A Brief Introduction

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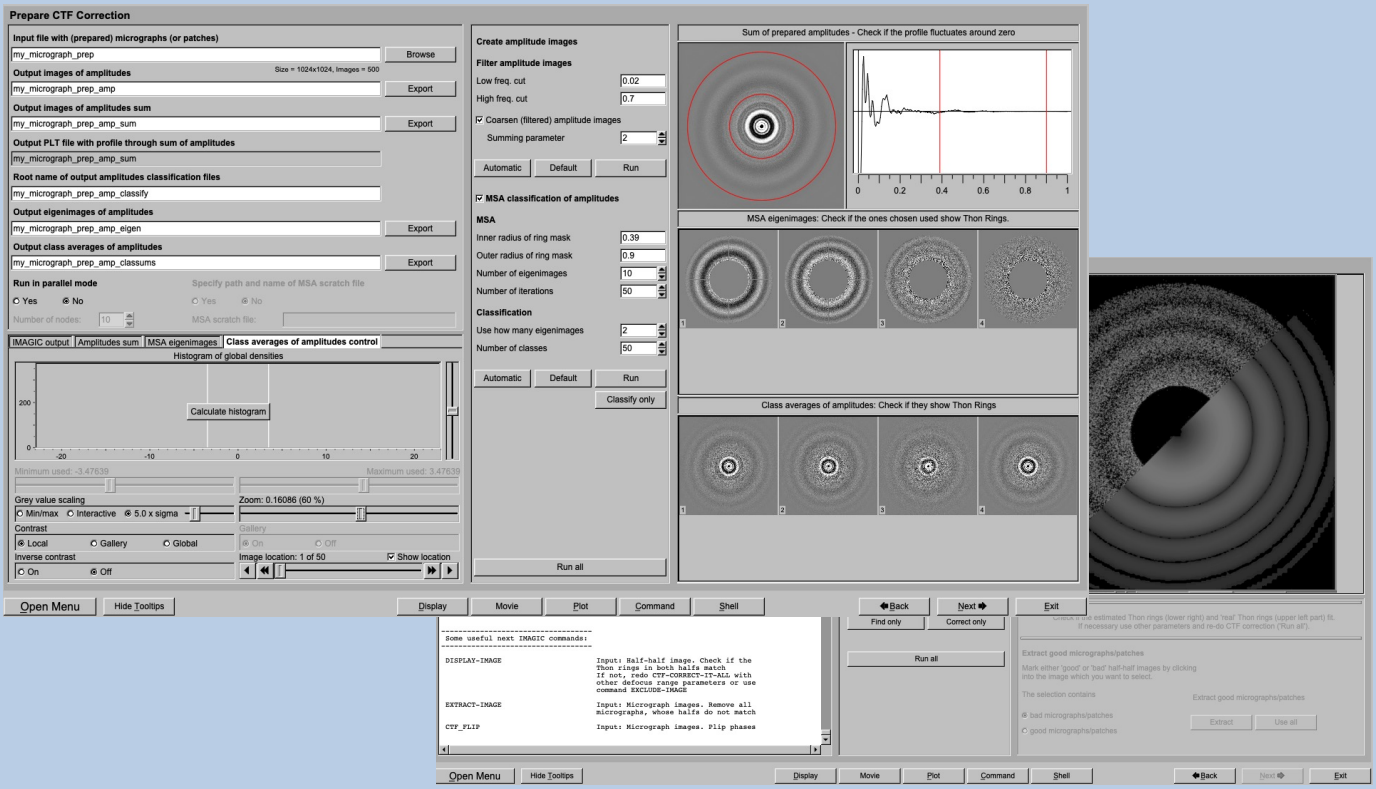
www.ImageScience.de

The IMAGIC guiCTF program

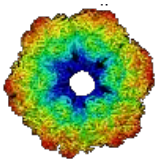


The **guiCTF** program follows a work-flow from Import Micrographs to CTF Correction.

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

CONTENT:

- IMAGIC GUI programs — How to use IMAGIC GUI programs
- guiCTF — How to CTF correct micrographs
  - > Import Micrographs
  - > Camera Correction
  - > Movie Alignment
  - > Prepare Micrographs
  - > Prepare CTF Correction
  - > CTF Correction
- Error hints — How to send us feedback



IMAGIC

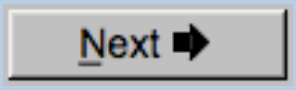
GUI Programs

Workflow

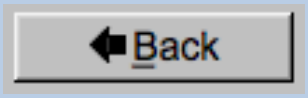
The idea of **guiCTF** is to guide you through a typical CTF correction of electron micrographs.

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

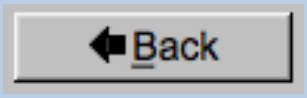
If **guiCTF** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

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

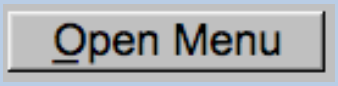
```
IMAGIC-COMMAND : guiCTF
```

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



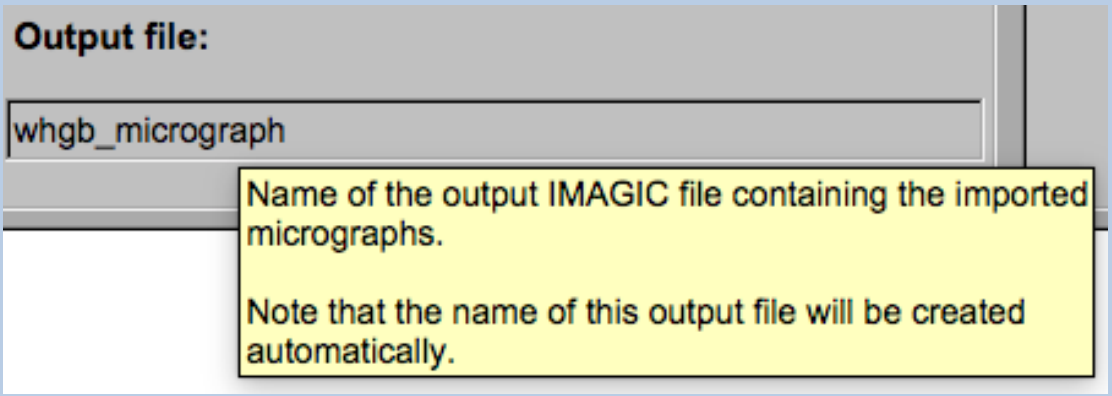
to navigate to the “Start” page where you can specify the working directory of **guiCTF**.

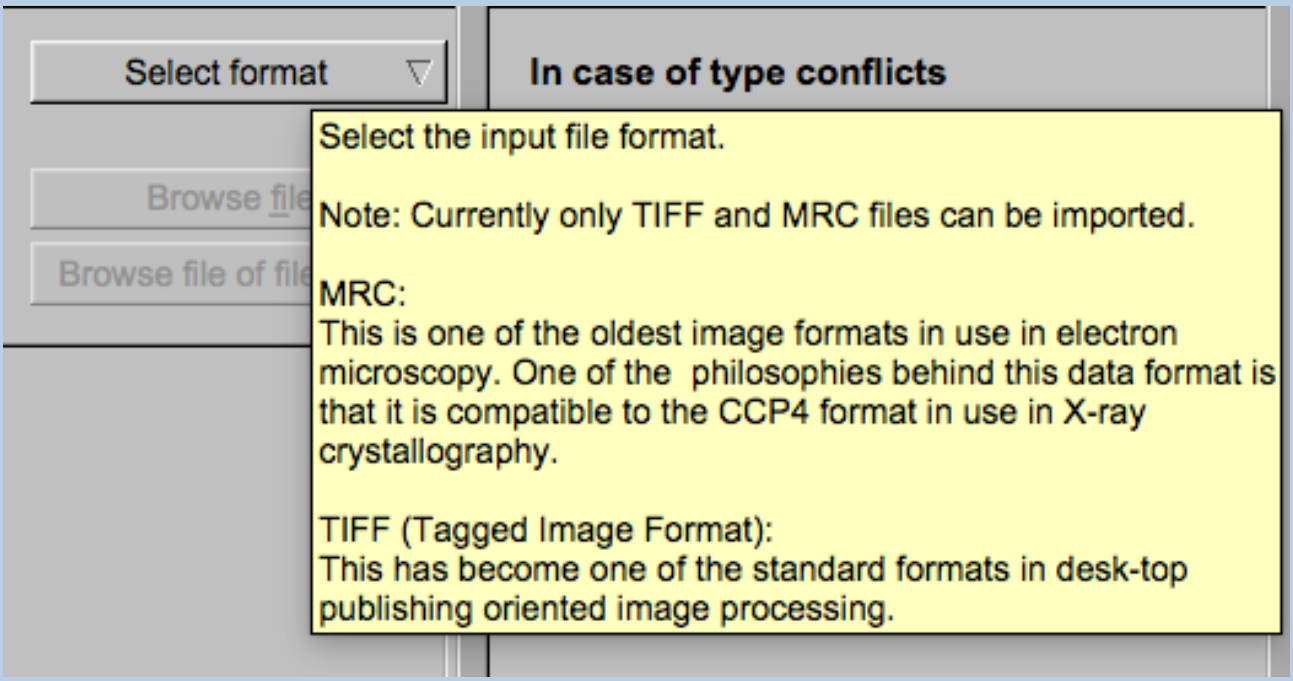
All output files will be stored in the working directory which you have specified on the start page.

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.





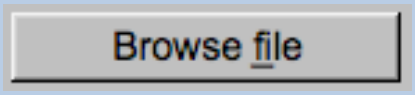
Input Files

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

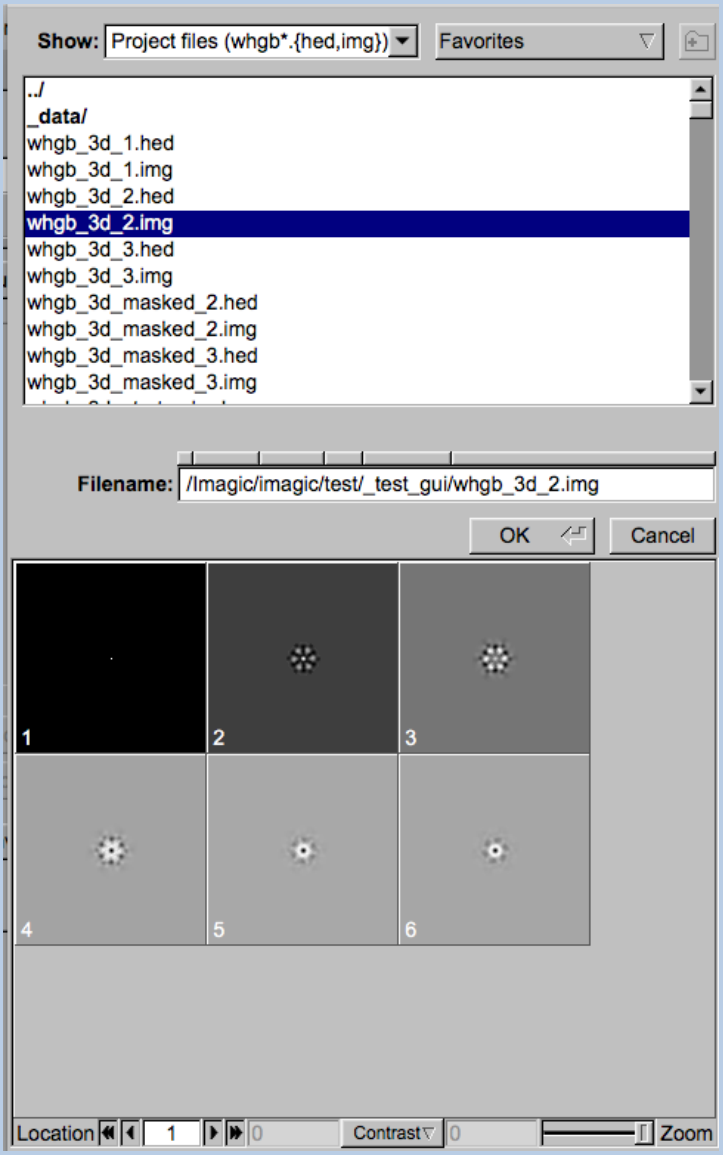
You can, of course, always use other input files names and even use other input directories.

Input File Chooser

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

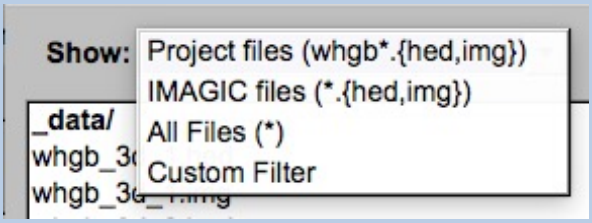


Pressing this button will open the IMAGIC file chooser:



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”.

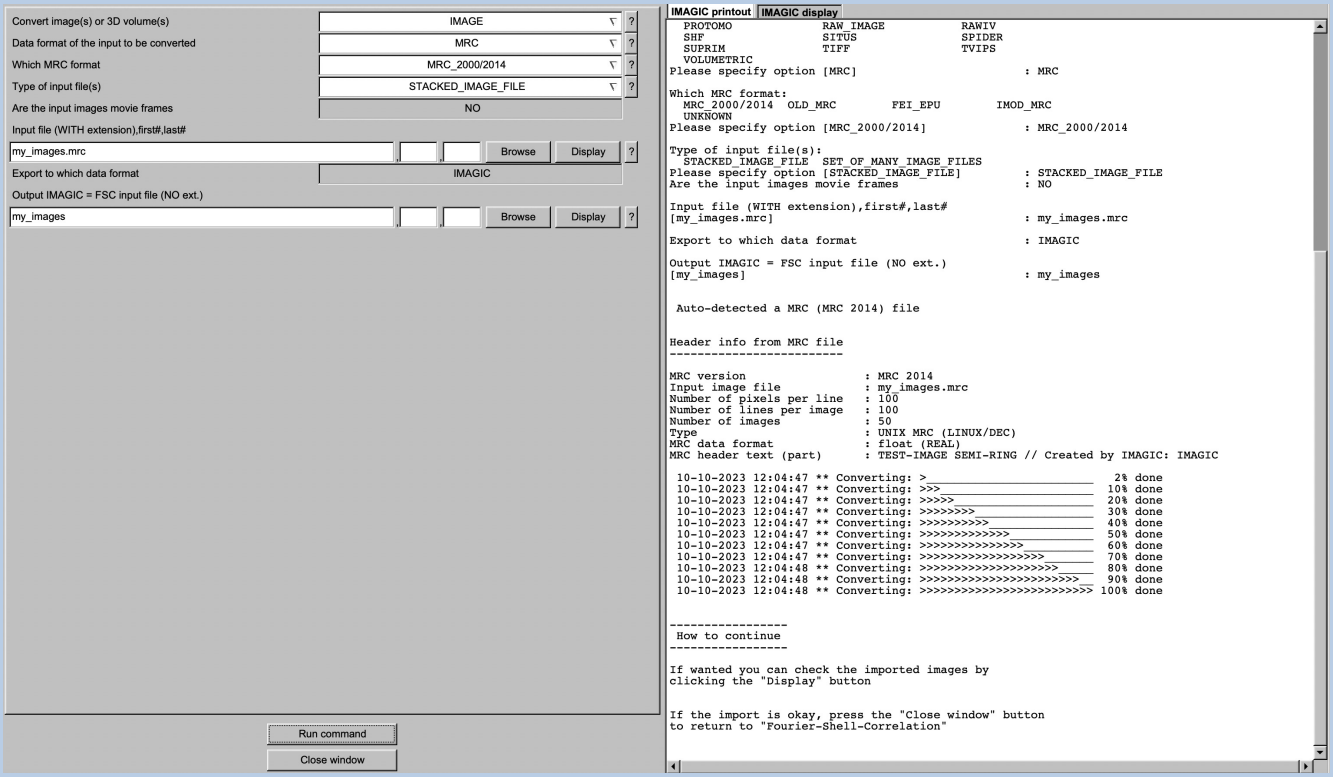
Output Files

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.

