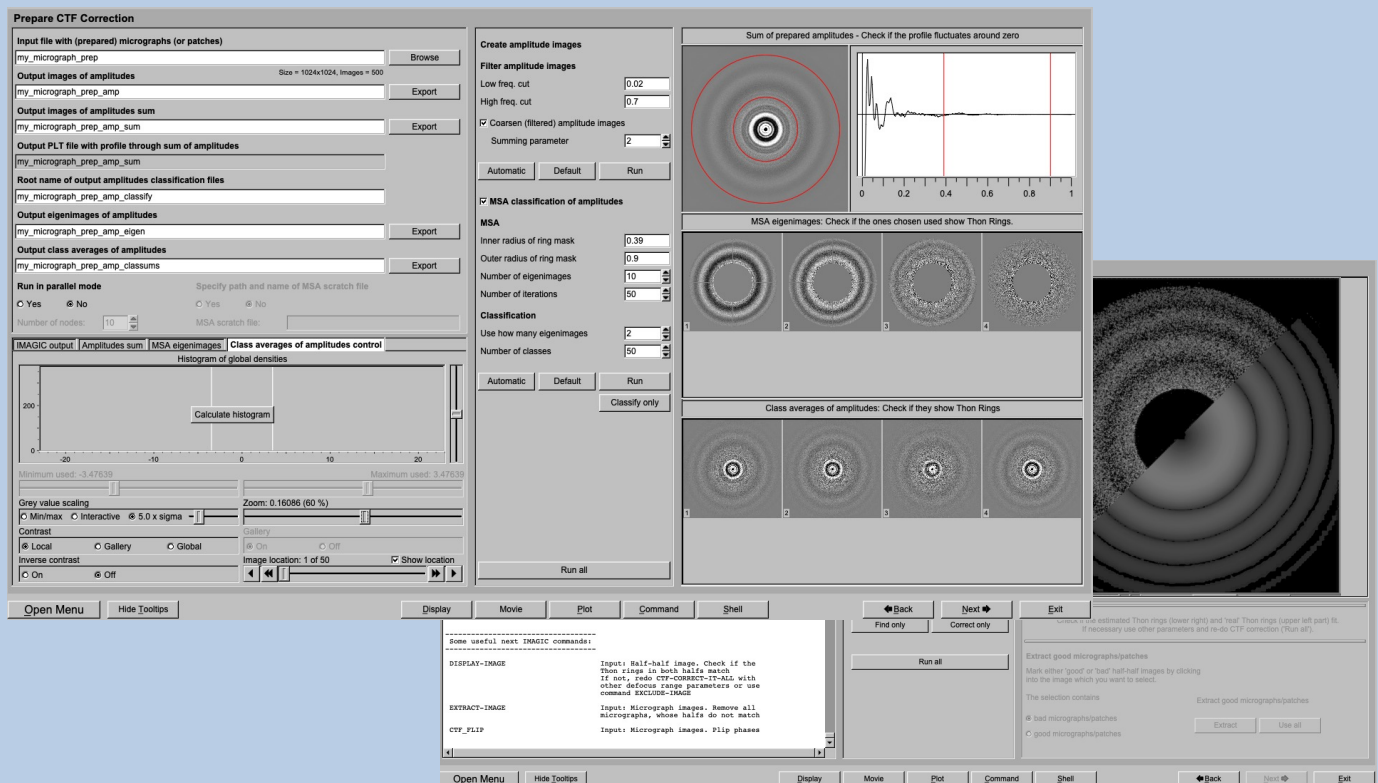


## A Brief Introduction

Version 18-Jan-2024  
[www.ImageScience.de](http://www.ImageScience.de)  
© Michael Schatz (Image Science)

# The IMAGIC guiCTF program



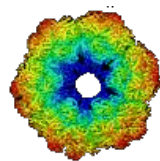
The **guiCTF** program follows a work-flow from Import Micrographs to CTF Correction.

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

## CONTENT:

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





**IMAGIC**

# **GUI Programs**

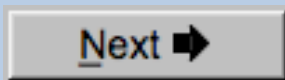


# Workflow

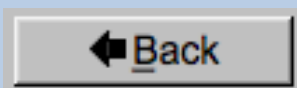
The idea of **guiCTF** is to guide you through a typical CTF correction of electron micrographs.

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

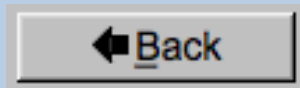
If **guiCTF** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

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

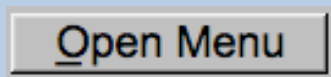
```
IMAGIC-COMMAND : guiCTF
```

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.

**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.

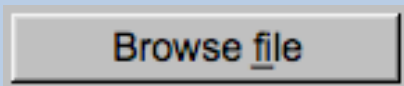
You can, of course, always use other input files names and even use other input directories.

<b>Input file with (raw) micrographs</b>	<b>Browse file</b>
my_micrographs	

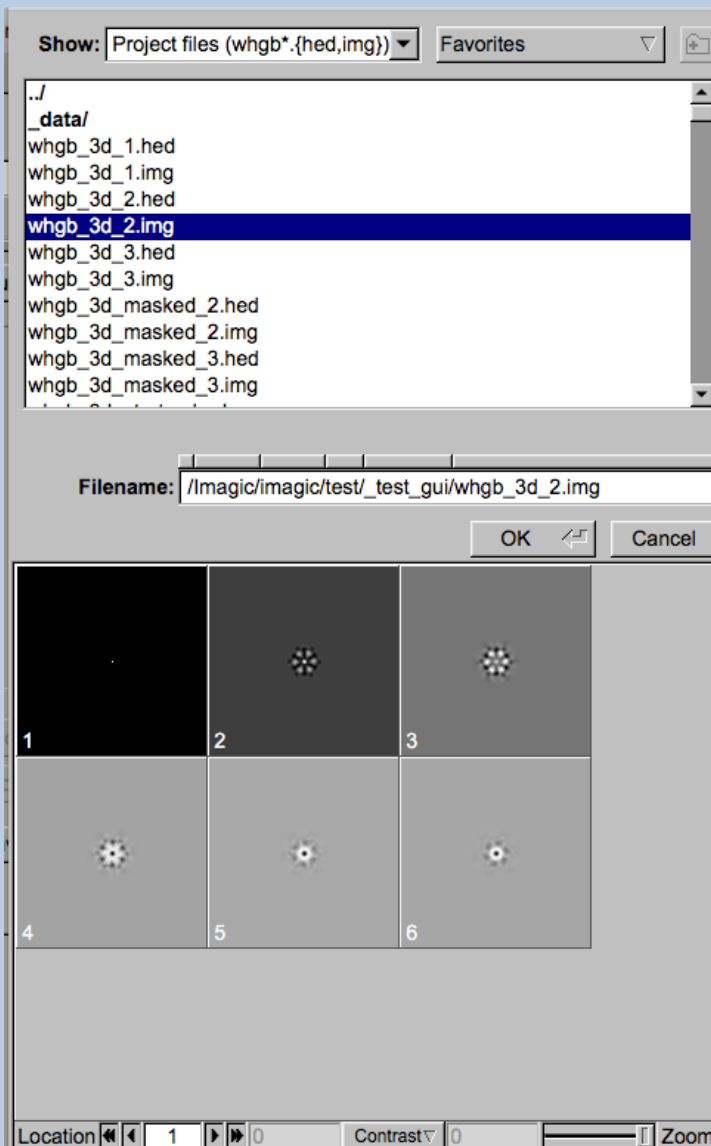


# Input File Chooser

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

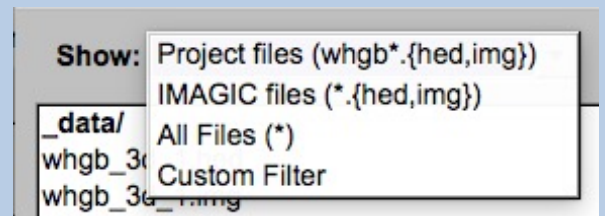


Pressing this button will open the IMAGIC file chooser:

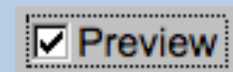


Choose the wanted file by clicking its name

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

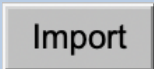
Usually the names of the output files are suggested but it is your choice, of course. On each page you can specify these output file names on the left hand side.

