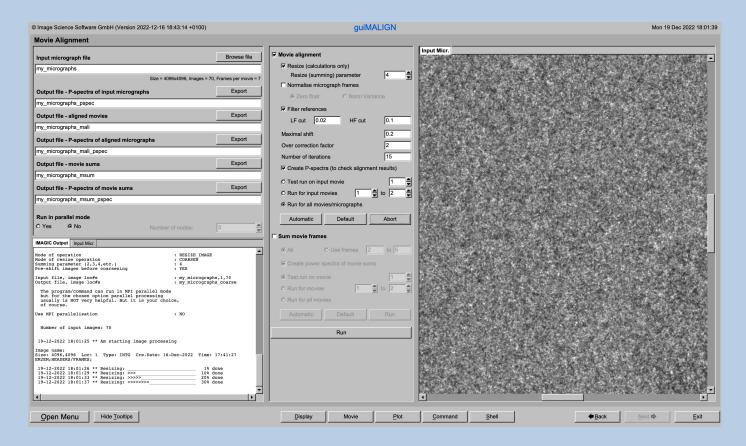


#### A Brief Introduction

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

### The IMAGIC guiMALIGN program



The **guiMALIGN** program follows a work-flow from Import Micrographs to Camera/Detector Correction.

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

#### **CONTENT:**

➤ IMAGIC GUI programs

guiMALIGN

> Import Micrographs

> Camera Correction

> Movie Alignment

> Error hints

How to use IMAGIC GUI programs How to align movie frames

How to send us feedback



# IMAGIC GUI Programs

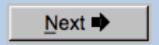


#### Workflow

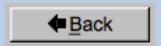
The idea of **guiMALIGN** is to guide you through a typical camera/detector correction measurement or camera.

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

IMAGIC-COMMAND : guimalign

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

Open Menu

to navigate to the "Start" page where you can specify the working directory of **guiMALIGN**.

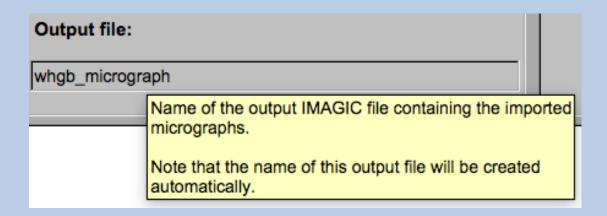
All output files will be stored in the working directory which you have specified on the start page.

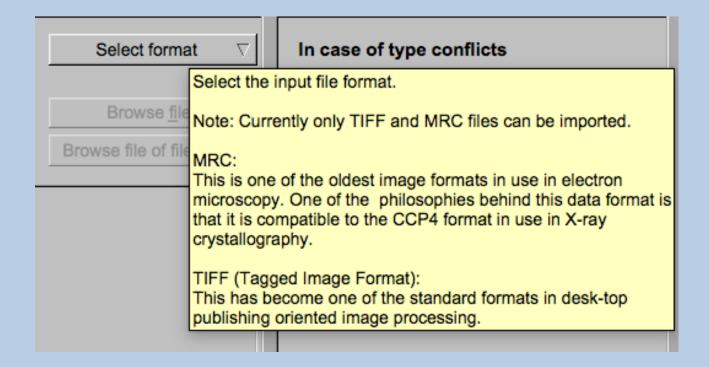
Input files can be chosen from other directories.



#### 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.

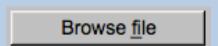
You can, of course, always use other input files names and even use other input directories.

