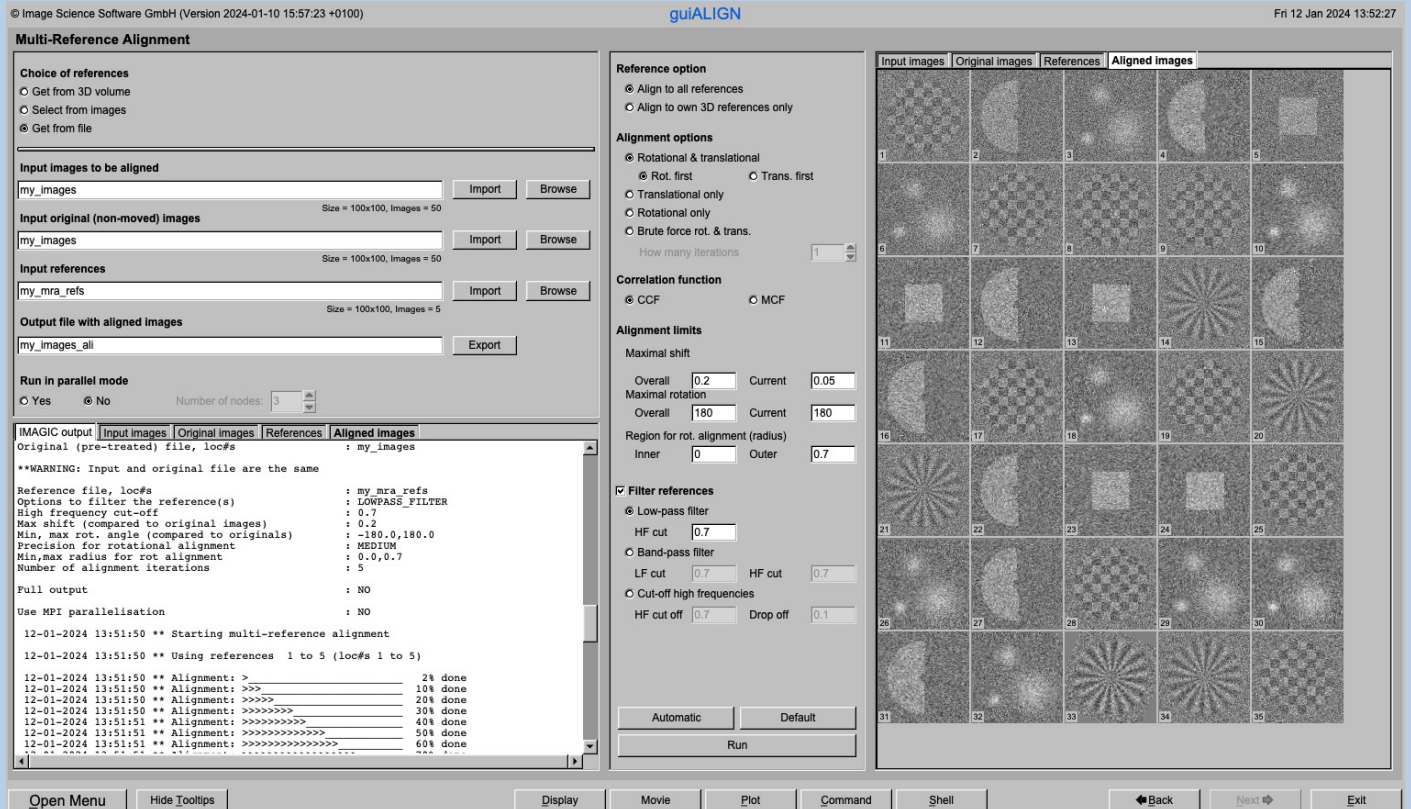


A Brief Introduction

Version 12-Jan-2024
www.ImageScience.de
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The IMAGIC guiMSA program



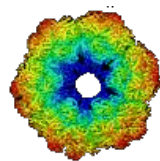
The **guiMSA** program performs a multivariate statistical analysis (MSA) followed by a classification to be able to create class averages (class-sums).

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

CONTENT:

- IMAGIC GUI programs How to use IMAGIC GUI programs
- **guiMSA** How to calculate MSA
- How to classify the data
- How to create class averages (class-sums)
- Error hints How to send us feedback





IMAGIC

GUI Programs

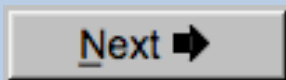


Workflow

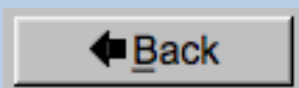
The idea of **guiMSA** is to guide you through a typical MSA and classification procedure .

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

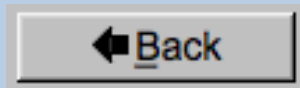
If **guiMSA** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

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

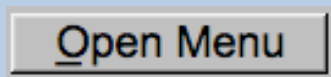
```
IMAGIC-COMMAND : guiMSA
```

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 **guiMSA**.

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.

Output file:

whgb_micrograph

Name of the output IMAGIC file containing the imported micrographs.

Note that the name of this output file will be created automatically.

Select format ▼

In case of type conflicts

Select the input file format.

Note: Currently only TIFF and MRC files can be imported.

MRC:
This is one of the oldest image formats in use in electron microscopy. One of the philosophies behind this data format is that it is compatible to the CCP4 format in use in X-ray crystallography.

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



Input Files

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

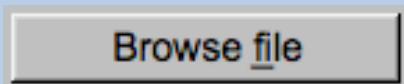
You can, of course, always use other input files names and even use other input directories.

