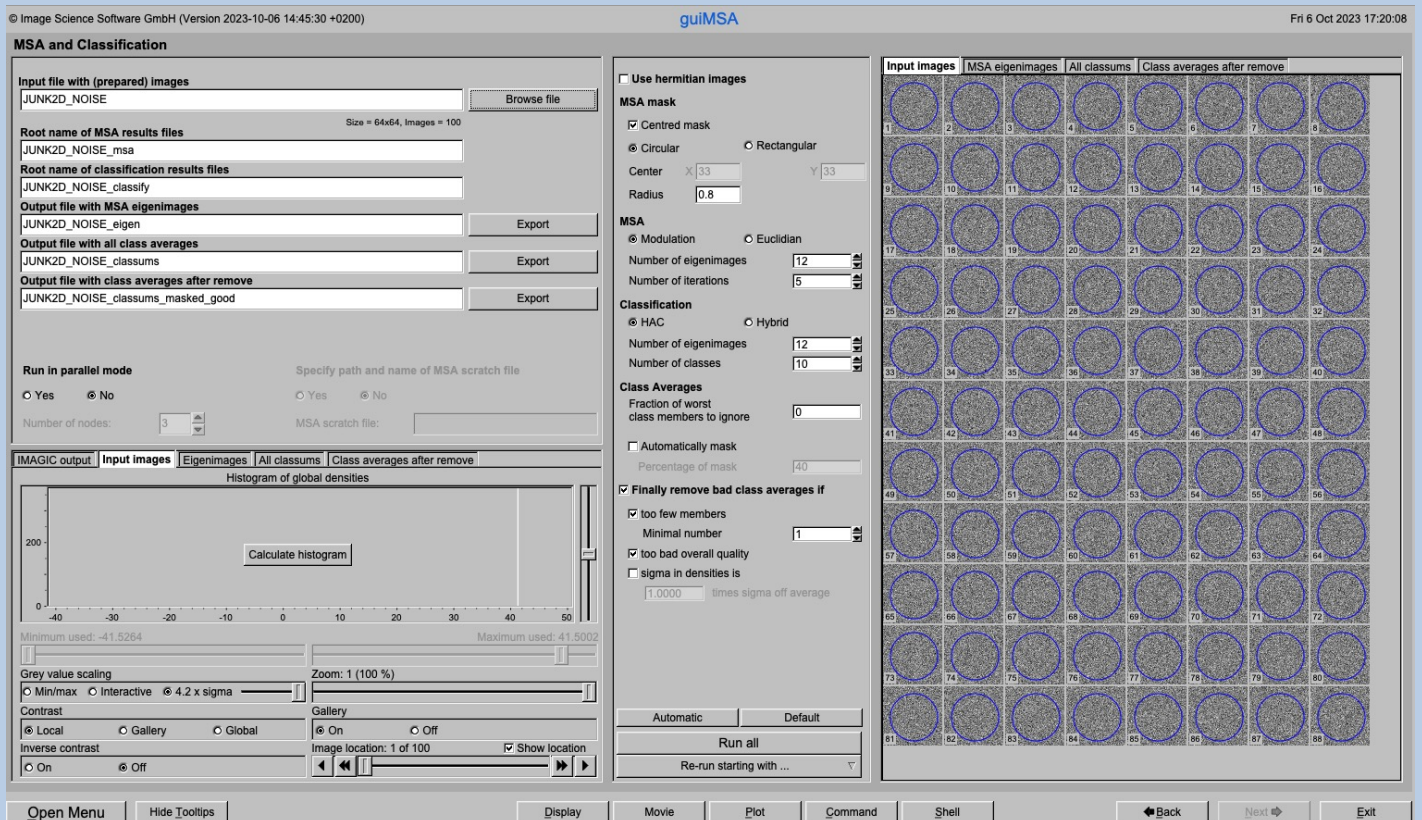




A Brief Introduction

Version 15-Jan-2024
www.ImageScience.de
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The IMAGIC guiALIGN program



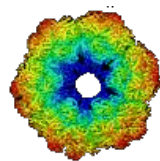
The **guiALIGN** aligns images (NOT 3D volumes) to single or a stack if references (multi-reference alignment). If wanted, the references can be selected from a stack of images or can be created from a 3D volume .

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

CONTENT:

- IMAGIC GUI programs How to use IMAGIC GUI programs
- **guiALIGN** How to Import images to IMAGIC
- How to prepare the images (filter, masks...)
- How to get the references
- How to multi—reference align the images
- How to send us feedback
- Error hints





IMAGIC

GUI Programs

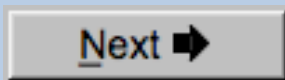


Workflow

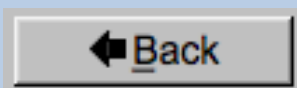
The idea of **guiALIGN** is to guide you through a typical camera/detector correction measurement or camera .

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

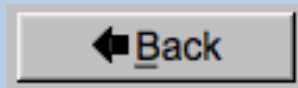
If **guiALIGN** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

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

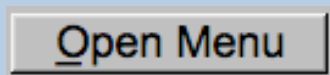
```
IMAGIC-COMMAND : gui-align-images
```

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 **guiALIGN**.

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.

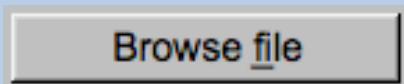
You can, of course, always use other input files names and even use other input directories.