Input file with (raw) micrographs	Browse file
my_micrographs	

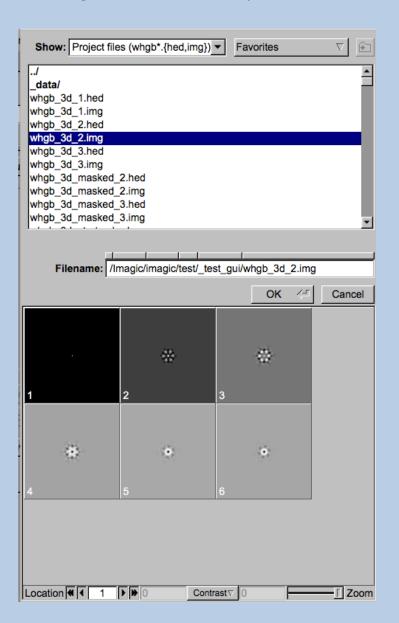


#### Input File Chooser

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

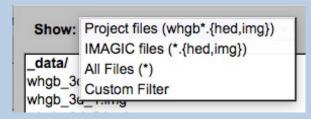


Pressing this button will open the IMAGIC file chooser:



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_micrographs	

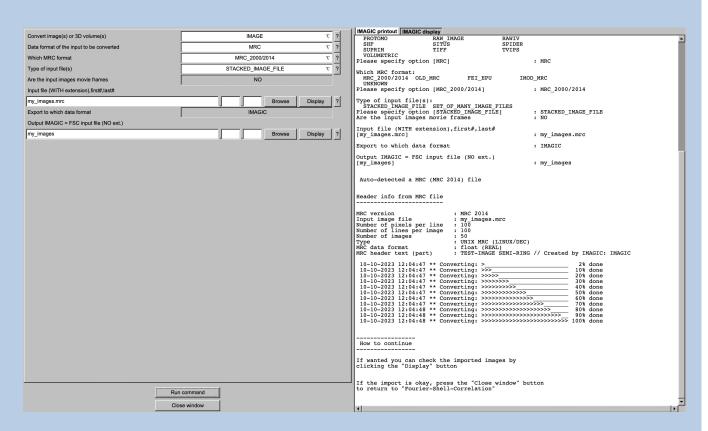


#### **Import Buttons**

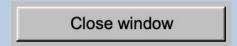
You do not want to use the "Import page" you can sometimes use an "Import" button to import the input images/3D volumes from any 3DEM format. The "Import" button which is located above the text field specifying the name of the related file.



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



Click the "Close window" button to exit this additional window:

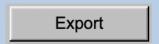


Refer to the guiEM2EM manual to get further help.

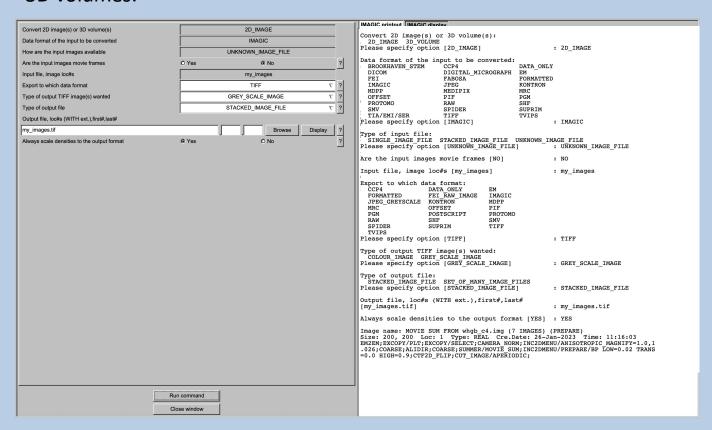


#### **Export Buttons**

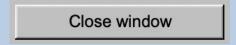
You can export output images/3D volumes to any 3DEM format. Click the "Export" button which is located above the text field specifying the name of the related file.



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



Click the "Close window" button to exit this additional window:



Refer to the guiEM2EM manual to get further help.