<b>Output file</b>	Export
my_micrographs	

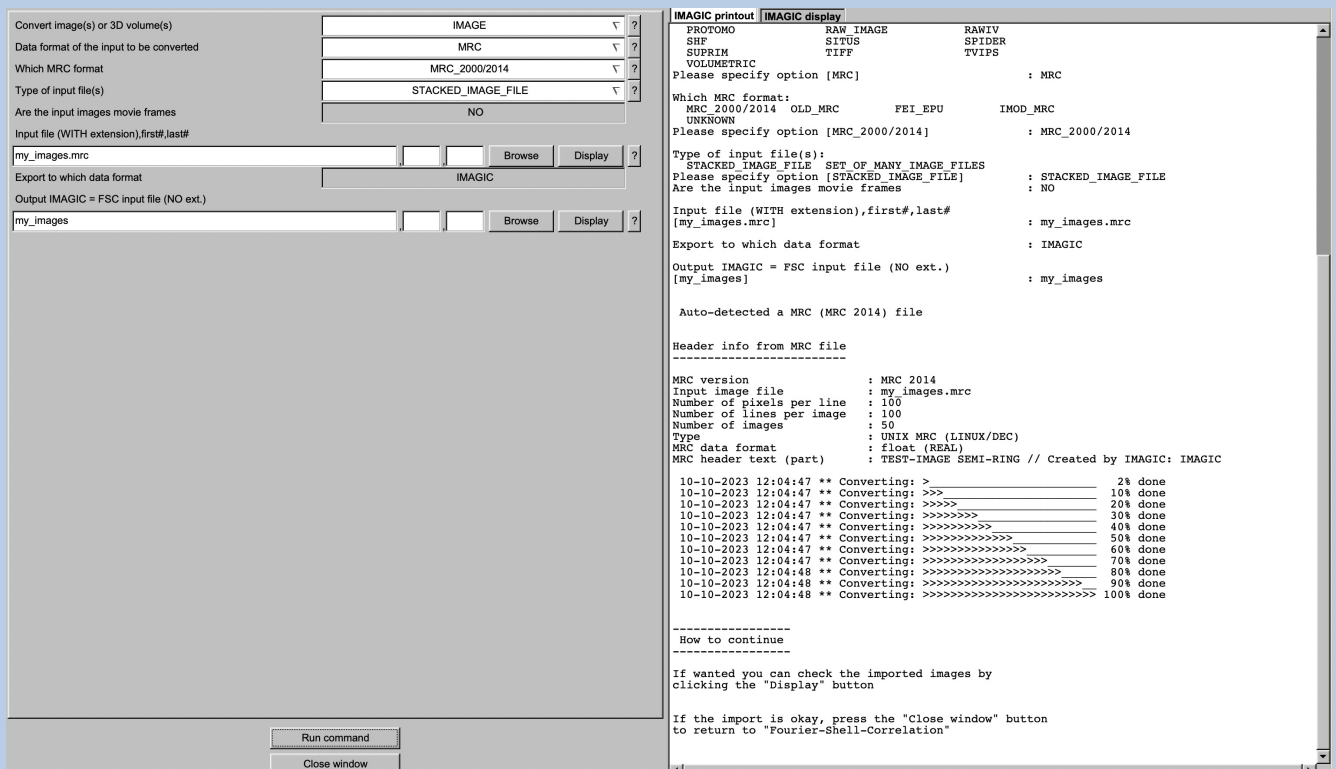


# Import Buttons

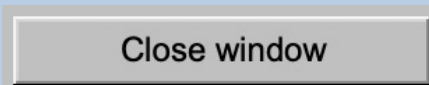
You do not want to use the “Import page” you can sometimes use an “Import” button to import the input images/3D volumes from any 3DEM format. The “Import” button which is located above the text field specifying the name of the related file.



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.

Export

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 displays the 'IMAGIC EM2EM' interface. On the left is a dialog box for configuring export parameters. On the right is a terminal window showing the command-line output of the 'EM2EM' process.