Input file
my_images

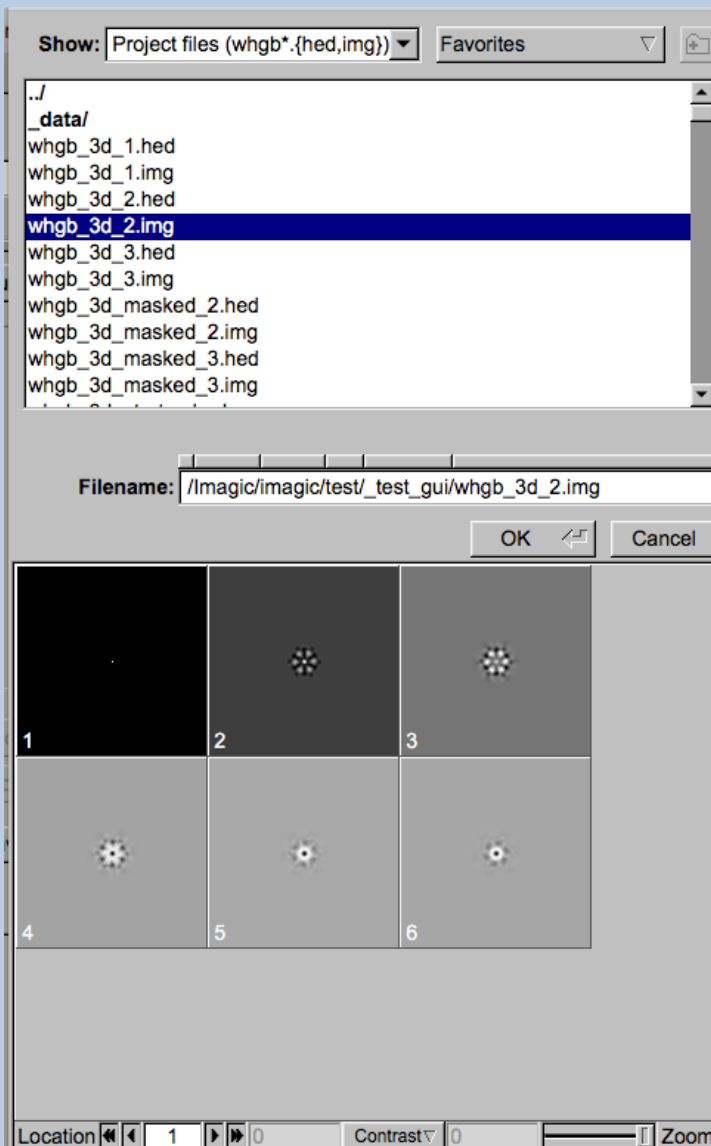


Input File Chooser

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

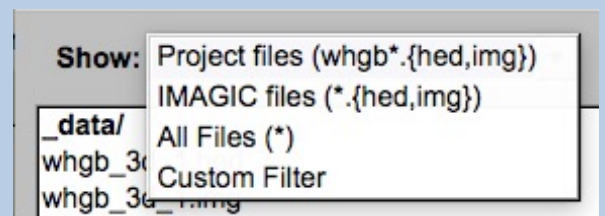


Pressing this button will open the IMAGIC file chooser:

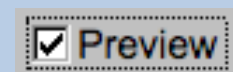


Choose the wanted file by clicking its name

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.

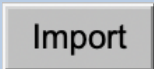
Output file

my_images

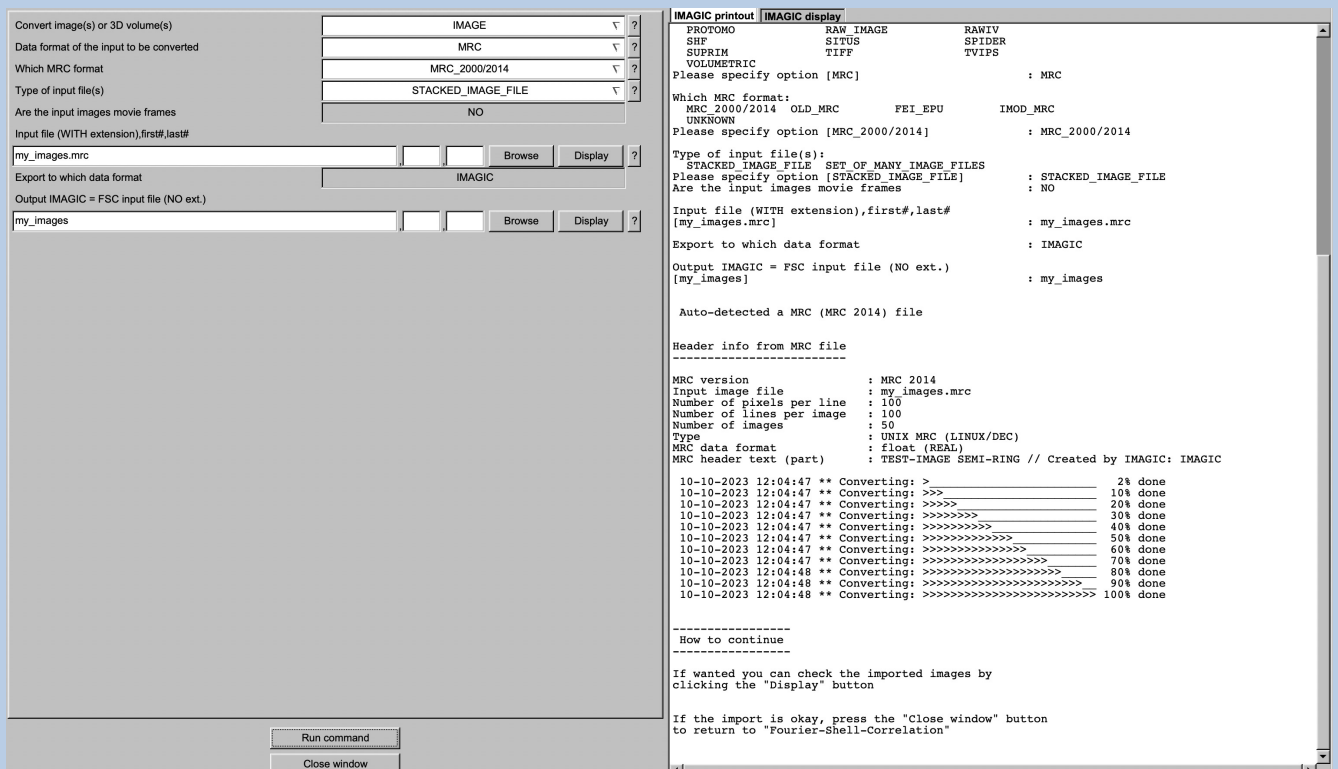


Import Buttons

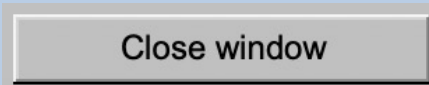
You do not want to use the “Import page” you can use the “Import” button to import the input images/3D volumes from any 3DEM format. The “Import” button which is located above the text field specifying the name of the related file.



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:

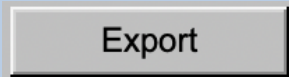


Refer to the **guiEM2EM** manual to get further help.



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:

A screenshot of the IMAGIC EM2EM software interface. The left pane shows a configuration window with various options for converting 2D images or 3D volumes. The right pane shows a terminal window with the command-line output of the same configuration. The configuration window includes fields for input file location, output format, and data format, with radio buttons for "Yes" and "No" options. The terminal window displays the corresponding command-line parameters and their values, such as "2D_IMAGE", "IMAGIC", "my_images", "TIFF", "GREY_SCALE_IMAGE", and "STACKED_IMAGE_FILE".