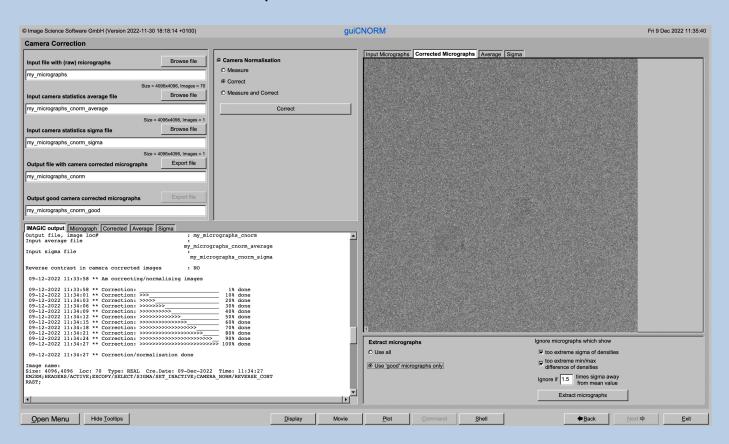
#### A Typical Page

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.





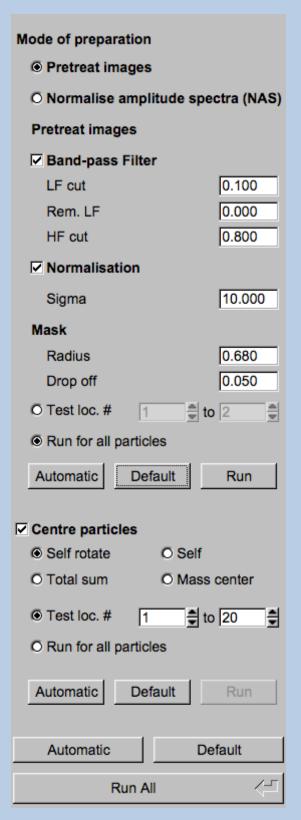
## A Typical Page - MPI Parallel

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
Yes	O Yes    ● No
Number of nodes: 3	MSA scratch file:

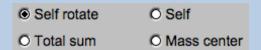


## A Typical Page - Program Parameters

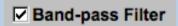


In the middle part of a typical **IMAGIC GUI program** page you will find the program parameters to be used.

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



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



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.

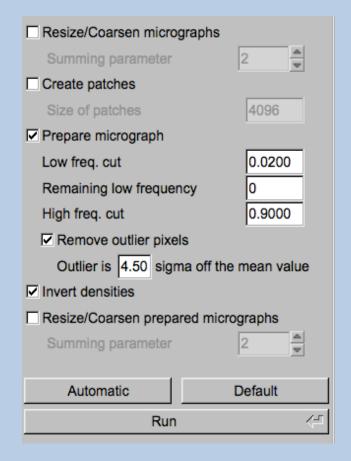
10.000

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



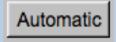


### A Typical Page - Automatic / Default

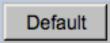


In the middle part of a typical **IMAGIC GUI program** page you will also find "Automatic" and "Default buttons.

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



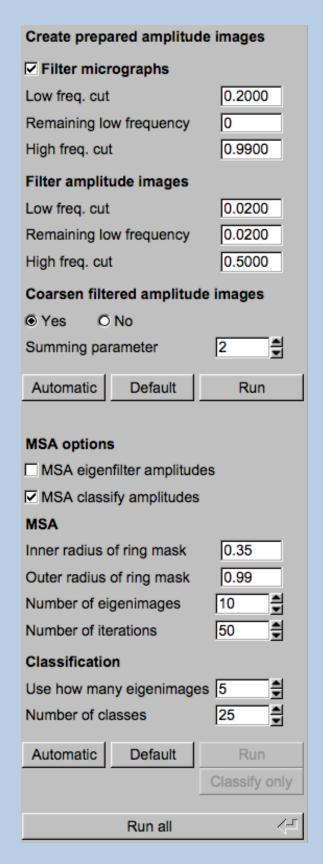
Pressing the "Default" button will fill in the values which you have used during the last "Run".



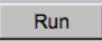
The values shown when entering a page are the default values (your last values given) if they are available. Else the automatic values are shown.



#### A Typical Page - Run buttons



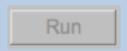
To run the calculations press the "Run" button.



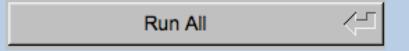
On a number of pages the calculations can be split. In this case you will find more than one single "Run" button.