**Export Dialog Box Parameters:**

- 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

**IMAGIC EM2EM Terminal Output:**

```
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               FABOSHA       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:

Close 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

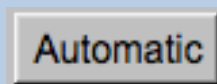
The screenshot shows a control panel with the following elements:

- Resize/Coarsen micrographs
  - Summing parameter: 2
- Create patches
  - Size of patches: 4096
- Prepare micrograph
  - Low freq. cut: 0.0200
  - Remaining low frequency: 0
  - High freq. cut: 0.9000
- Remove outlier pixels
  - Outlier is 4.50 sigma off the mean value
- Invert densities
- Resize/Coarsen prepared micrographs
  - Summing parameter: 2

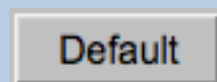
At the bottom, there are three buttons: "Automatic", "Default", and "Run".

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

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



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



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



# A Typical Page - Run buttons

**Create prepared amplitude images**

**Filter micrographs**

Low freq. cut

Remaining low frequency

High freq. cut

**Filter amplitude images**

Low freq. cut

Remaining low frequency

High freq. cut

**Coarsen filtered amplitude images**

Yes  No

Summing parameter

**MSA options**

MSA eigenfilter amplitudes

MSA classify amplitudes

**MSA**

Inner radius of ring mask

Outer radius of ring mask

Number of eigenimages

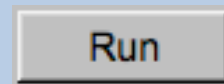
Number of iterations

**Classification**

Use how many eigenimages

Number of classes

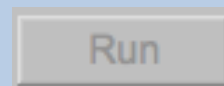
To run the calculations press the “Run” button.



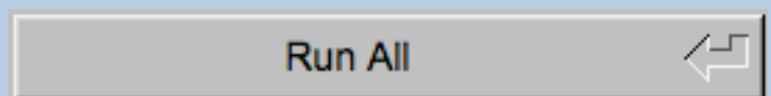
On a number of pages the calculations can be split. In this case you will find more than one single “Run” button.

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

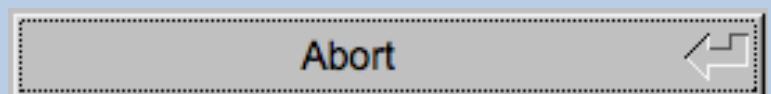
Maybe a certain “Run” button is not yet activated because it needs the results of calculations not yet done.



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



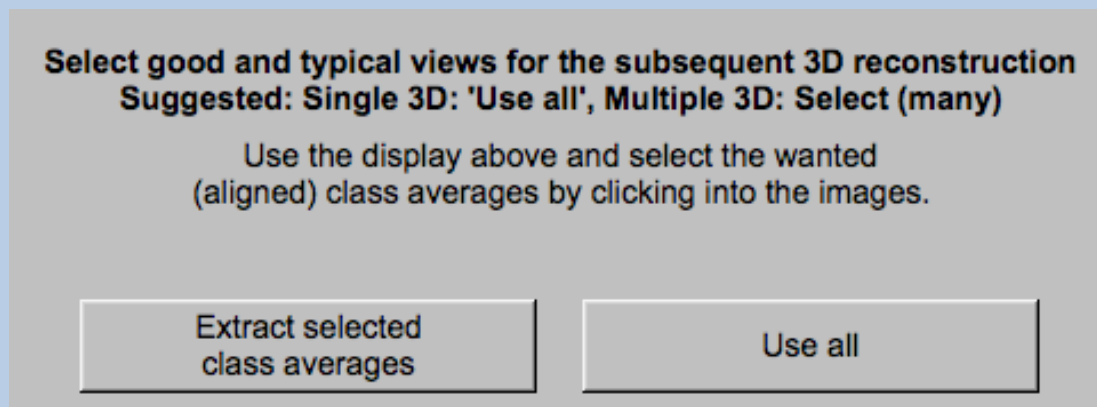
You can abort a running program by pressing the “Abort” button.





# A Typical Page - Additional Tasks

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

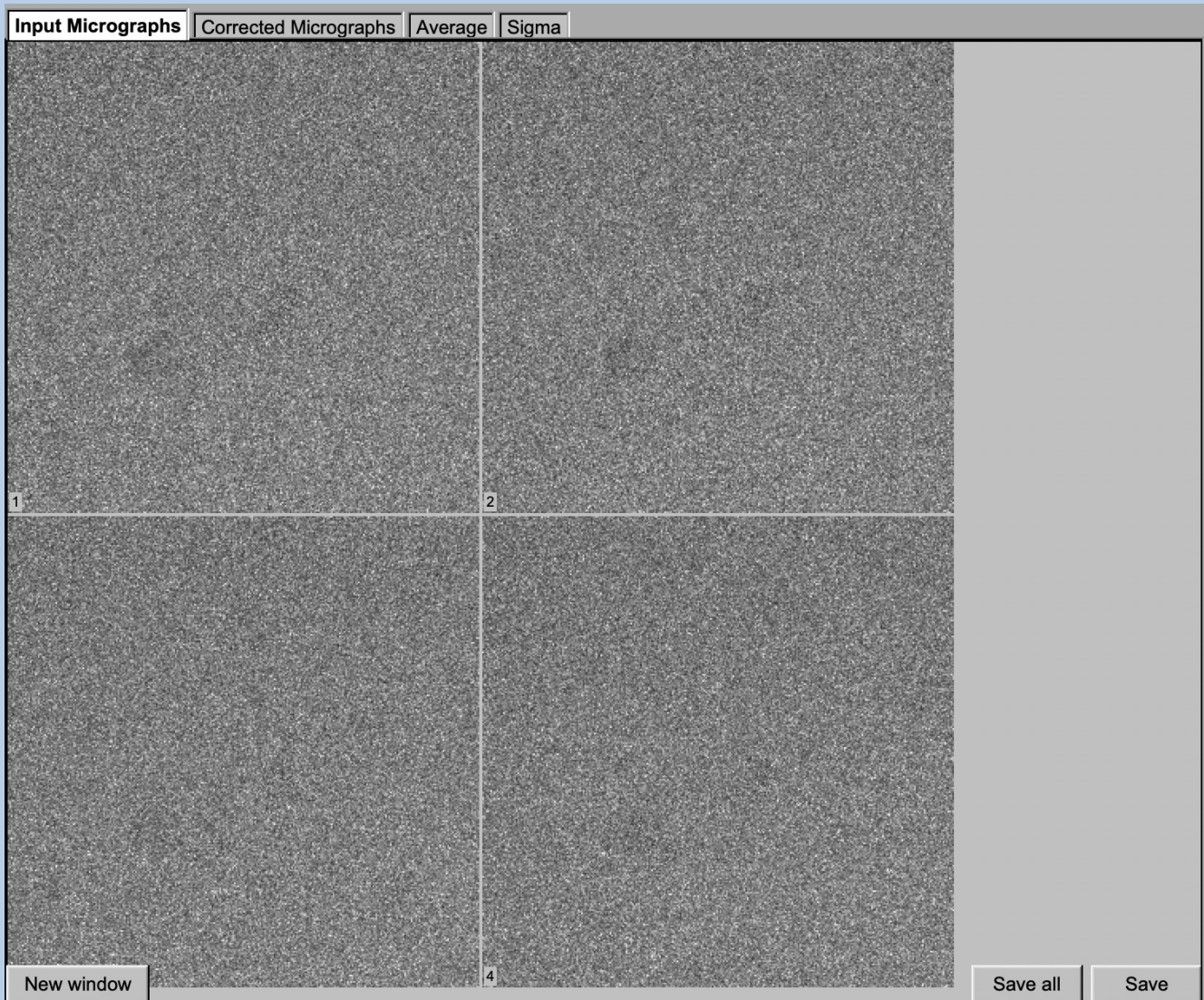


Note that the new output images are usually shown in a new display tab.





