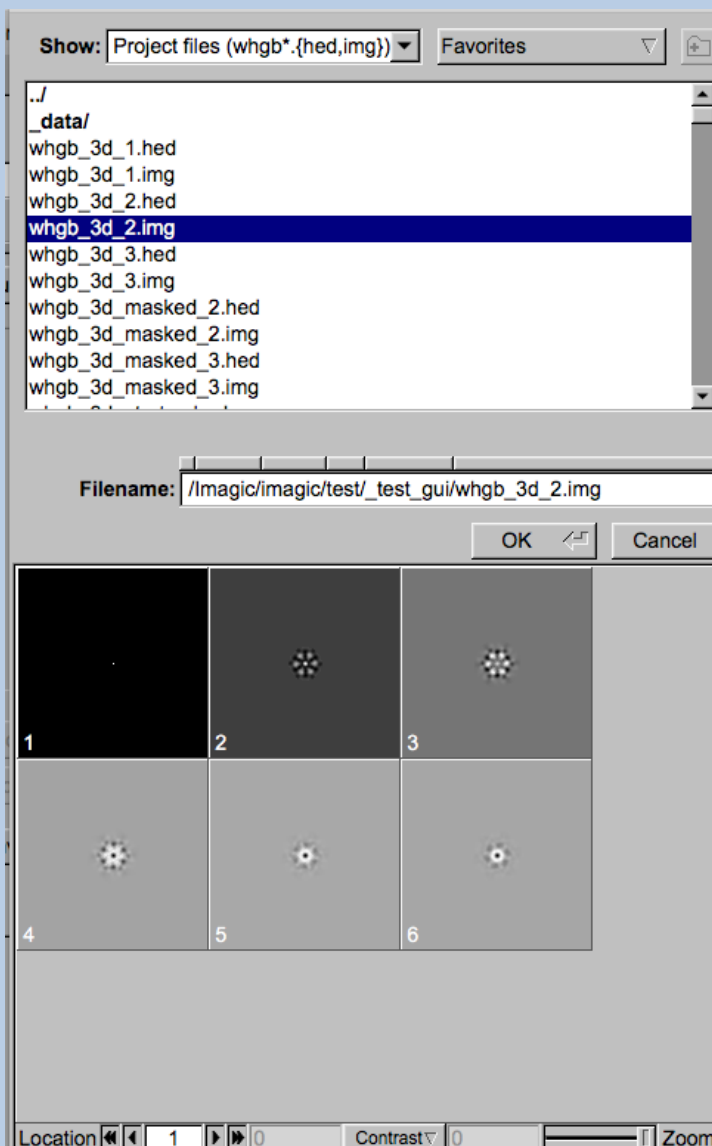


Input File Chooser

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

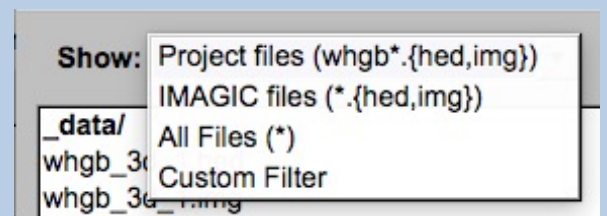


Pressing this button will open the IMAGIC file chooser:

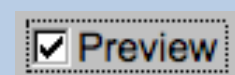


Choose the wanted file by clicking its name

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.

Output file	Export
my_spectra	



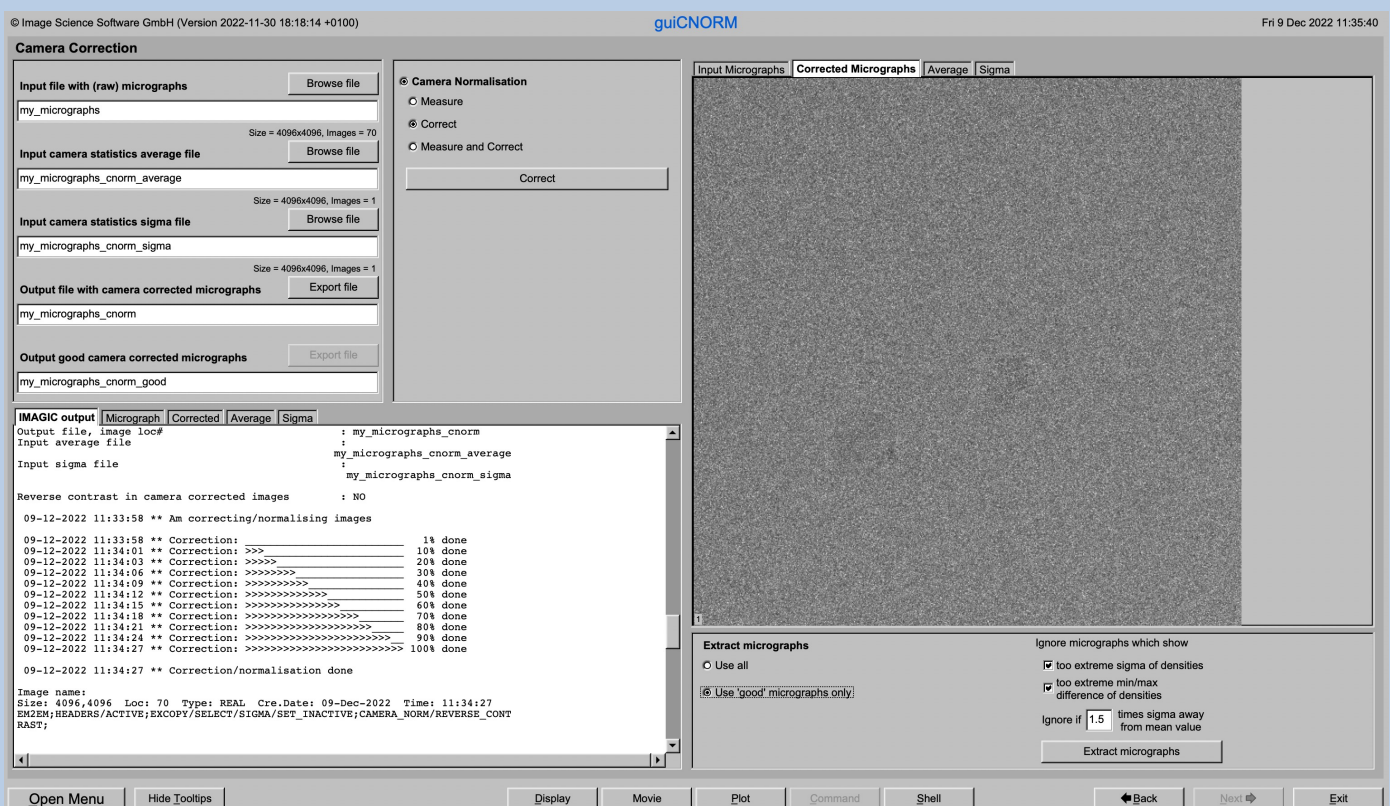
A Typical Page

A typical **IMAGIC GUI program** page has three columns.

The left part contains the file information and a kind of terminal window showing the print-out of the currently running IMAGIC program(s). In additional tabs you can find the control windows to adjust the displays on the left hand side.

The middle part usually contains parameters to be specified and a single or a number of “Run” buttons to start the calculation(s).

The right part displays input and output images. Sometimes it can also contain additional follow-up calculations and the related “Run” buttons.



A Typical Page - Program Parameters

Mode of preparation

☒ Pretreat images

☐ Normalise amplitude spectra (NAS)

Pretreat images

☒ **Band-pass Filter**

LF cut

Rem. LF

HF cut

☒ **Normalisation**

Sigma

Mask

Radius

Drop off

☐ Test loc. # to

☒ Run for all particles


☒ **Centre particles**

☒ Self rotate ☐ Self

☐ Total sum ☐ Mass center

☒ Test loc. # to

☐ Run for all particles



In the middle part of a typical **IMAGIC GUI program** page you will find the program parameters to be used.

Radio Buttons are showing options. One option only has to be used.

☒ Self rotate ☐ Self



☐ Total sum ☐ Mass center

Click buttons are showing options which you can use or not.

☒ **Band-pass Filter**

In text fields you can type in the wanted value. If the needed value is a number you can also move the cursor into this field, press the mouse key and keep it pressed and move the cursor to change the value.

There are also boxes where you can use up and down arrows to change the value.



A Typical Page - Automatic / Default

☐ Resize/Coarsen micrographs
Summing parameter 2

☐ Create patches
Size of patches 4096

☒ Prepare micrograph
Low freq. cut 0.0200
Remaining low frequency 0
High freq. cut 0.9000

☒ Remove outlier pixels
Outlier is 4.50 sigma off the mean value

☒ Invert densities

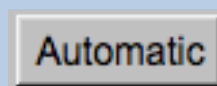
☐ Resize/Coarsen prepared micrographs
Summing parameter 2

Automatic Default

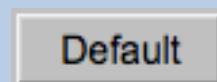
Run

In the middle part of a typical **IMAGIC GUI program** page you will also find “Automatic” and “Default buttons.

Pressing the “Automatic” button will fill in the values suggested by IMAGIC.



Pressing the “Default” button will fill in the values which you have used during the last “Run”.



The values shown when entering a page are the default values (your last values given) if they are available. Else the automatic values are shown.



A Typical Page - Run buttons

Create prepared amplitude images

☒ **Filter micrographs**

Low freq. cut

Remaining low frequency

High freq. cut

Filter amplitude images

Low freq. cut

Remaining low frequency

High freq. cut

Coarsen filtered amplitude images

☒ Yes ☐ No

Summing parameter

MSA options

☐ MSA eigenfilter amplitudes

☒ MSA classify amplitudes

MSA

Inner radius of ring mask

Outer radius of ring mask

Number of eigenimages

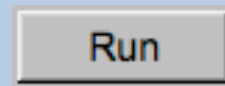
Number of iterations

Classification

Use how many eigenimages

Number of classes

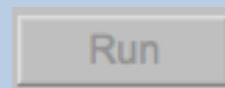
To run the calculations press the “Run” button.



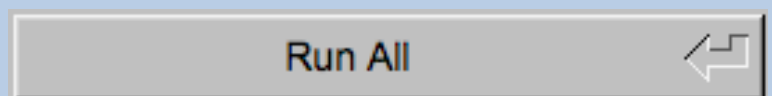
On a number of pages the calculations can be split. In this case you will find more than one single “Run” button.

Not running everything at once can be helpful when testing parameters.

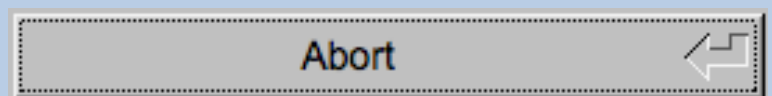
Maybe a certain “Run” button is not yet activated because it needs the results of calculations not yet done.



Pressing the “Run All” button starts all calculations currently activated on the page.

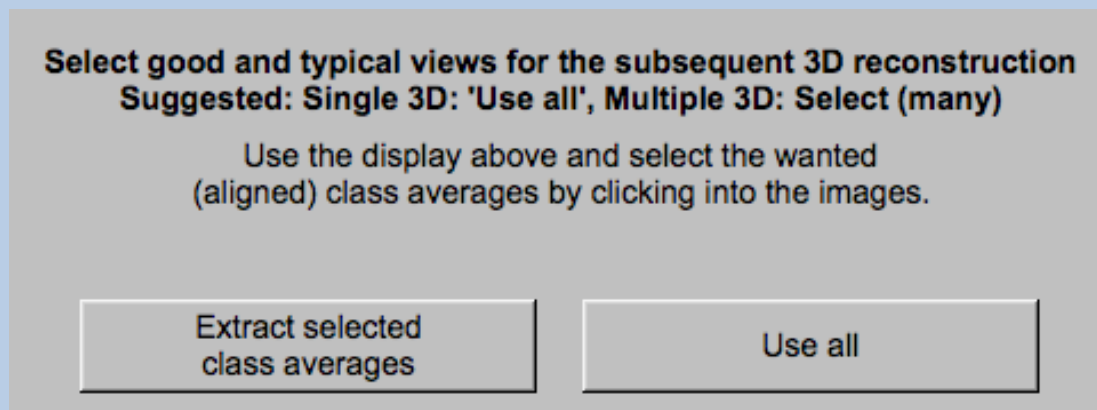


You can abort a running program by pressing the “Abort” button.