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

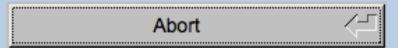
May be a certain "Run" button is not yet activated because it needs the results of calculations not yet done.



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



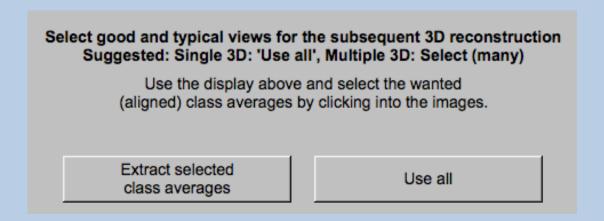
You can abort a running program by pressing the "Abort" button.





#### 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 - "Terminal Window"

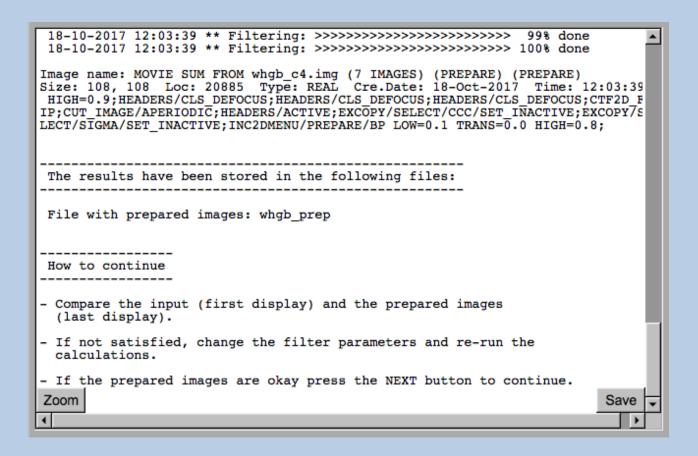
All print-out of an IMAGIC program started within the current **IMAGIC GUI program** page is shown in a kind of terminal window on the left hand side.

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

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

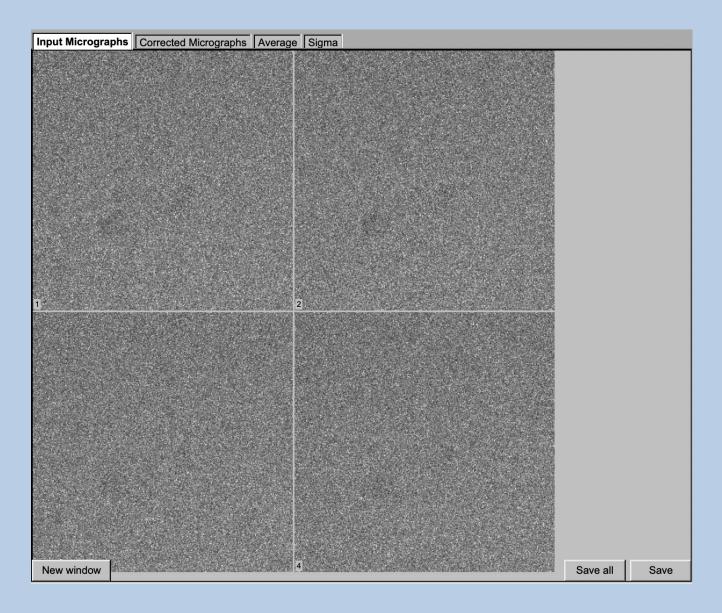
With the "Zoom" button you can open a separate larger window showing the print-out.