Input file
my_images

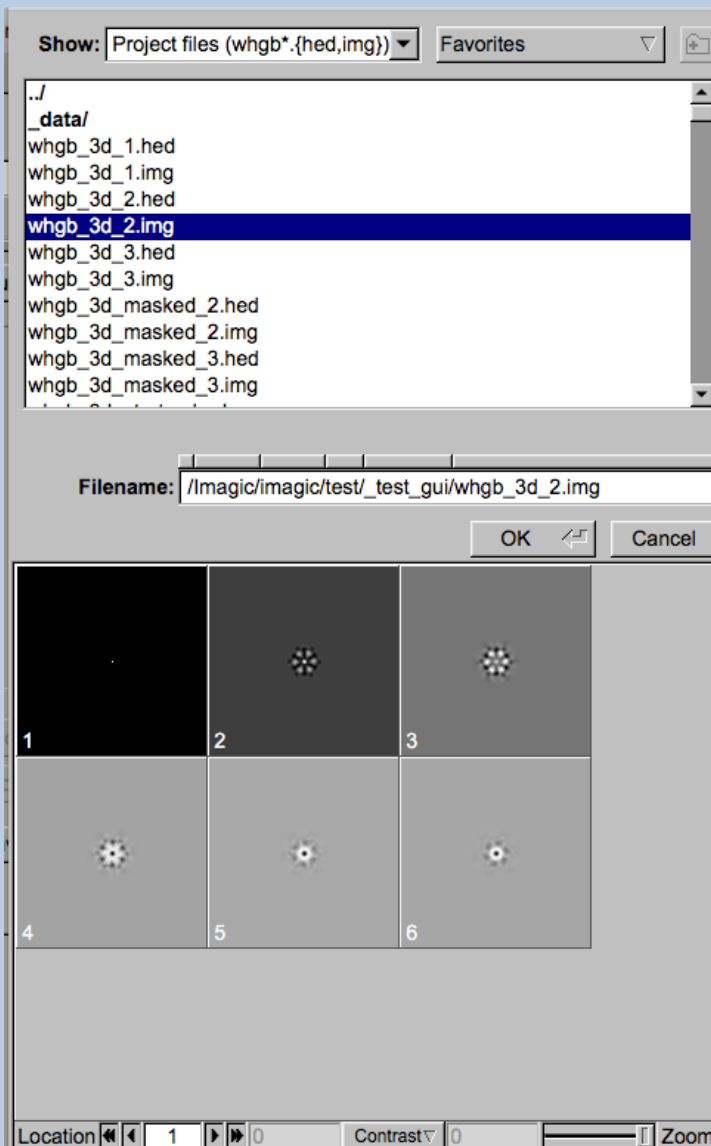


Input File Chooser

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

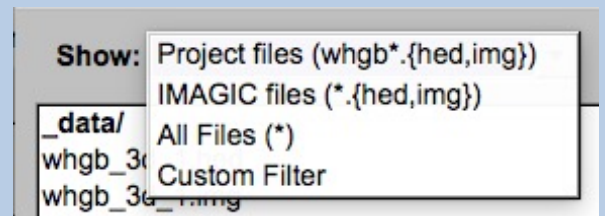


Pressing this button will open the IMAGIC file chooser:

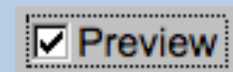


Choose the wanted file by clicking its name

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.

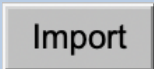
Output file

my_images

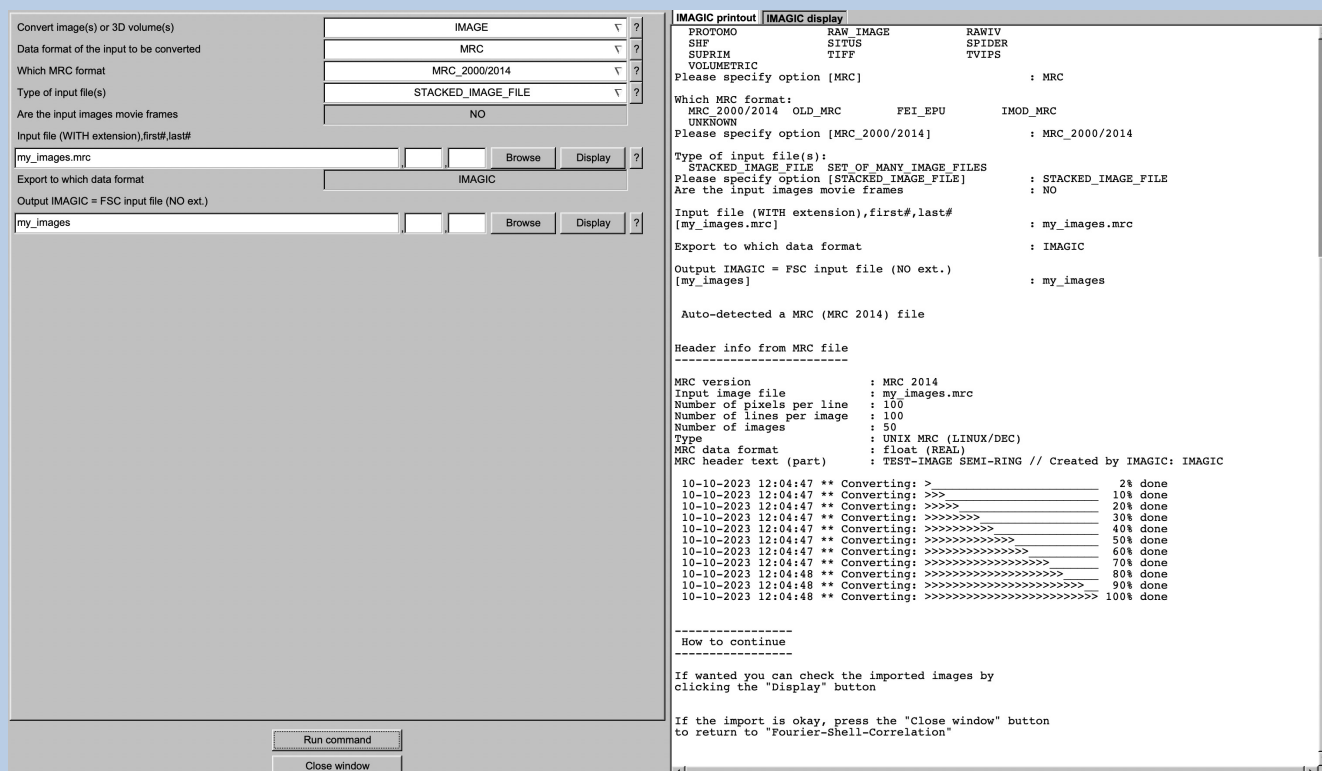


Import Buttons

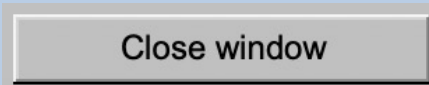
You do not want to use the “Import page” you can use the “Import” button to import the input images/3D volumes from any 3DEM format. The “Import” button which is located above the text field specifying the name of the related file.



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:

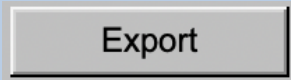


Refer to the **guiEM2EM** manual to get further help.