A Typical Page - Additional Tasks

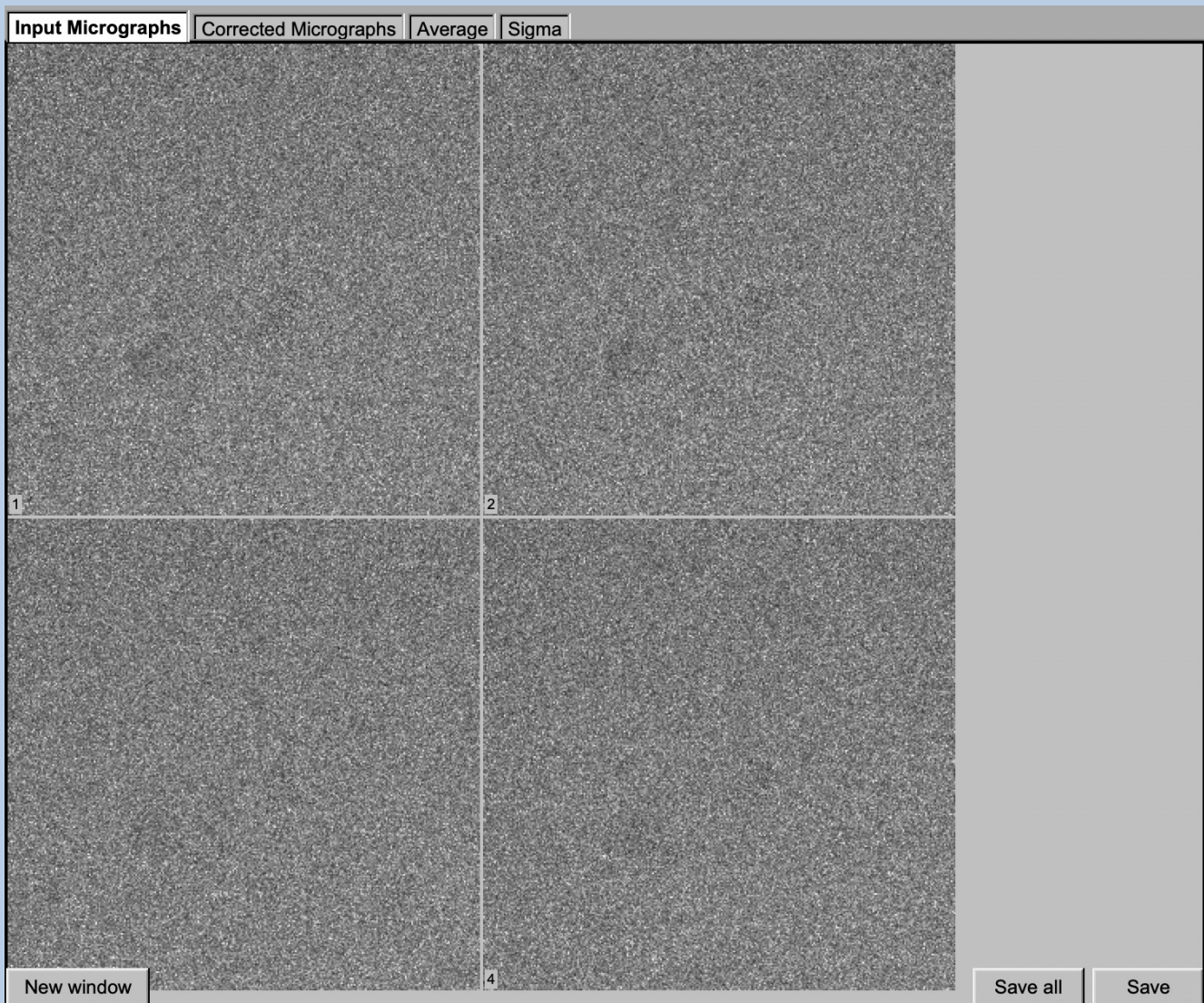
The main calculations on the page are done using the middle part of an typical **IMAGIC GUI program** page. But on a number of pages some additional calculations have to be done. Please follow the instructions given.



Note that the new output images are usually shown in a new display tab.



A Typical Page - Plot/Display of Spectra



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

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

Double click into the wanted plots or use the "New Window" button to get an enlarged plot window. Use "Save" to store the plot (JPG).

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



“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 what is input in the PLT file

Reset:. Reset to the automatic values

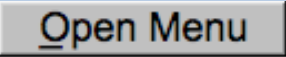
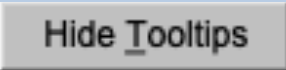
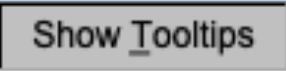
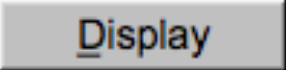
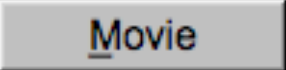


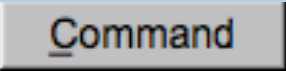
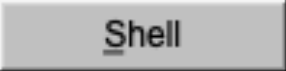
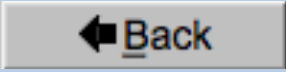
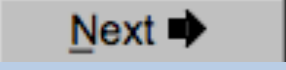
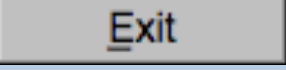
Style	Colour	Grid
Select curve style ▼	Select curve colour ▼	Select curve grid ▼
<hr/>		
Horizontal scaling	<input type="checkbox"/> Use for all plots	
1.00	32.00	Reset
<hr/>		
Vertical scaling	<input type="checkbox"/> Use for all plots	
-19.21	17.00	Reset
<hr/>		
Plot title	<input type="checkbox"/> Use for all plots	Reset
Fourier Ring Information - 1/2-bit		
<hr/>		
Text along horizontal axis	<input type="checkbox"/> Use for all plots	Reset
Radius in Fourier space		
<hr/>		
Text along vertical axis	<input type="checkbox"/> Use for all plots	Reset



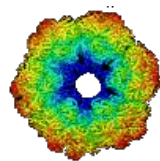
The Toolbar

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

The toolbar buttons:

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



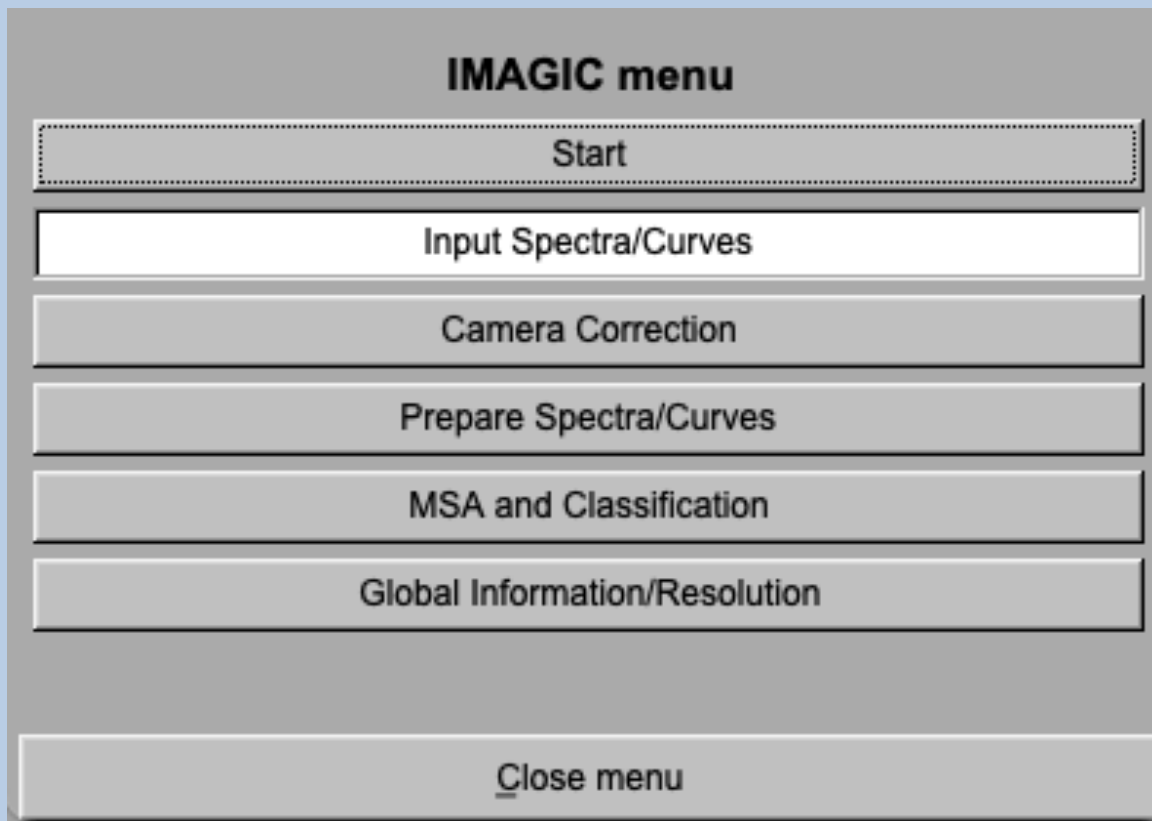


IMAGIC

guiSPECTRA



The guiSPECTRA Menu



PAGES:

Start	Page to adjust guiSPECTRA program parameters
Input Images	Import or specify the input spectra. Cut out a part, if wanted.
Camera Correction	Correct for camera errors/properties
Prepare Curves	Pre-treat images: Mask, filter, normalise variance, resize, sum ... Images
MSA and Classification	Classify the spectra to find typical spectra and typical differences in the data-set
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



The “Start” Page

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

Start

Working directory

Current working directory:
/Users/michael/workspace2/_brazil_school/

Browse directory

Click to close the program settings menu

Character/font size: 12

Window size: 1540 x 900

Start page picture / movie: Image ▾

File browser: Standard ▾

Save/Cancel Reset

Manuals & Papers

- Read +

Add PDF directory

On this page you can set some program parameters:

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



Start Working

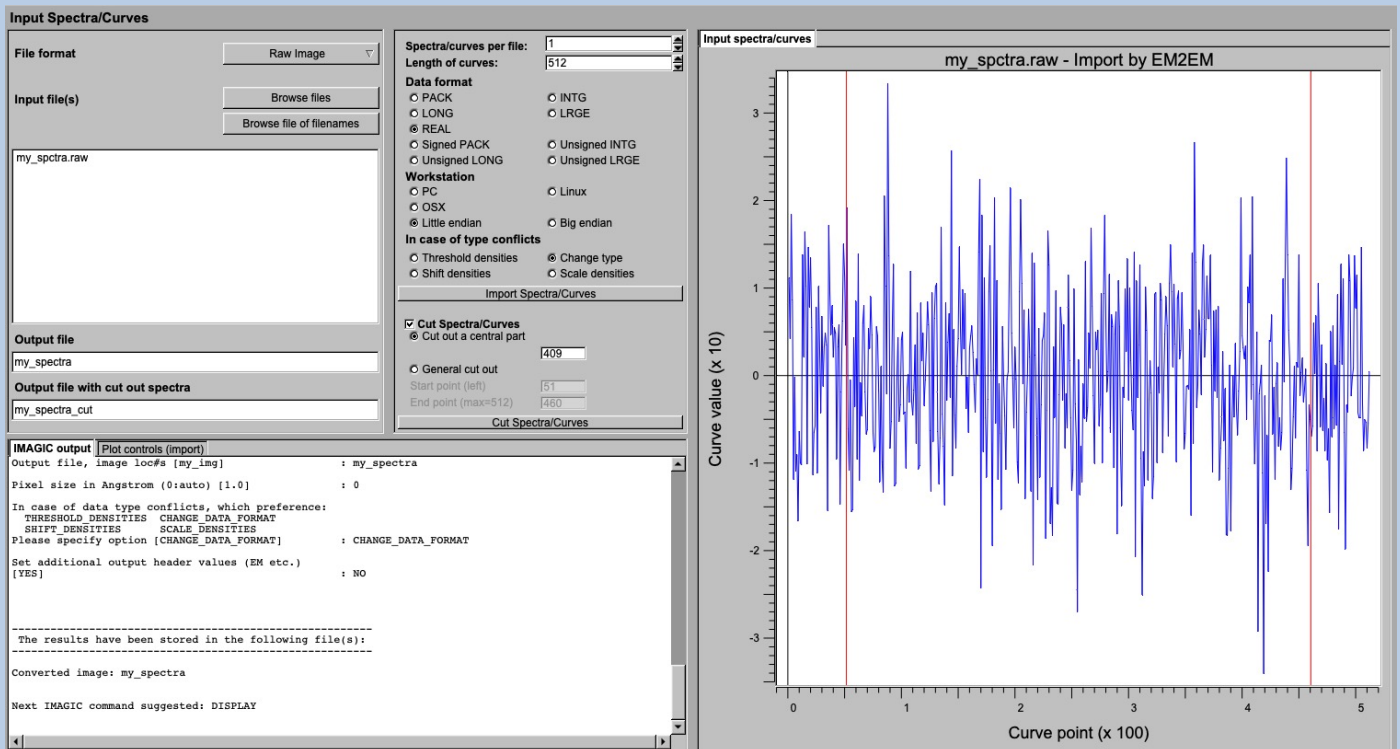
The page **guiSPECTRA** starts with the “Import Images” page.

The workflow using the “Next” button will guide you through all **guiSPECTRA** pages.

Use the “Back”, “Next” or “Open Menu” buttons to skip a page or to choose the wanted page.



The “Import Spectra” Page



DESCRIPTION:

Convert import image files using a number of typical formats (like DICOM or TIFF, for example) into a single (stacked) IMAGIC file.

The page can be skipped if your input images are already stored in IMAGIC format.

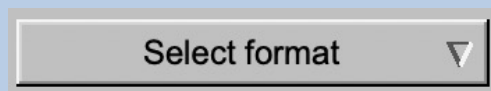
If wanted you can cut-out parts of the input spectra. Not suggested for detector correction.

Also refer to program **guiIMPORT**.



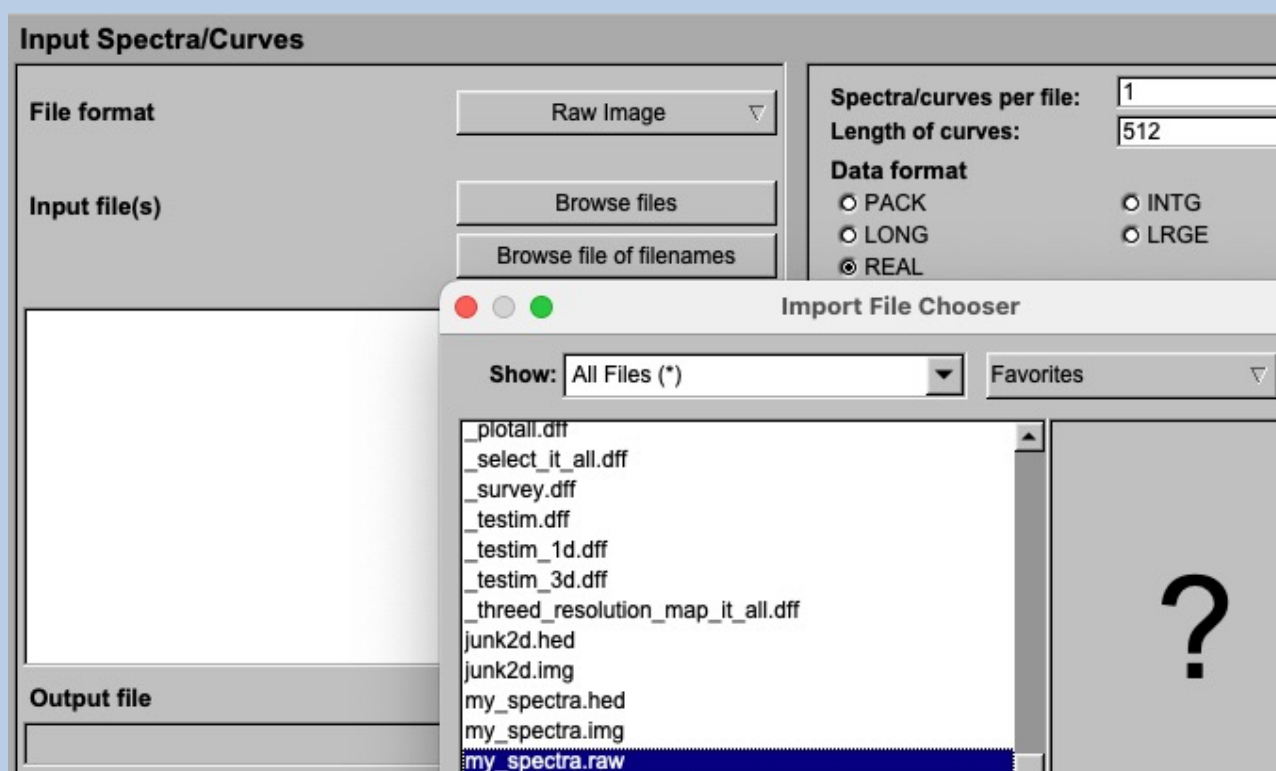
IMPORT SPECTRA:

Specify the file format in which your input 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.

```
my_spectra_1.raw  
my_spectra_2.raw
```

Next, you need to specify the name of the output file which is the IMAGIC file which will contain the imported spectra.

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

Format TIFF, for example:

In case of type conflicts

- | | |
|---|--|
| <input type="radio"/> Threshold densities | <input checked="" type="radio"/> Change type |
| <input type="radio"/> Shift densities | <input type="radio"/> Scale densities |

Having specified every information needed click the “Import Spectra/Curves” button to start the import of the Spectra.

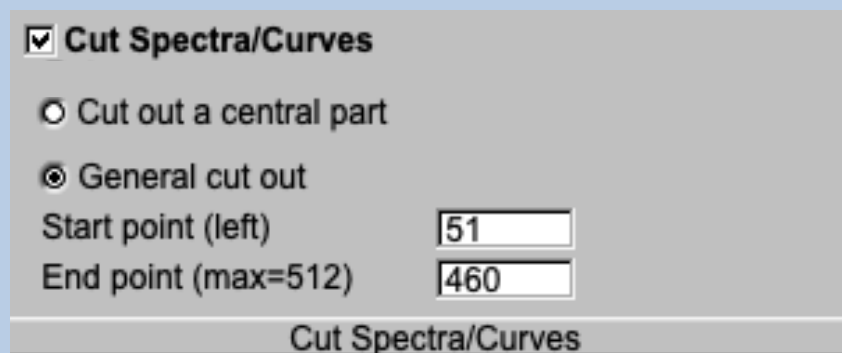
The imported images are shown in the plot/display tab on the right-hand side. See chapter “A Typical Page - Plot/Display control tabs”.



CUT SPECTRA / CURVES:

Having imported the input spectra, you may want to not use the full size of the curves but only a part of them.

Clicking the “Cut Spectra/Curves” option you can cut-out parts of the imported spectra:



☒ **Cut Spectra/Curves**

☐ Cut out a central part

☒ General cut out

Start point (left)

End point (max=512)

Cut Spectra/Curves

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

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

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



The “Detector Correction” Page

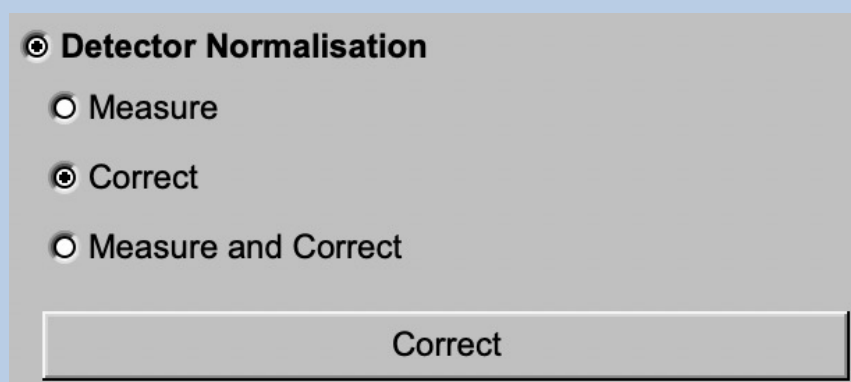
THIS OPTION IS STILL UNDER DEVELOPMENT

DESCRIPTION:

Get the detector statistics and /or detector correct/normalize the input spectra. Each output spectra is the input spectra minus the average image calculated from all spectra and divided by the standard deviation (again calculated from all spectra).



Choose one of the options



☒ **Detector Normalisation**

☐ Measure

☒ Correct

☐ Measure and Correct

Correct

- Measure:** Measure the detector statistics and create the detector statistics curves needed to detector correct spectra taken with this detector.
- Correct:** The detector statistics curves are already available. Detector correct the input spectra using these detector statistics curves.
- Measure and Correct:** Do both, measure the detector statistics and correct for it.



MEASURE:

Measure the detector statistics and create the detector statistics spectra needed to detector correct spectra taken with this detector.

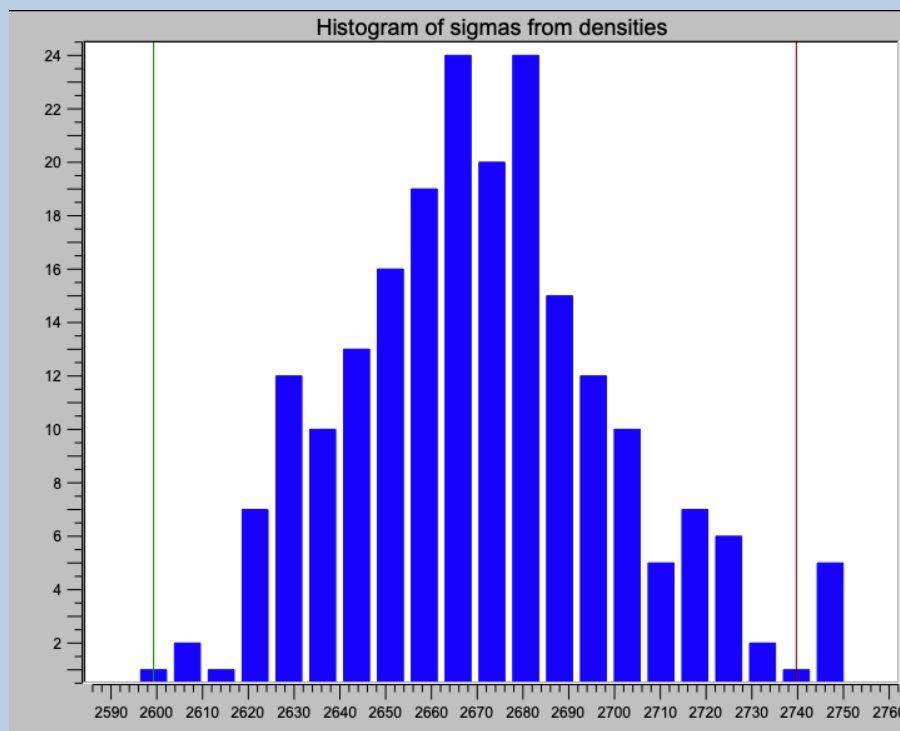
Mode

- ☒ Measure
- ☐ Correct
- ☐ Measure and Correct

Note that you usually need a huge number of input spectra to get a good statistics and a good subsequent detector correction.