Convert 2D image(s) or 3D volume(s) [?] 2D_IMAGE

Data format of the input to be converted [?] IMAGIC

How are the input images available [?] UNKNOWN_IMAGE_FILE

Are the input images movie frames [?] Yes No

Input file, image loc#s [?] my_images

Export to which data format [?] TIFF

Type of output TIFF image(s) wanted [?] GREY_SCALE_IMAGE

Type of output file [?] STACKED_IMAGE_FILE

Output file, loc#s (WITH ext.),first#,last# [?] my_images.tif

Always scale densities to the output format [?] Yes No

Run command

Close window

```
IMAGIC printout IMAGIC display
Convert 2D image(s) or 3D volume(s):
 2D_IMAGE 3D_VOLUME
Please specify option [2D_IMAGE] : 2D_IMAGE

Data format of the input to be converted:
BROOKHAVEN_STEM CCP4 DATA_ONLY
DICOM DIGITAL_MICROGRAPH EM
FEI FABOSA FORMATTED
IMAGIC JPEG KONTRON
MDPP MEDIPIX MRC
OFFSET PIF PGM
PROTOMO RAW SHF
SMV SPIDER SUPRIM
TIA/EMI/SER TIFF TVIPS
Please specify option [IMAGIC] : IMAGIC

Type of input file:
SINGLE_IMAGE_FILE STACKED_IMAGE_FILE UNKNOWN_IMAGE_FILE
Please specify option [UNKNOWN_IMAGE_FILE] : UNKNOWN_IMAGE_FILE

Are the input images movie frames [NO] : NO
Input file, image loc#s [my_images] : my_images

Export to which data format:
CCP4 DATA_ONLY EM
FORMATTED FEI_RAW_IMAGE IMAGIC
JPEG_GREYSCALE KONTRON MDPP
MRC OFFSET PIF
PGM POSTSCRIPT PROTOMO
RAW SHF SMV
SPIDER SUPRIM TIFF
TVIPS
Please specify option [TIFF] : TIFF

Type of output TIFF image(s) wanted:
COLOUR_IMAGE GREY_SCALE_IMAGE
Please specify option [GREY_SCALE_IMAGE] : GREY_SCALE_IMAGE

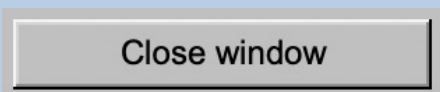
Type of output file:
STACKED_IMAGE_FILE SET_OF_MANY_IMAGE_FILES
Please specify option [STACKED_IMAGE_FILE] : STACKED_IMAGE_FILE

Output file, loc#s (WITH ext.),first#,last#
[my_images.tif] : my_images.tif

Always scale densities to the output format [YES] : YES

Image name: MOVIE SUM FROM whgb.c4.img (7 IMAGES) (PREPARE)
Size: 200, 200 Loc: 1 Type: REAL Cre.Date: 26-Jan-2023 Time: 11:16:03
EMEM;EXCOPY/PLT;EXCOPY/SELECT;CAMERA_NORM;INCDMENU/ANISOTROPIC_MAGNIFY=1.0,1
.026;COARSE;ALIDIR;COARSE;SUMMER/MOVIE SUM;INCDMENU/PREPARE/BP LOW=0.02 TRANS
=0.0 HIGH=0.9;CTF2D_FLIP;CUT_IMAGE/APERIODIC;
```

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



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



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.

© Image Science Software GmbH (Version 2022-11-30 18:18:14 +0100) **guiCNORM** Fri 9 Dec 2022 11:35:40

Camera Correction

Input file with (raw) micrographs
my_micrographs
Size = 4096x4096, Images = 70

Input camera statistics average file
my_micrographs_cnorm_average
Size = 4096x4096, Images = 1

Input camera statistics sigma file
my_micrographs_cnorm_sigma
Size = 4096x4096, Images = 1

Output file with camera corrected micrographs
my_micrographs_cnorm

Output good camera corrected micrographs
my_micrographs_cnorm_good

Camera Normalisation

Measure
 Correct
 Measure and Correct

Input Micrographs | **Corrected Micrographs** | Average | Sigma

Extract micrographs
 Use all
 Use "good" micrographs only

Ignore micrographs which show
 too extreme sigma of densities
 too extreme min/max difference of densities
Ignore if times sigma away from mean value

IMAGIC output | Micrograph | Corrected | Average | Sigma

```
Output file, image loc# : my_micrographs_cnorm
Input average file : my_micrographs_cnorm_average
Input sigma file : my_micrographs_cnorm_sigma
Reverse contrast in camera corrected images : NO
09-12-2022 11:33:58 ** Am correcting/normalising images
09-12-2022 11:33:58 ** Correction: _____ 1% done
09-12-2022 11:34:01 ** Correction: >>>>>>>> 10% done
09-12-2022 11:34:03 ** Correction: >>>>>>>> 20% done
09-12-2022 11:34:06 ** Correction: >>>>>>>> 30% done
09-12-2022 11:34:09 ** Correction: >>>>>>>> 40% done
09-12-2022 11:34:12 ** Correction: >>>>>>>> 50% done
09-12-2022 11:34:15 ** Correction: >>>>>>>> 60% done
09-12-2022 11:34:18 ** Correction: >>>>>>>> 70% done
09-12-2022 11:34:21 ** Correction: >>>>>>>> 80% done
09-12-2022 11:34:24 ** Correction: >>>>>>>> 90% done
09-12-2022 11:34:27 ** Correction: >>>>>>>> 100% done
09-12-2022 11:34:27 ** Correction/normalisation done
Image name:
Size: 4096,4096 Loc: 70 Type: REAL Cre.Date: 09-Dec-2022 Time: 11:34:27
EMZEM;HEADERS/ACTIVE;EXCOPY/SELECT/SIGMA/SET_INACTIVE;CAMERA_NORM/REVERSE_CONT
RAST;
```

Open Menu | Hide Tooltips | Display | Movie | Plot | Command | Shell | Back | Next | Exit



A Typical Page - MPI Parallel

If calculations can run in parallel mode the left part of a typical **IMAGIC GUI program** page also shows the buttons to specify the related parameters.

Run in parallel mode		Specify path and name of MSA scratch file	
<input checked="" type="radio"/> Yes	<input type="radio"/> No	<input type="radio"/> Yes	<input checked="" type="radio"/> No
Number of nodes:	<input type="text" value="3"/>	MSA scratch file:	<input type="text"/>



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

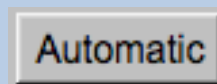
The screenshot shows a control panel with the following elements:

- 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

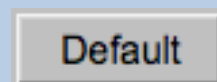
At the bottom, there are three buttons: "Automatic", "Default", and "Run".

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

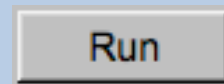
Number of iterations

Classification

Use how many eigenimages

Number of classes

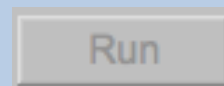
To run the calculations press the “Run” button.



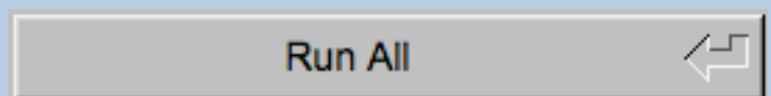
On a number of pages the calculations can be split. In this case you will find more than one single “Run” button.

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

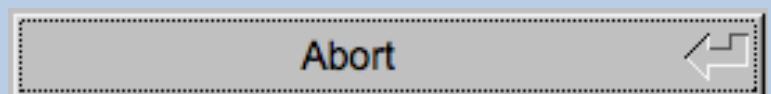
Maybe a certain “Run” button is not yet activated because it needs the results of calculations not yet done.



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

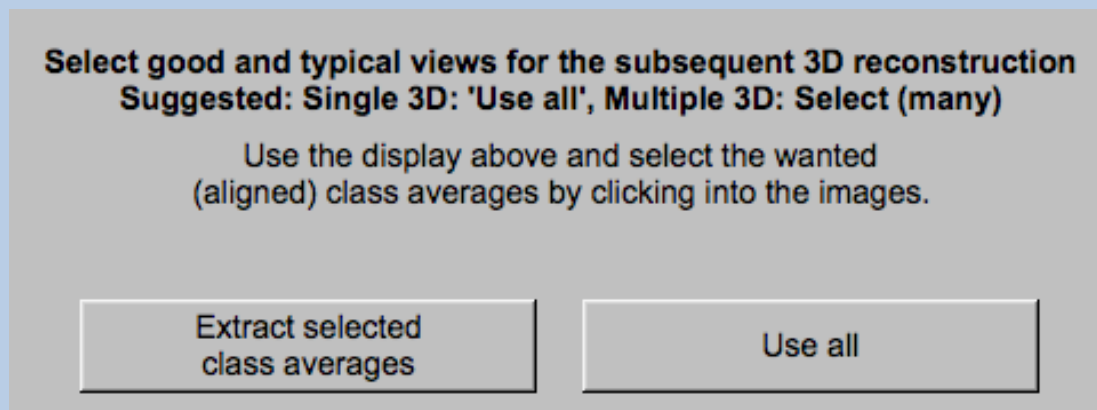


You can abort a running program by pressing the “Abort” button.