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:

The screenshot shows the IMAGIC EM2EM software interface. On the left is a configuration panel with various options and dropdown menus. On the right is a terminal window displaying the command-line output of the software. The configuration panel includes fields for input file name, output format, and output file name. The terminal window shows the same configuration being translated into a series of command-line arguments and options.

Configuration Panel:

- 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

Terminal Output:

```
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

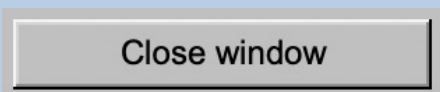
Type of output file:
  STACKED_IMAGE_FILE  SET_OF_MANY_IMAGE_FILES
Please specify option [STACKED_IMAGE_FILE] : STACKED_IMAGE_FILE

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 1.5 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

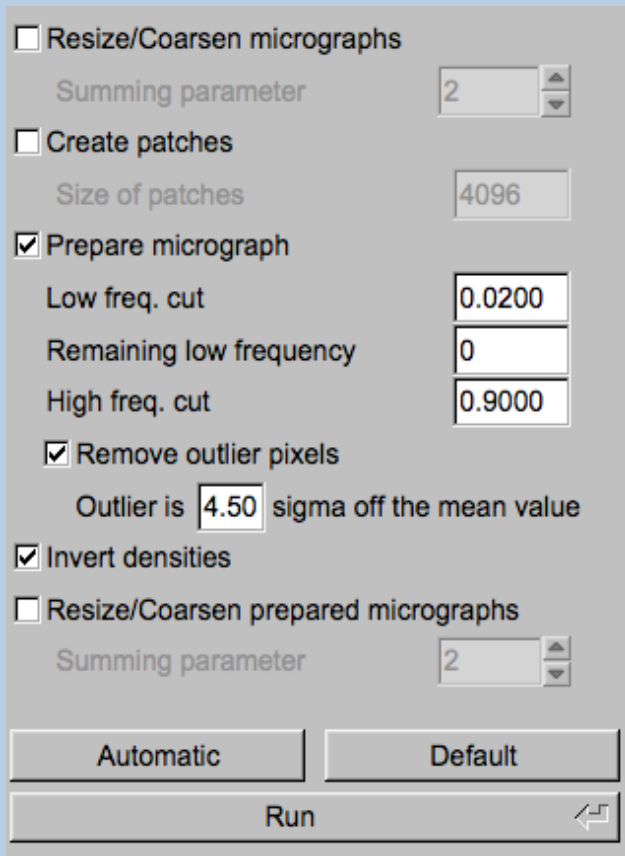
In text fields you can type in the wanted value. If the needed value is a number you can also move the cursor into this field, press the mouse key and keep it pressed and move the cursor to change the value.

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



A Typical Page - Automatic / Default



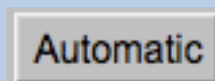
The screenshot shows a GUI window with the following elements:

- 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

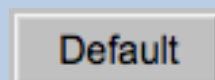
At the bottom, there are three buttons: "Automatic", "Default", and "Run".

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

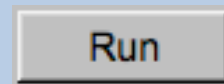
Number of iterations

Classification

Use how many eigenimages

Number of classes

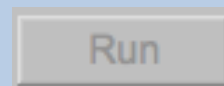
To run the calculations press the “Run” button.



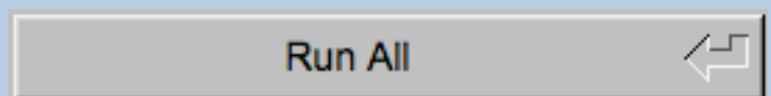
On a number of pages the calculations can be split. In this case you will find more than one single “Run” button.

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