First the statistics of the input spectra densities is needed. Normally you will use the “Calculate Statistics” button to calculate this statistics.

The histogram of sigma values is shown on the right-hand side. For a “good” dataset this histogram usually has a Gaussian like shape.



If the shape looks correct you can “Use all curves”.



Find Detector Statistics

- ☒ Use all images
- ☐ Ignore outliers

If wanted you can, of course, remove “outliers”.

Find Detector Statistics

- ☐ Use all images
- ☒ Ignore outliers

Minimum sigma	4279.2400	
Maximum sigma	4334.6800	

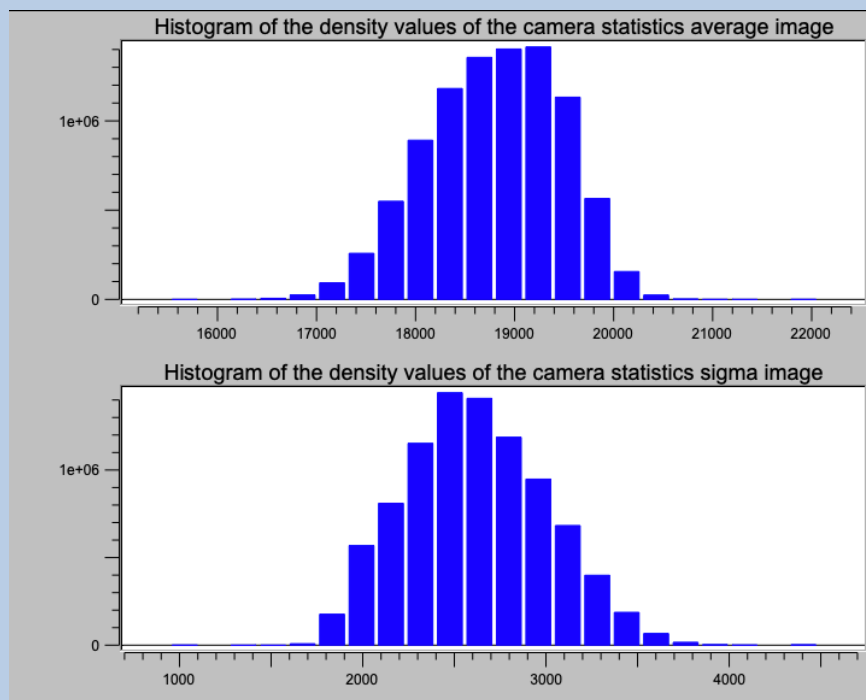
Use the up and down arrows to define a minimum and a maximum value for sigma (do NOT type the values). Only spectra corresponding to the histogram part between the red lines will be used for the detector correction. The red vertical lines in the histogram will help you to check the chosen values.

Do not forget to specify the names of the output files on the left hand side of the page.

Press the “Measure” button to start the calculations.

The measured detector statistics is shown in two histograms using the sigma of the densities in the detector statistics average and in the detector statistics sigma curves, respectively.





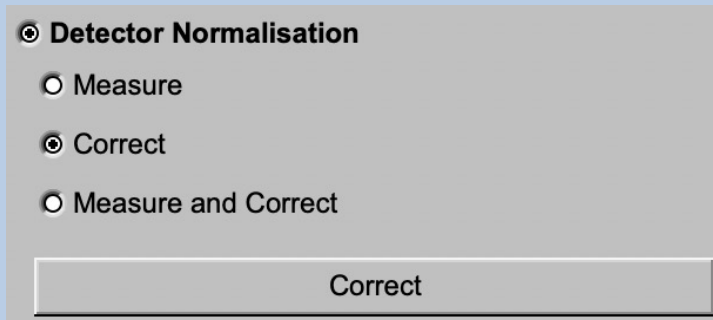
The histograms usually have a Gaussian like shape. In case of detector errors (blind or dark pixels, for example) these can easily be seen as vertical lines.

The detector statistics average and sigma curves are also displayed in tabs on the right hand side of the page.



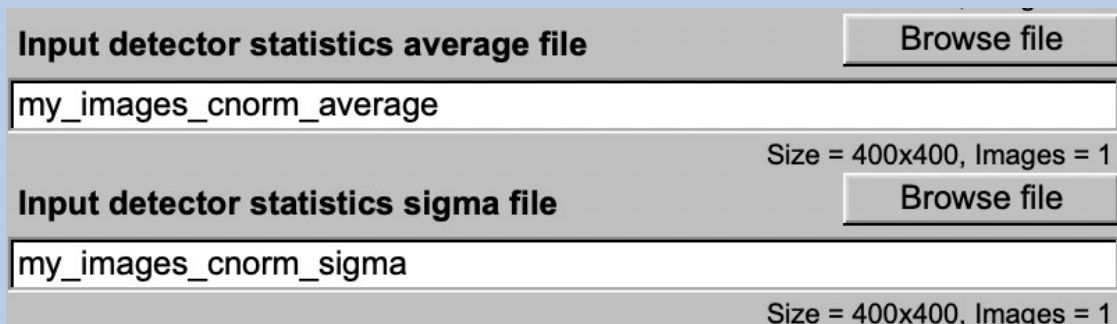
CORRECT:

Once having the detector statistics average and sigma spectra available you can correct all spectra taken with this detector (also the ones not used for getting the statistics).



A dialog box titled "Detector Normalisation" with three radio button options: "Measure", "Correct", and "Measure and Correct". The "Correct" option is selected. At the bottom is a button labeled "Correct".

Of course, you need to specify the input detector statistics average and sigma curves file needed for the detector correction



Two input fields for file selection. The first is labeled "Input detector statistics average file" with a "Browse file" button and the text "my_images_cnorm_average". Below it, the text "Size = 400x400, Images = 1" is displayed. The second is labeled "Input detector statistics sigma file" with a "Browse file" button and the text "my_images_cnorm_sigma". Below it, the text "Size = 400x400, Images = 1" is displayed.

and, as usual, the output file name



An output field labeled "Output file with detector corrected images" with an "Export file" button. The text "my_images_cnorm" is entered in the field.

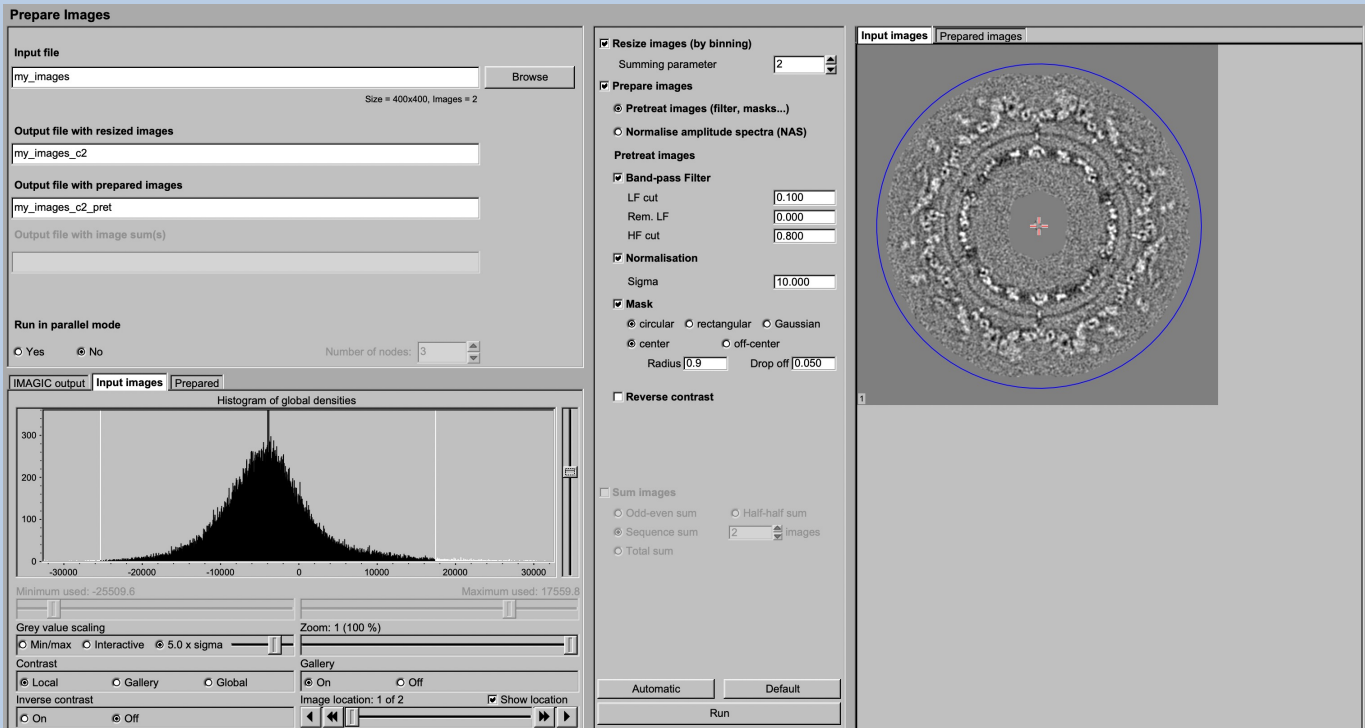
Click the "Correct" button to start the detector correction.

The detector corrected spectra are displayed on the right hand side of the page.

Note that the detector corrected spectra can be converted to other formats by clicking the "Export file" button which opens a separate "EM2EM" page.



The “Prepare Images” Page



DESCRIPTION:

It can be helpful to pre-treat the input images 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

☒ **Resize images (by binning)**

Summing parameter

You can pre-treat the images. Options are

☒ **Prepare images**

☒ **Pretreat images (filter, masks...)**

☐ **Normalise amplitude spectra (NAS)**

Pretreat images

☒ **Band-pass Filter**

LF cut

Rem. LF

HF cut

☒ **Normalisation**

Sigma

☒ **Mask**

☒ circular ☐ rectangular ☐ Gaussian

☒ center ☐ off-center

Radius Drop off

☒ **Reverse contrast**

Options are band-pass filtering

☒ **Band-pass Filter**

LF cut

Rem. LF

HF cut



normalise the variance in each image

☒ **Normalisation**

Sigma

imposing a mask

☒ **Mask**

☒ circular ☐ rectangular

☒ center ☐ off-center

Radius Drop off

if wanted you can also reverse the contrast.

☒ **Reverse 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:

☒ **Prepare images**

☐ Pretreat images (filter, masks...)

☒ **Normalise amplitude spectra (NAS)**

☐ **Mask**

☐ circular ☐ rectangular

☐ center ☐ off-center

Radius Drop off

☐ **Reverse contrast**

NAS Filter

LF cut

Rem. LF

HF cut

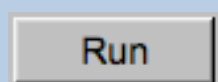
Imposing a mask and reversing the contrast are also options here.