# A Typical Page - Display



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

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

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

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



# A Typical Page - “Display Control” Tabs

The visualisation settings of the images shown on the right-hand side of each **IMAGIC GUI program** page can be adjusted in its own related “Display control” tab on the bottom left part of each page. Also refer to **guiDISPLAY**.

Grey value scaling: Adjust the contrast

Min/Max: Scale the grey-values to minimum/maximum

Interactive: Set the limits by giving numbers

Sigma: Use an amount of sigma to set the limits

Contrast

How to calculate the grey value scaling

Local: Calculated in each image separately

Global: Calculated using all image densities  
(as displayed in the histogram)

Gallery: Calculated in the currently displayed images

Inverse contrast:

Use one of the radio buttons

Zoom

Enlarge the displayed images

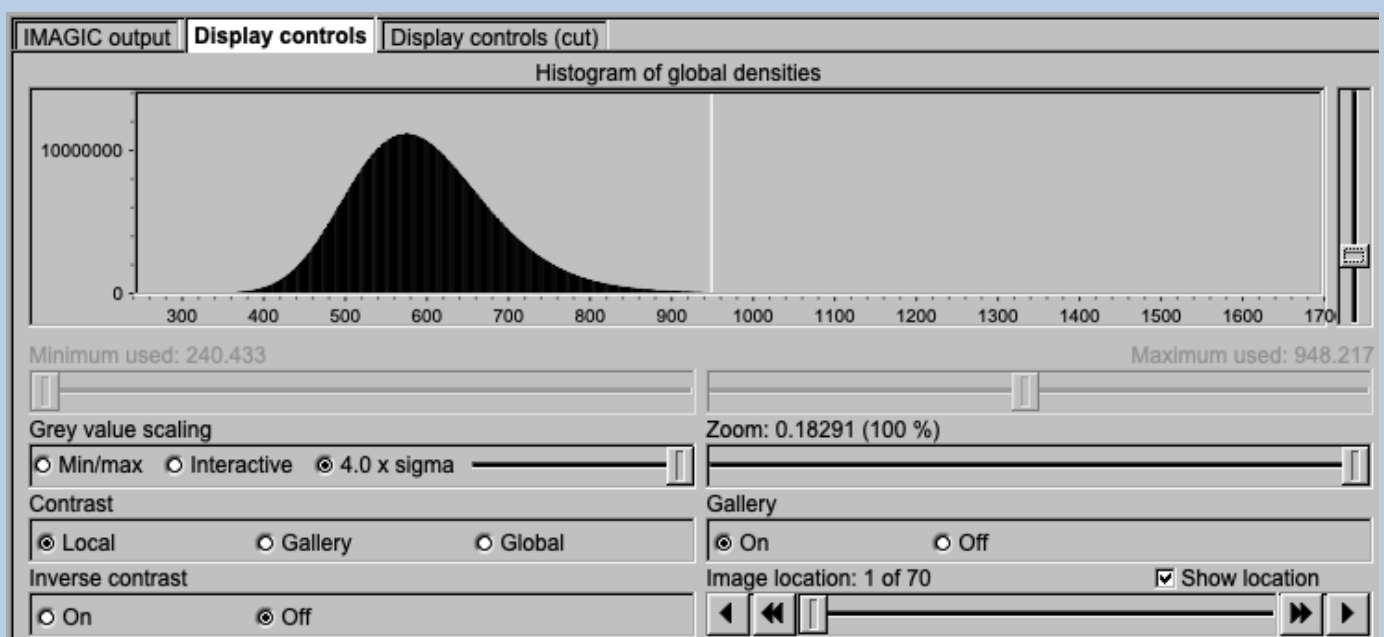
Gallery

On Display the images in a gallery  
(may be you need another zoom to see more than one image)

Off Show only one image

Image Locations.

Use the slider or the arrows to select image locations



# A Typical Page - “Plot Control” Tabs

The visualisation settings of curves/spectra is shown on the right-hand side of an **IMAGIC GUI program** page can be adjusted in its own related “Plot control” tab on the bottom left part of each page. Also refer to **guiPLOT**.

Style, Colour, Grid: Adjust the curve line style, the colour and add a grid if wanted

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

Plot title Set the text of the plot title

Text along ... Set the text along the given axis

Use for all plots: Use the setting for all plots in a file independent of what is input in the PLT file

Reset:. Reset to the automatic values

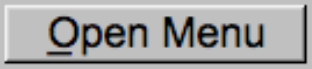
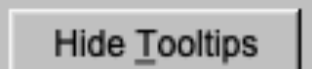
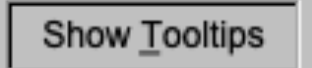
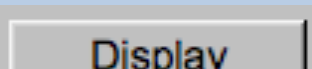
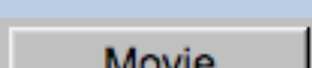
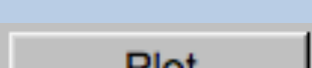
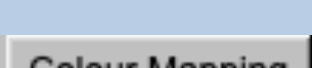

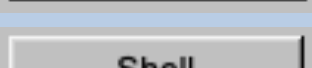
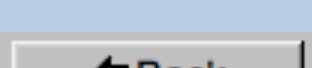
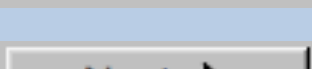
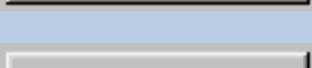
Style Select curve style ▾	Colour Select curve colour ▾	Grid Select curve grid ▾
Horizontal scaling 1.00	<input type="checkbox"/> Use for all plots 32.00	Reset
Vertical scaling -19.21	<input type="checkbox"/> Use for all plots 17.00	Reset