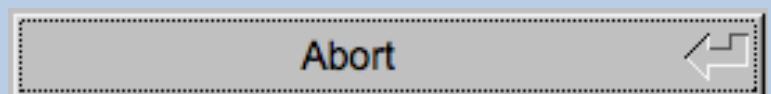
Maybe a certain “Run” button is not yet activated because it needs the results of calculations not yet done.



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

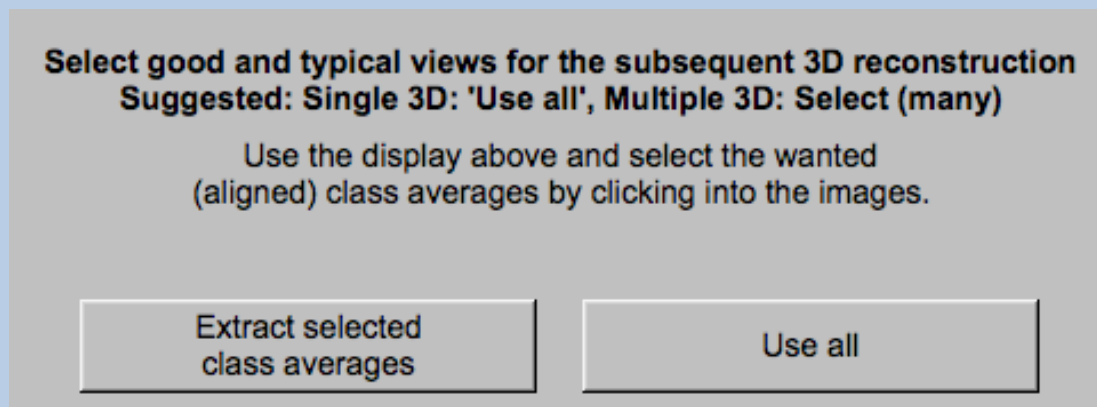


You can abort a running program by pressing the “Abort” button.



A Typical Page - Additional Tasks

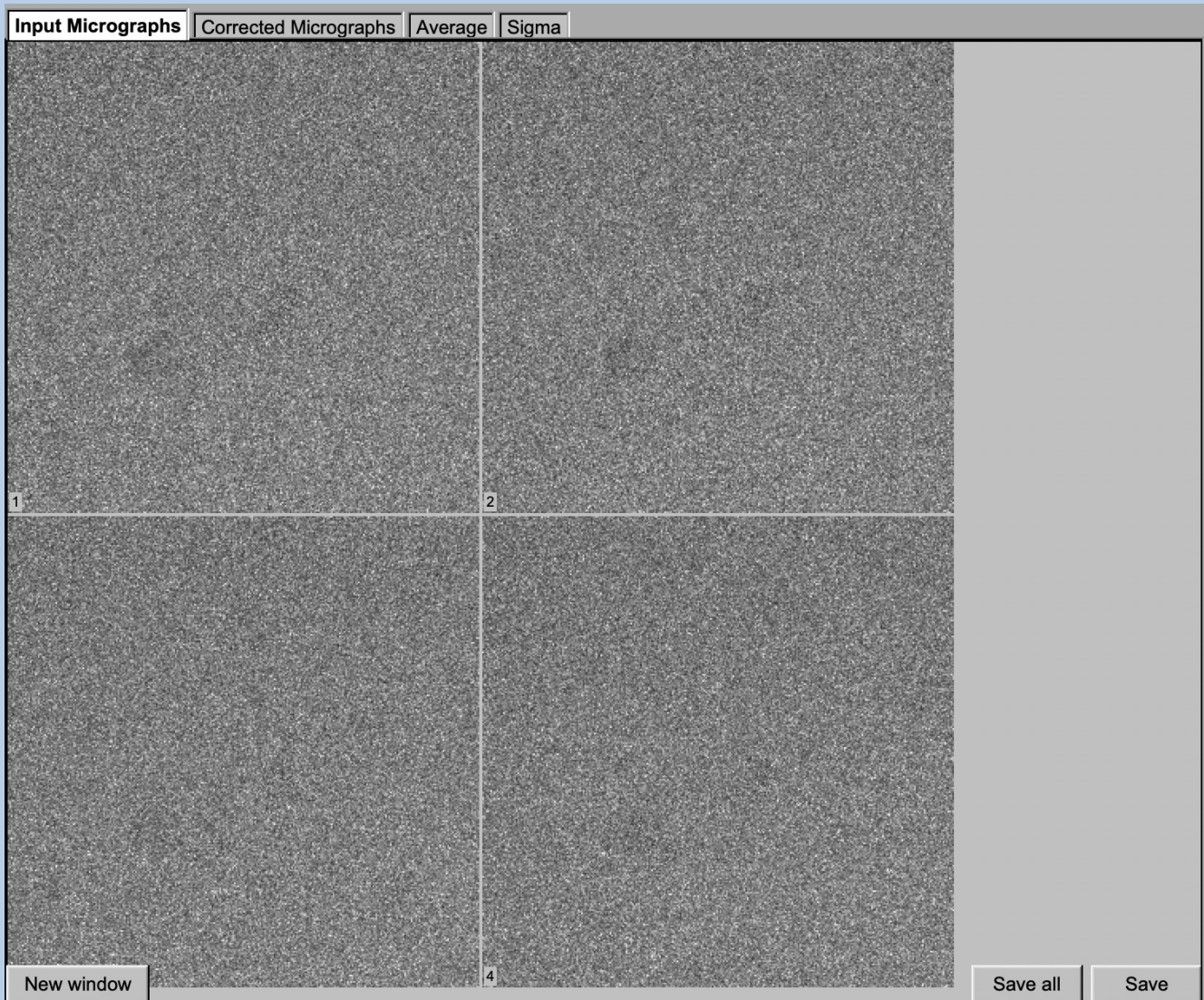
The main calculations on the page are done using the middle part of an typical **IMAGIC GUI program** page. But on a number of pages some additional calculations have to be done. Please follow the instructions given.



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

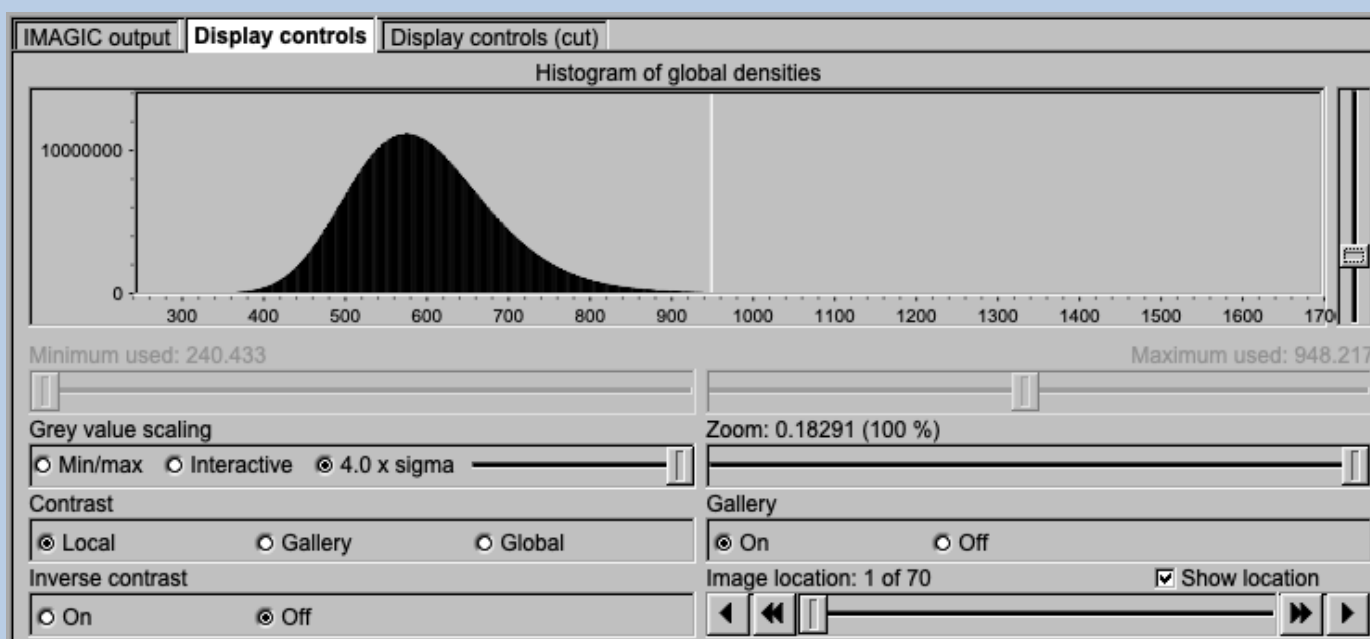
Gallery

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.

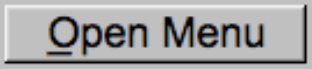
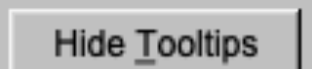
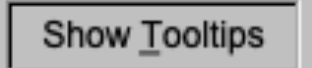
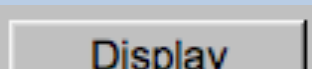
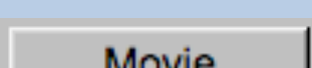
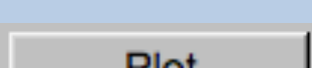
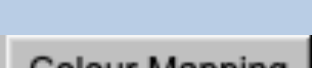

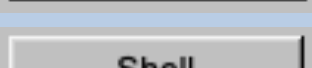
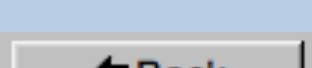
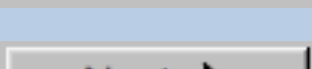
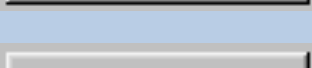
Use the slider or the arrows to select image locations



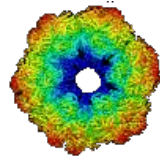
A Typical Page - The Toolbar

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



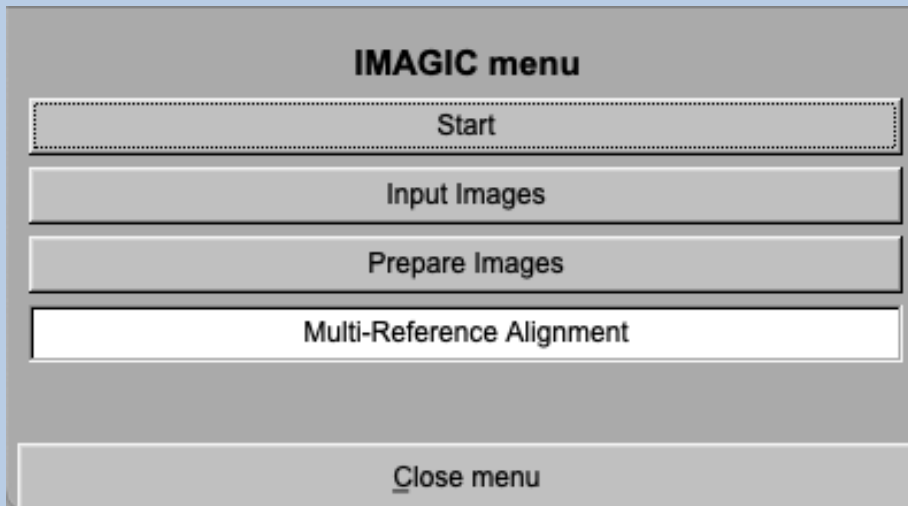


IMAGIC

guiALIGN



The guiALIGN Menu



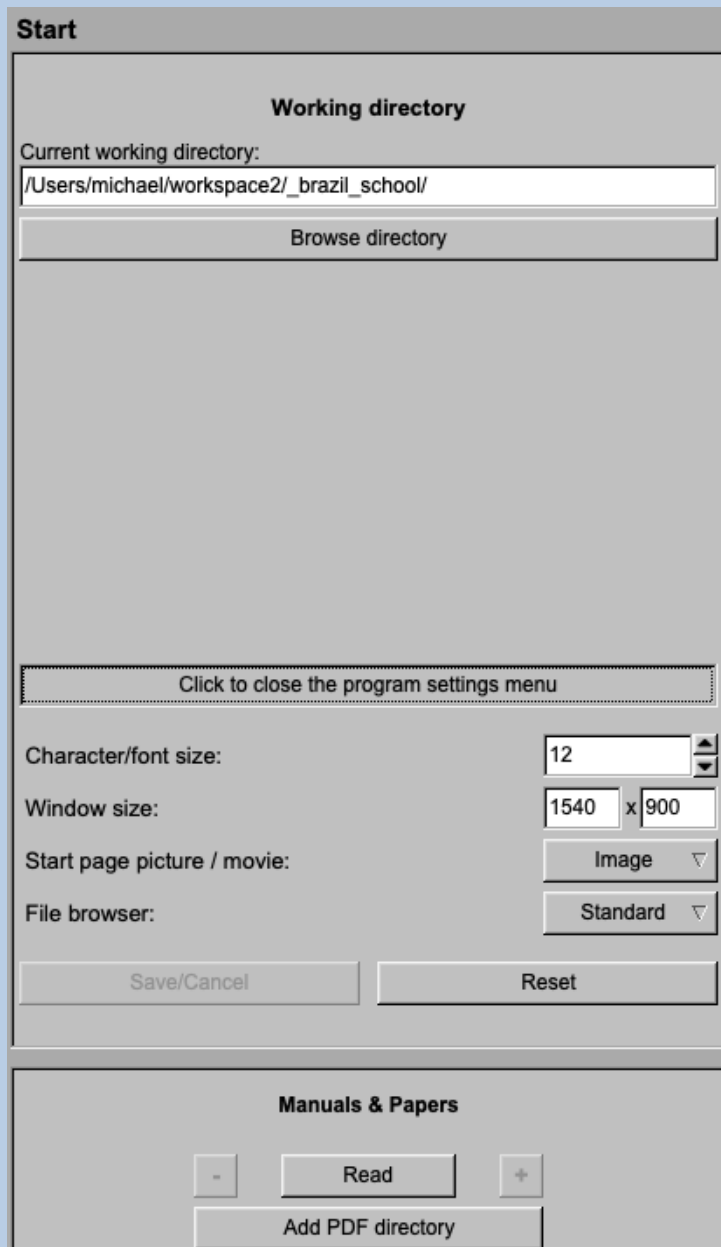
PAGES:

Start	Page to adjust guiALIGN program parameters
Import Images...	Import or specify the input.
Prepare Images...	Pre-treatment: Mask, filter, normalise variance, resize, summing ...
Multi-Reference Alignment	Multi-reference alignment of images. If wanted references can be selected from a stack of images or from (a) 3D volume(s).



The “Start” Page

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



On this page you can set some program parameters:

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



Start Working