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

Input file	
<input type="text" value="my_images"/>	<input type="button" value="Browse"/>
Size = 400x400, Images = 2	
Output file with resized images	
<input type="text" value="my_images_c2"/>	
Output file with prepared images	
<input type="text" value="my_images_c2_pret"/>	

Start the calculations by clicking the “Run” button:



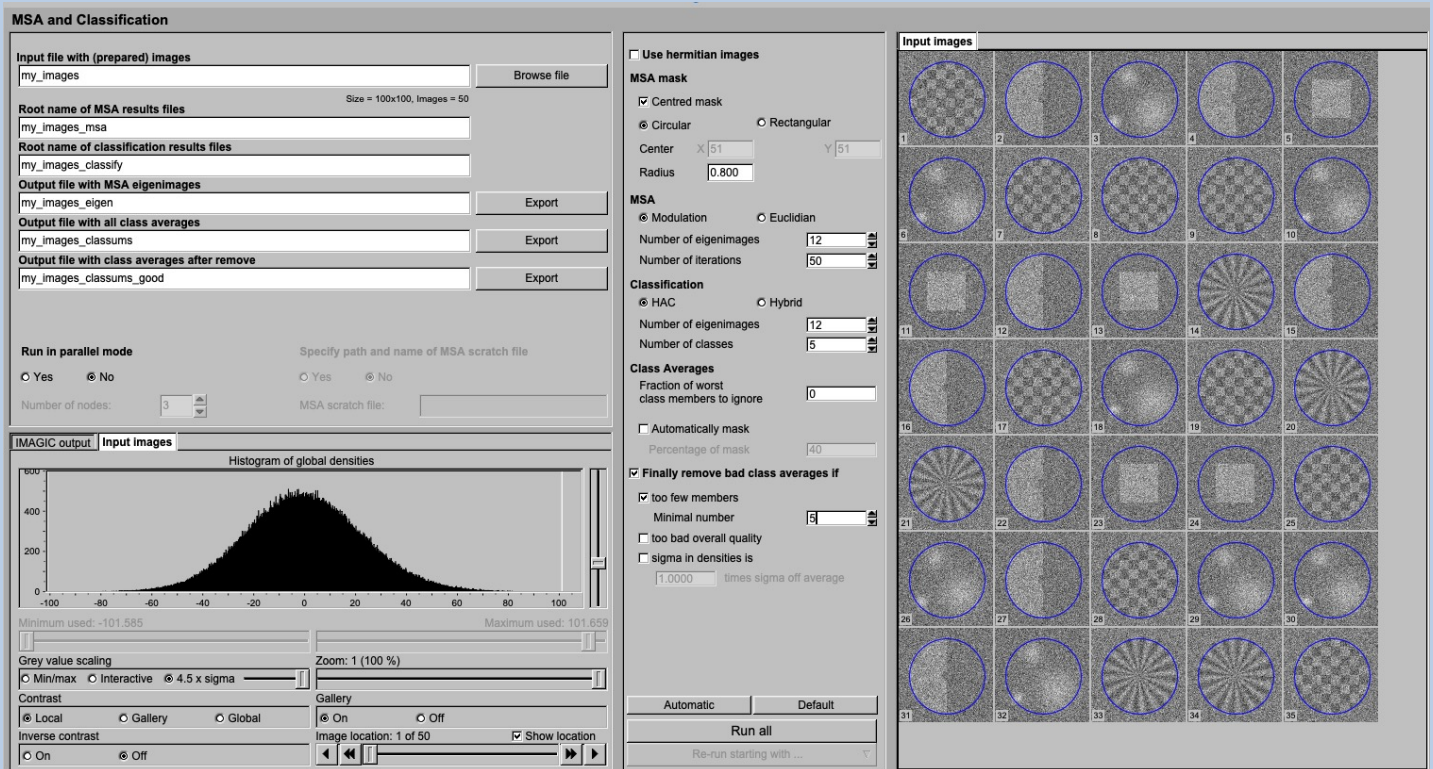
You can also create various image sums which you may need for the subsequent calculations of the global and local information content in your images.

<input checked="" type="checkbox"/> Sum images	
<input checked="" type="radio"/> Odd-even sum	<input type="radio"/> Half-half sum
<input type="radio"/> Sequence sum	<input type="text" value="2"/> <input type="button" value="▲"/> <input type="button" value="▼"/> images
<input type="radio"/> Total sum	

Output file with image sum(s)
<input type="text" value="my_images_c2_pret_odd_even_sum"/>



The “MSA and Classification” Page



DESCRIPTION:

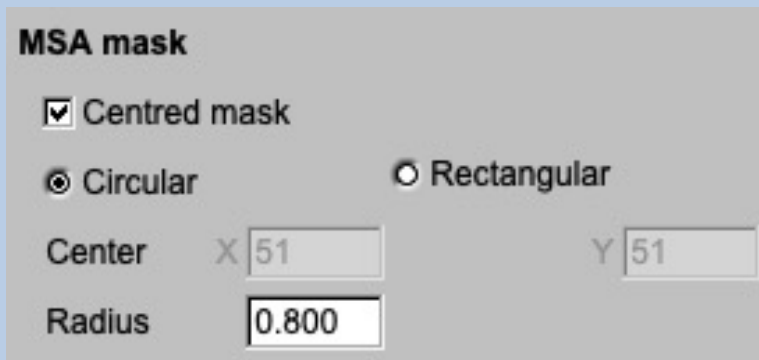
The **guiMSA** program performs a multivariate statistical analysis (MSA) on the spectra followed by a classification to be able to create class averages (class-sums).

Looking at the eigenspectra and the class averages can be useful to find typical spectra, to find the differences in the spectra and to estimate the information content of the data-set.



Usually Input are the spectra which were prepared on the previous page.

Specify a mask (if wanted you can use the mouse and the display on the righthand side) to specify the mask). The mask defines which part of the spectra are to be analysed (“area of interest”). Only pixels falling within this mask are actually contributing to the analysis.



MSA mask

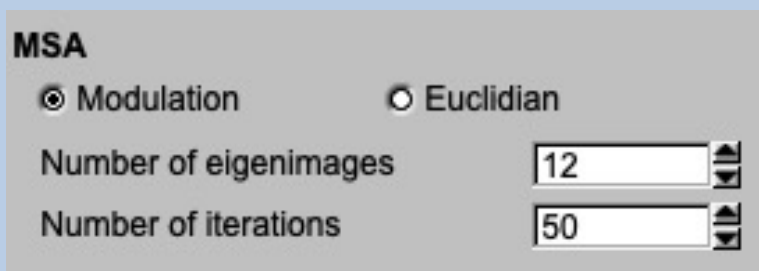
☒ Centred mask

☒ Circular ☐ Rectangular

Center X Y

Radius

You can use the metric of the the MSA eigenimage-eigenvalue calculations. in nearly all cases this is “Modulation”. MSA is an iterative procedure. You have to define the number of eigenimages and the number of iterations (usually large).



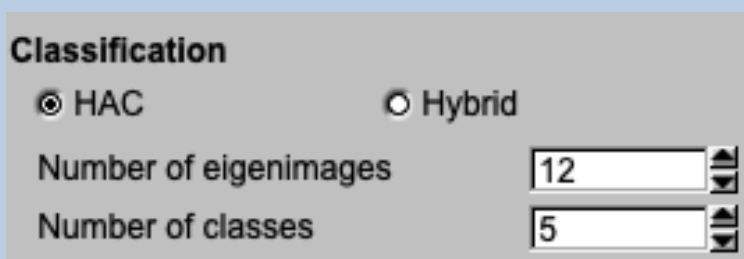
MSA

☒ Modulation ☐ Euclidian

Number of eigenimages

Number of iterations

You can have to specify the classification option (“HAC” or “Hybrid”) and especially the number of classes.



Classification

☒ HAC ☐ Hybrid

Number of eigenimages

Number of classes



The number of classes is related to the average number of images per class you would like. You can play with this value to see how the quality of the classes is affected. Ideally, you would have as few members per class as possible whilst still obtaining high contrast class averages.

If wanted you can use less eigenimages for classification than calculated in MSA.

If wanted “bad” class members can be ignored when creating the class averages.

Class Averages

Fraction of worst class members to ignore

In cryo-EM it can be good idea to automatically mask the class averages. In most other analysis you will probably ignore this option.

☒ Automatically mask

Percentage of mask

In cases, like in cryo-EM, where you would like to get the best class averages it is good idea to remove bad class averages.

☒ **Finally remove bad class averages if**

☒ too few members

Minimal number

☒ too bad overall quality

☒ sigma in densities is

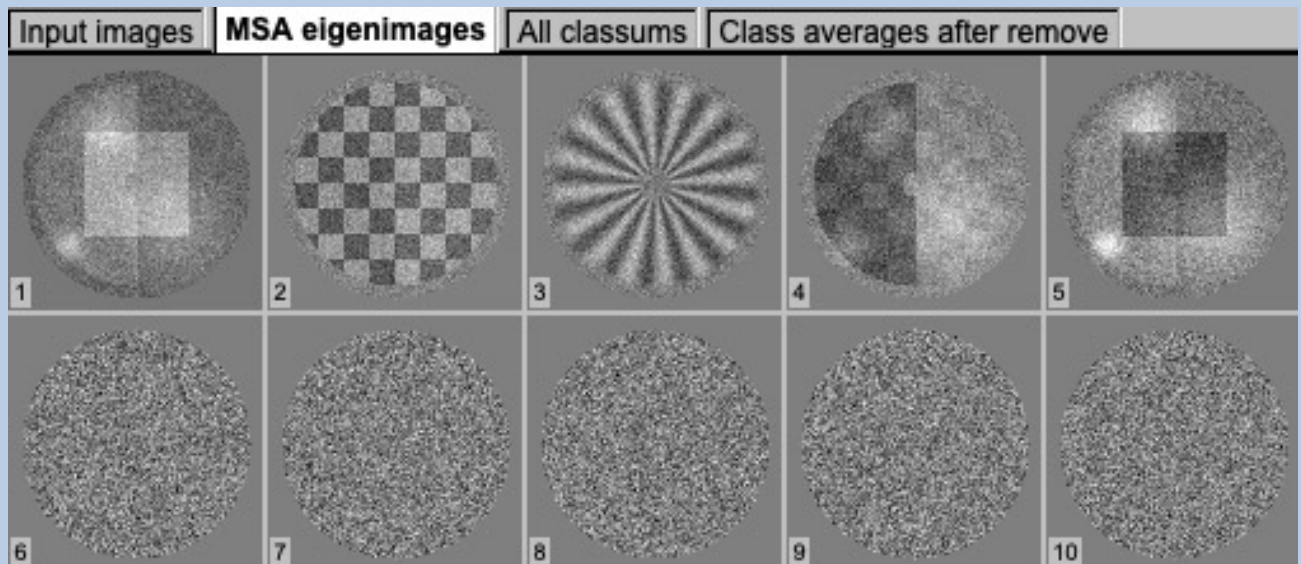
times sigma off average

If you want to classify all you input into classes you will, of course, ignore this option.



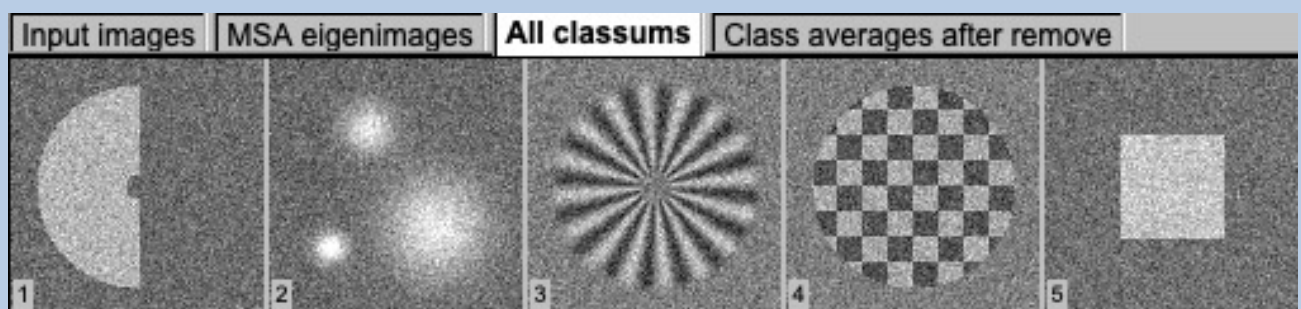
As usual results are displayed on the right hand side, the related display controls are on the left hand side. Carefully check the results:

First have a look at the eigenimages. The eigenimages of a (centred) dataset are a good way of examining the information content of a dataset.