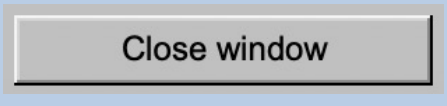
Import Buttons

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:



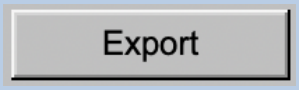
Click the “Close window” button to exit this additional window:



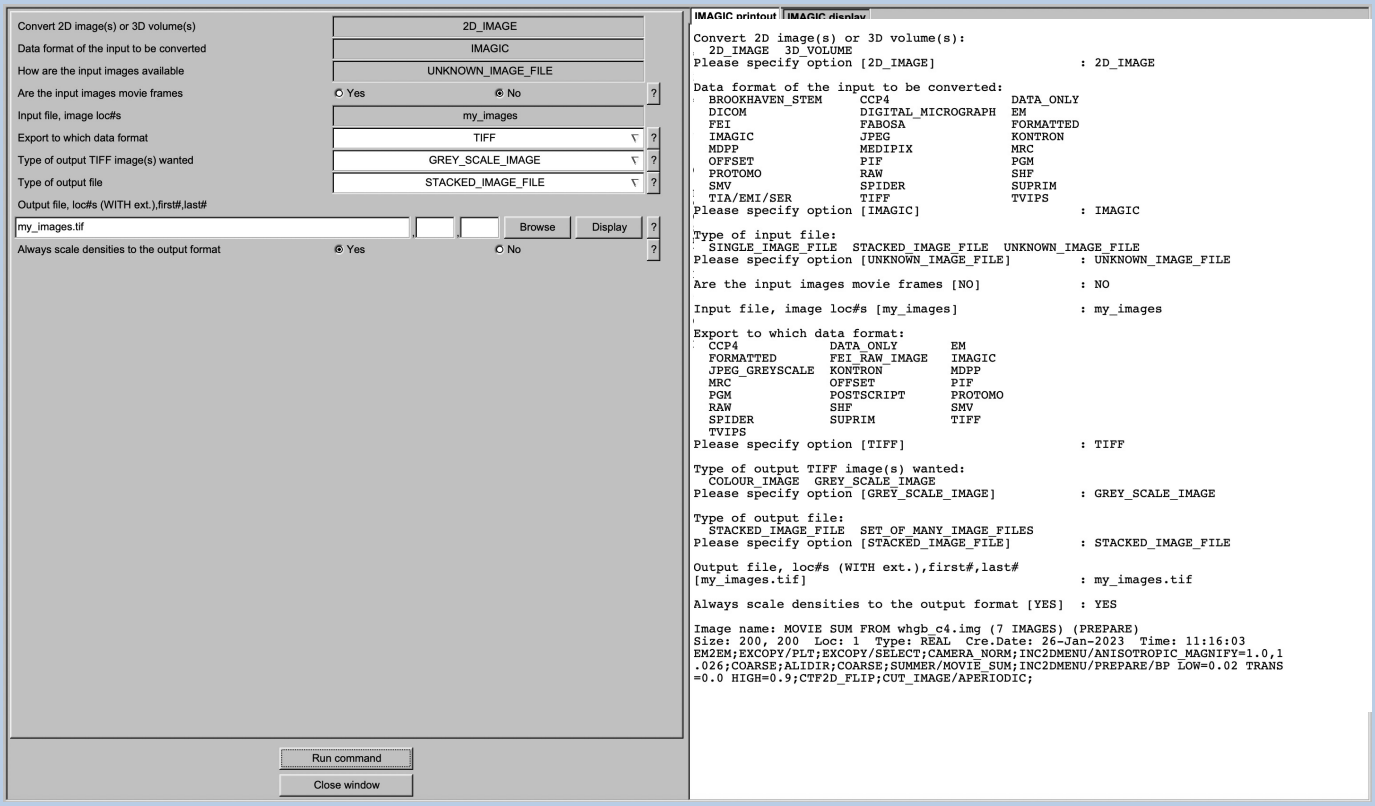
Refer to the **guiEM2EM** manual to get further help.

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.

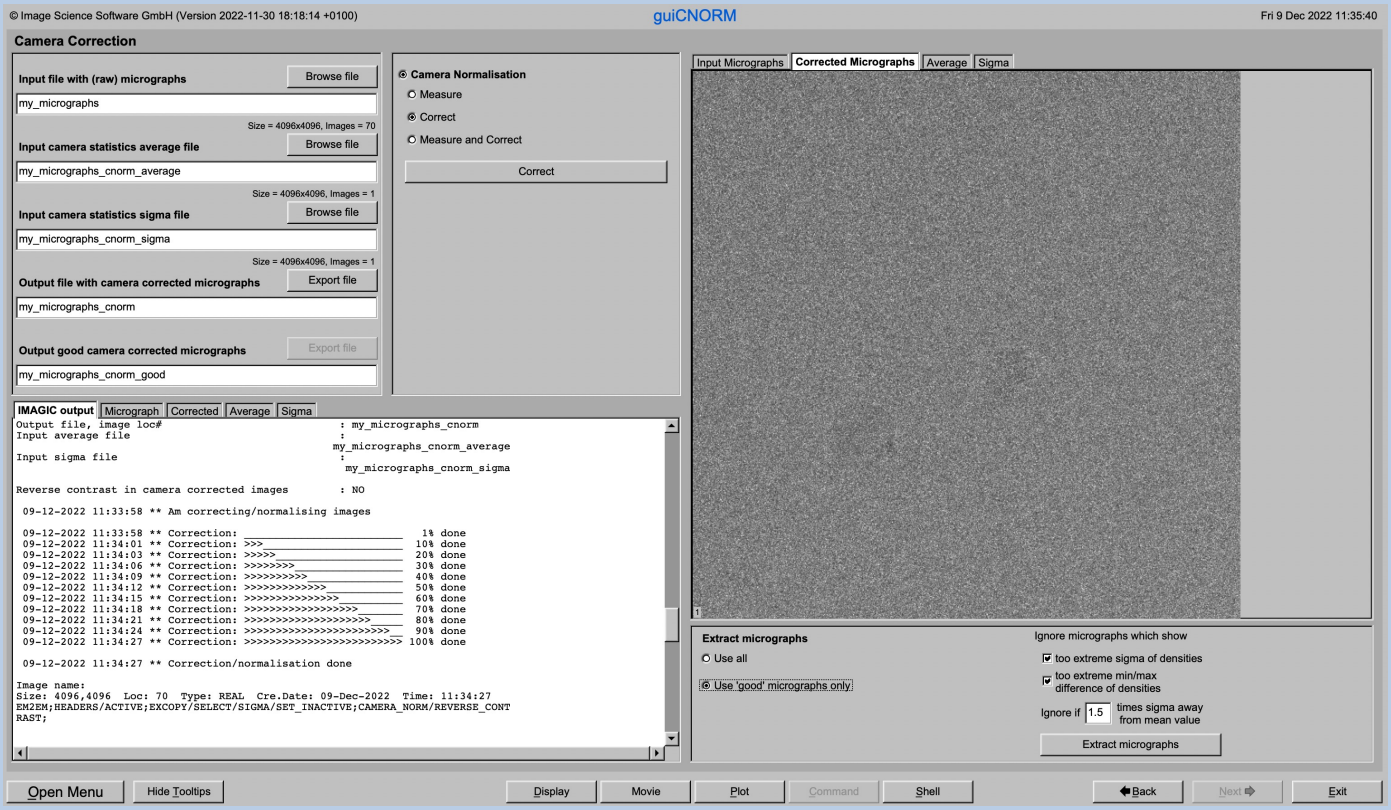
A Typical Page

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.

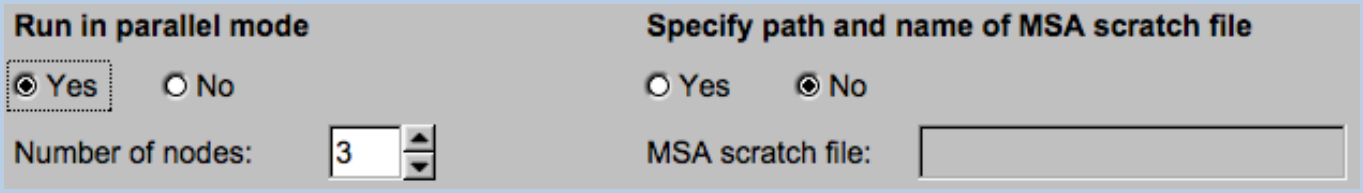
The middle part usually contains parameters to be specified and a single or a number of “Run” buttons to start the calculation(s).

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

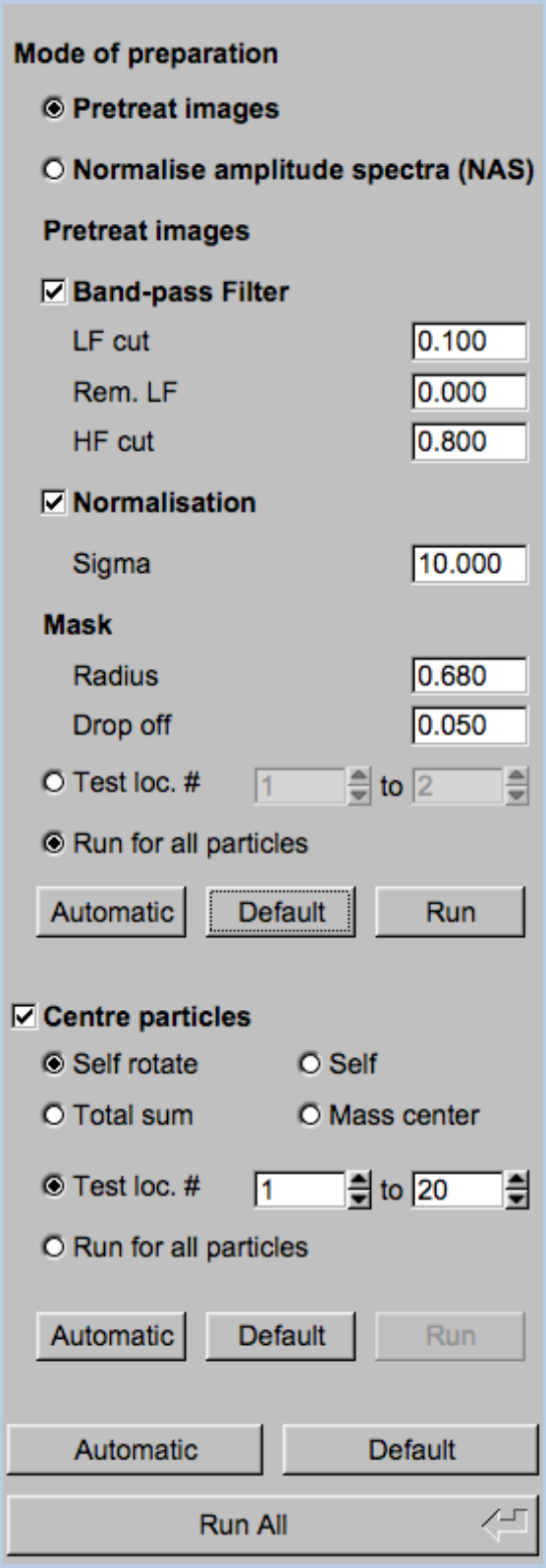


A Typical Page - MPI Parallel

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.

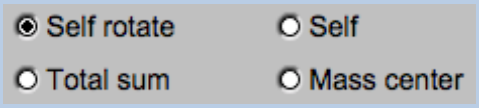


A Typical Page - Program Parameters

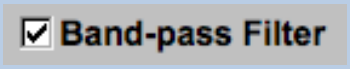


In the middle part of a typical **IMAGIC GUI program** page you will find the program parameters to be used.

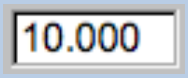
Radio Buttons are showing options.
One option only has to be used.



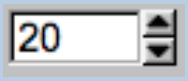
Click buttons are showing options which you can use or not.



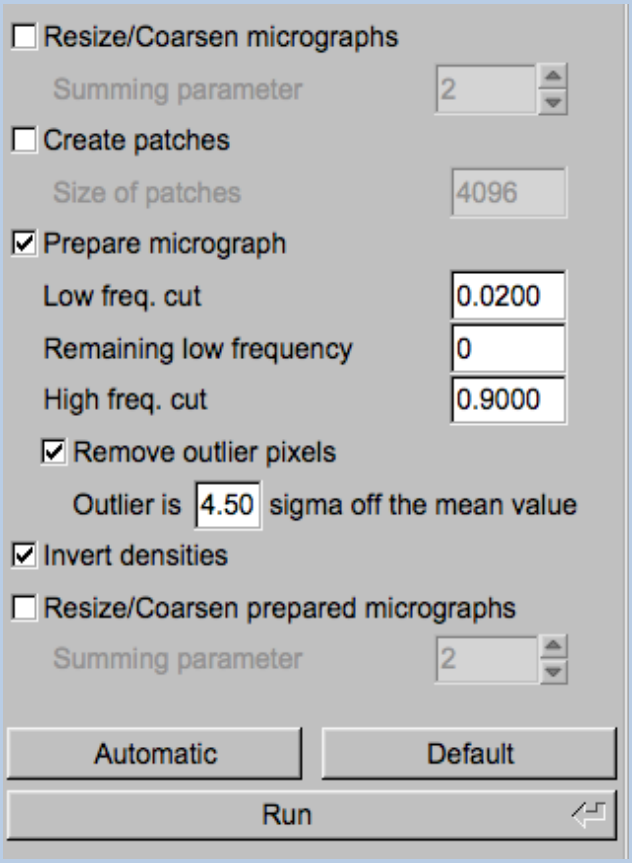
In text fields you can type in the wanted value. If the needed value is a number you can also move the cursor into this field, press the mouse key and keep it pressed and move the cursor to change the value.



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

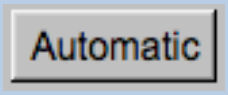


A Typical Page - Automatic / Default

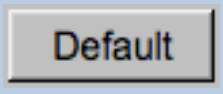


In the middle part of a typical **IMAGIC GUI program** page you will also find “Automatic” and “Default buttons.

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

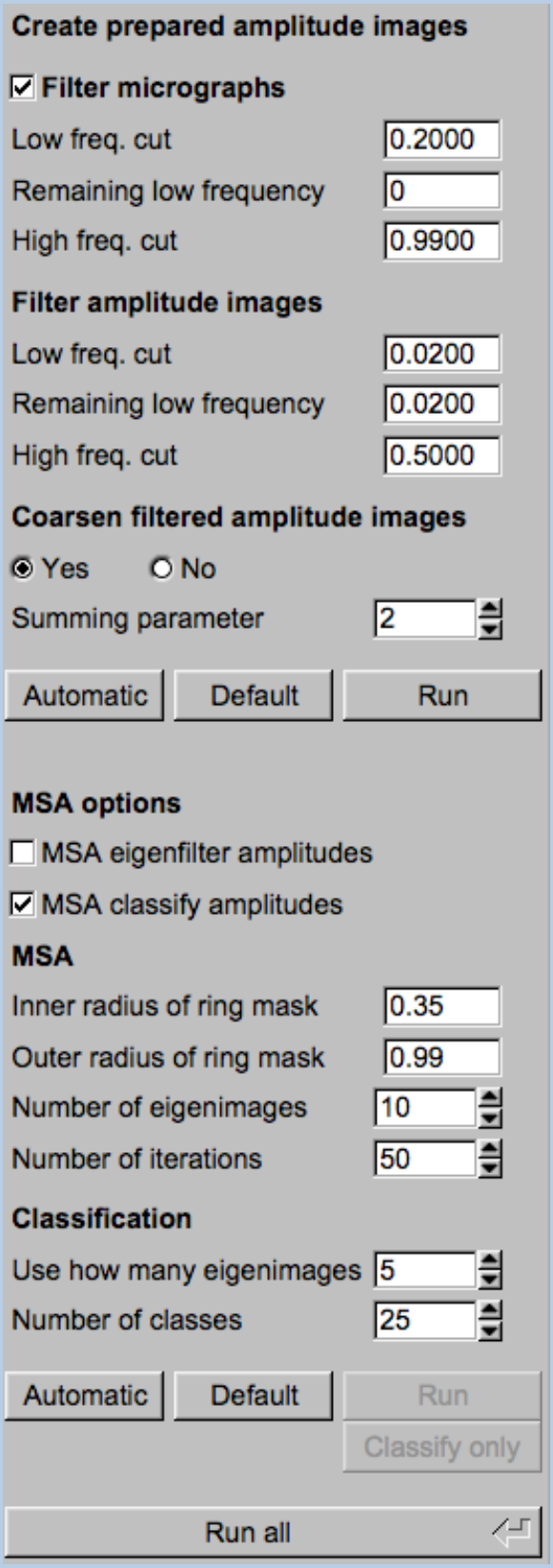


Pressing the “Default” button will fill in the values which you have used during the last “Run”.

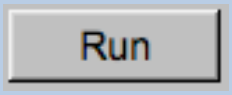


The values shown when entering a page are the default values (your last values given) if they are available. Else the automatic values are shown.

A Typical Page - Run buttons



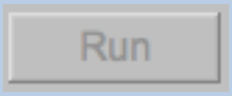
To run the calculations press the “Run” button.



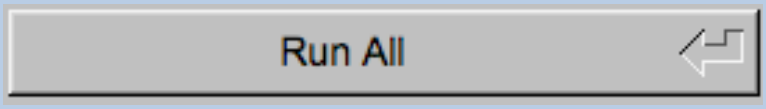
On a number of pages the calculations can be split. In this case you will find more than one single “Run” button.

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.



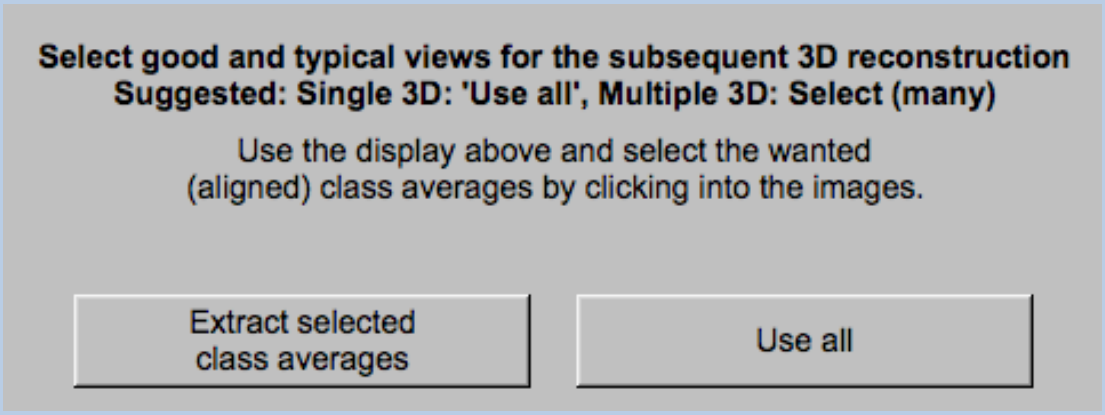
Pressing the “Run All” button starts all calculations currently activated on the page.