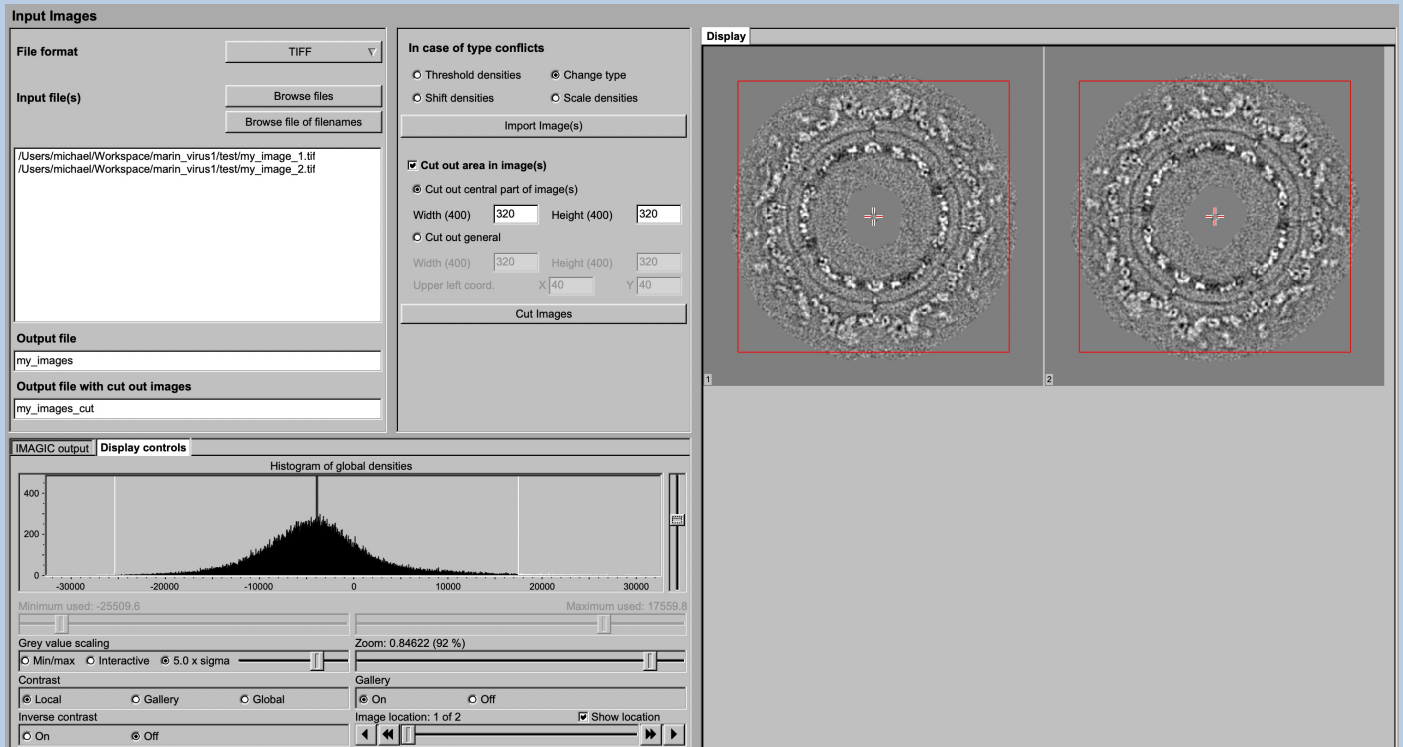
guiALIGN starts with the “Import” page.

The workflow using the “Next” button will guide you through all **guiALIGN** 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 files using any 3D-EM format (or TIFF) into a single (stacked) IMAGIC image file.

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

Also refer to program **guiIMPORT**.



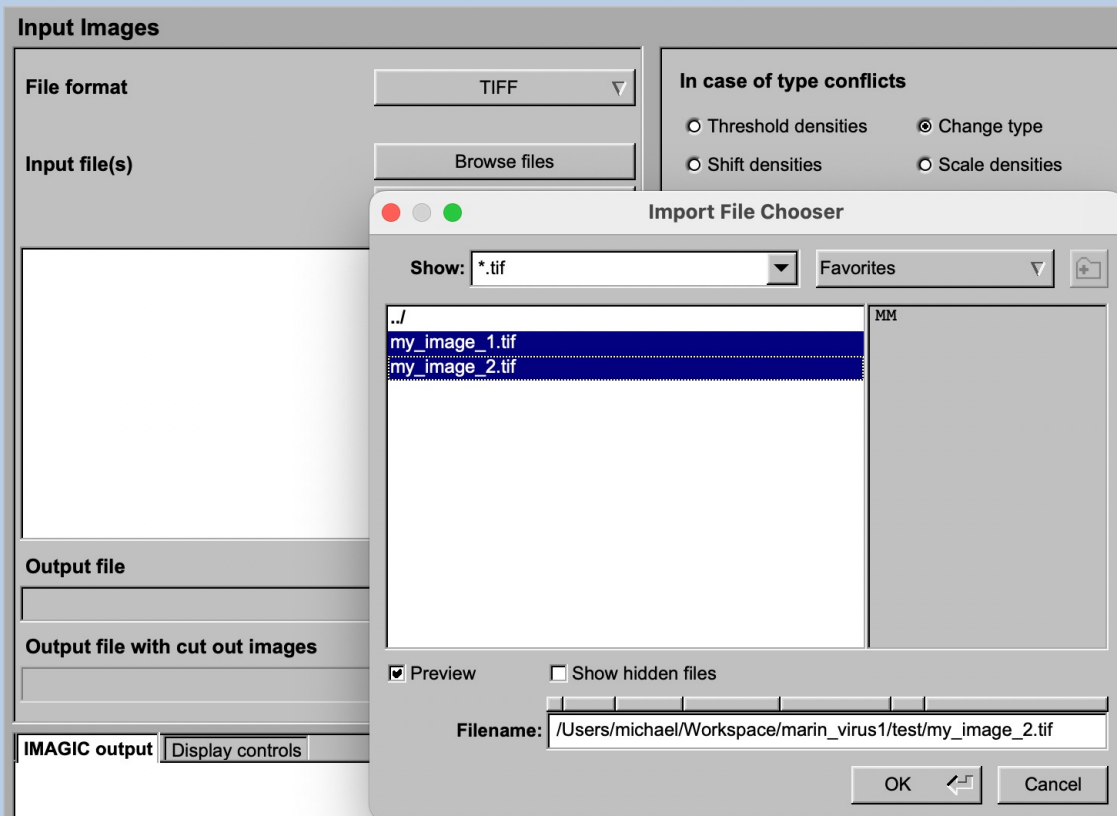
IMPORT :

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.

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



The “Prepare Images” Page

Prepare Images

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: Yes 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: Sum Images (Odd-even sum, Half-half sum, Sequence sum: 2, Total sum)

Automatic Default Run

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:

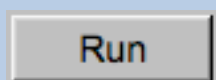
Input file

Size = 400x400, Images = 2

Output file with resized images

Output file with prepared images

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

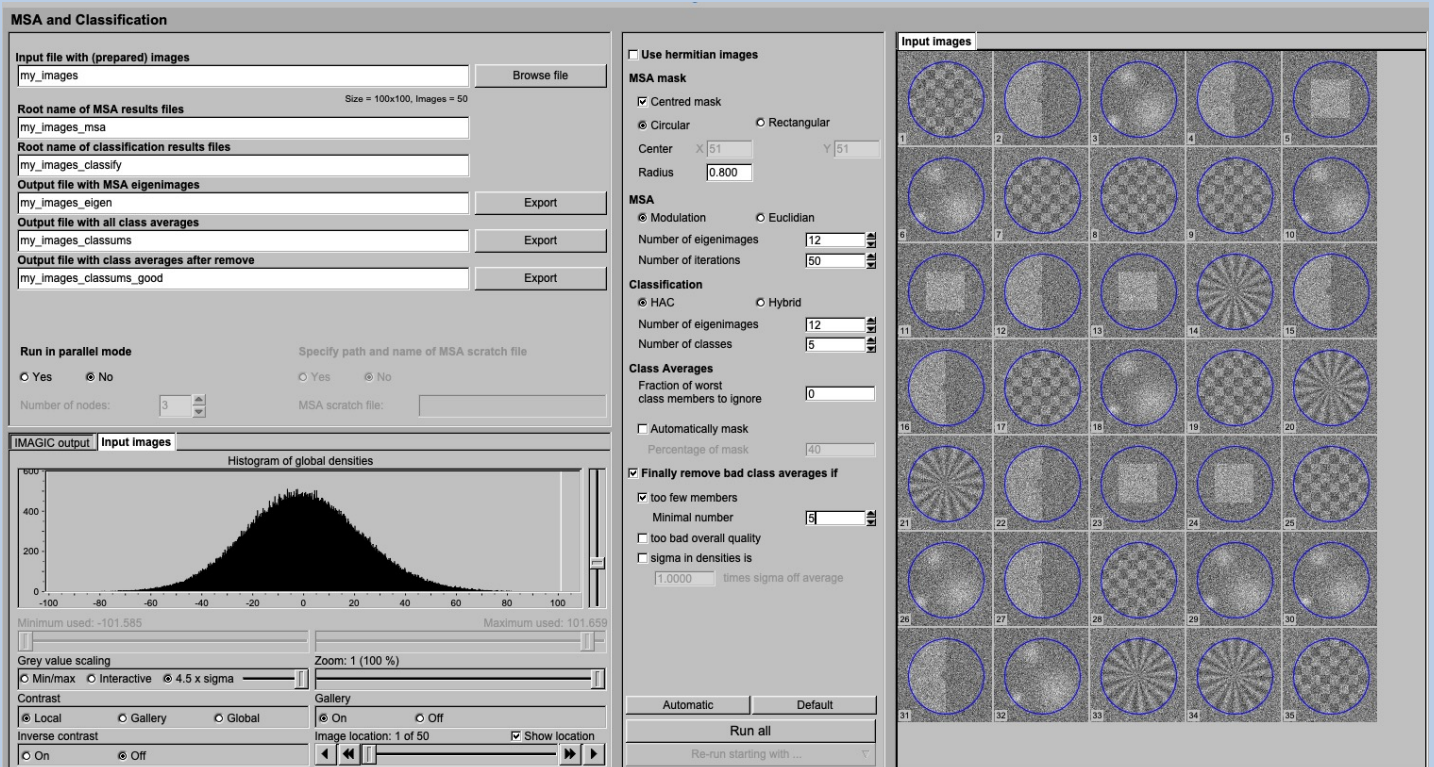
Sequence sum images

Total sum

Output file with image sum(s)



The “Multi-Reference Alignment” Page



DESCRIPTION:

The **guiALIGN** aligns images to a stack of references (multi-reference alignment).

If wanted references can be selected from a stack of images or can be generated as forward projections from (a) 3D volume(s).

FULL IMAGE DATA-SET ANALYSIS:

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



To align the particle images you need references. In principle, there are three options to get these references:

GET REFERENCES FROM A 3D VOLUME:

The first option is to generate the references from a 3D volume which is related to the images to be aligned.

Choice of references

Get from 3D volume
 Select from images
 Get from file

Input 3D volume file

my_3d

Size = 64x64x64, Volumes = 5

Output file with generated references

my_3d_ref