Plot title Fourier Ring Information - 1/2-bit	<input type="checkbox"/>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



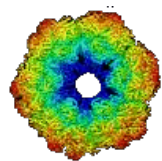
# A Typical Page - The Toolbar

There is a toolbar at the bottom of each **guiCTF** 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 <b>guiDISPLAY</b> .
	Open a MOVIE page (display in an endless loop). Refer to <b>guiDISPLAY</b>
	Open a PLOT page to show IMAGIC curves. Refer to <b>guiPLOT</b>
	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 <b>guiIMAGIC</b> .
	Run a shell / terminal page. command
	Go to the previous page
	Continue with the next page
	Exit <b>guiCTF</b>



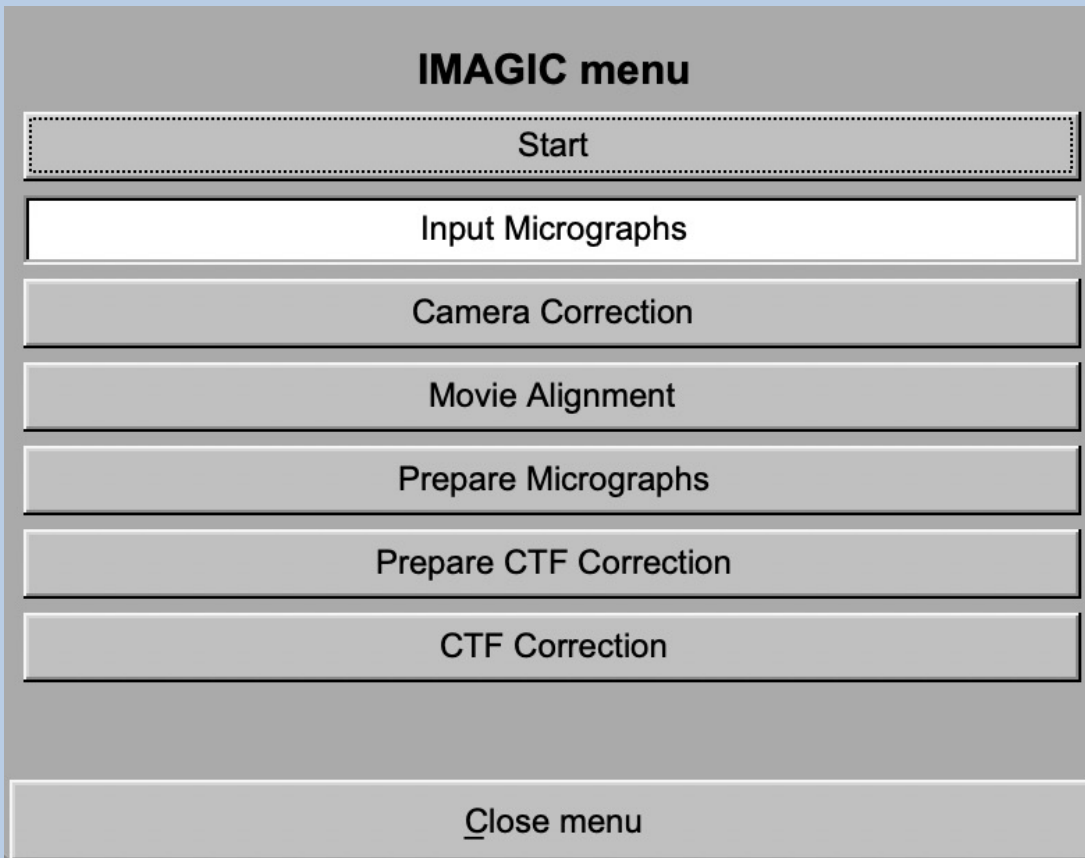


**IMAGIC**

**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:

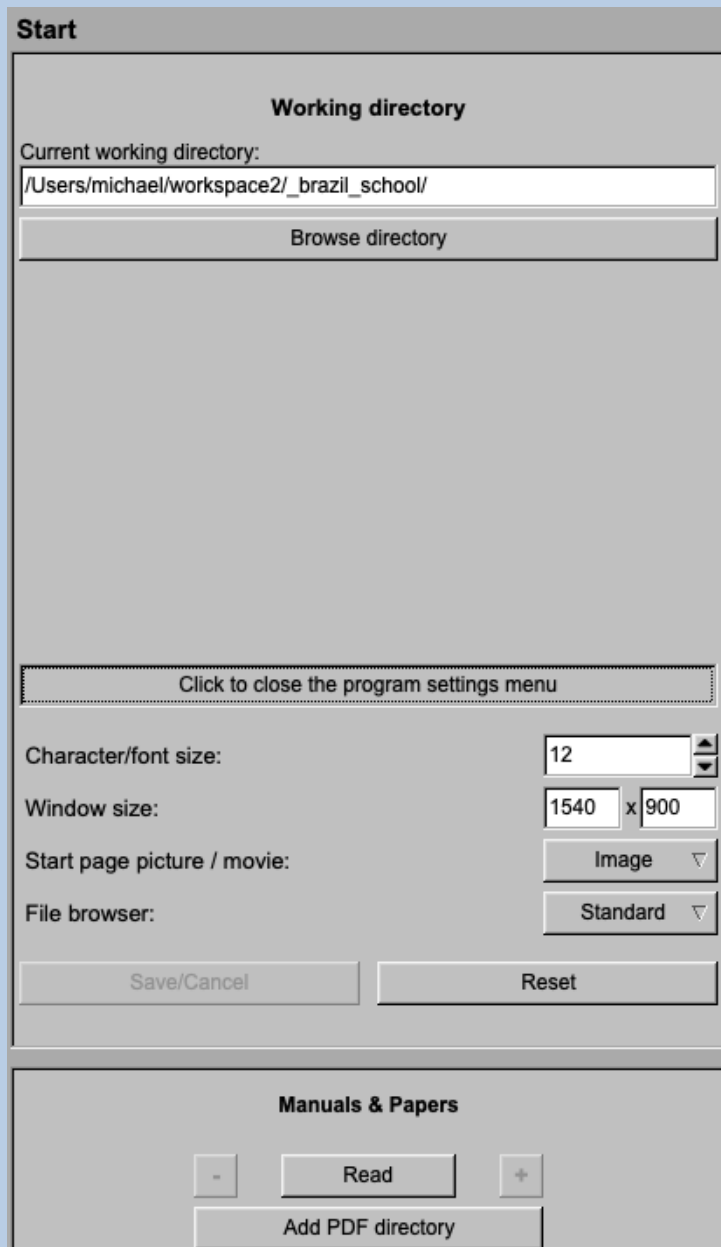
Start:	Page to adjust some program parameters
Close menu:	Close this menu and return to last page.





# 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

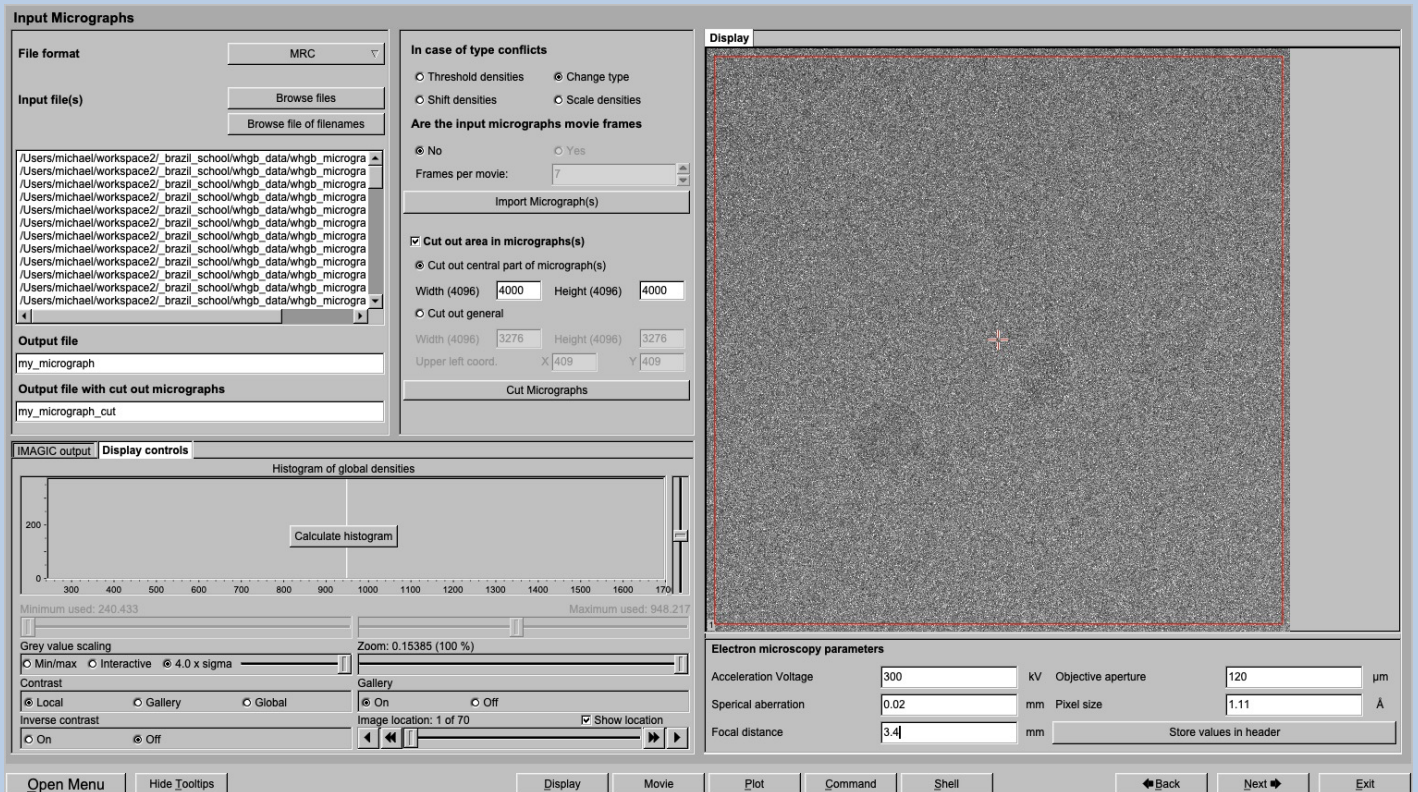
The page **guiCTF** starts with the “Import Micrograph” page.

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**.



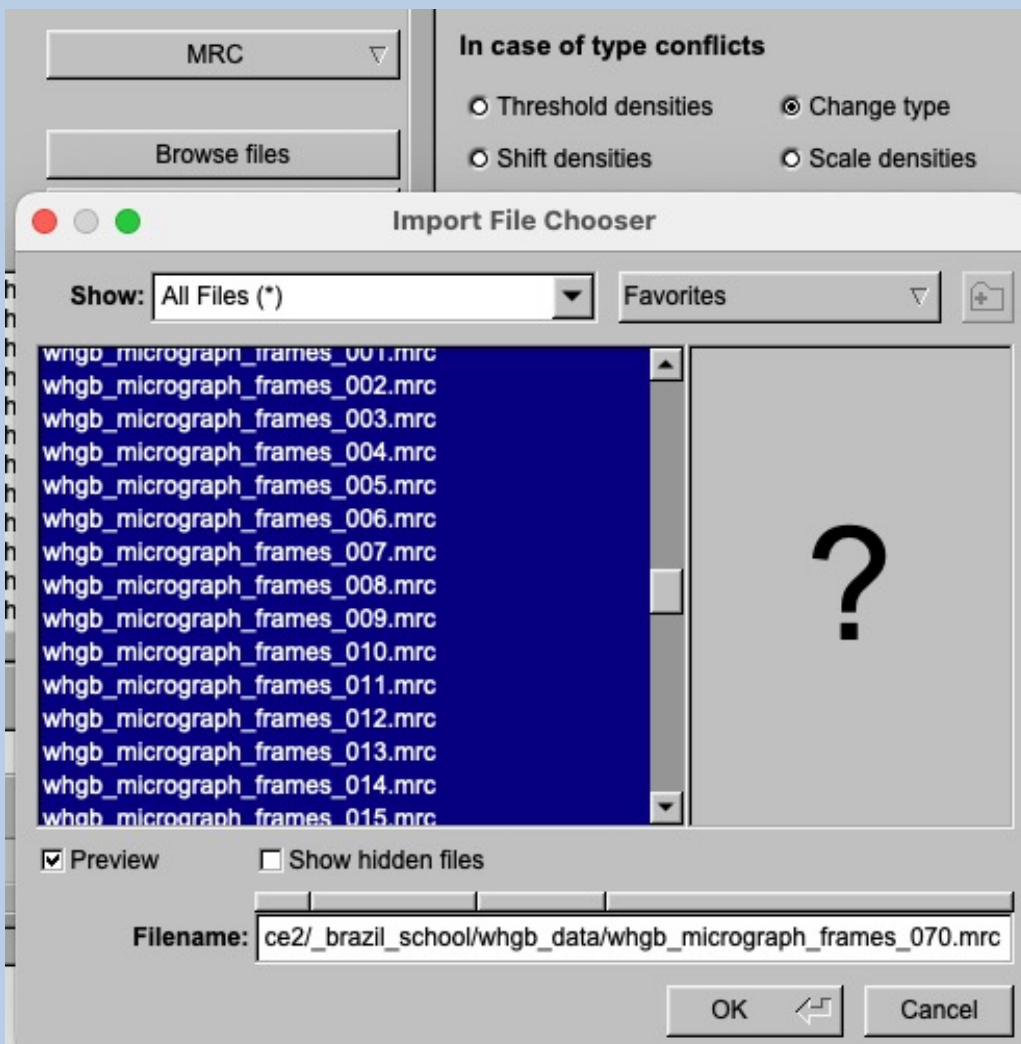
## IMPORT MICROGRAPHS:

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

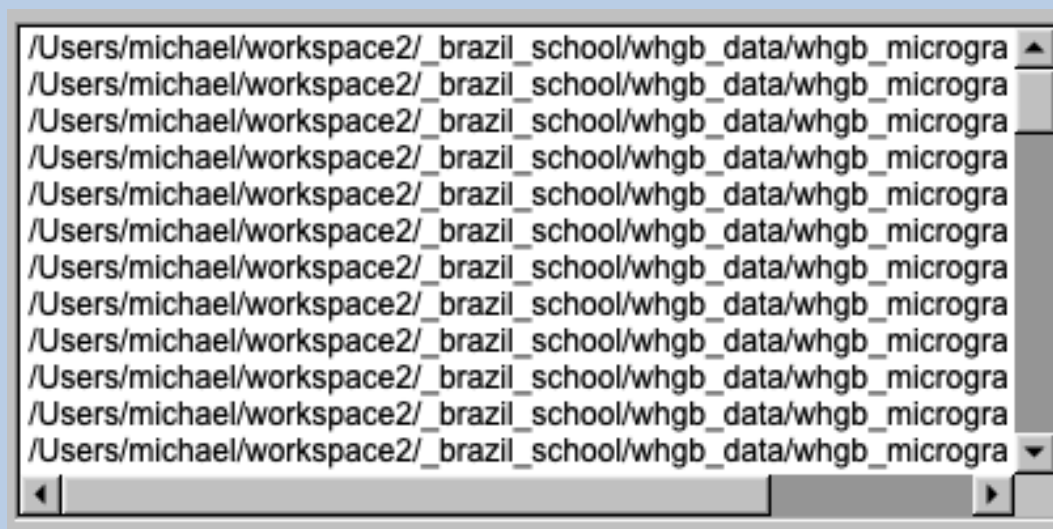


and choose one of the formats in the listing.

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.



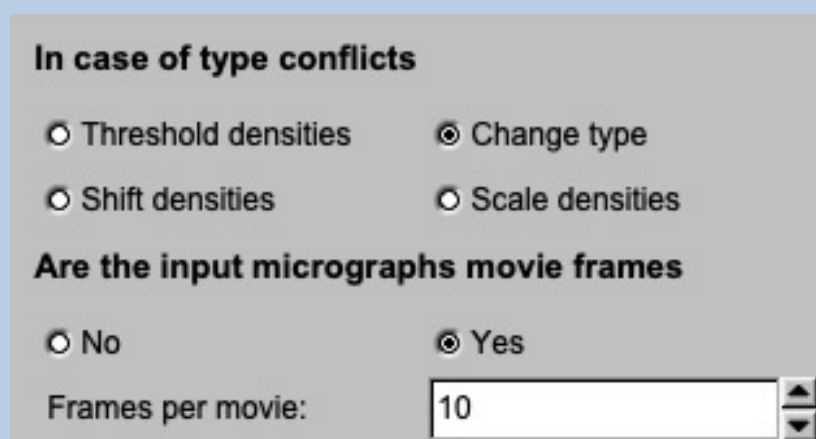
If wanted you can edit the list of files. But be careful there is no automatic control of file names in this list.



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”.

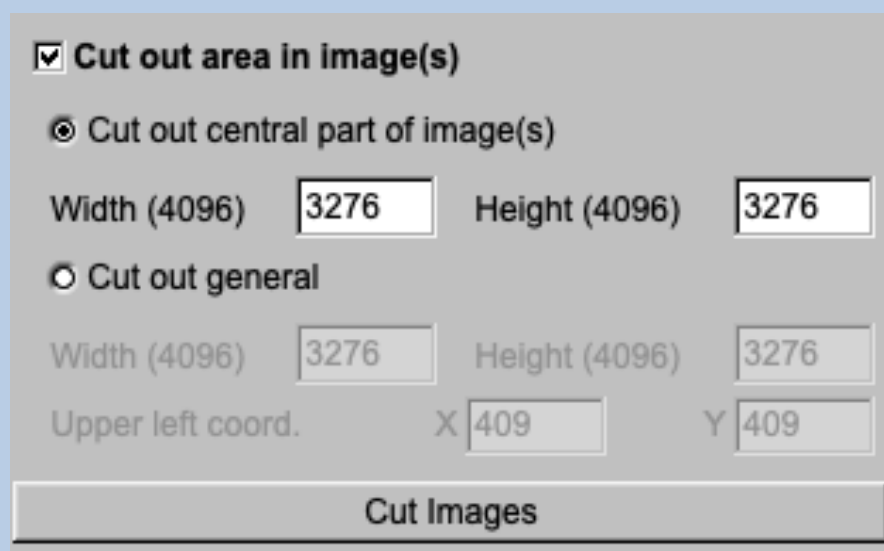


## CUT MICROGRAPHS / IMAGES

(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:



**Cut out area in image(s)**

Cut out central part of image(s)

Width (4096)  Height (4096)

Cut out general

Width (4096)  Height (4096)

Upper left coord. X  Y

**Cut Images**

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

Electron microscopy parameters					
Acceleration Voltage	<input type="text" value="300"/>	kV	Objective aperture	<input type="text" value="120"/>	$\mu\text{m}$
Spherical aberration	<input type="text" value="0.02"/>	mm	Pixel size	<input type="text" value="1.11"/>	$\text{\AA}$
Focal distance	<input type="text" value="3.4"/>	mm	<input type="button" value="Store values in header"/>		

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.

**Camera Normalisation**

Measure

Correct

Measure and Correct

Correct

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

**Input camera statistics average file** Browse file

my\_micrographs\_cnorm\_average