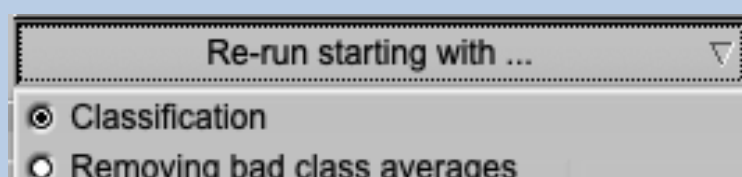


Note that the first eigenimage always shows (a sort of) average of all images and that higher eigenimages describe less important variance than lower numbered eigenimages.

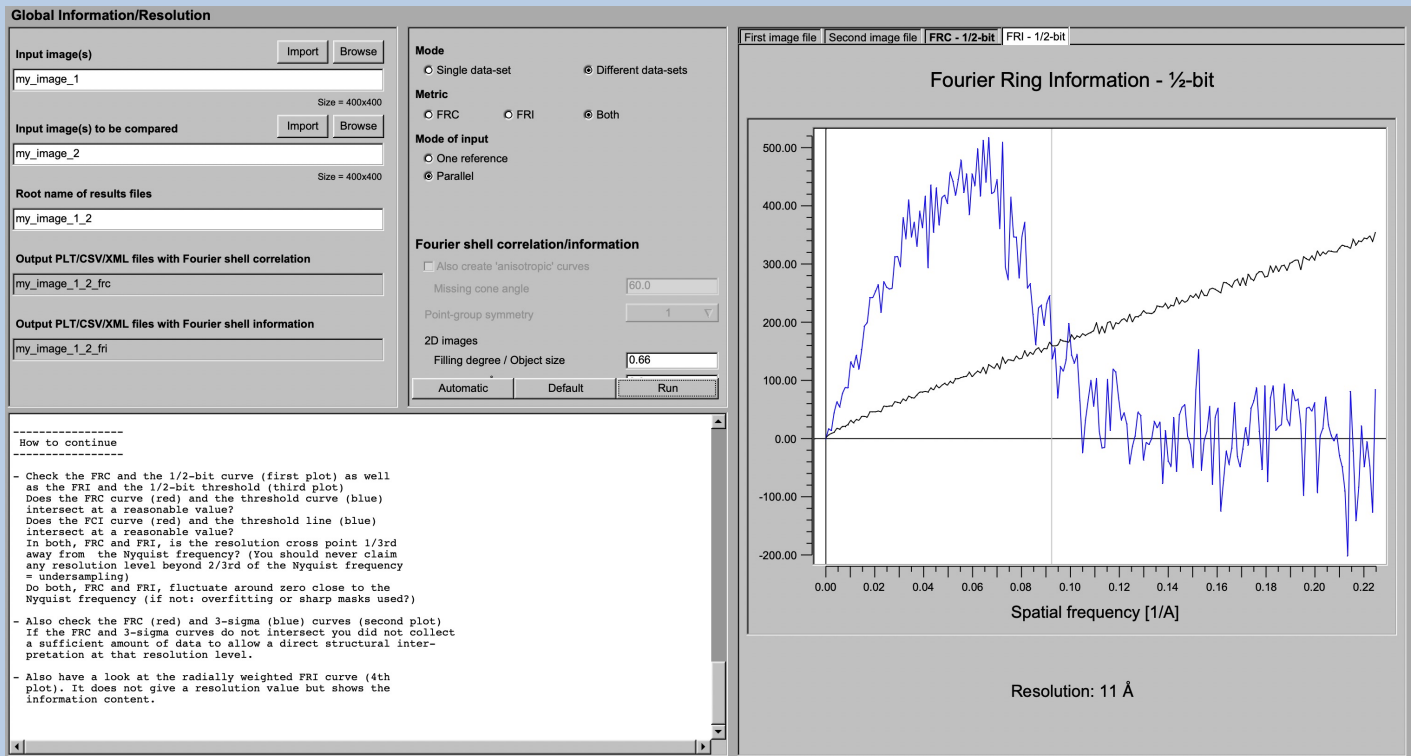
Of course, also check the class averages:



You can play around with the classification parameters and check the new results. If you do not want to change the MSA parameters you can use the “Classify only” and /or the “Removing bad class averages” button.



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.

Mode

☒ Single data-set ☐ Different data-sets

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

Mode of input

☐ One reference ☐ Sequential
☐ Parallel ☐ Sequential pairs
☒ Self

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

Mode of input

☐ One reference
☒ Parallel

Modes of input are:

➤ One reference:

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

Input image(s) to be compared	Import	Browse
<input type="text" value="my_images"/>		
Size = 400x400		
Reference image	Import	Browse
<input type="text" value="my_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.

Input 1st half data-set 3D volume(s)	Import	Browse
<input type="text" value="my_images_1"/>		
Size = 400x400		
Input 2nd half data-set 3D volume(s)	Import	Browse
<input type="text" value="my_images_2"/>		
Size = 400x400		

➤ 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.

Input images to be compared	Import	Browse
<input type="text" value="my_images"/>		
Size = 400x400		

➤ 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.

Input image(s)	Import	Browse
<input type="text" value="my_image"/>		
Size = 400x400		



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

Mode of input

☐ One reference ☐ Sequential

☐ Parallel ☐ Sequential pairs

☒ Self

LF cut HF cut

Next you are expected to choose the wanted metric:

Metric

☐ FRC ☐ FRI ☒ Both

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:

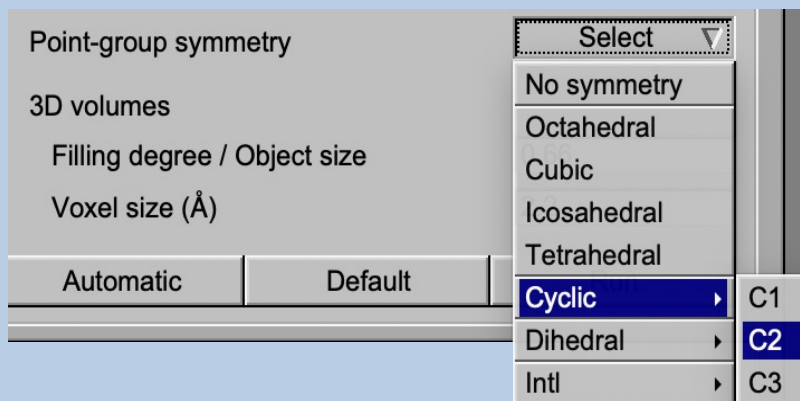
2D images

Filling degree / Object size

Pixel size (Å)



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



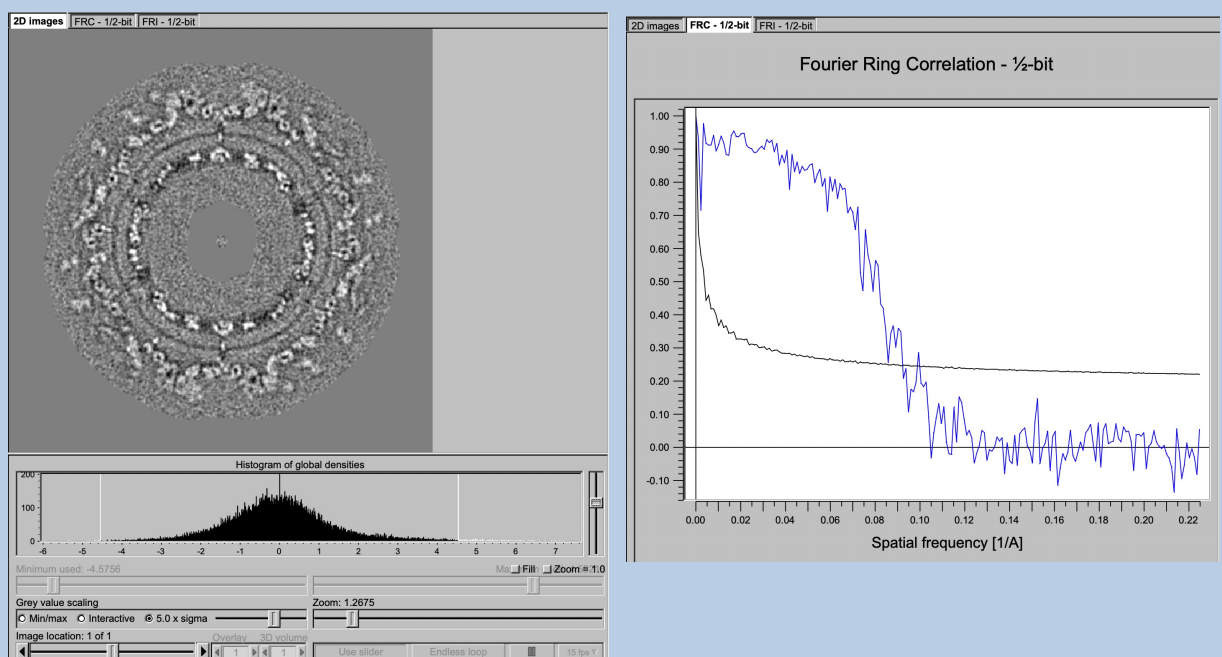
Move the cursor to an input fields to get context sensitive help.

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**.

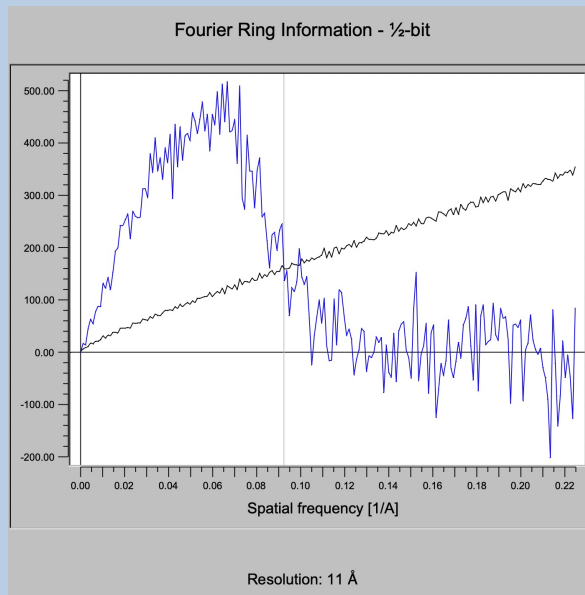


Clicking the “Run” button will start the calculations.

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.

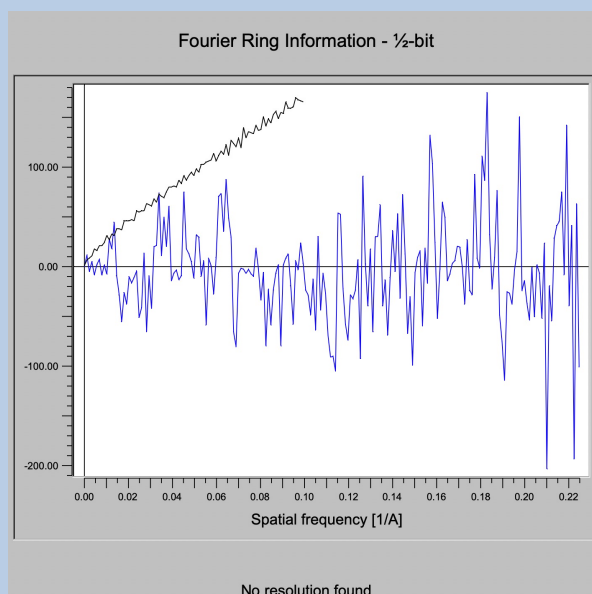


In contrast to the FRC/FSC curve the FRI/FSI plots also show the estimated resolution value in case FRI/FSI curve and the ½-bit threshold curve intersect:



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).