A Typical Page - Additional Tasks

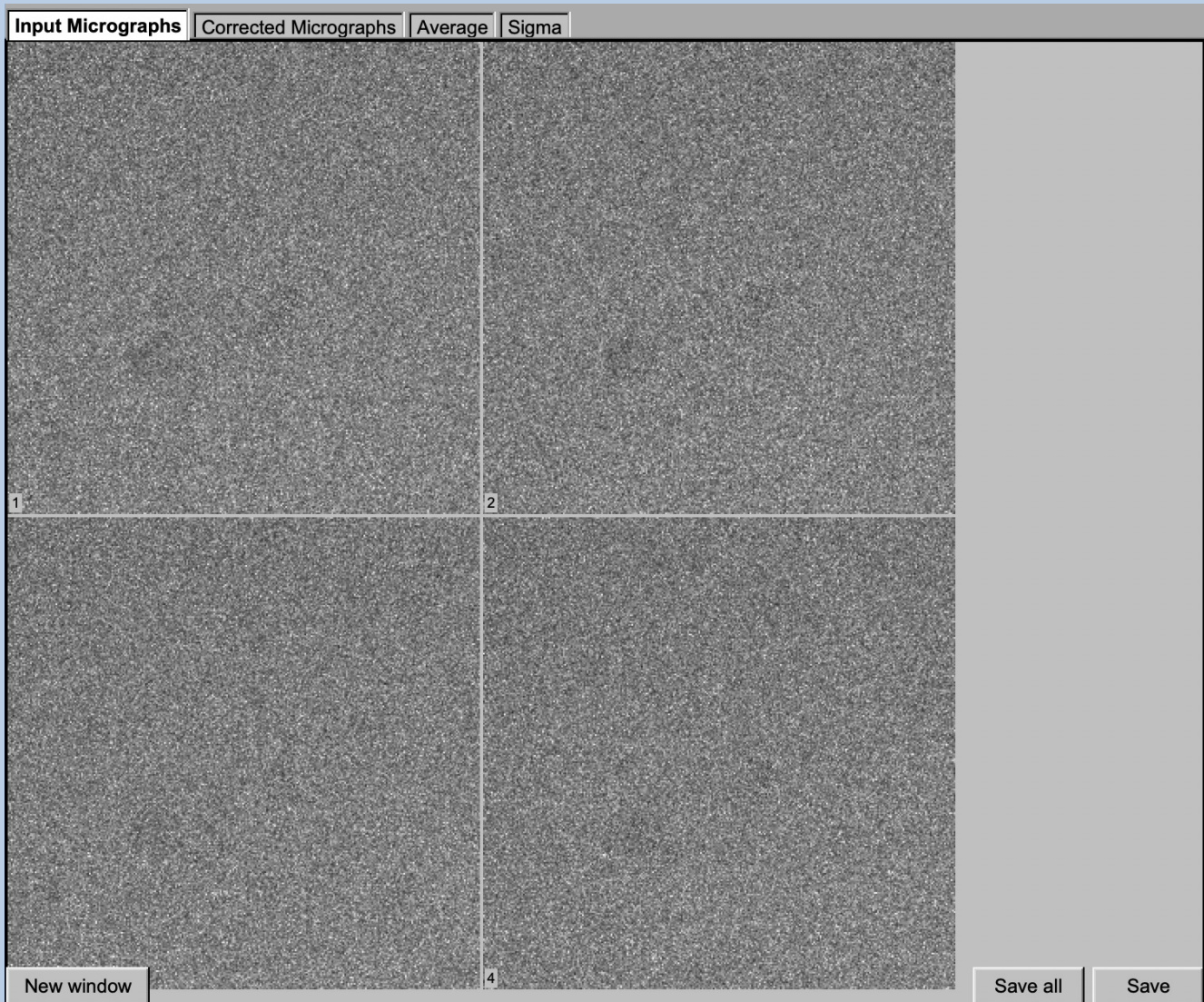
The main calculations on the page are done using the middle part of an typical **IMAGIC GUI program** page. But on a number of pages some additional calculations have to be done. Please follow the instructions given.



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



A Typical Page - 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**.



A Typical Page - “Display Control” Tabs

The visualisation settings of the images 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

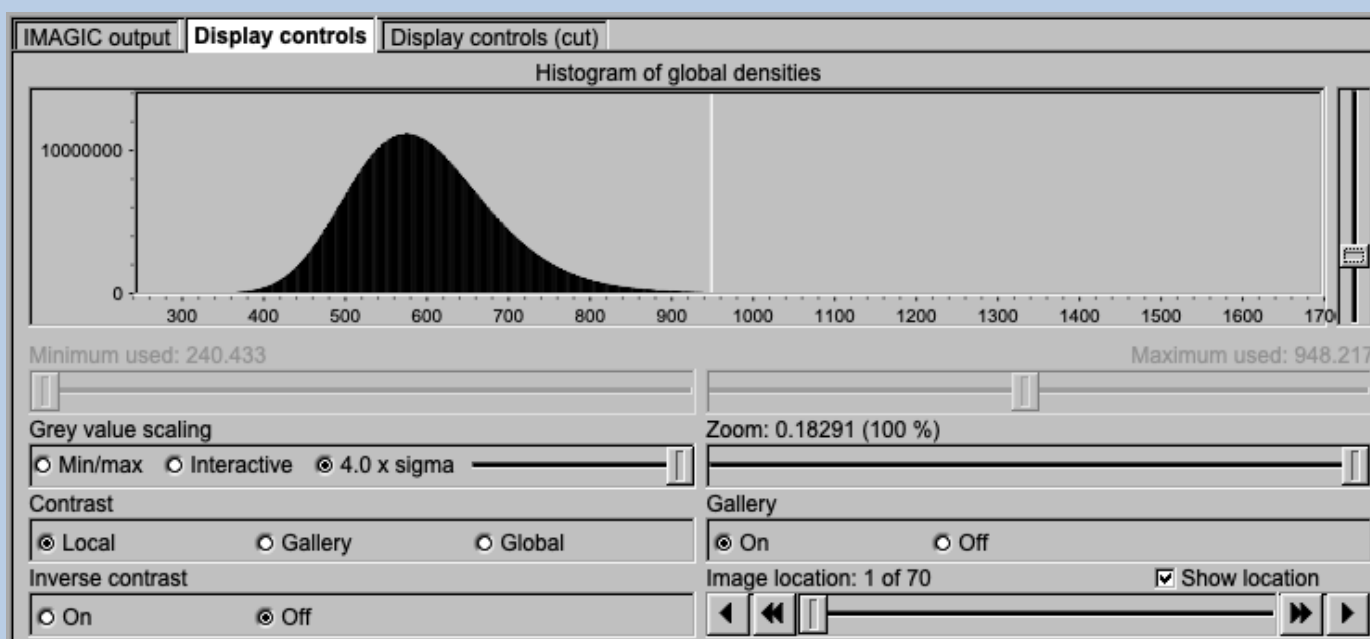
Gallery

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



A Typical Page - “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

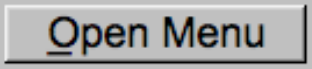
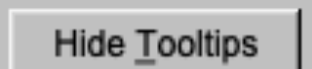
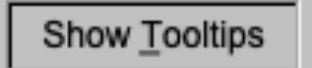
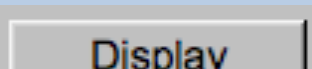
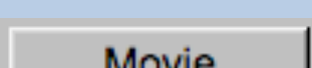
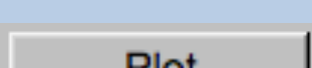
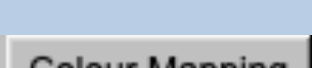