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”

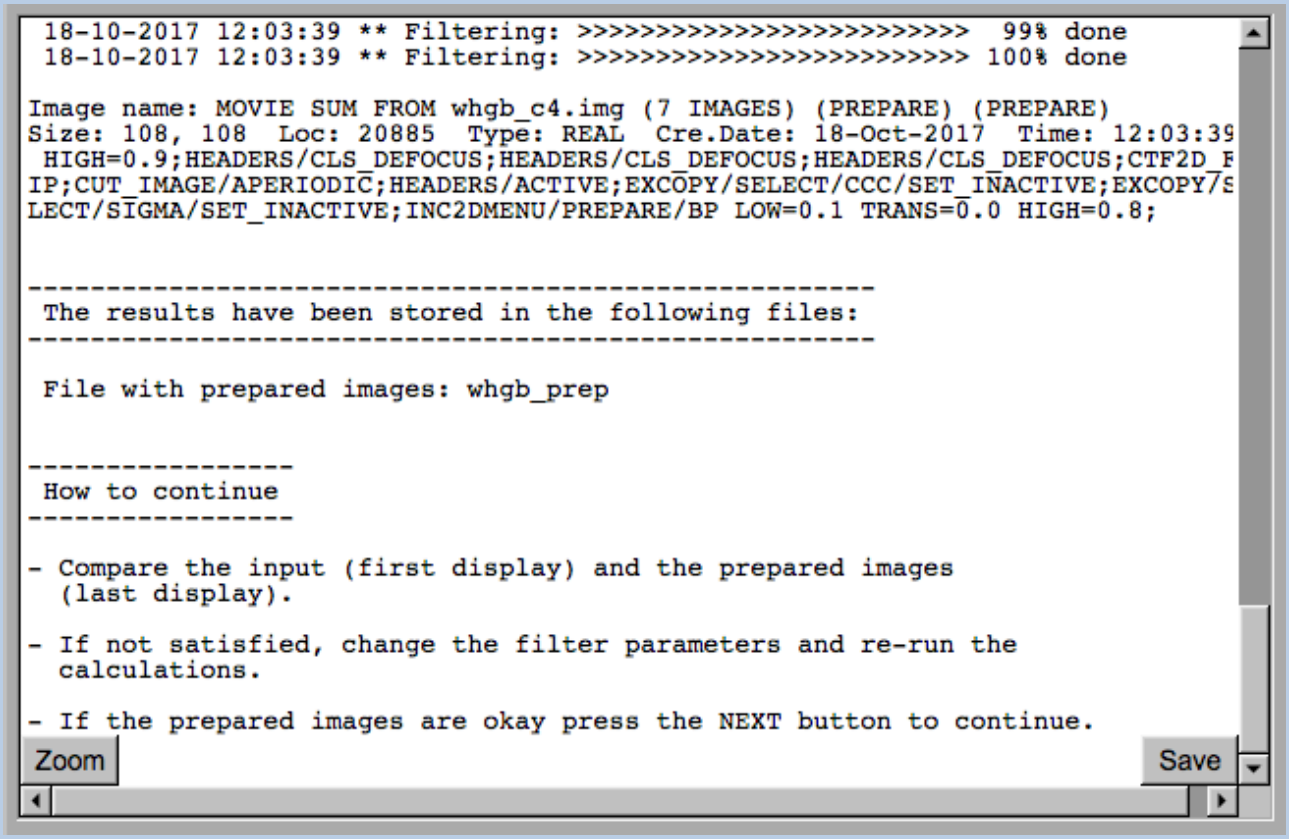
All print-out of an IMAGIC program started within the current **IMAGIC GUI program** page is shown in a kind of terminal window on the left hand side.

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.

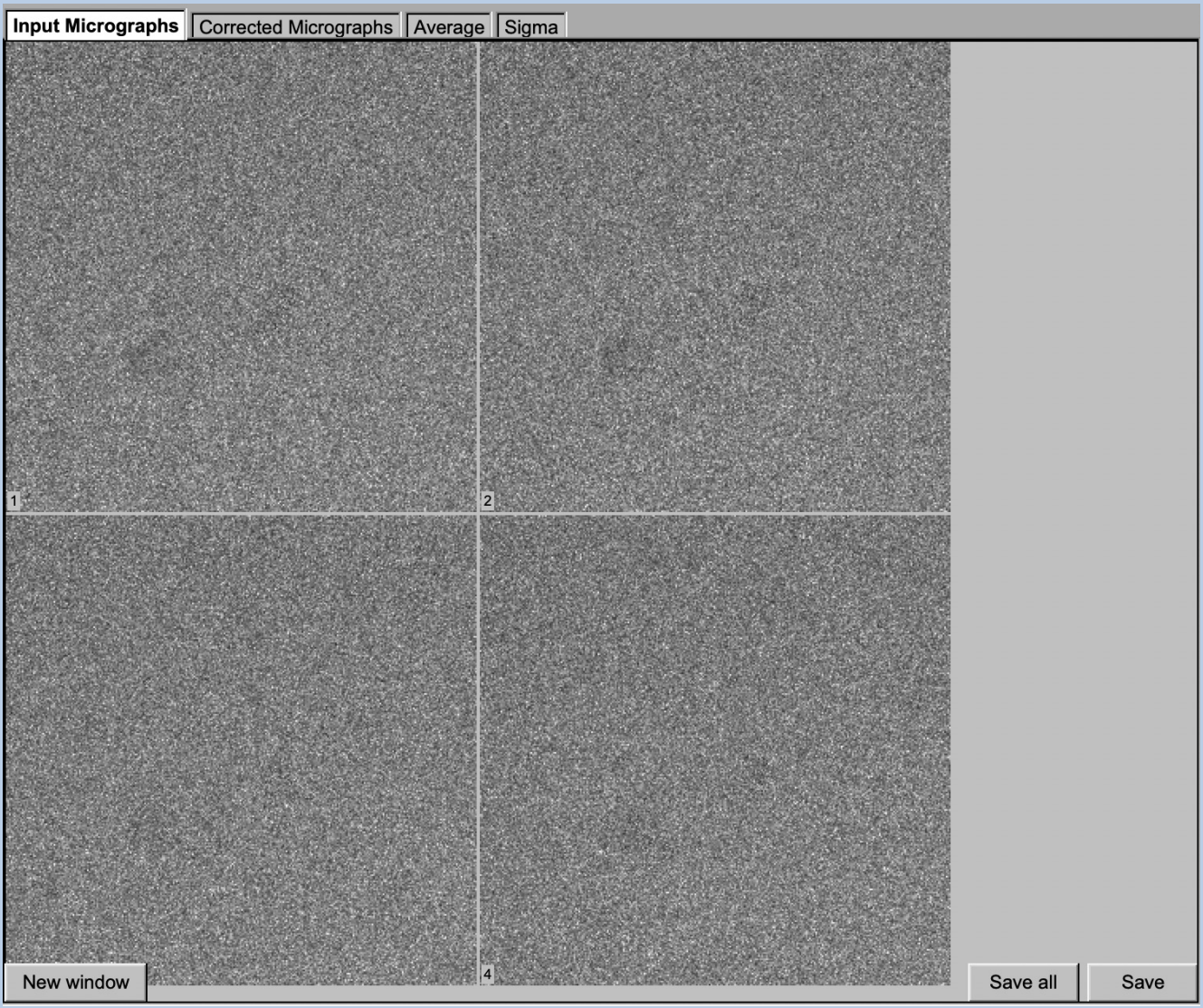
You can use the “Save” button to store the print-out in a text file.

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.

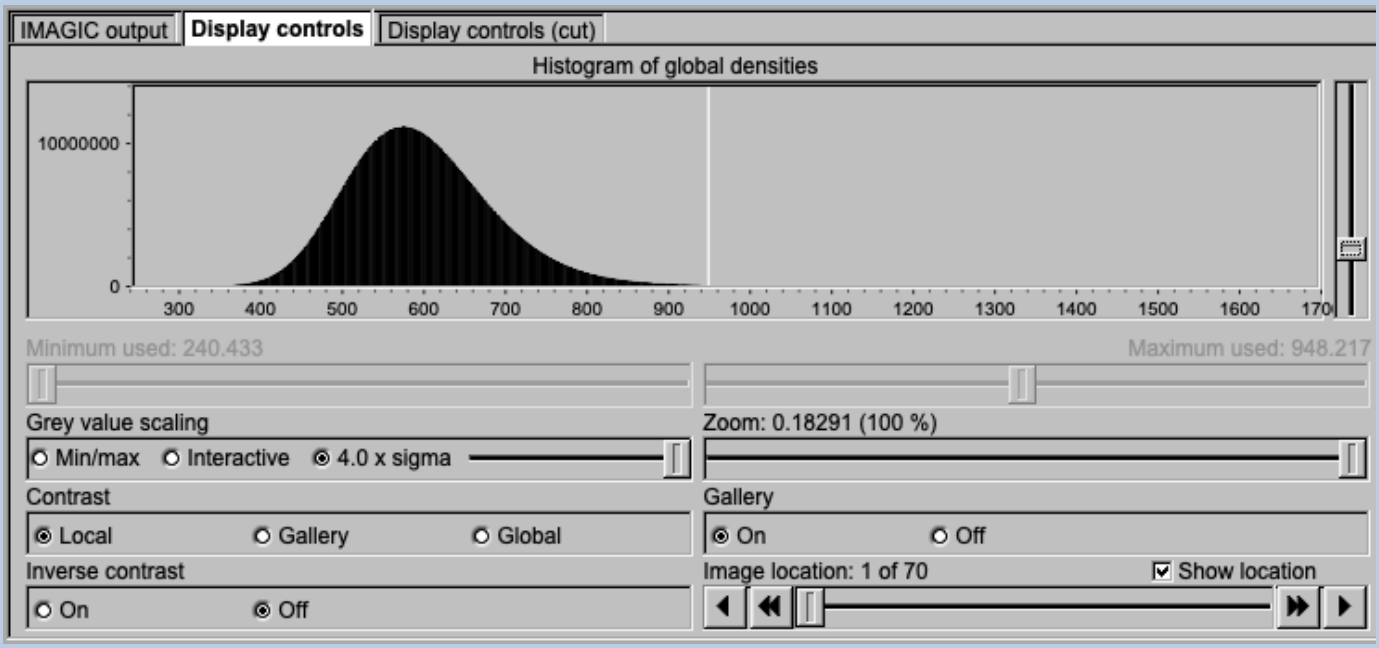
Double click into the wanted images or use the “New Window” button to get an enlarged display window. Use “Save” to store the display (JPG).

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 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

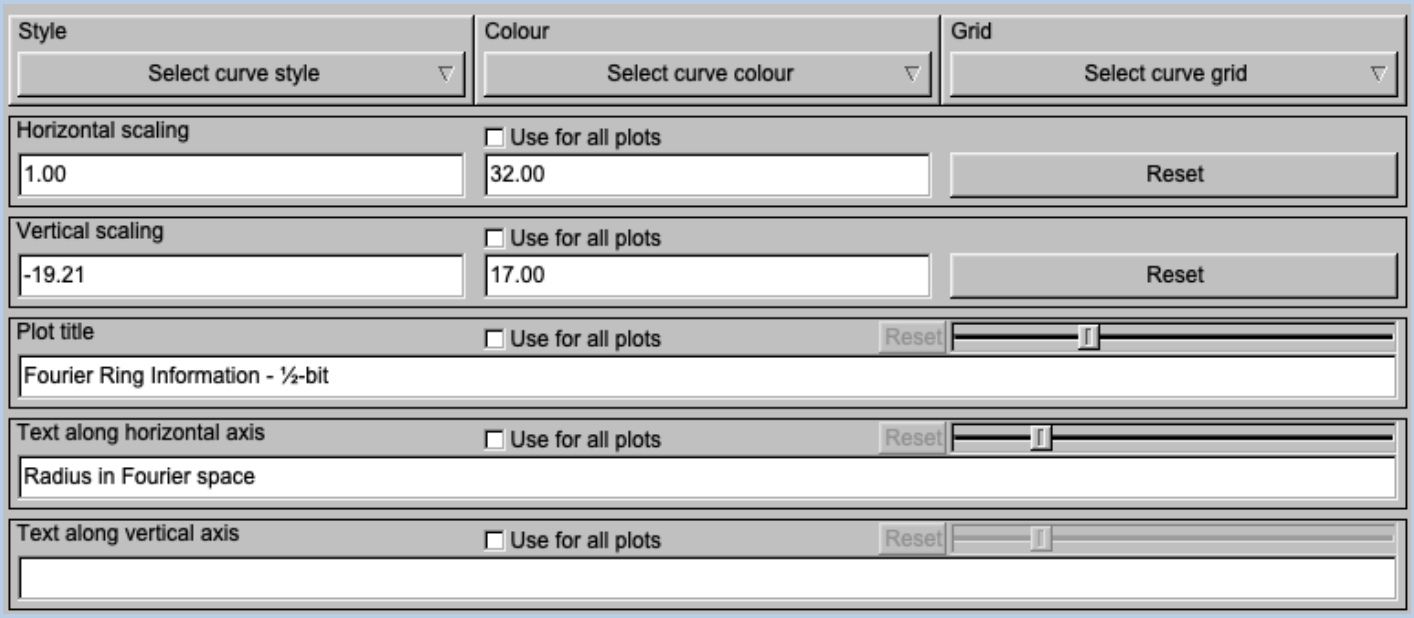
Horizontal, vertical scaling: Set minimal and maximal horizontal or vertical limits

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 of what is input in the PLT file

Reset:. Reset to the automatic values

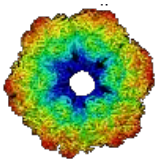


A Typical Page - The Toolbar

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

The toolbar buttons:

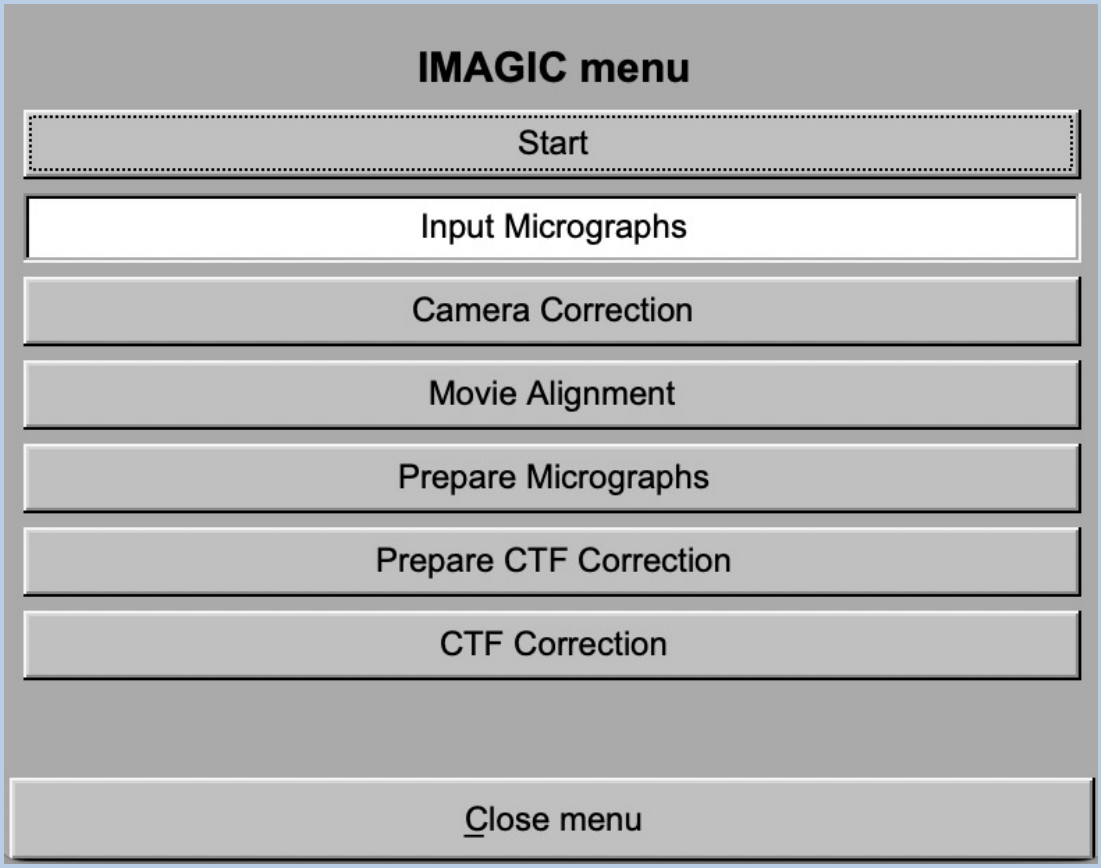
Open Menu	Open the MENU to navigate to each page wanted
Hide Tooltips Show Tooltips	Show or hide the context sensitive tooltips (the help text may sometimes disturb)
Display	Open a DISPLAY page to visualize IMAGIC images. Refer to **guiDISPLAY**.
Movie	Open a MOVIE page (display in an endless loop).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