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





### 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

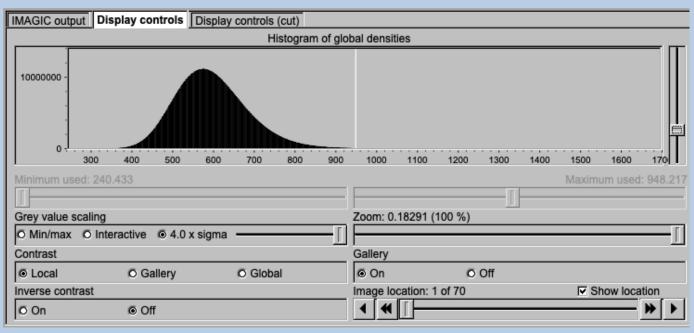
Zoom Enlarge the displayed images

Gallery On Display the images in a gallery

(may be you need another zoom to see

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 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
Horizontal scaling	☐ Use for all plots	
1.00	32.00	Reset
Vertical scaling	☐ Use for all plots	
-19.21	17.00	Reset
Plot title	☐ Use for all plots Rese	
Fourier Ring Information - 1/2-bit		
Text along horizontal axis	☐ Use for all plots Rese	
Radius in Fourier space		
Text along vertical axis	☐ Use for all plots Rese	



#### A Typical Page - The Toolbar

There is a toolbar at the bottom of each guiMALIGN page.

The toolbar buttons:

Display

Movie

Plot

Colour Mapping

Command

Shell

Back

Next

Exit

Open Menu Open the MENU to navigate to each page wanted

Show Tooltips

Show 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 **guilMAGIC**.

Run a shell / terminal page. command

Go to the previous page

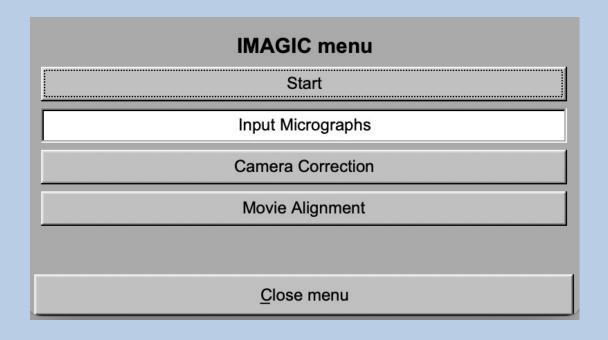
Continue with the next page

Exit **guiMALIGN** 

# IMAGIC guiMALIGN



#### The guiMALIGN Menu



PAGES:

guiMALIGN:

Import Micrographs: Convert micrographs/images into

IMAGIC image format

Camera Correction: Measure and/or correct for camera

statistics

Movie Alignment: Align movie frames

General:

Start: Page to adjust some program parameters Close menu: Close this menu and return to last page.



# The "Start" Page

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



#### **Start Working**

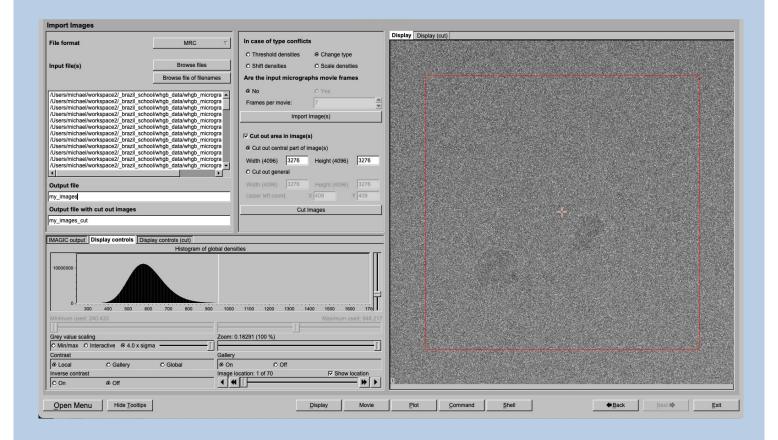
The page guiMALIGN starts with the "Import Micrograph" page.

The workflow using the "Next" button will guide you through all **guiMALIGN** pages.

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



## The "Import Micrographs" Page



#### **DESCRIPTION:**

Convert import micrograph 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.

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

Also refer to program guilMPORT.