The advantage is that you can get all possible directions (all possible "views") and that the references are perfectly (3-D) aligned to each other. So, when using this option do NOT centre or align these references any more.

As usual, specify the input file containing the 3D volume(s) to be used the generate the references as well as the name of the references output file.

Please also specify the number of references/forward projections wanted and the threshold value to be used for the forward projections. I wanted, you can also generate mirror references.

Number of references

Threshold

Also use mirror views



There are three ways to calculate the forward projections:

The projection direction to create the references are chosen ad random:

Mode of projection

Random

Asymmetric triangle

Tomography

The references can re-present all typical views within the asymmetric triangle)in case the object has a certain 3D point-group symmetry):

Mode of projection

Random

Asymmetric triangle

Tomography

In certain cases you may want forward projection in an angular range around the equator of the object:

Mode of projection

Random

Asymmetric triangle

Tomography

Threshold

Angular limit

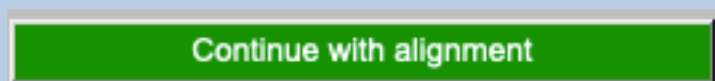
Min Max

Click the "Generate references" button to generate the references:



Check the references in the display tab on the right hand side. If needed, change the parameters and re-generate the references.

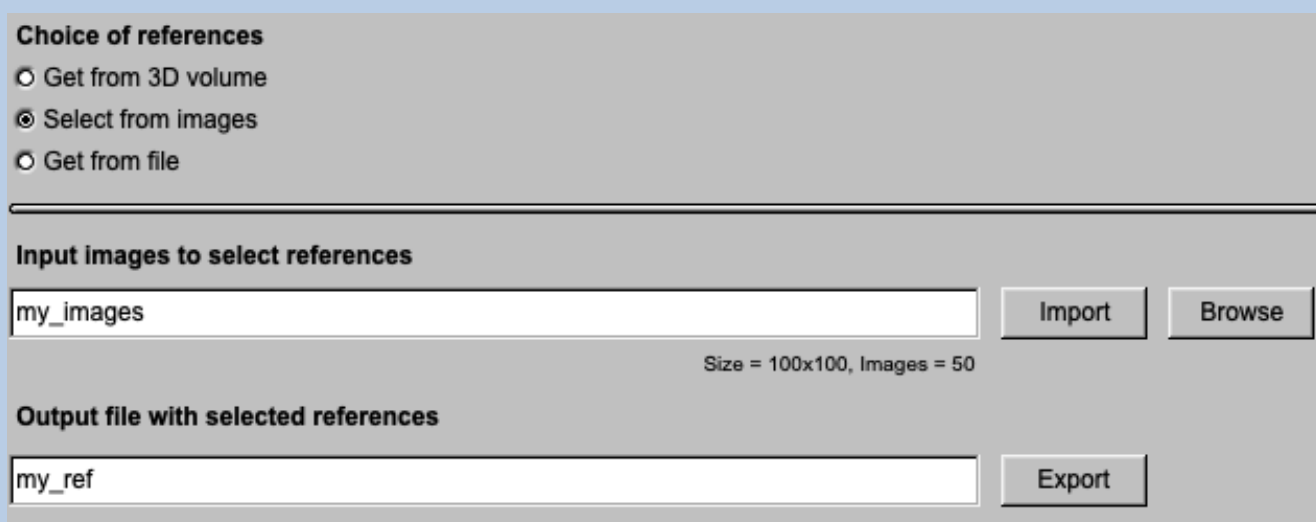
If the references are alright click the “Continue with alignment” button



and the page will skip to the “Get from file” option to run the alignment.

SELECT REFERENCES FROM A STACK OF IMAGES:

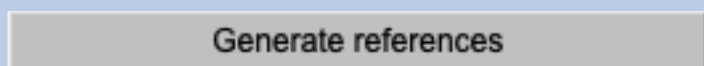
The second option is to select the references from a stack of images (usual "typical" and noise-free images):

A screenshot of a software dialog box titled "Choice of references". It contains three radio button options: "Get from 3D volume", "Select from images" (which is selected), and "Get from file". Below the options is a section titled "Input images to select references" with a text input field containing "my_images", an "Import" button, and a "Browse" button. Below that, it shows "Size = 100x100, Images = 50". The next section is titled "Output file with selected references" with a text input field containing "my_ref" and an "Export" button.