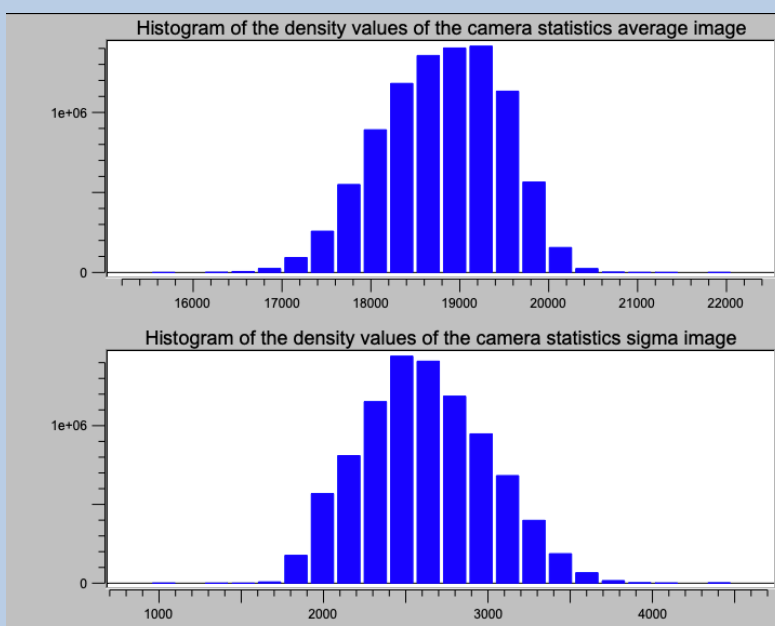
Size = 4096x4096, Images = 1

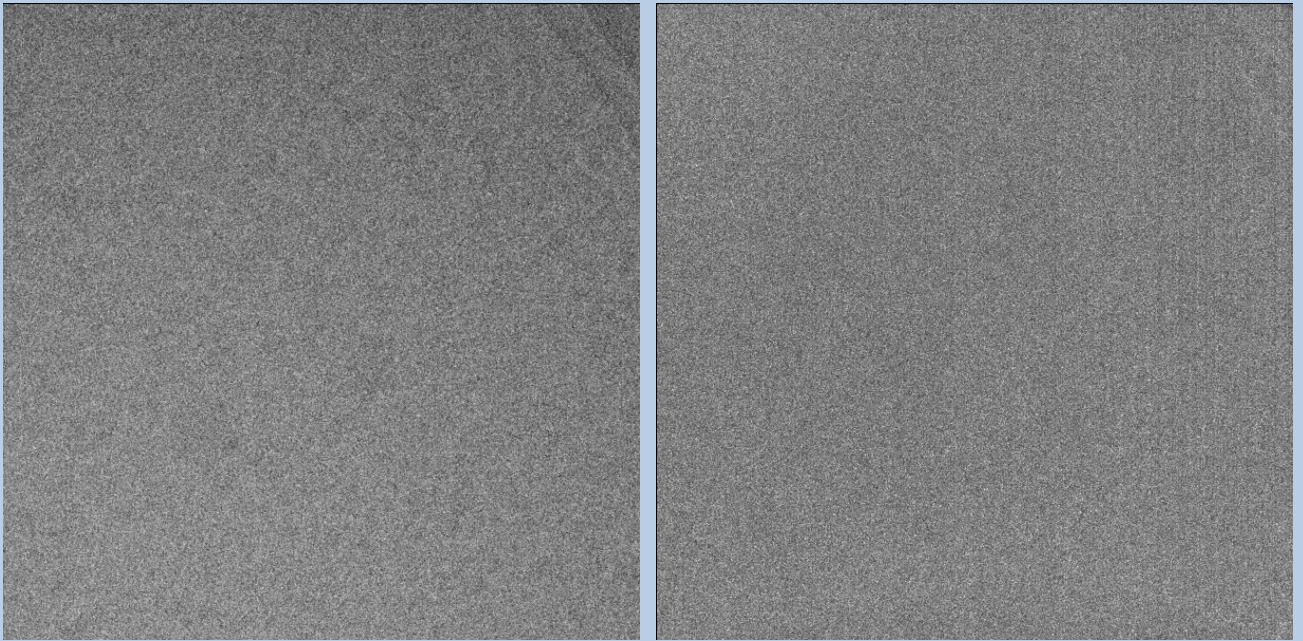
**Input camera statistics sigma file** Browse file

my\_micrographs\_cnorm\_sigma

Size = 4096x4096, Images = 1

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

<b>Input camera statistics average file</b>	<input type="button" value="Browse file"/>
<input type="text" value="my_micrographs_cnorm_average"/>	
Size = 4096x4096, Images = 1	
<b>Input camera statistics sigma file</b>	<input type="button" value="Browse file"/>
<input type="text" value="my_micrographs_cnorm_sigma"/>	
Size = 4096x4096, Images = 1	

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

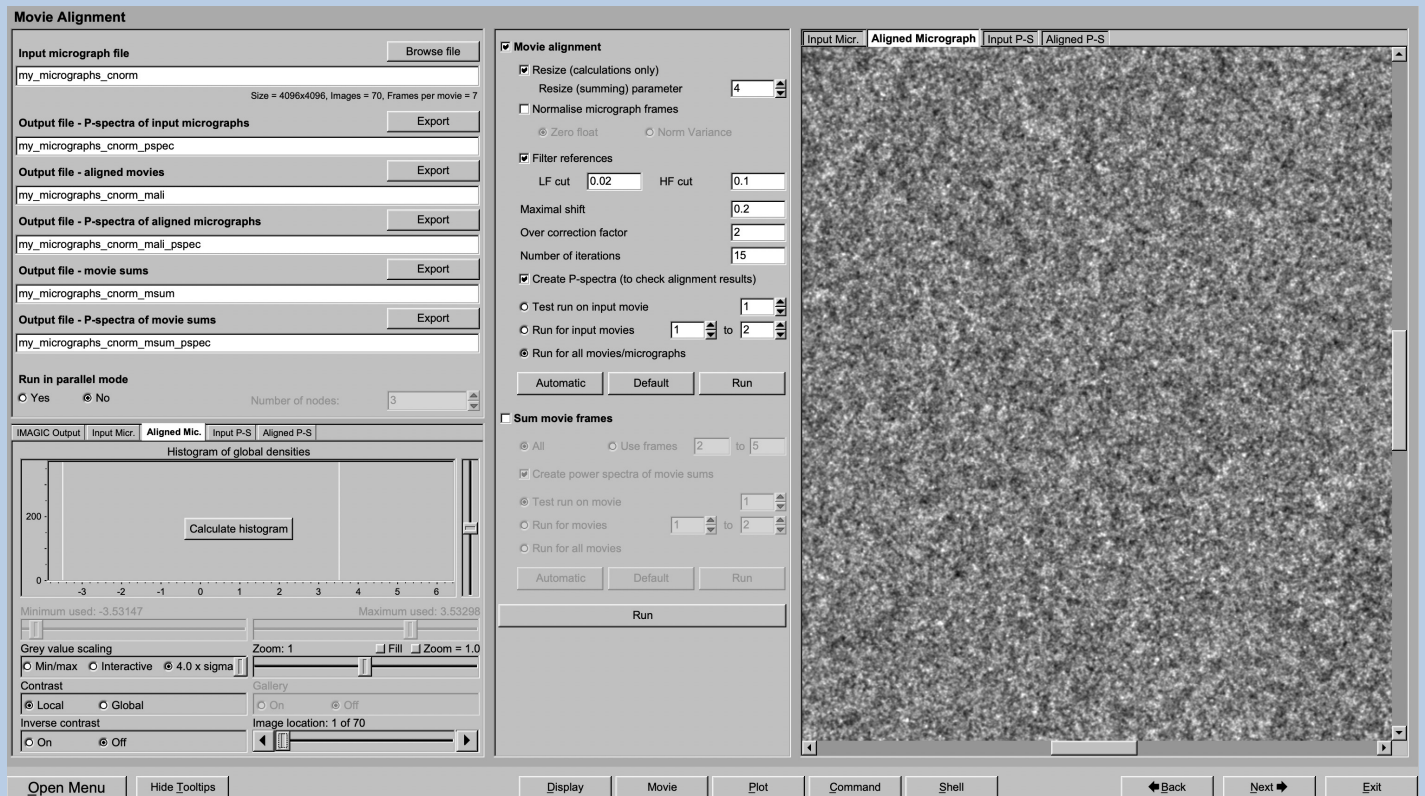
<b>Output file with camera corrected micrographs</b>	<input type="button" value="Export file"/>
<input type="text" value="my_micrographs_cnorm"/>	

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 these 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 file containing the micrograph movie frames (usually the camera corrected micrographs)

<b>Input micrograph file</b>	Browse file
my_micrographs_cnorm	
Size = 4096x4096, Images = 70, Frames per movie = 7	

and the names of the output files.

<b>Output file - P-spectra of input micrographs</b>	Export
my_micrographs_cnorm_pspec	
<b>Output file - aligned movies</b>	Export
my_micrographs_cnorm_mali	
<b>Output file - P-spectra of aligned micrographs</b>	Export
my_micrographs_cnorm_mali_pspec	
<b>Output file - movie sums</b>	Export
my_micrographs_cnorm_msum	
<b>Output file - P-spectra of movie sums</b>	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

<input checked="" type="checkbox"/> Resize (calculations only)
Resize (summing) parameter <input type="text" value="4"/>

You can normalize the densities (usually not needed):

<input checked="" type="checkbox"/> Normalise micrograph frames
<input checked="" type="radio"/> Zero float <input type="radio"/> Norm Variance



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):

Filter references  
LF cut  HF cut

Subsequently, specify the parameters for the movie alignment:

Maximal shift   
Over correction factor

Movie alignment is an iterative procedure. Give the number of iterations wanted:

Number of iterations

To later check the quality of the alignment it is suggested to also create the power spectra 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.

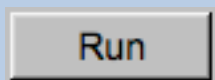
Test run on input movie   
 Run for input movies  to   
 Run for all movies/micrographs