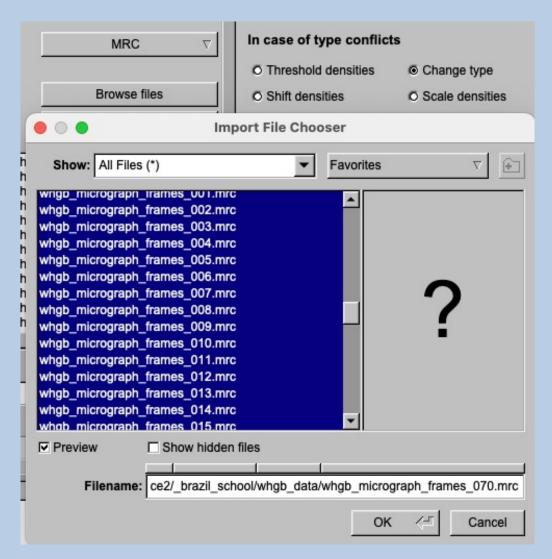
#### **IMPORT MICROGRAPHS:**

Specify the file format in which your input micrographs/images are stored. Click the "Select format" button



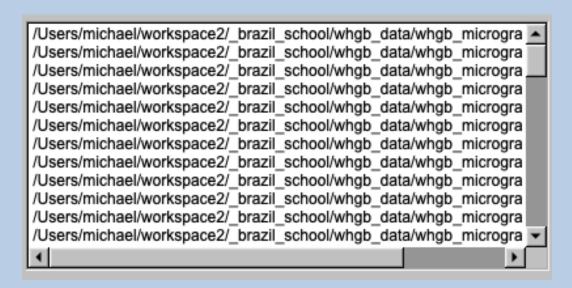
and choose one of the formats in the listing.

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





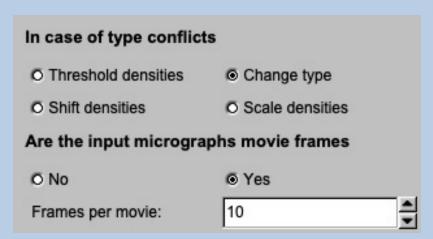
If wanted you can edit the list of files. But be careful there is no automatic control of file names in this list.



Next, you need to specify the name of the output file which is the IMAGIC image file which will contain the imported image(s).

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

#### Format MRC, for example:



Having specified every information needed click the "Import Micrograph" button to start the import of the image(s).

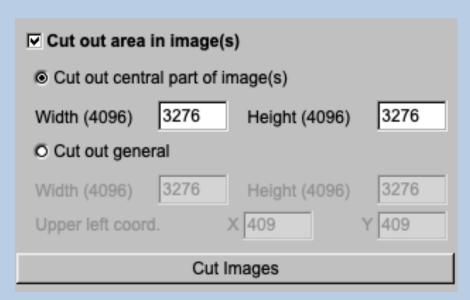
The imported images are shown in the display tab on the right-hand side. See chapter "A Typical Page - Display control tabs".



#### CUT MICROGRAPHS / IMAGES - Not suggested

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

Clicking the "Cut out area of image(s)" option you can cut-out parts of the imported images:



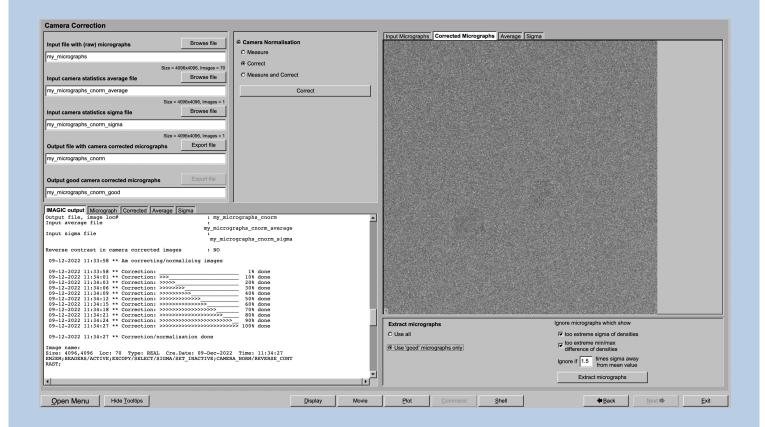
The chosen part is shown in the display window. You can cut-out a central part or any part wanted. The cut-out part is the same in all images, of course.