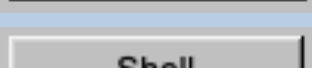
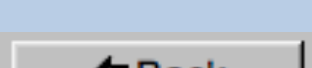
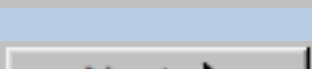
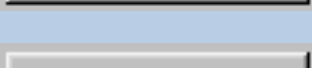
Style Select curve style ▾	Colour Select curve colour ▾	Grid Select curve grid ▾
Horizontal scaling 1.00	<input type="checkbox"/> Use for all plots 32.00	Reset
Vertical scaling -19.21	<input type="checkbox"/> Use for all plots 17.00	Reset
Plot title Fourier Ring Information - 1/2-bit	<input type="checkbox"/>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



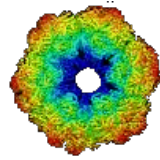
A Typical Page - The Toolbar

There is a toolbar at the bottom of each **guiMSA** 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 guiMSA



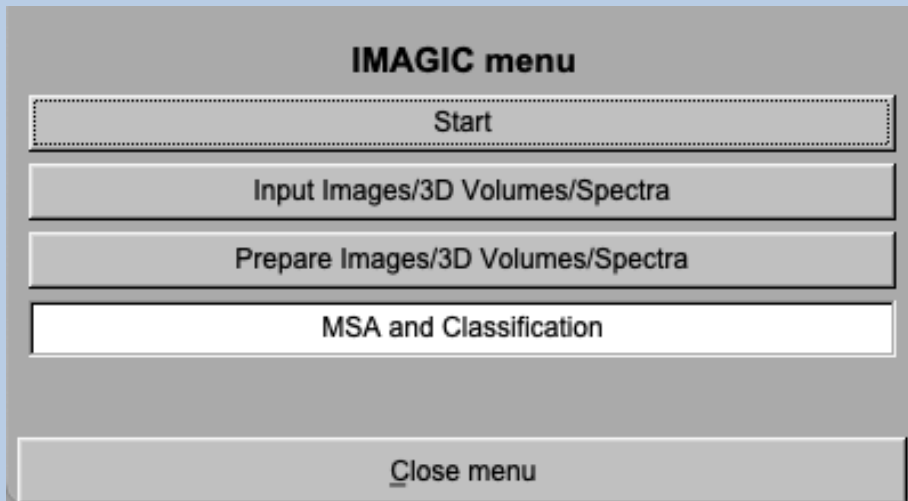


IMAGIC

guiMSA



The guiMSA Menu



PAGES:

Start	Page to adjust guiMSA 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 ...
MSA and Classification	Multivariate Statistical Analysis (MSA), classification and creation of class averages



The “Start” Page

This page is not part of the **guiMSA** 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 **guiMSA** program windows and/or text
(a re-start is needed)
- c) the type of file browser



Start Working

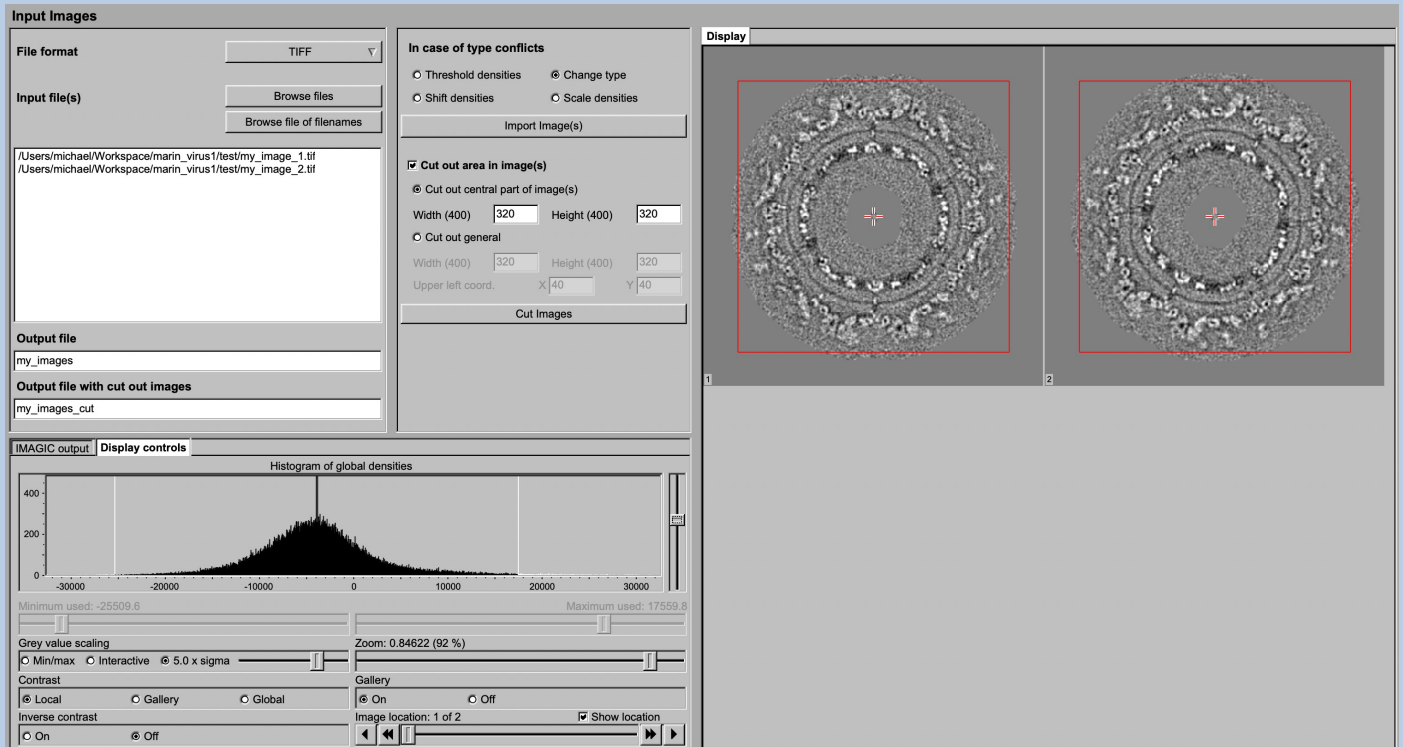
guiMSA starts with the “Import” page.