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

Test run on movie   
 Run for movies  to   
 Run for all movies



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:

**Sum aligned movie frames**

All       Use frames  to

Create power spectra of movie sums

Test run on movie  

Run for movies   to  

Run for all movies



# The “Prepare Micrograph” Page

The screenshot shows the 'Prepare Micrographs' window in IMAGIC. On the left, there are fields for 'Input file with (aligned) micrographs' (my\_micrograph) and 'Output file with prepared micrographs' (my\_micrograph\_prep). The window size is 1024x1024, Images = 500. Below these are options for 'Run in parallel mode' (Yes/No) and 'Number of nodes' (3). The main control area has several checkboxes: 'Resize/Coarsen micrographs' (unchecked), 'Create patches' (unchecked), 'Prepare/filter micrographs' (checked), 'Remove outlier pixels' (checked), and 'Invert densities' (checked). There are also input fields for 'Low freq. cut' (0.0200), 'High freq. cut' (0.9000), and 'Outlier is' (4.50 sigma off the mean value). At the bottom left, a log window shows the progress of preparing 7 images, with the last one at 100% done. The log also displays image metadata and the next suggested command: DISPLAY.

## 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

Resize/Coarsen micrographs

Summing parameter

or create patches

Create patches

Size of patches

to speed up the CTF calculations.

Imposing a band-pass filter is always suggested:

Prepare/filter micrographs

Low freq. cut

Remaining low frequency

High freq. cut

Remove outlier pixels

Outlier is  sigma off the mean value

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

Invert densities

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

Resize/Coarsen prepared micrographs

Summing parameter





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

**Input file with (aligned) micrographs** Browse file

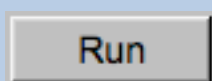
my\_micrograph

Size = 1024x1024, Images = 5

**Output file with prepared micrographs**

my\_micrograph\_prep

Start the calculations by clicking the “Run” button:



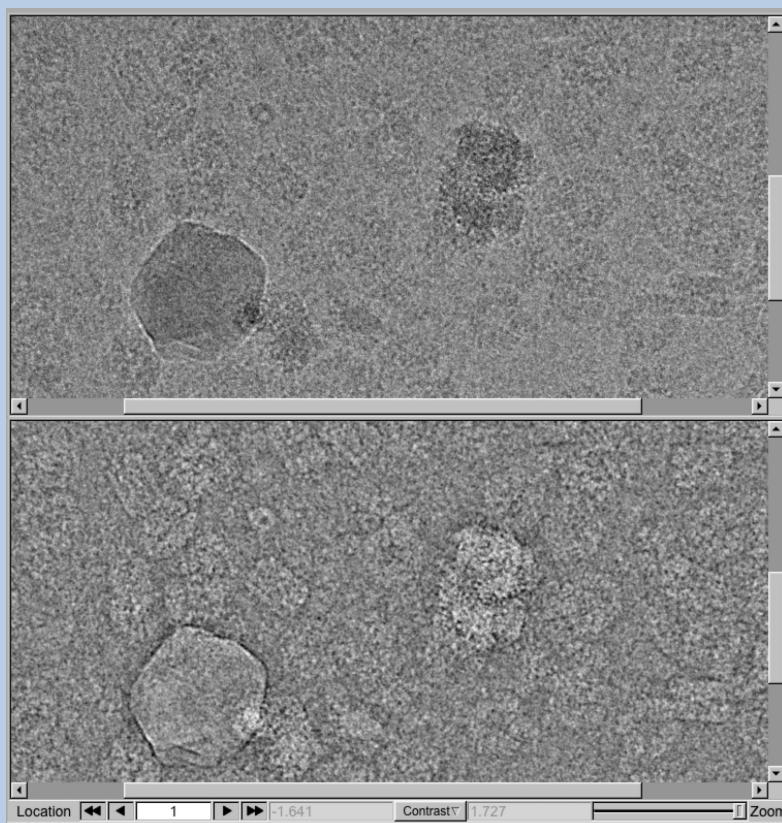
Play around with the parameters running movie alignment on a single or a small range of micrographs.

Test run on micrograph

Test run on micrographs 1 to 2

Run for all micrographs

Check the pre-treated micrographs:



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

Test run on micrograph

Test run on micrographs

Run for all micrographs

1 to 2