IMAGIC

guiCTF

The guiCTF Menu



PAGES:

guiCTF:

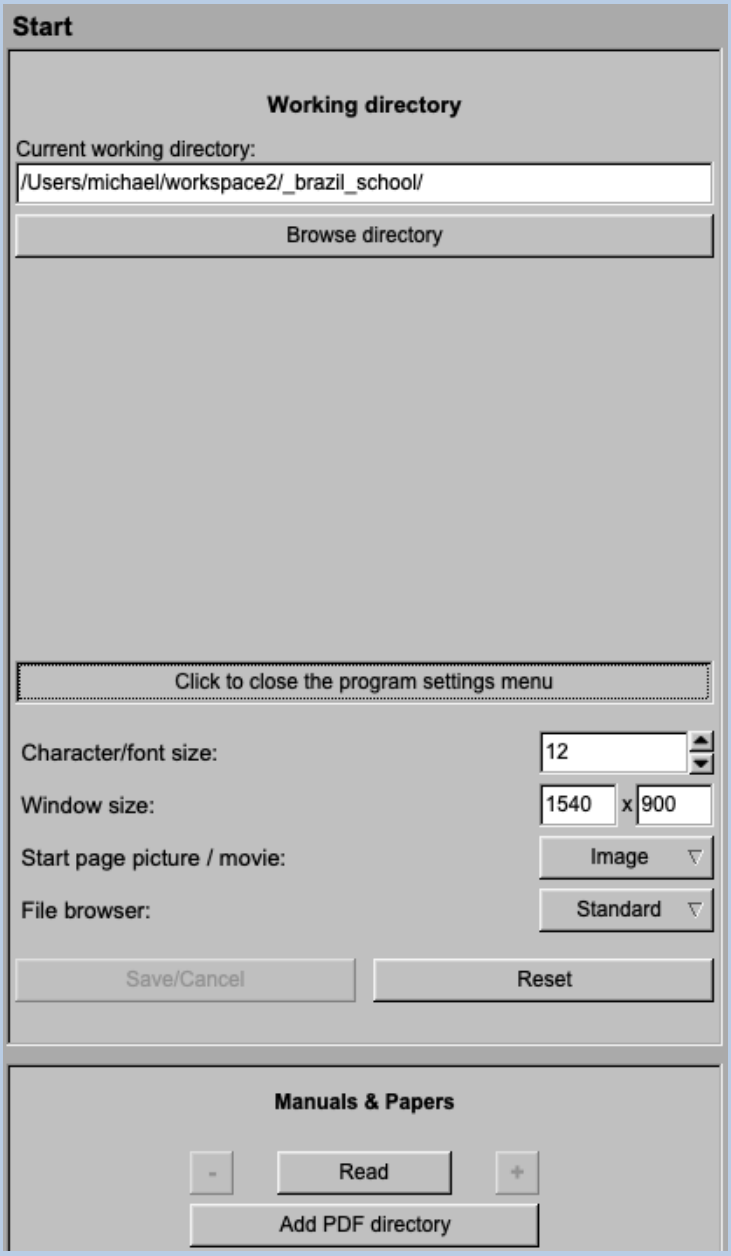
Import Micrographs:	Convert micrographs/images into IMAGIC image format
Camera Correction:	Measure and/or correct for camera statistics
Movie Alignment:	Align movie frames
Prepare Micrographs	Pre-treat Micrographs
Prepare CTF Correction	Prepare the CTF determination and correction
CTF Correction	Find CTF and correct micrographs for it

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 **guiCTF** 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 **guiCTF** program windows and/or text (a re-start is needed)
c) the type of file browser

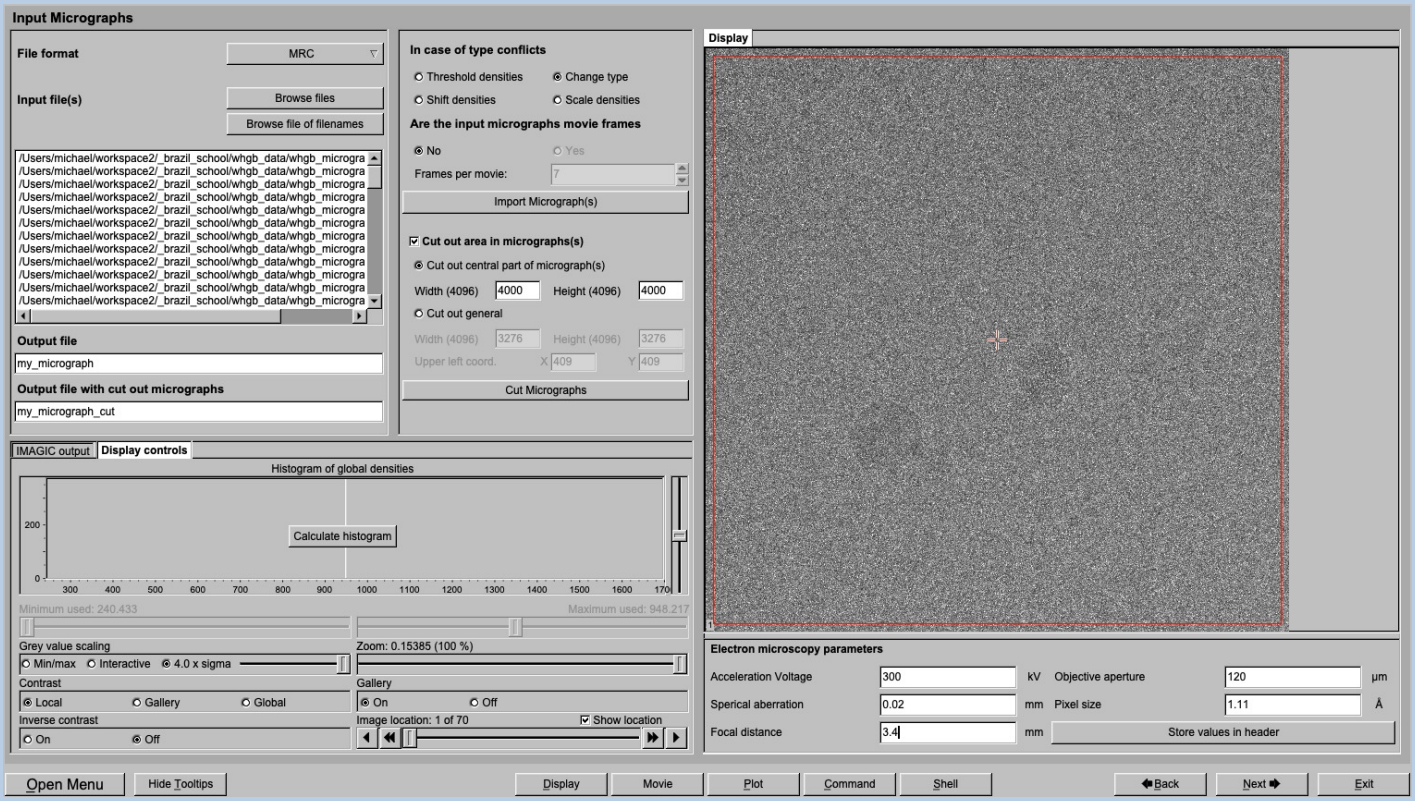
Start Working

The page **guiCTF** starts with the “Import Micrograph” page.

The workflow using the “Next” button will guide you through all **guiCTF** 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 and if all electron microscopy parameters are already stored in the input headers.

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

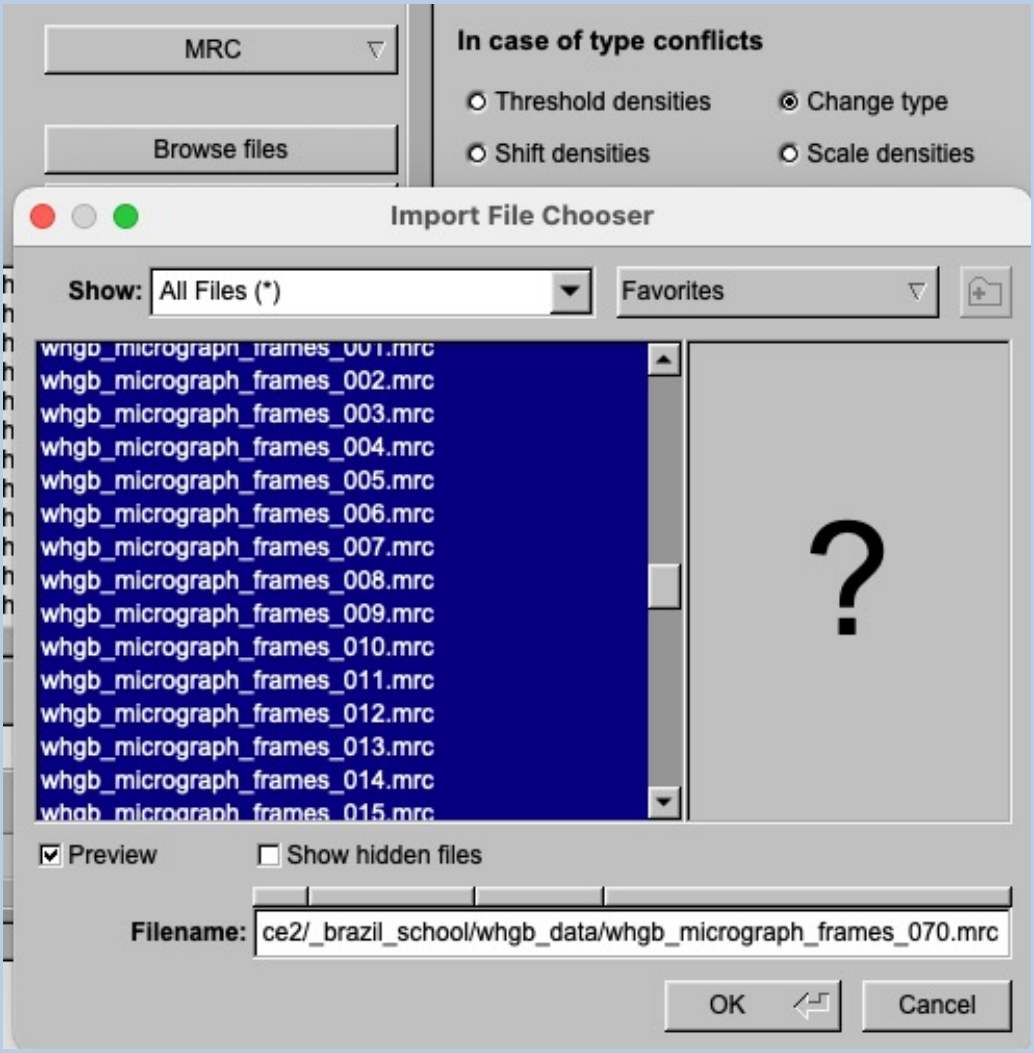
Also refer to program **guiIMPORT**.

IMPORT MICROGRAPHS:

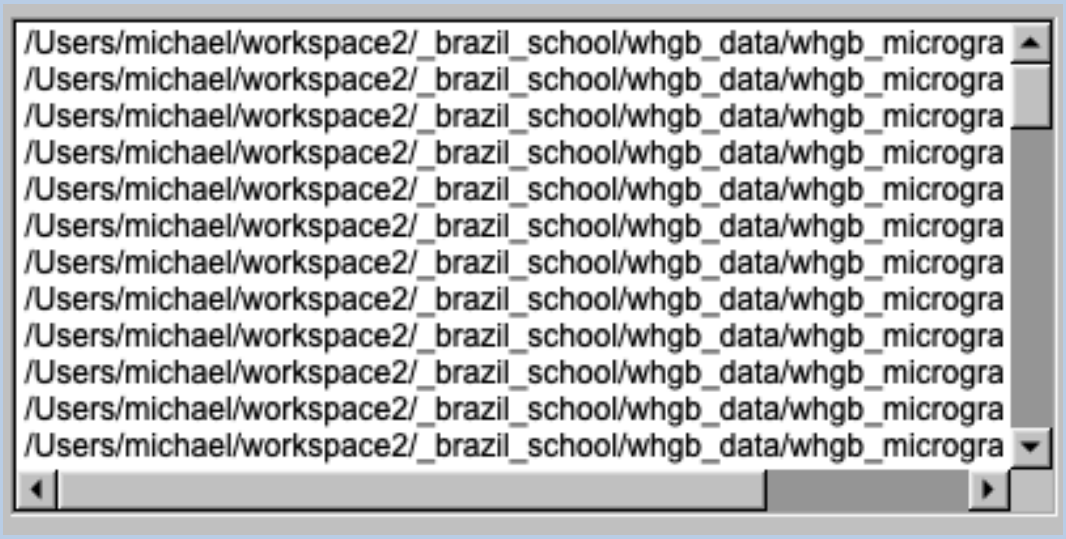
Specify the file format in which your input micrographs/images are stored. Click the “Select format” button

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.



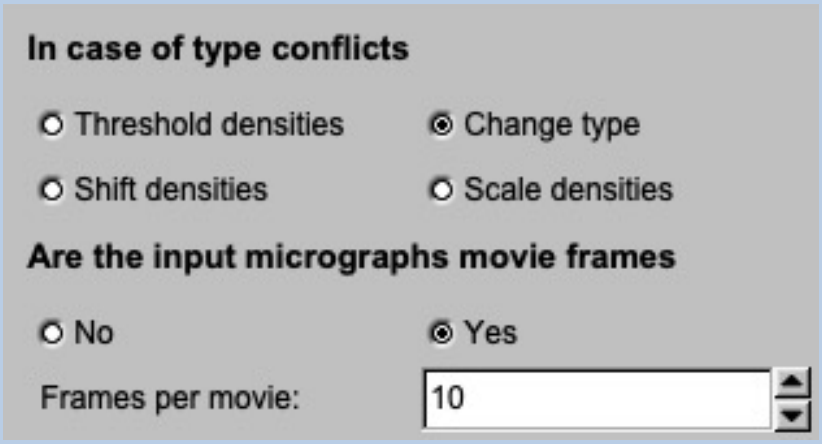
If wanted you can edit the list of files. But be careful there is no automatic control of file names in this list.



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:



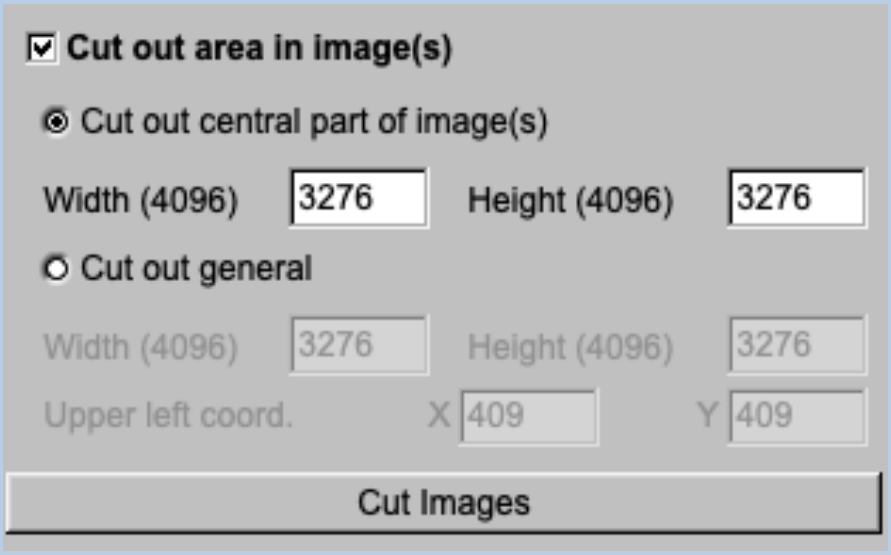
Having specified every information needed click the “Import Micrograph” button to start the import of the image(s).

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 in **guiCTF**):

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:



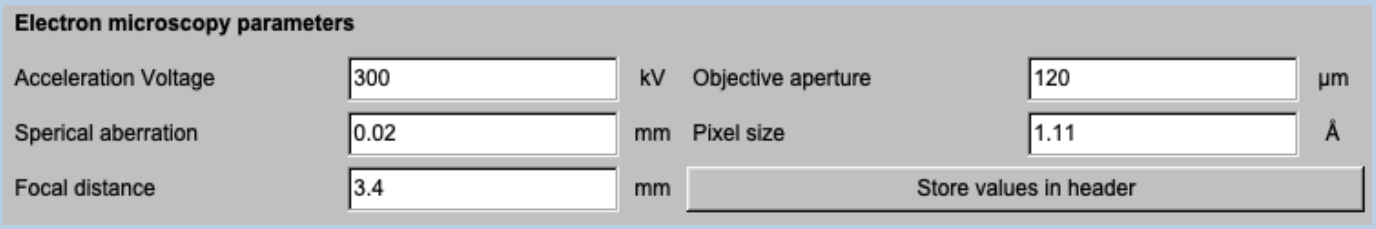
The chosen part is shown in the display window. You can cut-out a central part or any part wanted. The cut-out part is the same in all images, of course.