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

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



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**.



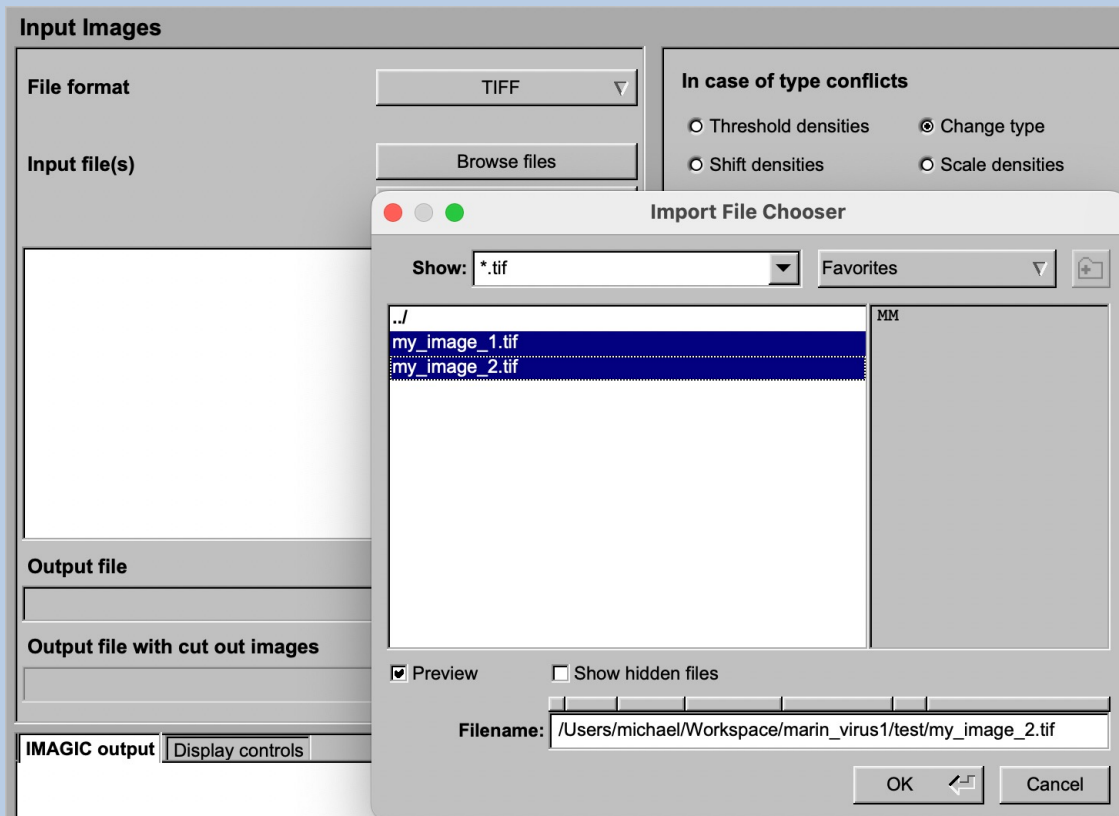
IMPORT :

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.

```
/Users/michael/Workspace/marin_virus1/test/my_image_1.tif  
/Users/michael/Workspace/marin_virus1/test/my_image_2.tif
```

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:

In case of type conflicts

- Threshold densities
- Change type
- Shift densities
- Scale densities

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

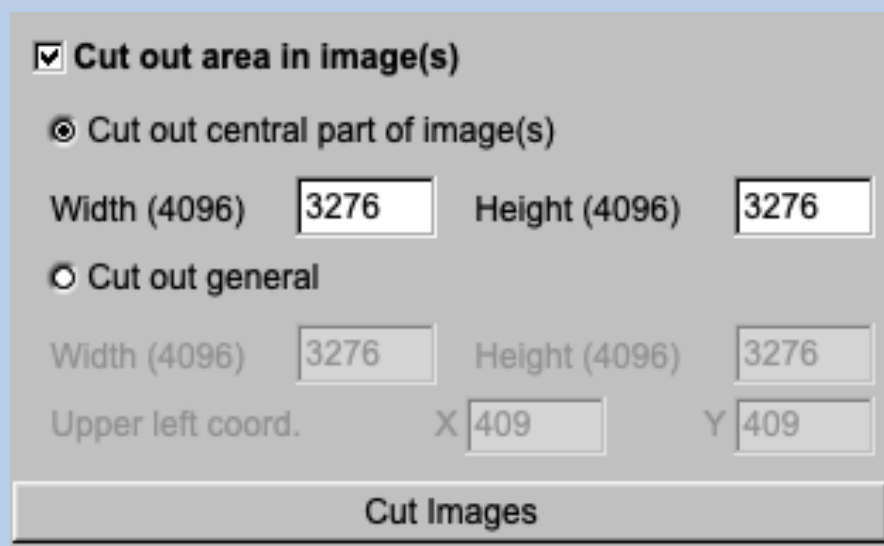
The imported images/3D volumes/spectra are shown in the display/plot tab on the right-hand side. See chapters “A Typical Page - Display control tabs” or “A Typical Page - plot control tabs”.



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 :



Cut out area in image(s)

Cut out central part of image(s)

Width (4096) Height (4096)

Cut out general

Width (4096) Height (4096)

Upper left coord. X Y

Cut Images

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 Images” Page

The screenshot shows the 'Prepare Images' GUI with the following details:

- Input file:** my_images (Size = 400x400, Images = 2)
- Output file with resized images:** my_images_c2
- Output file with prepared images:** my_images_c2_pret
- Run in parallel mode:** No (Number of nodes: 3)
- Histogram of global densities:** Minimum used: -25509.6, Maximum used: 17559.8
- Processing options:**
 - Resize images (by binning): Summing parameter 2
 - Prepare images
 - Pretreat images (filter, masks...)
 - Normalise amplitude spectra (NAS)
 - Band-pass Filter
 - LF cut: 0.100
 - Rem. LF: 0.000
 - HF cut: 0.800
 - Normalisation: Sigma 10.000
 - Mask
 - circular
 - rectangular
 - Gaussian
 - center
 - off-center
 - Radius: 0.9, Drop off: 0.050
 - Reverse contrast
- Sum Images:**
 - Odd-even sum
 - Half-half sum
 - Sequence sum (2 Images)
 - Total sum
- Buttons:** Automatic, Default, Run

DESCRIPTION:

It can be helpful to pre-treat the input 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

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:

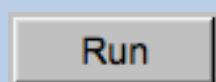
Input file
my_images Browse

Size = 400x400, Images = 2

Output file with resized images
my_images_c2

Output file with prepared images
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.