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

Having specified everything click the "Cut Images" button to run the calculations.



### The "Camera Correction" Page



#### **DESCRIPTION:**

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

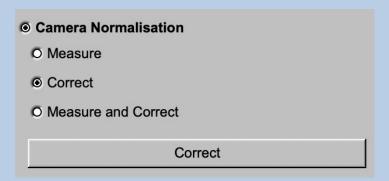
Also refer to program guiCNORM.

#### NOTE:

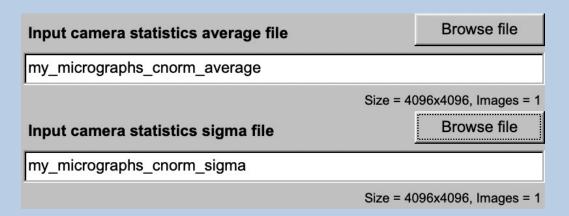
Skip this page if no camera correction is wanted/needed.



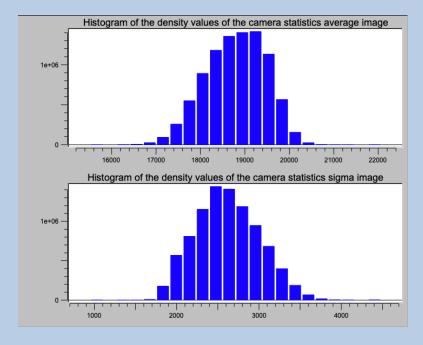
Use the "Correct" option to camera correct the input micrographs using the camera statistics images (average and sigma) of the camera/detector the input macrographs were imaged with.



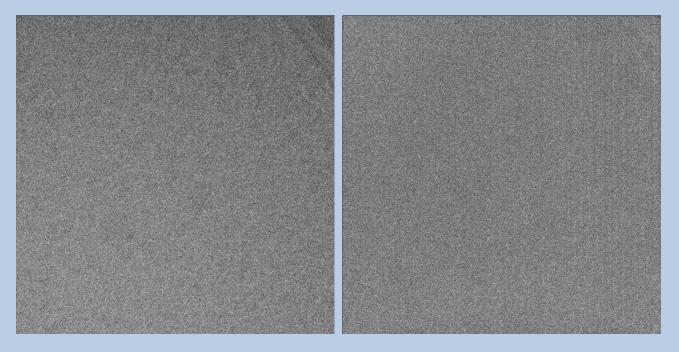
Specify the input camera statistics average and sigma image file needed for the camera correction



The input camera statistics is shown in two histograms using the sigma of the densities in the camera statistics average and in the camera statistics sigma image, which are displayed in display tabs on the right hand side.

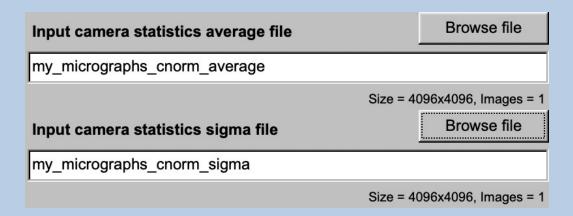






It is always a good idea to check these histograms and images.

Specify the input camera statistics average and sigma image file needed for the camera correction



and, as usual, the file name of the output file which will contain the camera corrected micrographs.

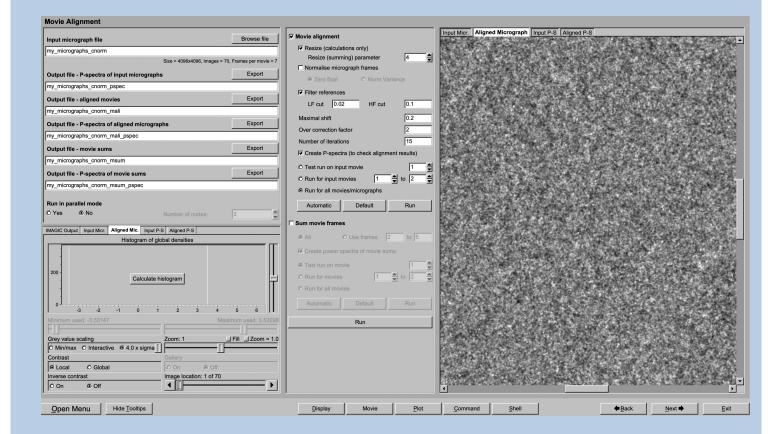


Click the "Correct" button to start the camera correction.