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.

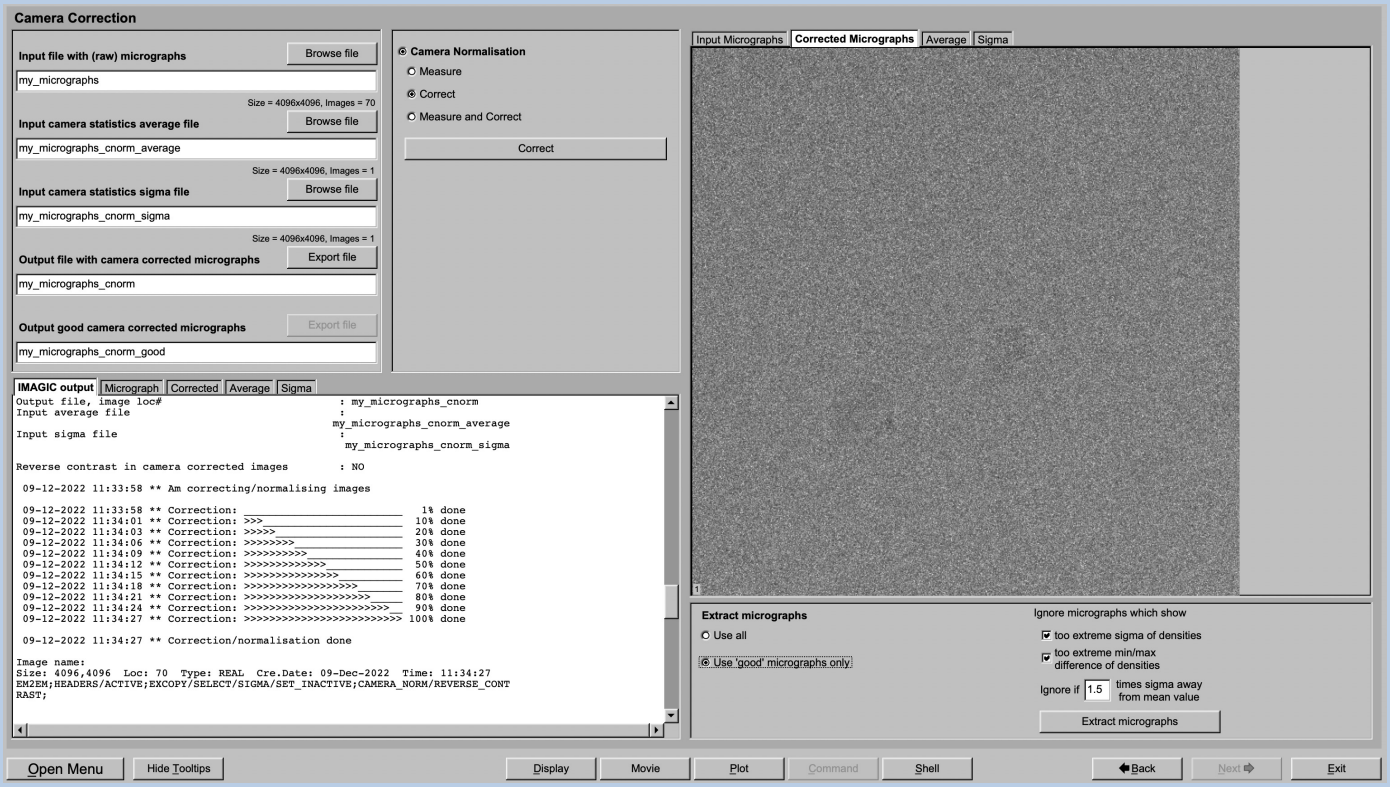
SPECIFY THE ELECTRON MICROSCOPY PARAMETERS):

If not yet stored in the input headers you have to specify the the parameters of the electron microscope the micrographs were imaged with.



Having specified all parameters use the button “Store values in header” to write the given parameters into the input headers:

The “Camera Correction” Page



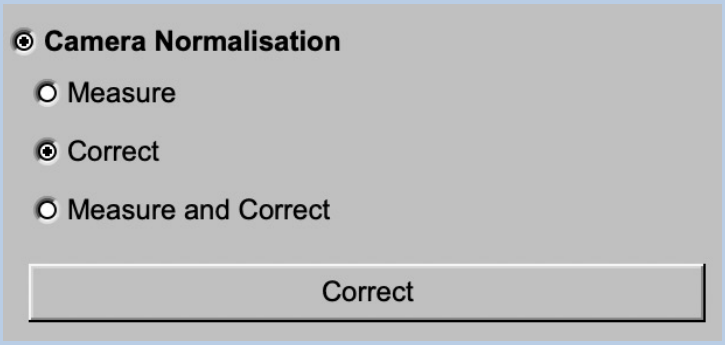
DESCRIPTION:

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).

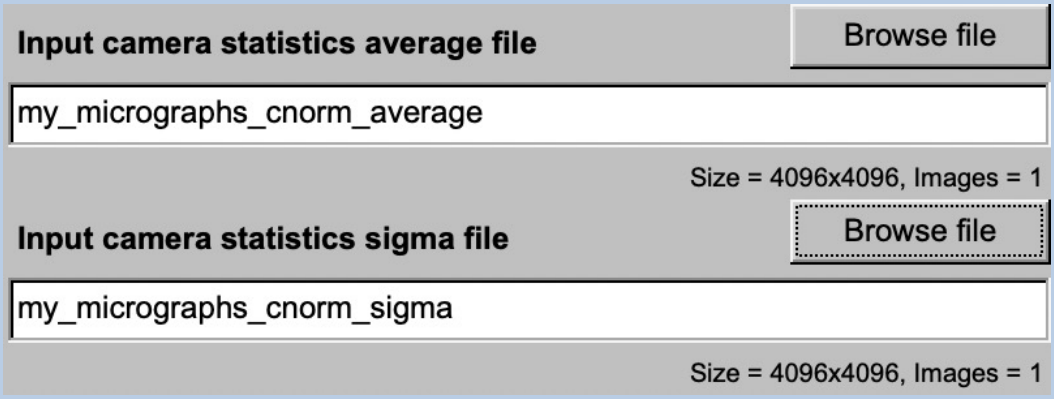
Also refer to program **guiCNORM**.

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

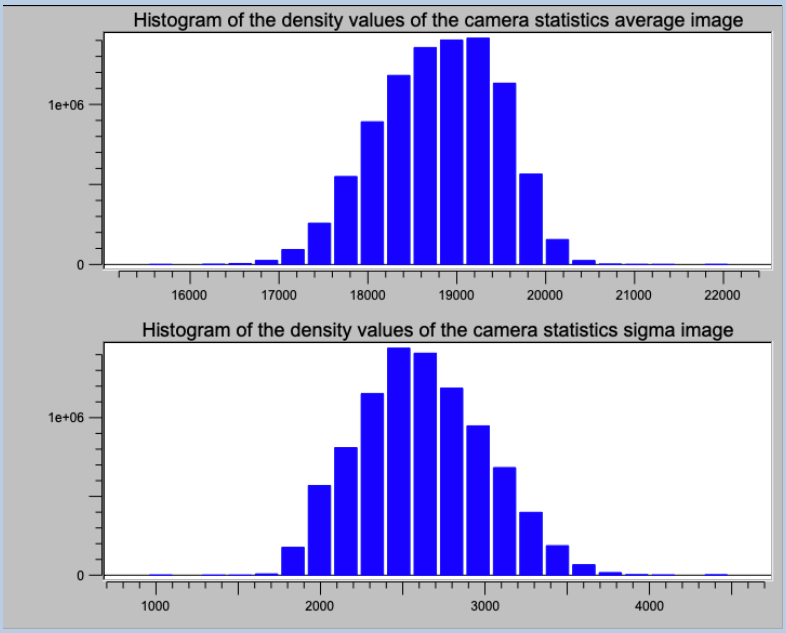
Use the “Correct” option to camera correct the input micrographs using the camera statistics images (average and sigma) of the camera/detector the input macrographs were imaged with.

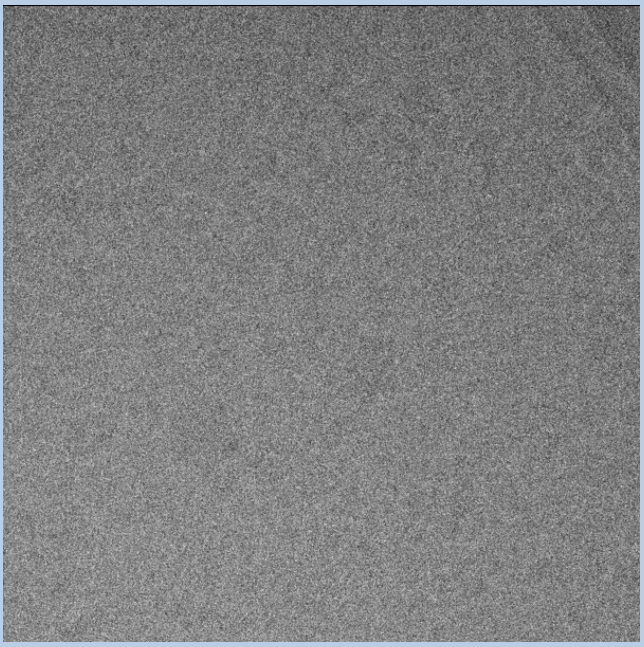


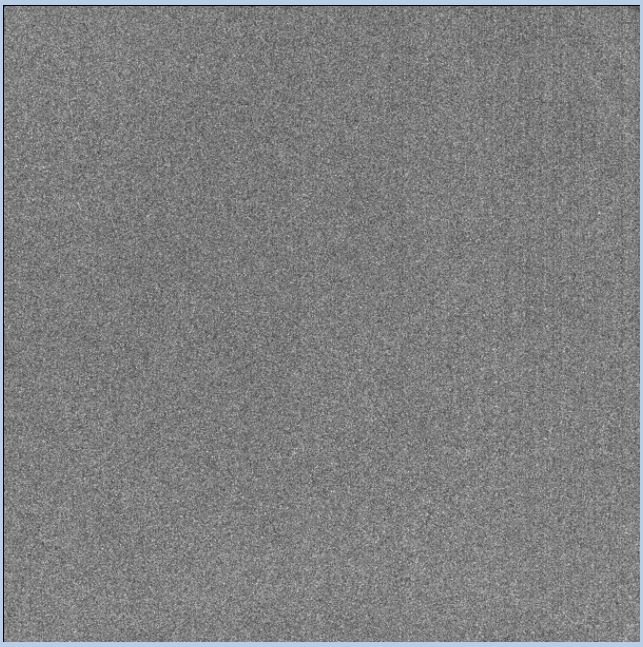
Specify the input camera statistics average and sigma image file needed for the camera correction



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.

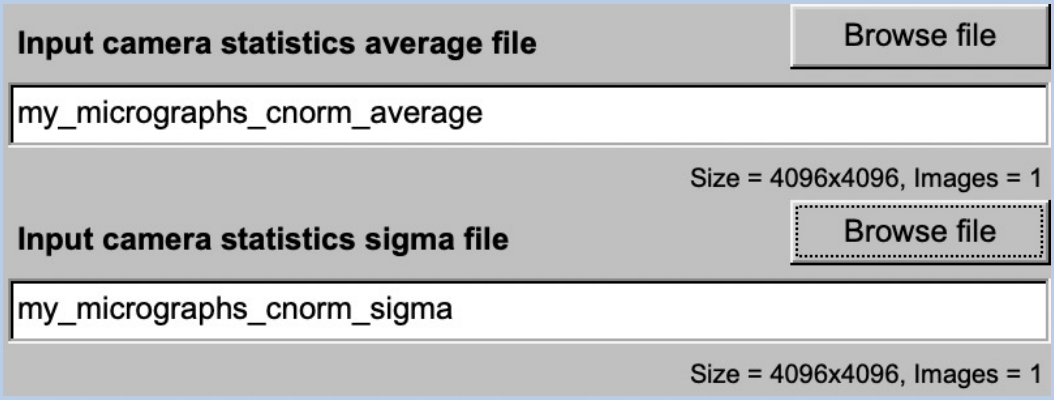




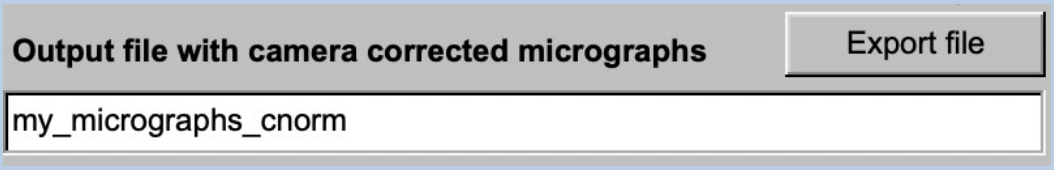


It is always a good idea to check these histograms and images.

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



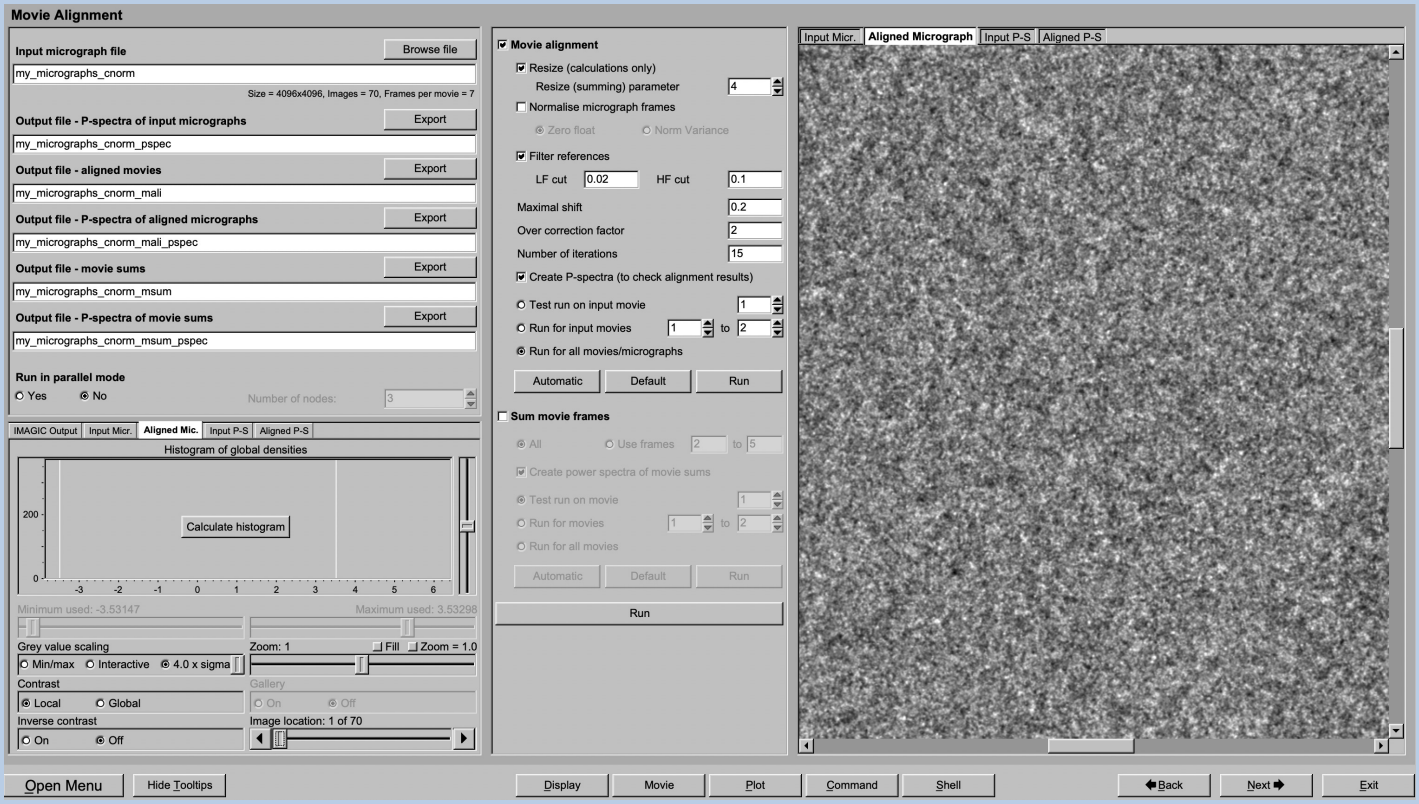
and, as usual, the file name of the output file which will contain the camera corrected micrographs.