Sum images

Odd-even sum Half-half sum

Sequence sum images

Total sum

Output file with image sum(s)
my_images_c2_pret_odd_even_sum

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 “MSA and Classification” Page

MSA and Classification

Input file with (prepared) images
my_images Browse file

Root name of MSA results files
my_images_msa Size = 100x100, Images = 50

Root name of classification results files
my_images_classify

Output file with MSA eigenimages
my_images_eigen Export

Output file with all class averages
my_images_classsums Export

Output file with class averages after remove
my_images_classsums_good Export

Run in parallel mode
Specify path and name of MSA scratch file
 Yes No Yes No
Number of nodes: 3 MSA scratch file:

IMAGIC output Input Images
Histogram of global densities
Minimum used: -101.585 Maximum used: 101.659
Zoom: 1 (100 %)
Grey value scaling
 Min/max Interactive 4.5 x sigma
Contrast
 Local Gallery Global
Inverse contrast
 On Off
Gallery
 On Off
Image location: 1 of 50 Show location

Use hermitian images

MSA mask
 Centred mask Rectangular
 Circular Euclidian
Center X: 51 Y: 51
Radius: 0.800

MSA
 Modulation Euclidian
Number of eigenimages: 12
Number of iterations: 50

Classification
 HAC Hybrid
Number of eigenimages: 12
Number of classes: 5

Class Averages
Fraction of worst class members to ignore: 0
 Automatically mask
Percentage of mask: 40
 Finally remove bad class averages if
 too few members
Minimal number: 5
 too bad overall quality
 sigma in densities is
1.0000 times sigma off average

Automatic Default
Run all
Re-run starting with ...

Input Images
35 images in a grid

DESCRIPTION:

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

FULL IMAGE DATA-SET ANALYSIS:

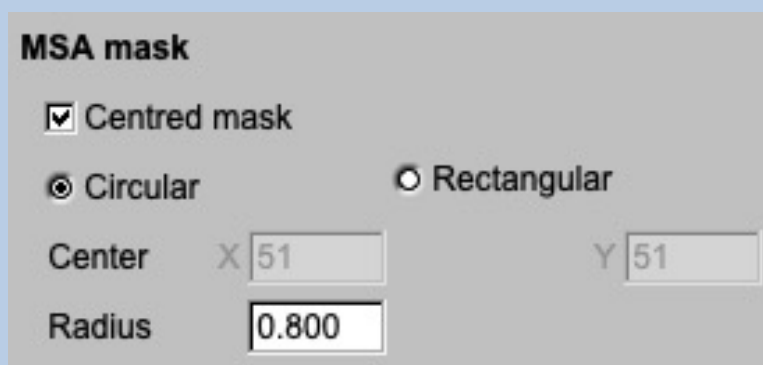
Refer to the manual: “Analysis of Wormhemoglobin - IMAGIC GISP“



The aim of Multivariate Statistical Analysis (MSA) and classification is to find similar images (in cryo-EM views of the particle) so that one can average them to reduce the noise level and to find the “typical” views.

Usually Input are the images/3D volumes/spectra which were prepared on the previous page.

Specify a circular or rectangular mask (if wanted you can use the mouse and the display on the righthand side) to specify the mask). The mask defines which parts of the images/3D volumes/spectra are to be analysed (“area of interest”). Only pixels falling within this mask are actually contributing to the analysis.



MSA mask

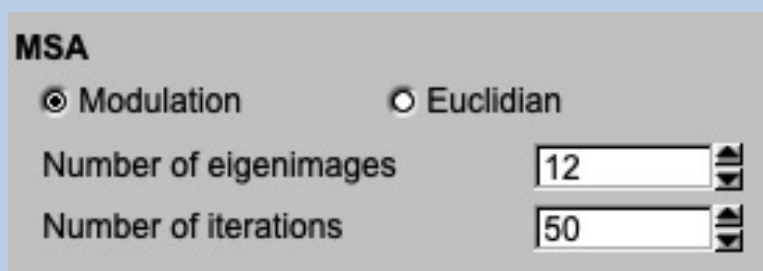
Centred mask

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).



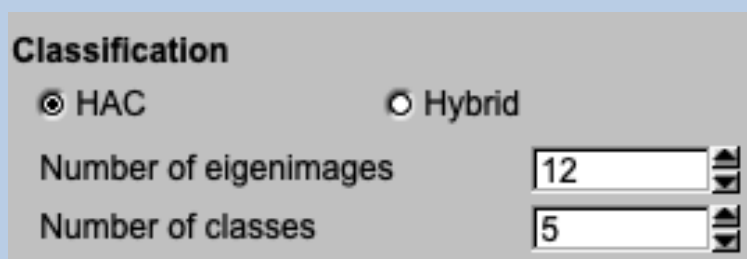
MSA

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. Giving “0” means that all class members are used.

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

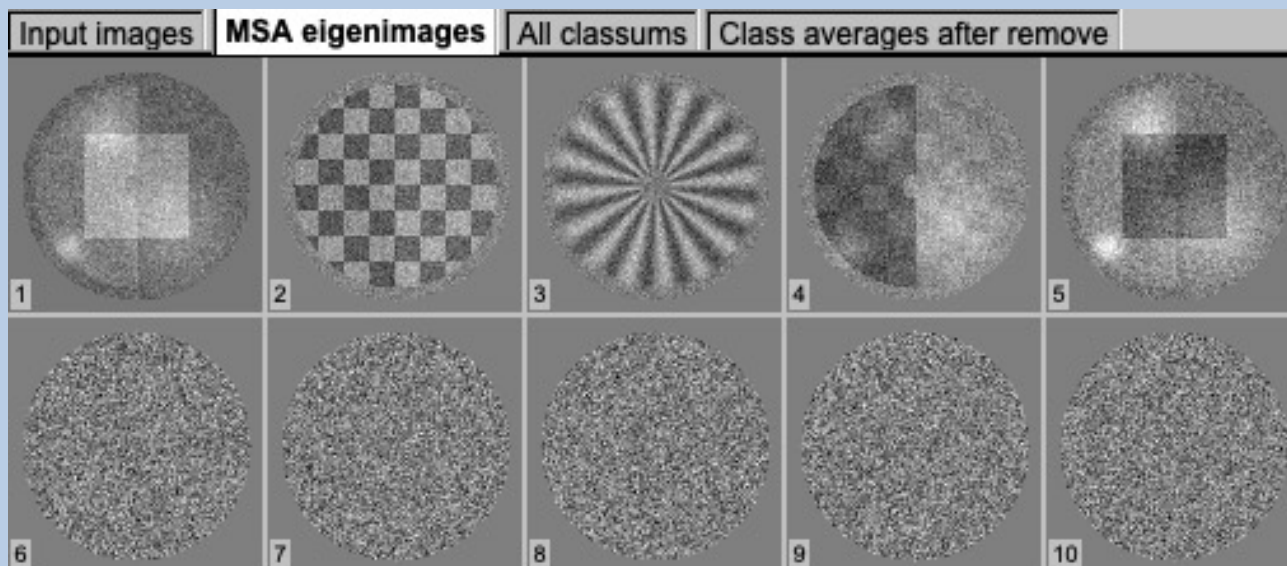
- too few members
Minimal number
- too bad overall quality
- sigma in densities is
 times sigma off average

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



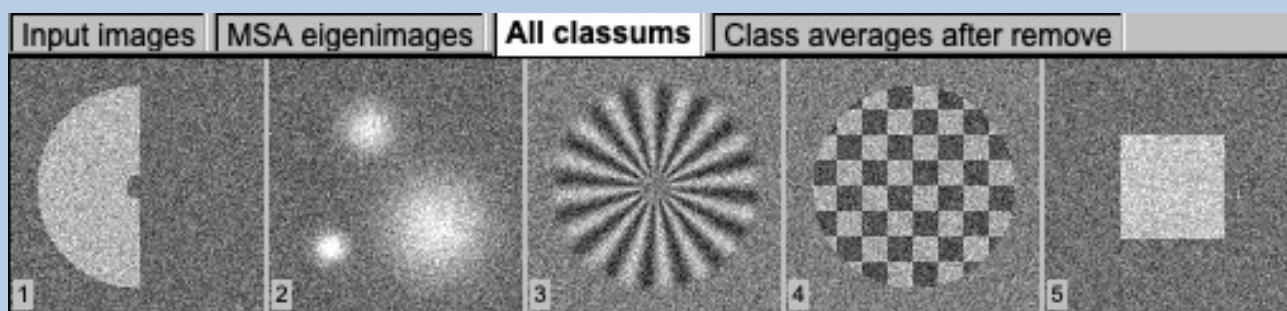
As usual results are displayed on the right hand side, the related display controls are on the left hand side. Carefully check the results:

First have a look at the eigenspectra. The eigenspectra are a good way of examining the information content of a dataset.