As usual, specify the input file containing the 3D volume(s) to be used the generate the references as well as the name of the references output file.

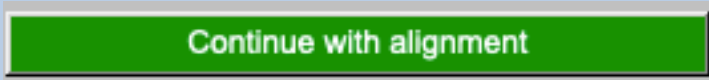
Now check the images in the display tab on the right hand side and select the wanted references (all typical views with good contrast, if possible) by clicking into the image. A second click will de-select it.

Having chosen all wanted images click the “Generate references” button to extract them and generate the references image file:



Check the references in the display tab on the right hand side. If needed, select or de-select images and re-generate the references.

If the references are alright click the “Continue with alignment” button and the page will skip to the “Get from file” option.



and the page will skip to the “Get from file” option to run the alignment.

NOTE: This option bears the risk of reference bias!

GET REFERENCES FROM FILE:

If your reference images are already stored in an IMAGIC image file use the “Get from file” option to run the the multi-reference alignment

Choice of references

- Get from 3D volume
- Select from images
- Get from file

As usual, specify the input file containing the images to be aligned, the "original" images and the references. Also give the name of the output file to contain the aligned images.

Input images to be aligned		
<input type="text" value="my_images"/>	<input type="button" value="Import"/>	<input type="button" value="Browse"/>
	Size = 100x100, Images = 50	
Input original (non-moved) images		
<input type="text" value="my_images"/>	<input type="button" value="Import"/>	<input type="button" value="Browse"/>
	Size = 100x100, Images = 50	
Input references		
<input type="text" value="my_ref"/>	<input type="button" value="Import"/>	<input type="button" value="Browse"/>