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



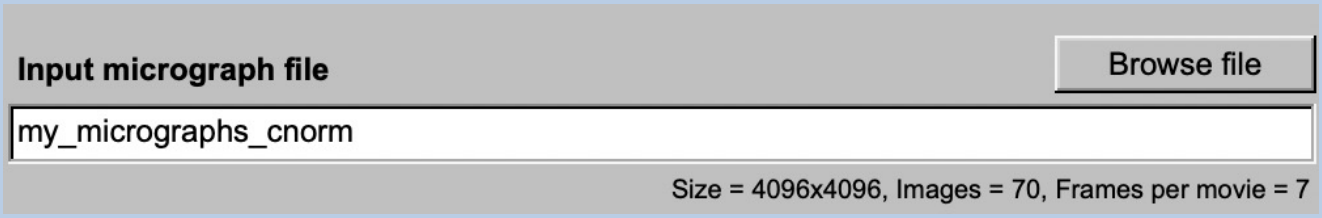
DESCRIPTION:

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

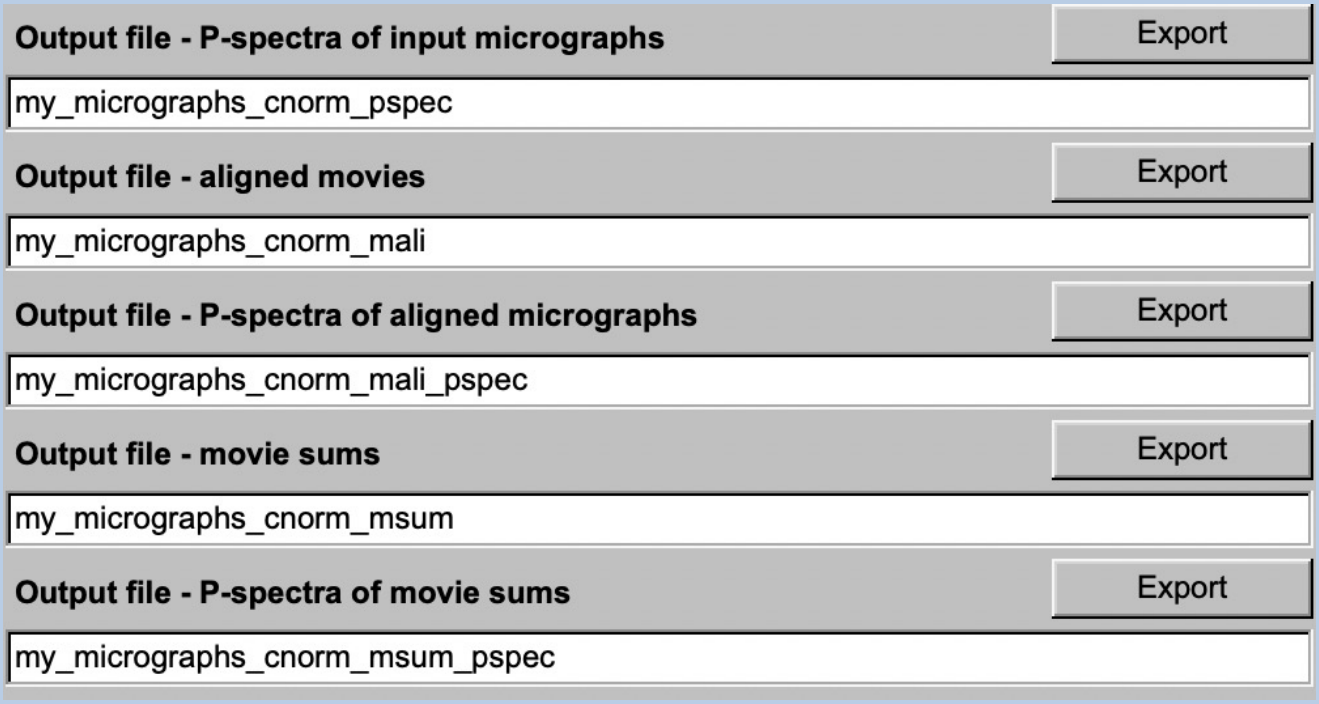
Also refer to program **guiMALIGN**.

NOTE:
Skip this page if no movie alignment is wanted/needed.

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

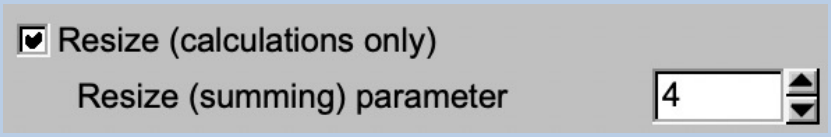


and the names of the output files.

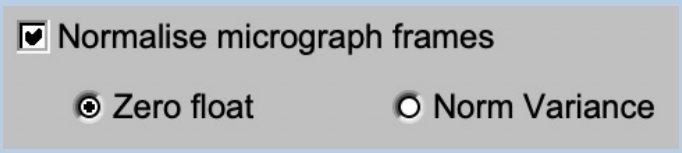


As usual **guiMALIGN** suggests names but it is your choice, of course:

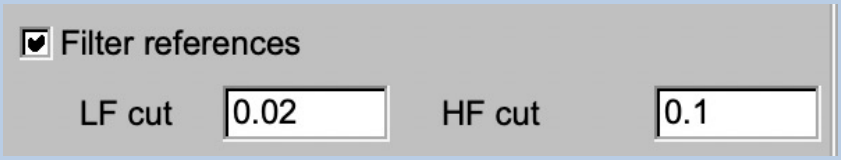
You can resize the micrographs to be used in movie alignment to speed-up the calculations



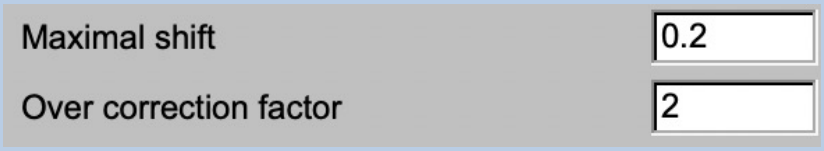
You can normalize the densities (usually not needed):



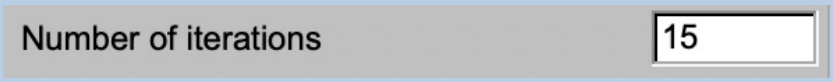
Usually it is a good idea to low-pass filter the references. Note that these references are intermediate sums of the aligned movie frames and are created automatically):



Subsequently, specify the parameters for the movie alignment:

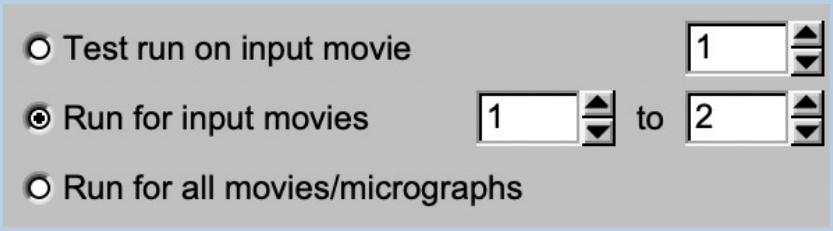


Movie alignment tis an iterative procedure. Give the number of iteration wanted:



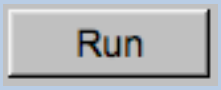
To later check the quality of the alignment it is suggested to also create the p-spectra of the input and the aligned movie sums:

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



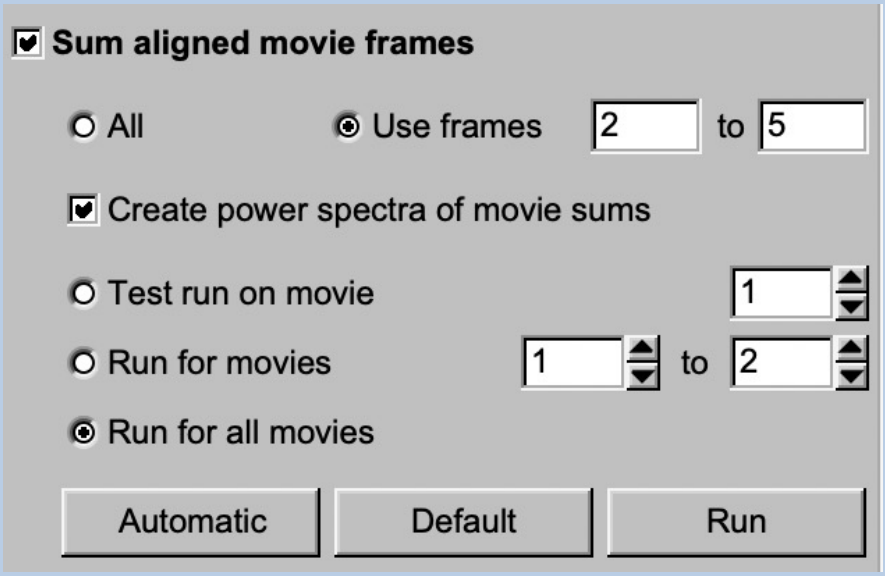
If all parameters are adjusted and the movie alignment of the test micrographs is okay run movie alignment for all input micrographs:

Start the calculations by clicking the “Run” button:

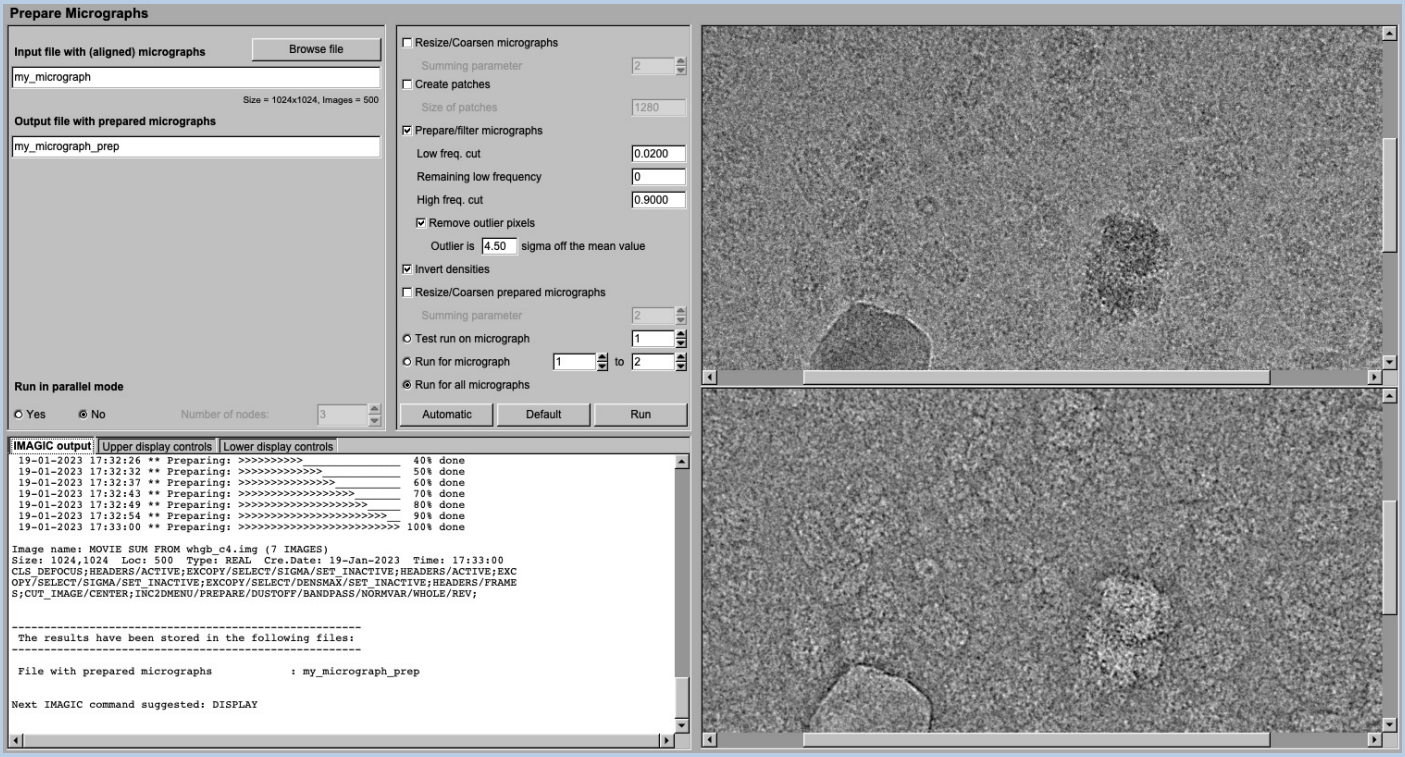


Compare the input and the aligned movie frames. Also compare the related P-spectra. As usual these images are displayed in tabs on the right hand side

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:



The “Prepare Micrograph” Page



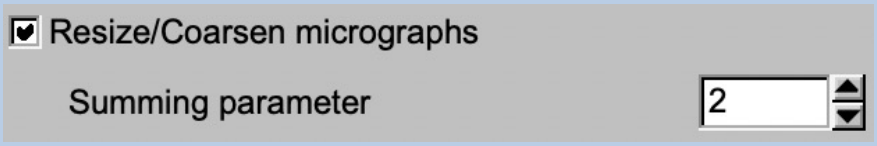
DESCRIPTION:

Usually it is necessary to pre-treat the input micrographs by imposing a band-pass filter. filter

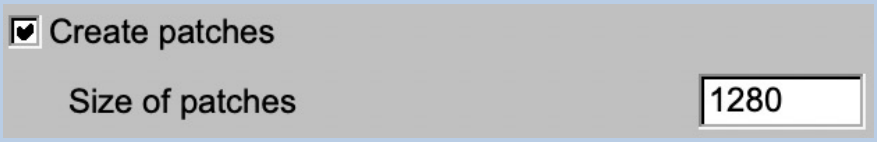
NOTE:
Of course, you can skip this page if no such treatment is wanted/needed.

Prepare the micrograph images for CTF determination

You can resize the micrograph images

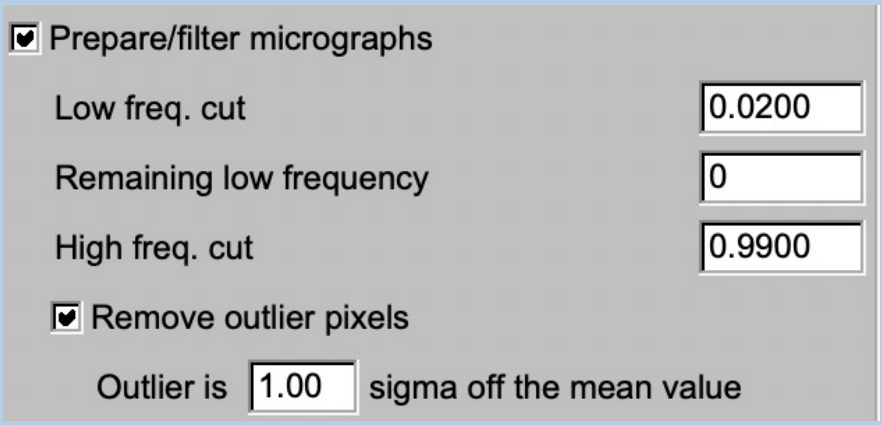


or create patches

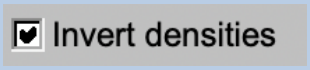


to speed up the CTF calculations.

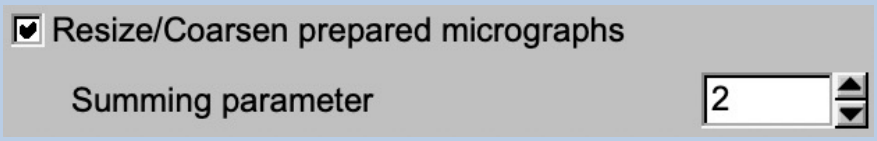
Imposing a band-pass filter is always suggested:



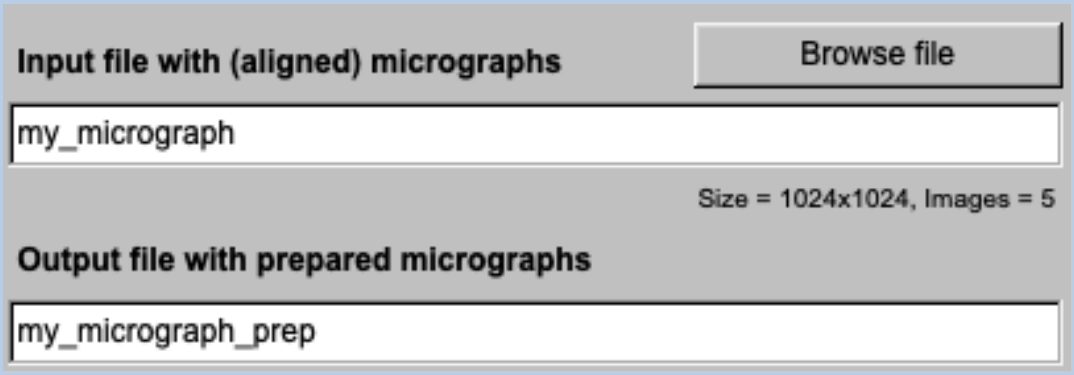
If wanted you can also invert the contrast although this does not change any CTF calculation.