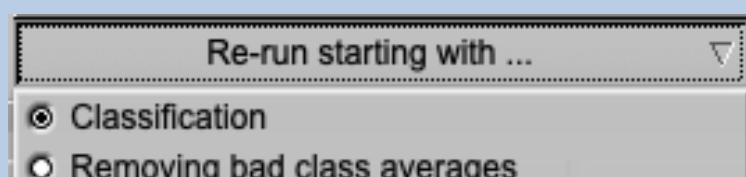


Note that the first eigenimage always shows (a sort of) average of all images and that higher eigenimages describe less important variance than lower numbered eigenimages.

Of course, also check the class averages:



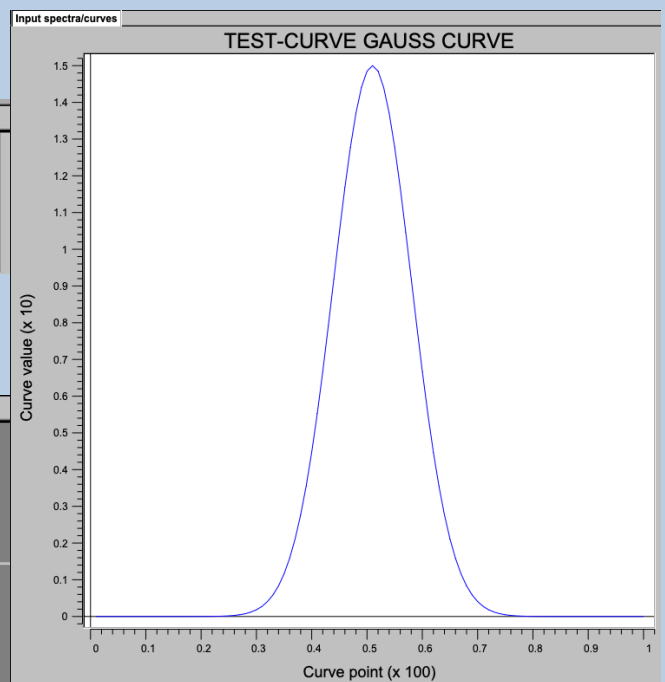
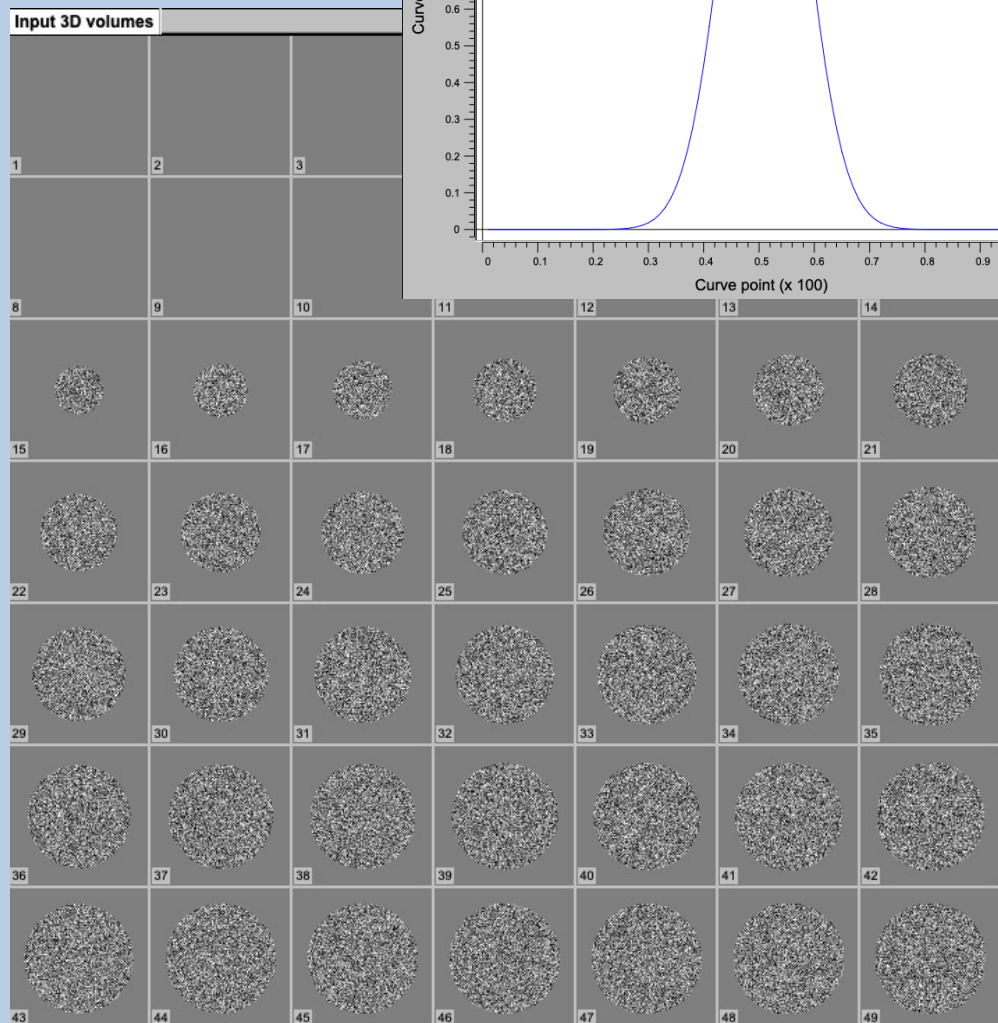
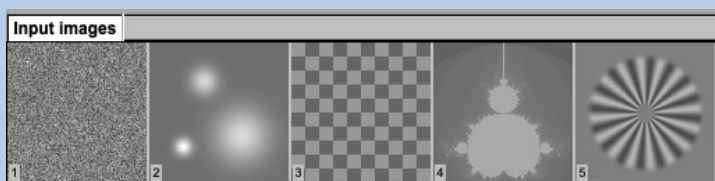
You can play around with the classification parameters and check the new results. If you do not want to change the MSA parameters you can use the “Classify only” and /or the “Removing bad class averages” button.

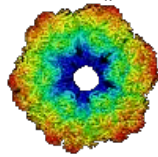


Dimensions

In the previous pages **guiMSA** 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.





IMAGIC

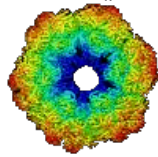
guiMSA

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

guiMSA

Feedback / Error hints

We intensively tested the **guiMSA** 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