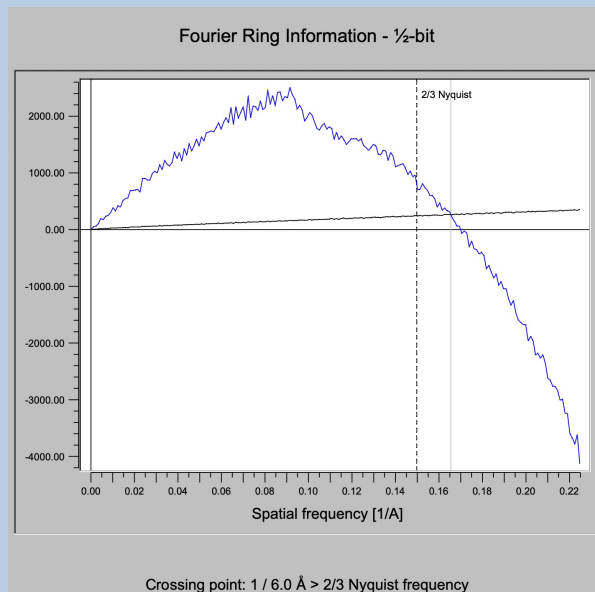
No resolution is printed if the estimated resolution value is too small :



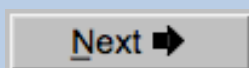
No resolution but a warning is printed if the estimated resolution value is too small or too close to the Nyquist frequency.

PLEASE NOTE:

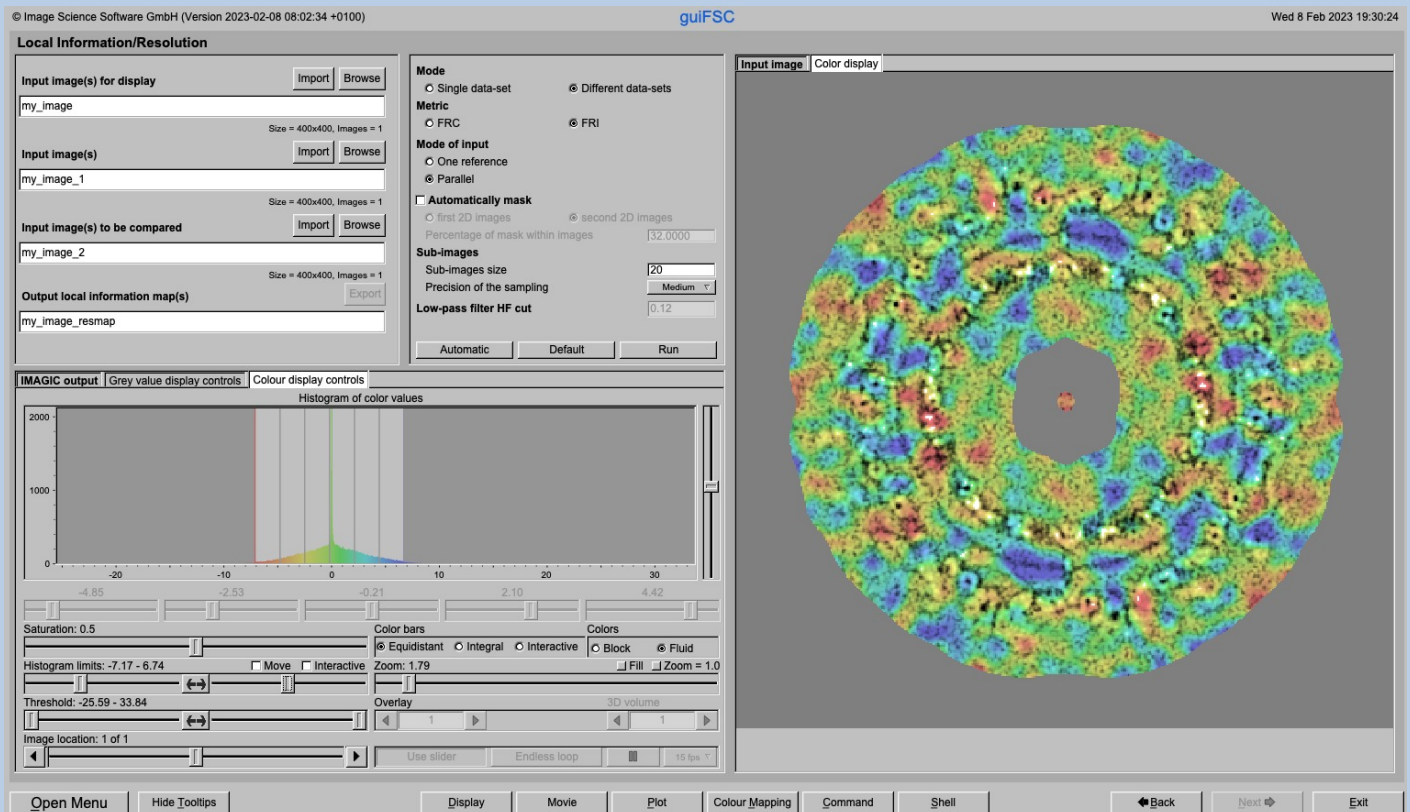
Under-sampling remains one of the worst sins one can commit in estimating the resolution / information content in images / 3D volumes. You should never claim any resolution level beyond 2/3rd of the Nyquist frequency.



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



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.

Mode

☒ Single data-set ☐ Different data-sets

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

Mode of input

☐ One reference ☐ Sequential
☐ Parallel ☐ Sequential pairs
☒ Self

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

Mode of input

☐ One reference
☒ Parallel

Modes of input are:

➤ One reference:

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

Input image(s) to be compared	Import	Browse
<input type="text" value="my_images"/>		
Size = 400x400		
Reference image	Import	Browse
<input type="text" value="my_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.

Input 1st half data-set 3D volume(s)	Import	Browse
<input type="text" value="my_images_1"/>		
Size = 400x400		
Input 2nd half data-set 3D volume(s)	Import	Browse
<input type="text" value="my_images_2"/>		
Size = 400x400		

➤ 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.

Input images to be compared	Import	Browse
<input type="text" value="my_images"/>		
Size = 400x400		

➤ 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.

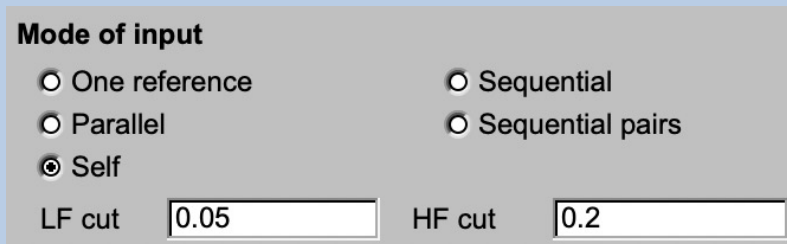
➤ Self

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.

Input image(s)	Import	Browse
<input type="text" value="my_image"/>		
Size = 400x400		



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



Mode of input

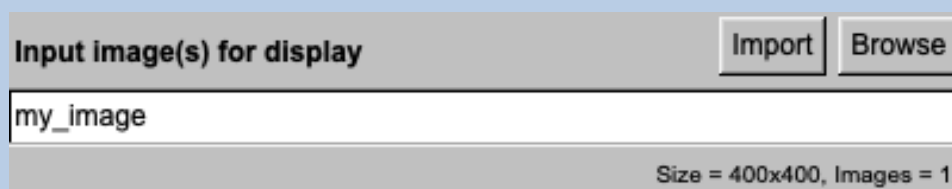
☐ One reference ☐ Sequential

☐ Parallel ☐ Sequential pairs

☒ Self

LF cut HF cut

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.



Input image(s) for display

Size = 400x400, Images = 1

Next you are expected to choose the wanted metric:



Metric

☐ FRC ☒ FRI

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 high frequency cut of the low-pass filter I calculated automatically:

Sub-images	
Sub-images size	<input type="text" value="20"/>
Precision of the sampling	<input type="button" value="Medium"/>
Low-pass filter HF cut	<input type="text" value="0.12"/>

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.

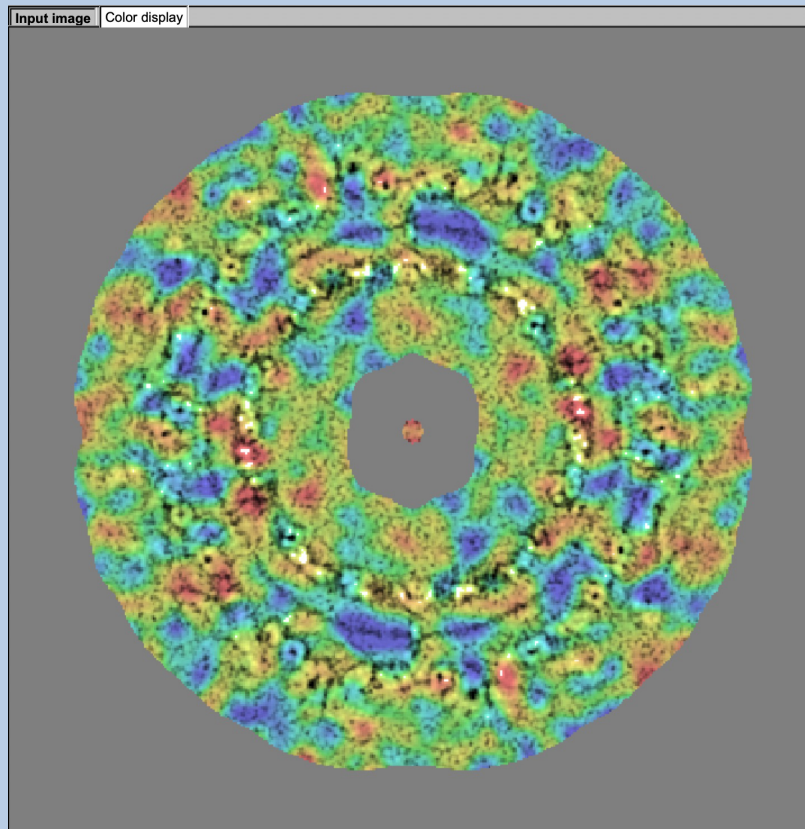
As usual you can reset all parameters to the last values you have used by clicking the “Default” button, clicking the “Automatic” button will reset all parameters to the values suggested by **guiFSC**.

<input type="button" value="Automatic"/>	<input type="button" value="Default"/>	<input type="button" value="Run"/>
--	--	------------------------------------

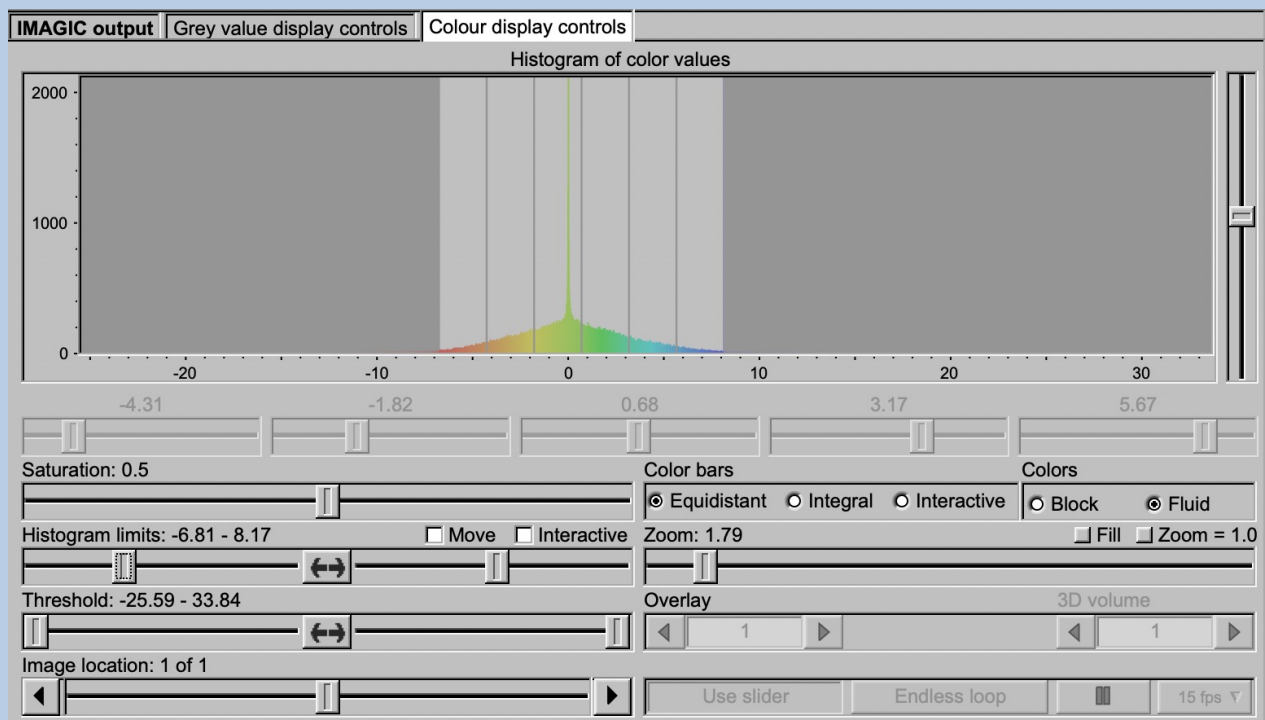
Clicking the “Run” button will start the calculations.