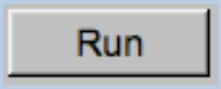
Finally, you can once more resize the pre-treated micrographs:



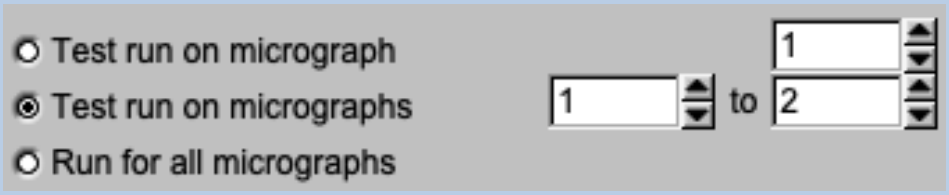
As usual, specify the names of the input and the output files:



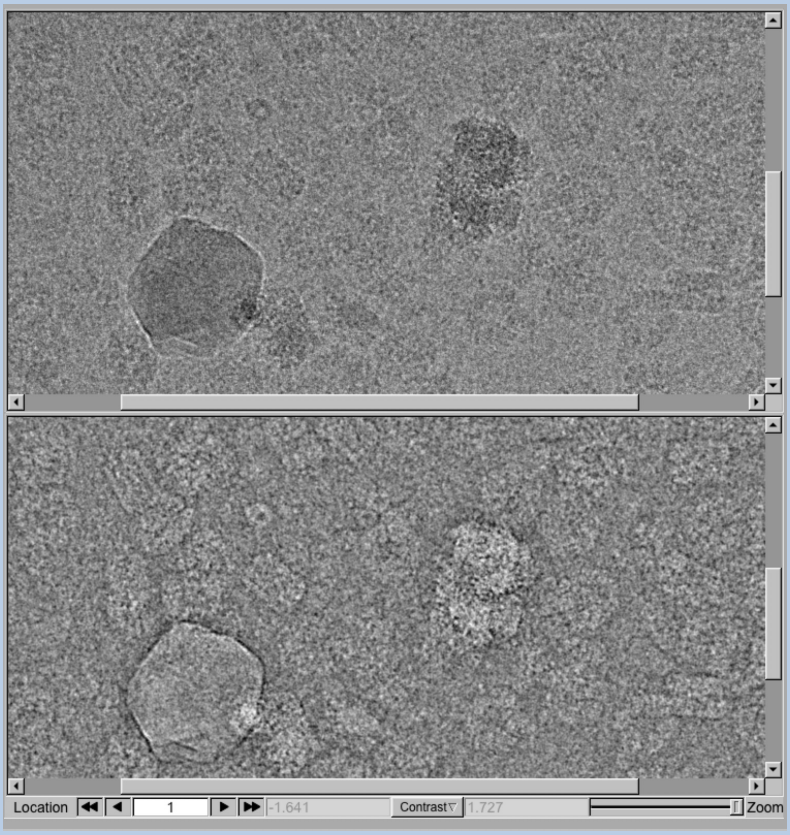
Start the calculations by clicking the “Run” button:



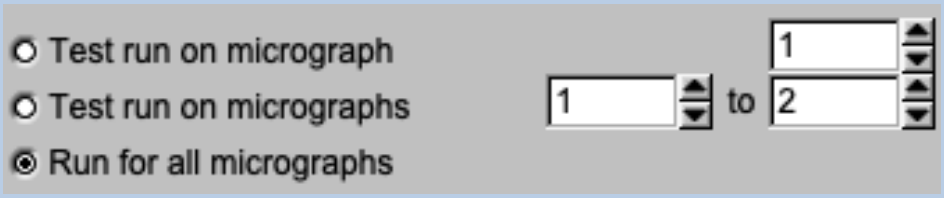
Play around with the parameters running movie alignment on a single or a small range of micrographs.



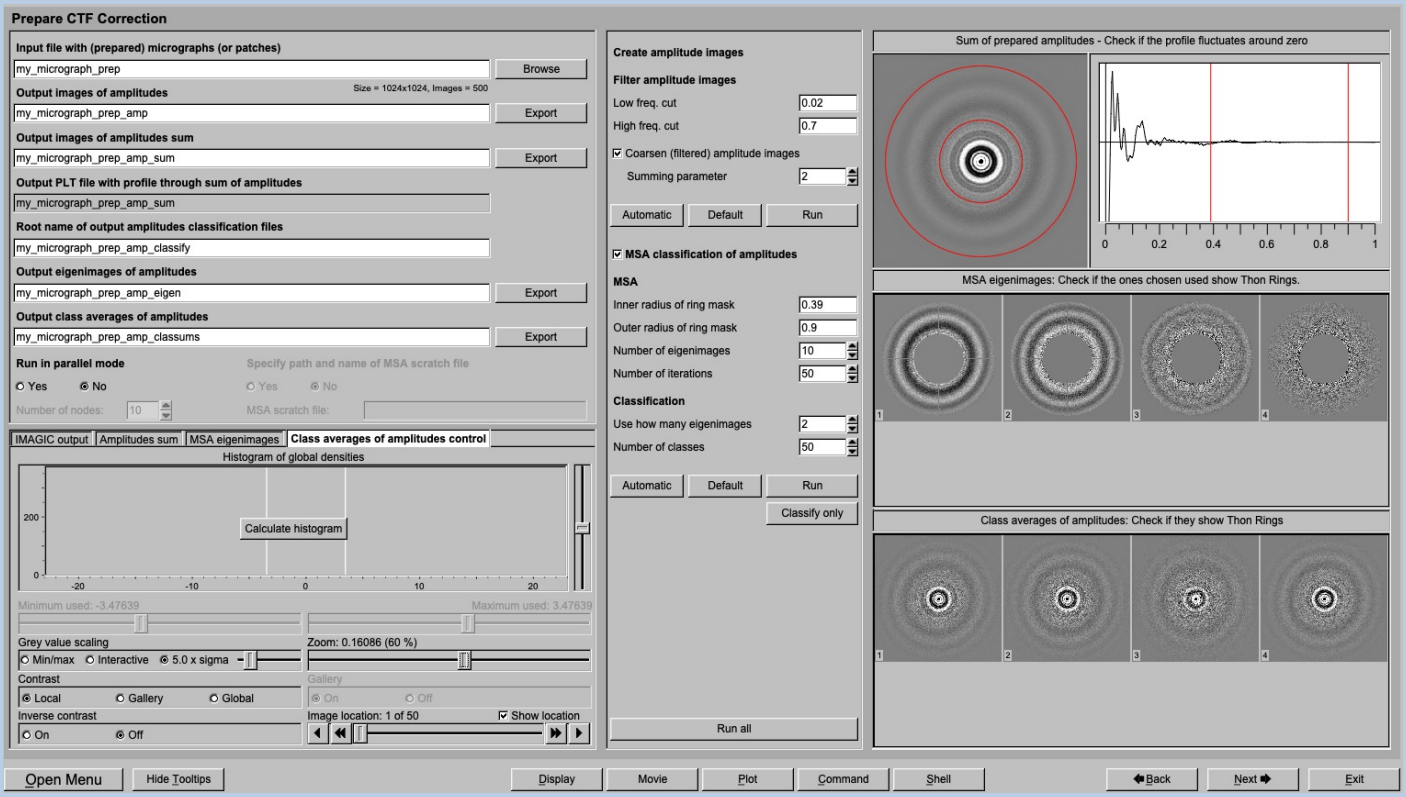
Check the pre-treated micrographs:



If all parameters are adjusted and the pre-treated micrographs look okay run the calculations for all micrographs:



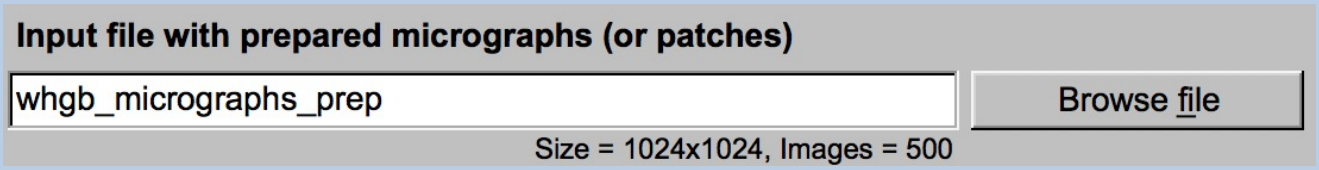
The “Prepare CTF Correction” Page



DESCRIPTION:

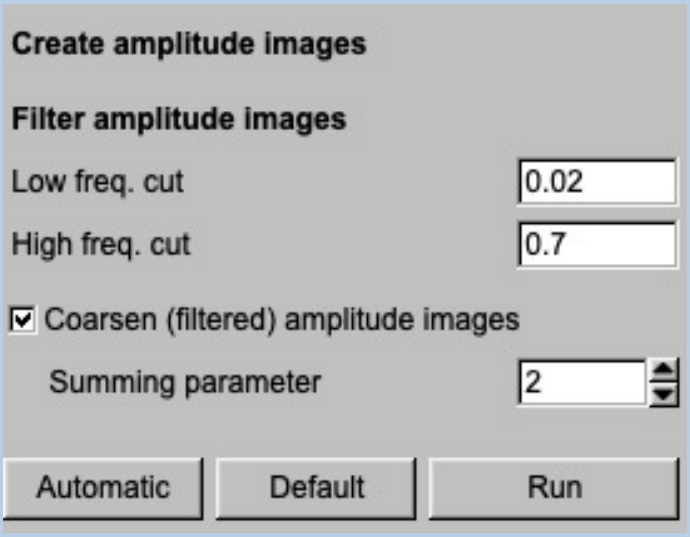
Create amplitude images of the input micrographs with good contrast and enhanced Thon rings so that the subsequent CTF algorithm can find these rings to calculate the defocus values.

Input are the prepared micrograph images which file name you have to specify



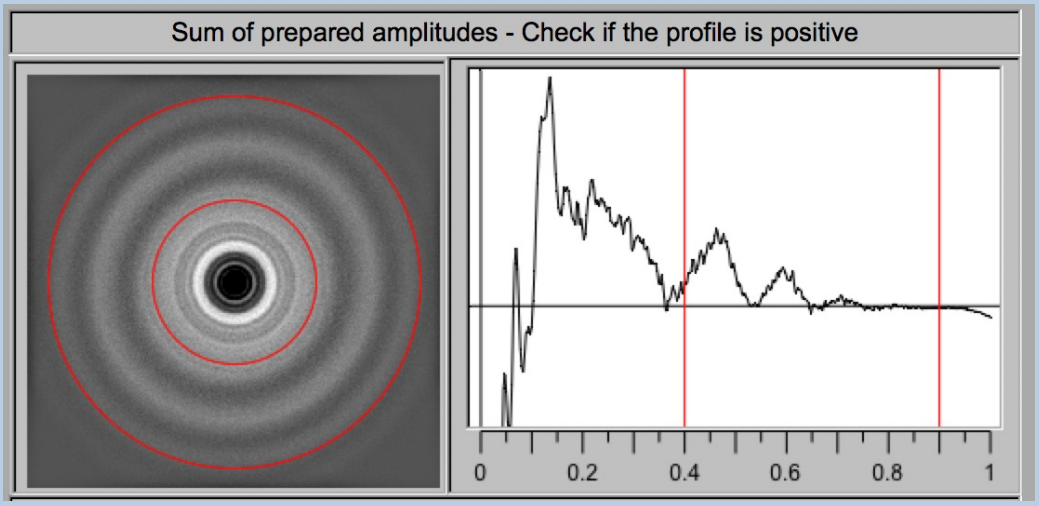
First the amplitudes of the (pre-treated) micrographs are calculated. These amplitudes are treated like real images which will be will masked and band-pass filtered (especially the background has to be removed by reducing the low frequencies). All this treatment is done to enhance the contrast of the amplitude images so that the Thon rings are better visible.

Play around with the filter parameters (to speed up the CTD estimation you can additionally resize the amplitude images):



Now it is necessary to check if the filter parameters were chosen correctly. Therefore, all pre-treated amplitudes will be averaged and displayed on the right-hand side. The red lines indicate the chosen MSA mask radii (see below).

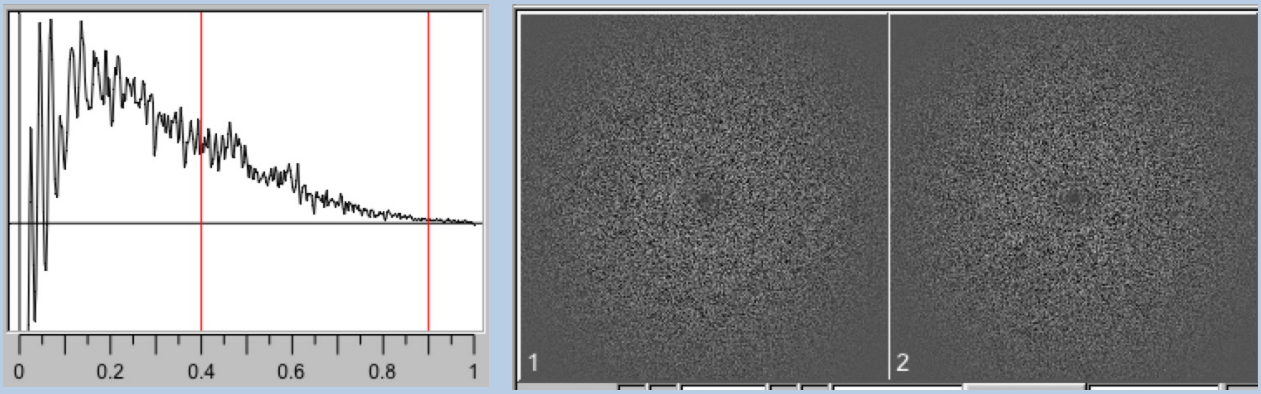
Also, a profile along the central line is shown



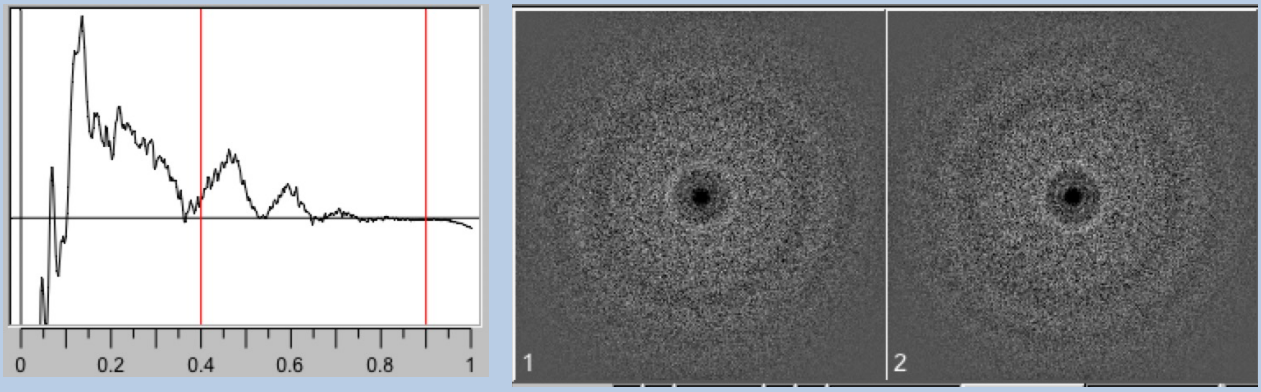
The second display row shows the created amplitude images. If the filter parameters are chosen correctly you should see Thon rings.

Play around with the filter parameters and see how they influence the amplitude images.

If the CTF curve does not converge to zero, the low frequencies are not yet reduced enough and the filter parameters should be enhanced:



If the CTF curve approaches zero for high frequencies the band-pass parameters were chosen correctly:



If the Thon rings are clearly visible one can use these amplitude images to find the CTF and determine the defocus values.

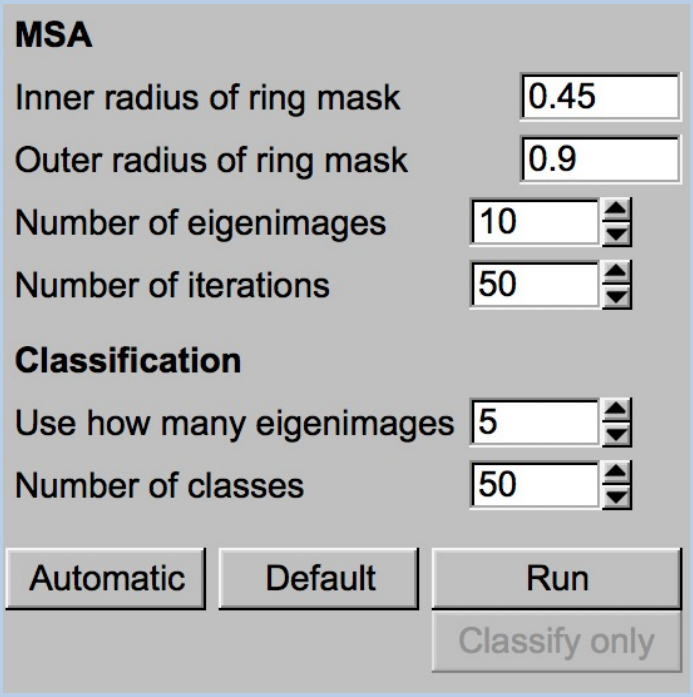
In this case do not use MSA and classification and click the “Next” button.

But usually MSA and classification is used to enhance the Thon rings:

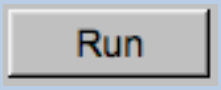
In contrast to the individual amplitude images the class averages will show the Thon rings much better which are needed to find the defocus values.

Certain radial areas are not of interest and should not be taken into consideration for MSA and classification. Check if the ring mask correctly masks out the unwanted inner and outer parts. Use the red rings in the amplitude average image and the red horizontal line in the related profile for help.