The camera corrected images are displayed on the right hand side of the page.



# The "Movie Alignment" Page

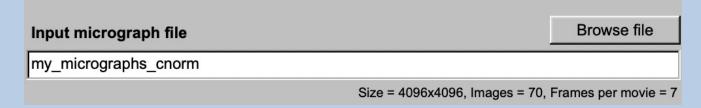


#### **DESCRIPTION:**

If the input micrographs are movie frames you can align theses frames within each movie.



Specify the names of the Input fille containing the micrograph movie frames (usually the camera corrected micrographs)

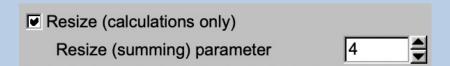


and the names of the output files.

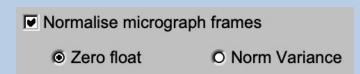
Output file - P-spectra of input micrographs	Export
my_micrographs_cnorm_pspec	
Output file - aligned movies	Export
my_micrographs_cnorm_mali	
Output file - P-spectra of aligned micrographs	Export
my_micrographs_cnorm_mali_pspec	
Output file - movie sums	Export
my_micrographs_cnorm_msum	
Output file - P-spectra of movie sums	Export
my_micrographs_cnorm_msum_pspec	

As usual guiMALIGN suggests names but it is your choice, of course:

You can resize the micrographs to be used in movie alignment to speed-up the calculations



You can normalize the densities (usually not needed):





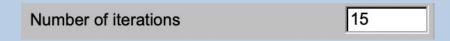
Usually it is a good idea to low-pass filter the references. Note that these references are intermediate sums of the aligned movie frames and are created automatically):



Subsequently, specify the parameters for the movie alignment:

Maximal shift	0.2
Over correction factor	2

Movie alignment tis an iterative procedure. Give the number of iteration wanted:

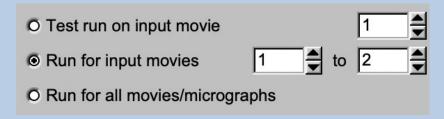


To later check the quality of the alignment it is suggested to also create the pspectra of the input and the aligned movie sums:

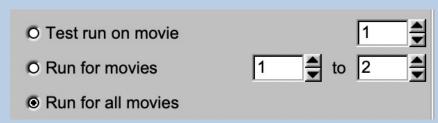
■ Create power spectra of movie sums

#### NOTE:

Play around with the parameters running movie alignment on a single or a small range of micrographs.

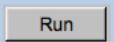


If all parameters are adjusted and the movie alignment of the test micrographs is okay run movie alignment for all input micrographs:



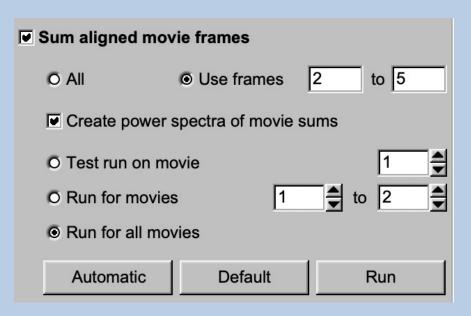


Start the calculations by clicking the "Run" button:



Compare the input and the aligned movie frames. Also compare the related P-spectra. As usual these images are displayed in tabs on the right hand side

To check the alignment is is also a good idea to have a look at the movie sums and the P-spectra of the aligned movie frames:



As usual, you can export the resulting output files to any 3DEM format by clicking the related "Export" button.





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.





Feedback / Error hints

We intensively tested the **guiMALIGN** 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**

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