	Size = 100x100, Images = 5	
Output file with aligned images		
<input type="text" value="my_images_ali"/>	<input type="button" value="Export"/>	



Note:

In case the input images are already moved (in a previous **IMAGIC** alignment, for example) the "original" non-moved images will be used for an "equivalent move" to avoid multiple interpolation artefacts.

If your references were generated from multiple 3D volumes it can be a good idea to align each particle image either to all references or to its own 3D volume references only. It is your choice:

Reference option

- Align to all references
- Align to own 3D references only

There is a number of parameters which you can specify to adjust the alignment::

Alignment options

- Rotational & translational
 - Rot. first
 - Trans. first
- Translational only
- Rotational only
- Brute force rot. & trans.

How many iterations

Correlation function

- CCF
- MCF

Alignment Parameters

Max. shift for overall/current MRA

Overall Current

Max. rot. for overall/current MRA

Overall Current

Region for rot. alignment (radius)

Inner Outer

You can get detailed help by moving the cursor over the related boxes.



You can get help by moving the cursor over the related boxes.

Do not forget to strongly suppress the high frequencies during alignments to avoid bias and over-fitting:

Filter references

Low-pass filter

HF cut

Filter references

Low-pass filter

HF cut

Band-pass filter

LF cut HF cut

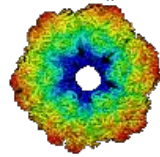
Cut-off high frequencies

HF cut off Drop off

Run the alignment and check the printout in the terminal window as well as the aligned images in the display on the right-hand side.

If necessary, change parameters and re-run the alignment,





IMAGIC

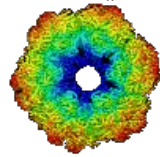
guiALIGN

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

guiALIGN

Feedback / Error hints

We intensively tested the **guiALIGN** 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.



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