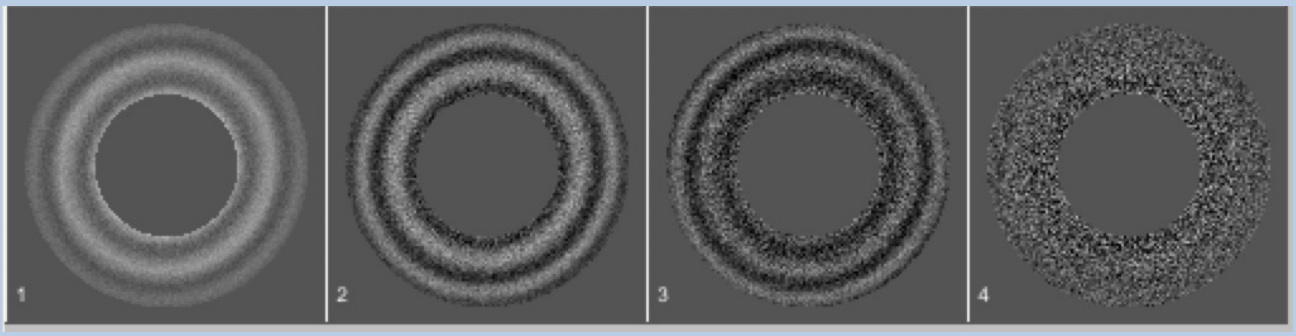
Start with the automatically given MSA and classification parameters. You can later adjust the parameters when checking the first results (eigenimages, class averages etc.).



Start the MSA and classification calculations by clicking the “Run” button:

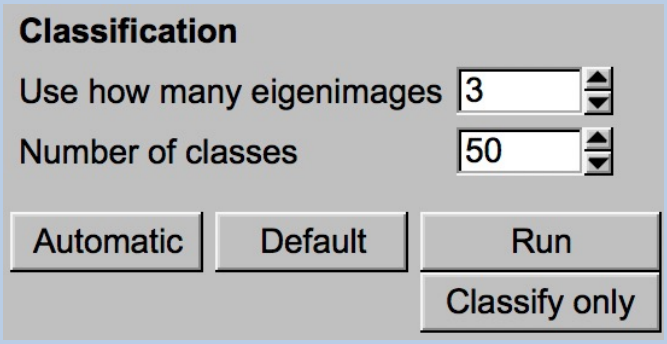


Check the MSA eigenimages and find out the range of MSA eigenimages showing Thon rings:

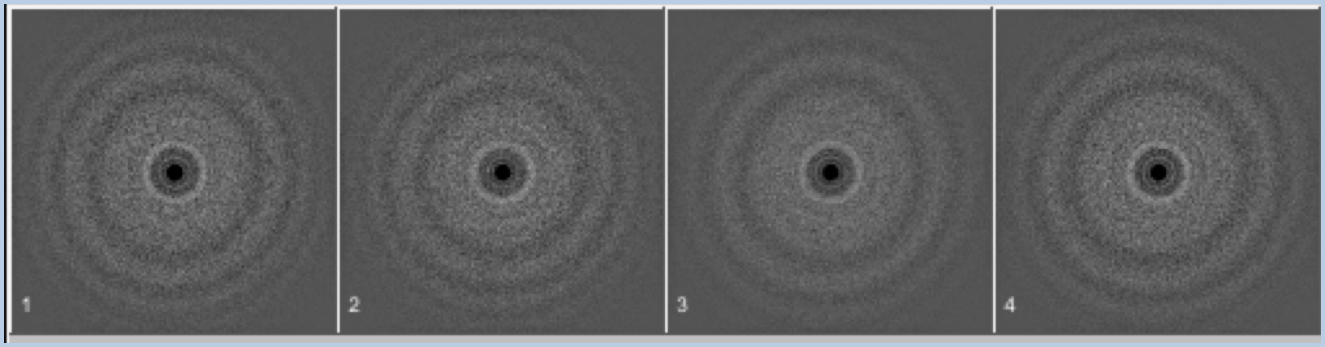


For classification only use the eigenimages showing Thon rings.

You don’t have to re-calculate the full MSA and classification (“Run”) when only changing the number of eigenimages to be used for classification or the number of classes. You can click the “Classify only” button.

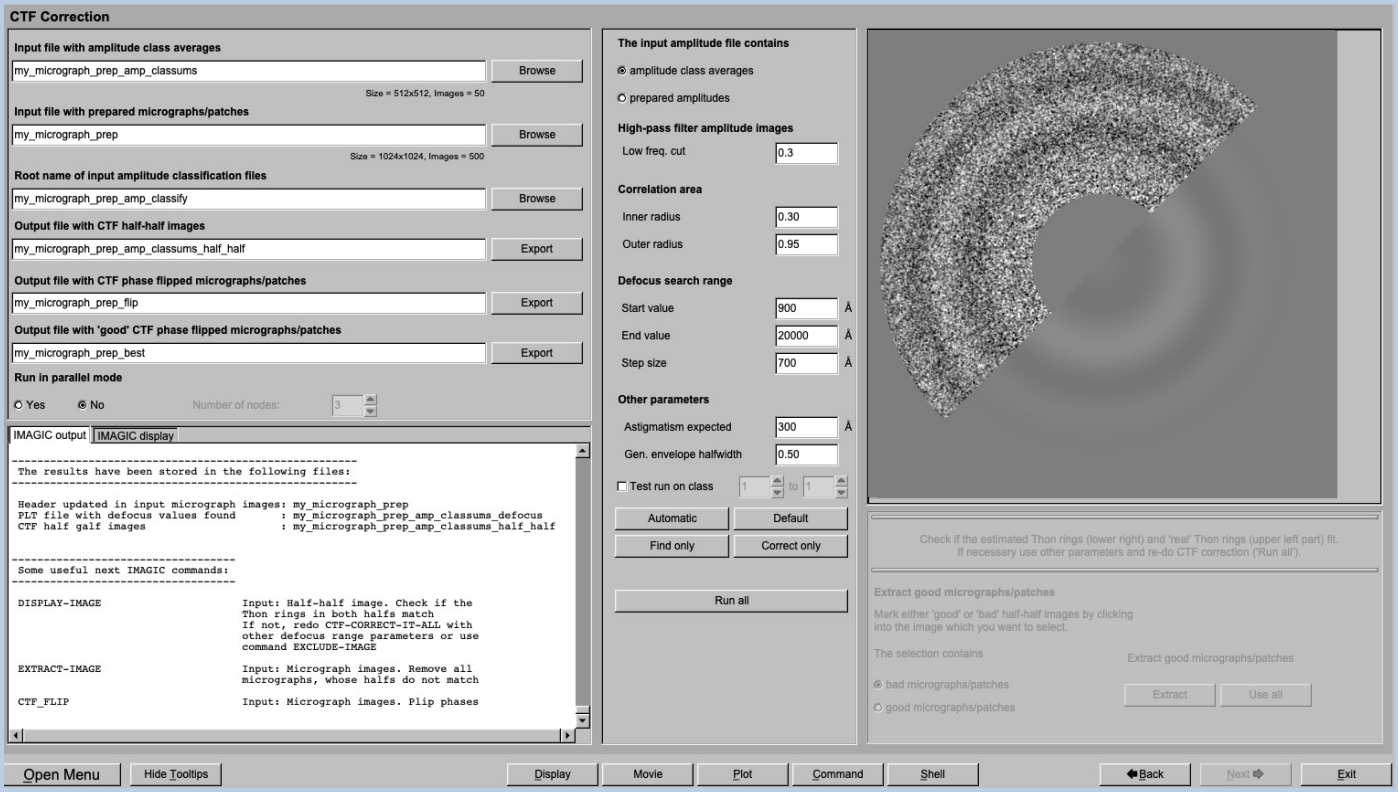


The finally created class averages of the amplitude images should clearly show Thon rings.



As usual click the “Next” button to continue.

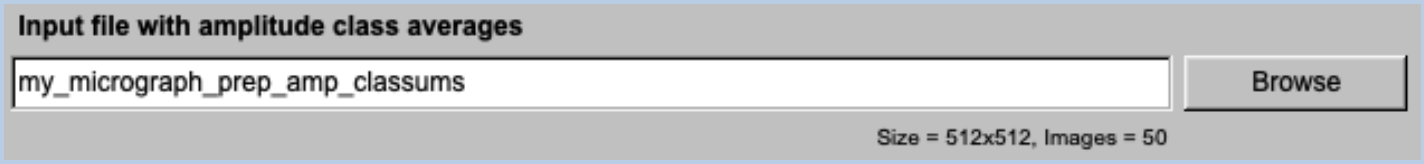
The “CTF Correction” Page

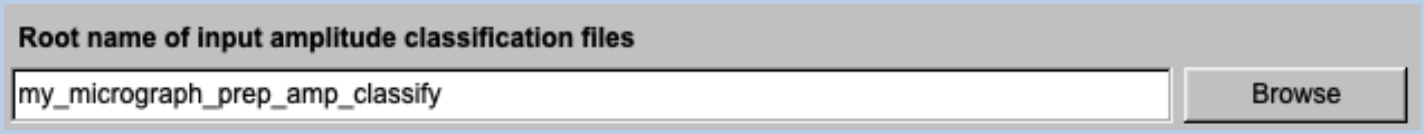


DESCRIPTION:

Find the CTF using the amplitude images created on the previous page (“Prepare CTF Correction”) and CTF correct the input micrographs.

Depending on the options chosen on the “Prepare CTF Correction” page the defocus values are either calculated from the amplitude class averages (using the classification results files)

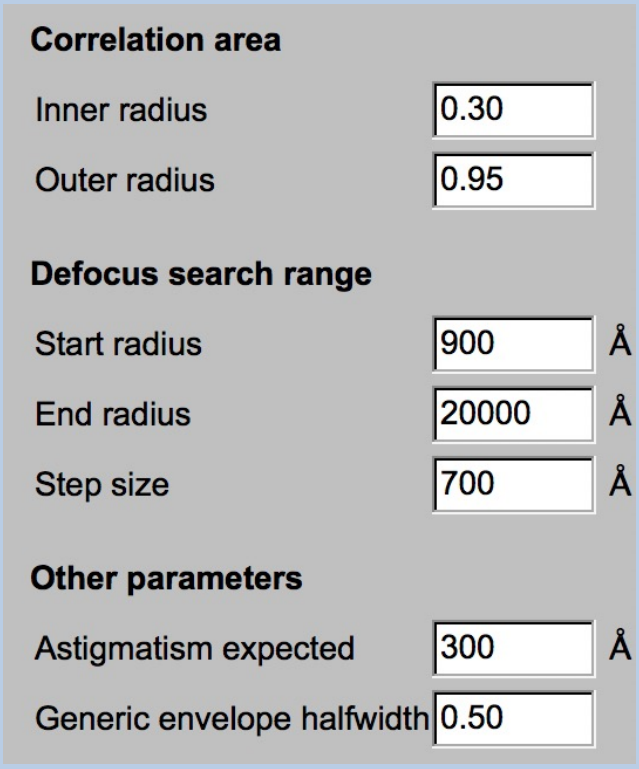




or from the amplitude images

In both cases input for the CTF correction are the micrograph images, of course:

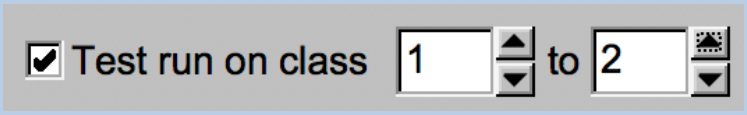
You can adjust some CTF search parameters:



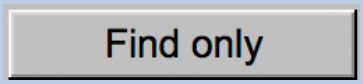
Important parameters are the following:

- Inner and outer correlation radius: A normalized cross correlation is used to compare the filtered experimental amplitude image to the theoretical CTFs. The centre and periphery of the amplitude image do not contain rings and are not important in the estimation. Therefore, the cross-correlation is only computed over a ring area specified by two radii. You can play with these parameters to obtain the best estimation - or simply try the suggested values.
- Defocus range and step size: Here you can set the parameters for the initial brute force search. The first parameter is the start of the search, the second is the end of the search and the third is the step size over which the search is conducted. You can play with these parameters to obtain the best estimation - or simply try the suggested values.

You can test the parameters by using a single or a range of micrograph(s) as test only:



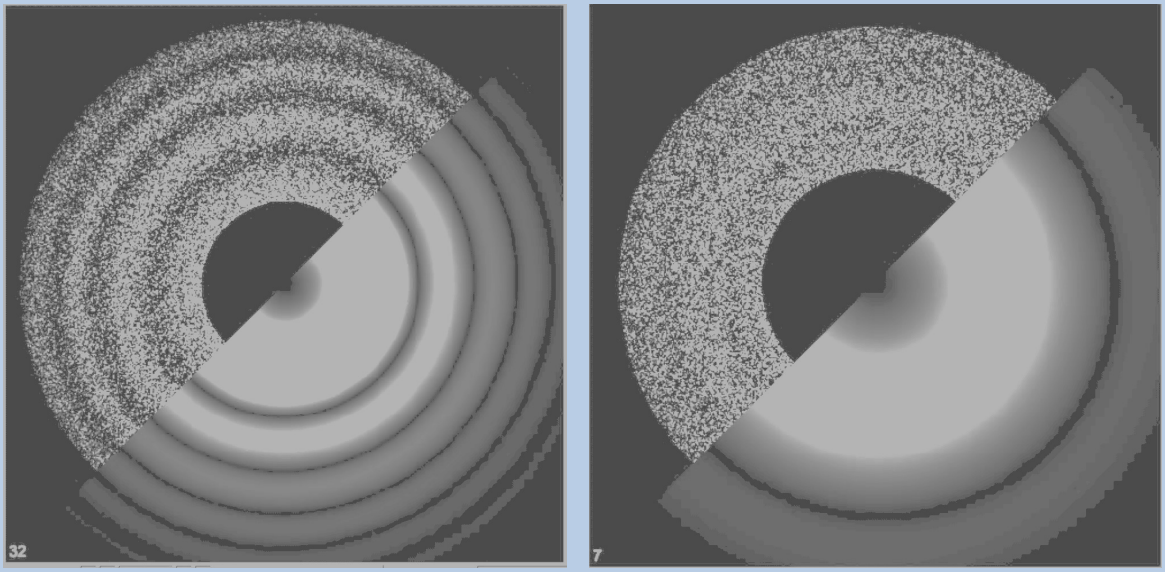
Click the “Find only” button to start the CTF search:



Check the defocus values found in the IMAGIC output on the left-hand side.

Also have a look at the half-half images in the display on the right-hand side. Each image will contain a) in the left half the input amplitude image, and b) in the right half the “estimated” CTF.

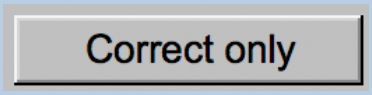
Use these "half-half" images to check the accuracy of the CTF estimation. The Thon rings of both halves should fit.



Good fit Bad fit

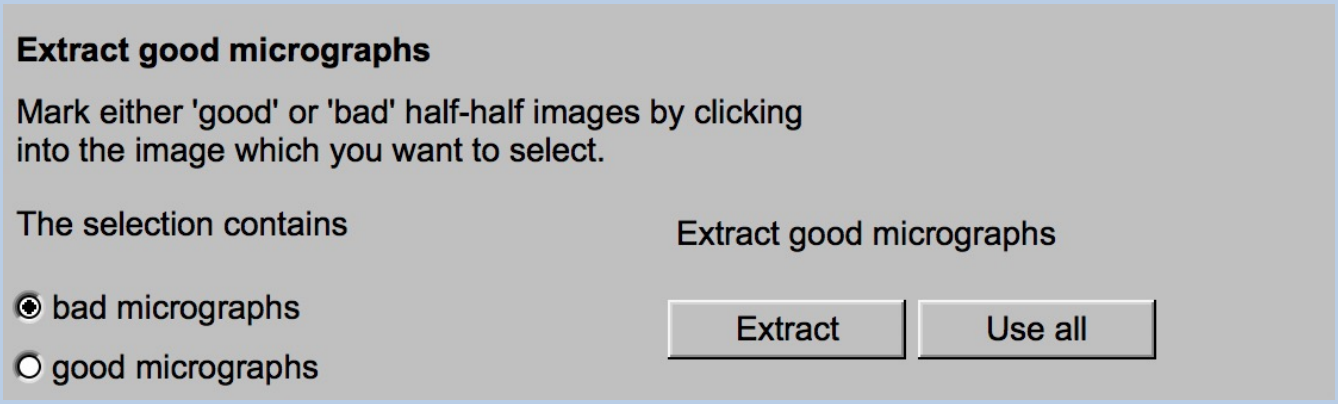
Having found the correct CTF parameters you can now CTF correct the micrographs (flip the phases).

Click the “Correct only” button to start the CTF correction:

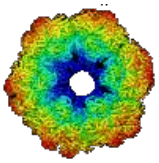


After the CTF correction you can select ‘good’ or ‘bad’ micrograph by clicking into the displayed CTF half-half images.

Having finished this selection specify if you have chosen the ‘good” or ‘bad’ micrographs and click the “Extract” to extract the ‘good’ micrographs.



Of course, you can use all micrographs. In this case click the “Use all” button.



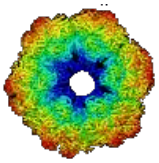
IMAGIC

guiCTF

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

guiCTF

Feedback / Error hints

We intensively tested the **guiCTF** 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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