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

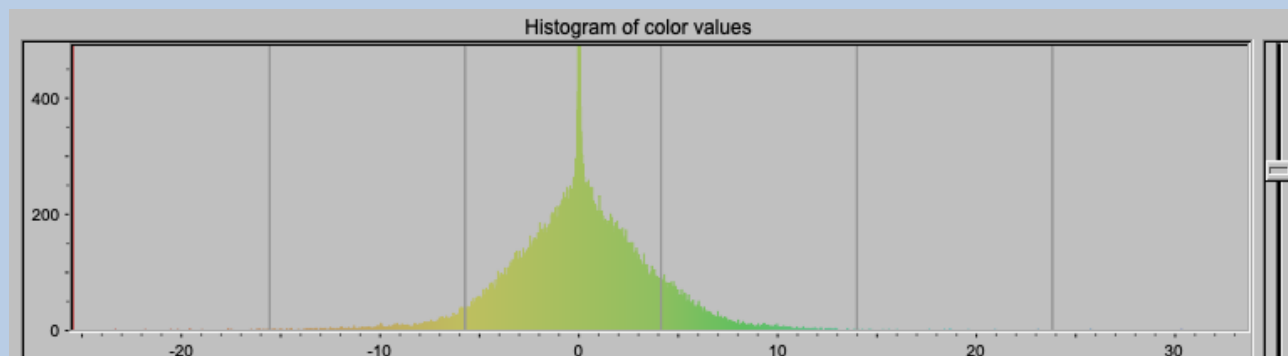




Use the “Colour display controls” to adjust the coloured display on the right-hand side.



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



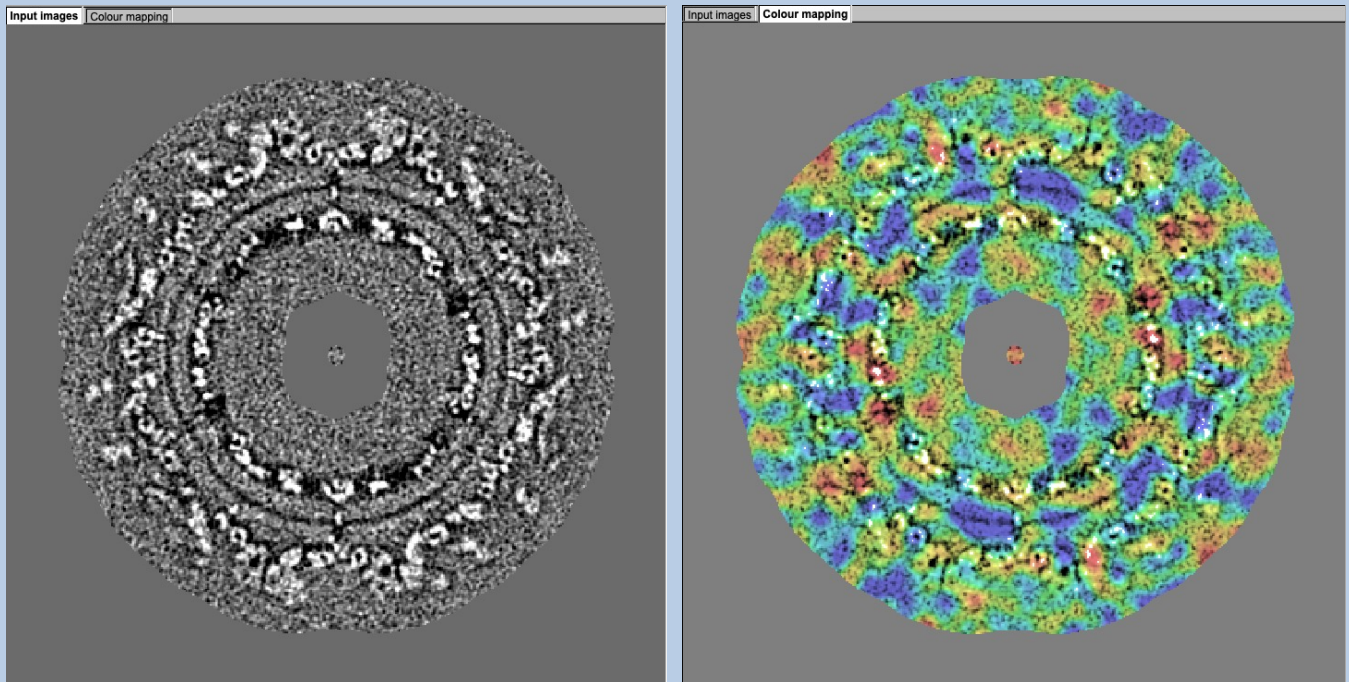
COMPARE TWO DIFFERENT LOCAL INFORMATION / RESOLUTION MAPS:

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.


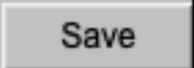
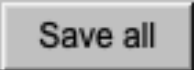


Display Windows

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

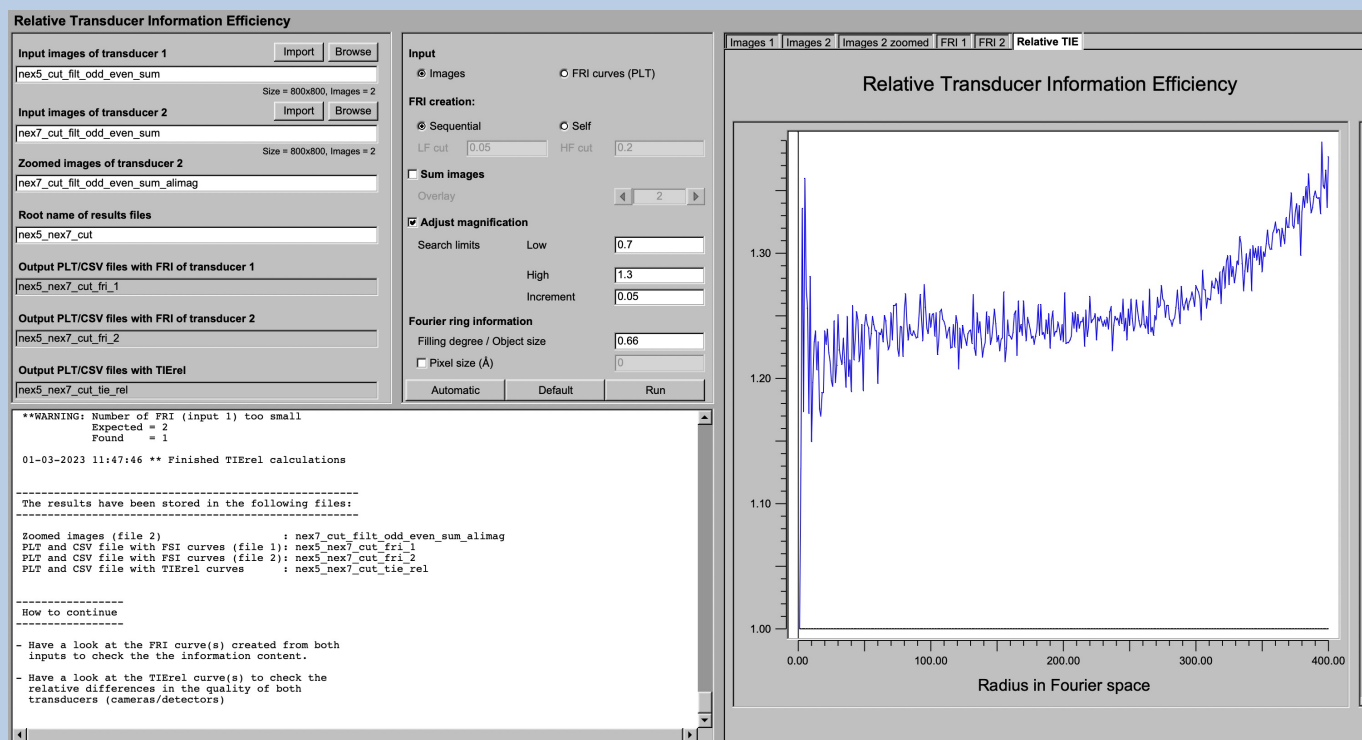


Moving the cursor into the display window there are a few options:

- | | |
|---|--|
|  | Open the display window a larger separate display window |
|  | Save the current displayed image in a JPG image |
|  | Save the whole window in a JPG image |



Relative Transducer Information Efficiency (TIE_{rel})



DESCRIPTION:

Calculate the relative transducer information efficiency to compare (the quality of) two detectors.



Input are two data sets each one taken by another detector. Usually the input are images or images sums which are used to calculate the global information (FRI) in each of the input data-sets

Input

☒ Images

☐ FRI curves (PLT)

As usual, specify the related file names:

Input images of transducer 1	<input type="button" value="Import"/>	<input type="button" value="Browse"/>
<input type="text" value="nex5_cut_filt_odd_even_sum"/>		
Size = 800x800, Images = 2		
Input images of transducer 2	<input type="button" value="Import"/>	<input type="button" value="Browse"/>
<input type="text" value="nex7_cut_filt_odd_even_sum"/>		
Size = 800x800, Images = 2		

Also specify the root name to be used to create the names of the various output files.

Root name of results files
<input type="text" value="nex5_nex7_cut"/>
Output PLT/CSV files with FRI of transducer 1
<input type="text" value="nex5_nex7_cut_fri_1"/>
Output PLT/CSV files with FRI of transducer 2
<input type="text" value="nex5_nex7_cut_fri_2"/>
Output PLT/CSV files with TIErel
<input type="text" value="nex5_nex7_cut_tie_rel"/>



There are a few parameters and options you can adjust:

Fourier ring information

Filling degree / Object size	<input type="text" value="0.66"/>
<input type="checkbox"/> Pixel size (Å)	<input type="text" value="0"/>

If wanted input images can be summed before the FRI is calculated

☒ **Sum images**

Overlay

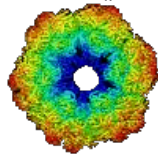
The magnification (the pixel size) in the input files can be different. In this case a magnification alignment is useful:

☒ **Adjust magnification**

Search limits	Low	<input type="text" value="0.7"/>
	High	<input type="text" value="1.3"/>
	Increment	<input type="text" value="0.05"/>

As usual, the resulting FRI and the TIE_{rel} curves are shown on the right hand side.





IMAGIC

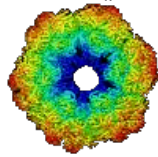
guiDETECTOR

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

guiDETECTOR

[Feedback / Error hints](#)

We intensively tested the **guiDETECTOR** program and tried to find all possible errors and inconsistencies. But the current 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

www.ImageScience.de
imagic@ImageScience.de