# The “Prepare CTF Correction” Page

**Prepare CTF Correction**

**Input file with (prepared) micrographs (or patches)**  
my\_micrograph\_prep [Browse]

**Output images of amplitudes** (Size = 1024x1024, Images = 500)  
my\_micrograph\_prep\_amp [Export]

**Output images of amplitudes sum**  
my\_micrograph\_prep\_amp\_sum [Export]

**Output PLT file with profile through sum of amplitudes**  
my\_micrograph\_prep\_amp\_sum [Export]

**Root name of output amplitudes classification files**  
my\_micrograph\_prep\_amp\_classify

**Output eigenimages of amplitudes**  
my\_micrograph\_prep\_amp\_eigen [Export]

**Output class averages of amplitudes**  
my\_micrograph\_prep\_amp\_classums [Export]

**Run in parallel mode** (Specify path and name of MSA scratch file)  
 Yes  No  Yes  No  
Number of nodes: 10 MSA scratch file: [ ]

**IMAGIC output** | Amplitudes sum | MSA eigenimages | Class averages of amplitudes control

**Histogram of global densities**  
[Calculate histogram]

Minimum used: -3.47639 Maximum used: 3.47639

**Grey value scaling**  
 Min/max  Interactive  5.0 x sigma Zoom: 0.18086 (60 %)

**Contrast**  
 Local  Gallery  Global  
 On  Off

**Inverse contrast**  
 On  Off

Image location: 1 of 50  Show location

**Create amplitude images**

**Filter amplitude images**  
Low freq. cut: 0.02  
High freq. cut: 0.7  
 Coarsen (filtered) amplitude images  
Summing parameter: 2 [ ]

Automatic Default Run

**MSA classification of amplitudes**

**MSA**  
Inner radius of ring mask: 0.39  
Outer radius of ring mask: 0.9  
Number of eigenimages: 10  
Number of iterations: 50

**Classification**  
Use how many eigenimages: 2  
Number of classes: 50

Automatic Default Run

Classify only

Run all

**Sum of prepared amplitudes - Check if the profile fluctuates around zero**

**MSA eigenimages: Check if the ones chosen used show Thon Rings.**

**Class averages of amplitudes: Check if they show Thon Rings**

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

## 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

**Input file with prepared micrographs (or patches)**

whgb\_micrographs\_prep Browse file

Size = 1024x1024, Images = 500

First the amplitudes of the (pre-treated) micrographs are calculated. These amplitudes are treated like real images which will be 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):

**Create amplitude images**

**Filter amplitude images**

Low freq. cut

High freq. cut

Coarsen (filtered) amplitude images

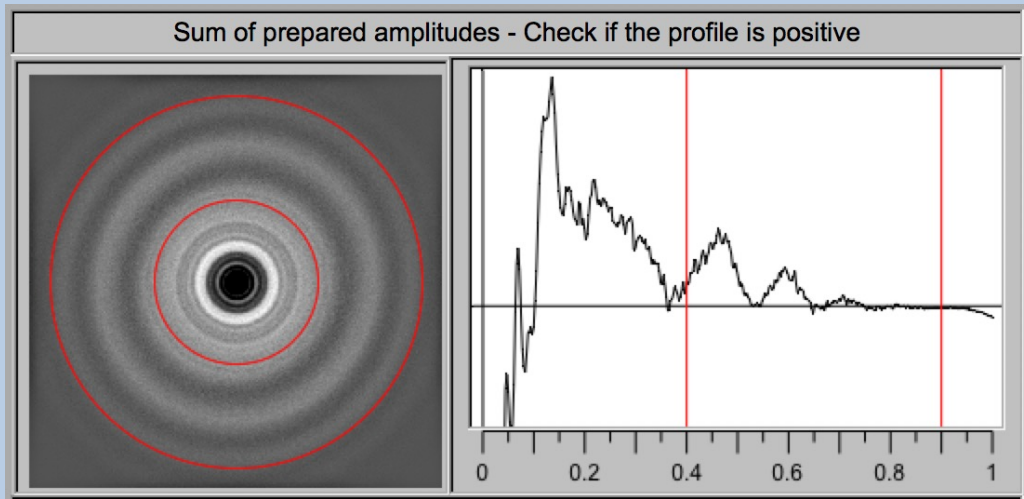
Summing parameter

Automatic Default Run

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

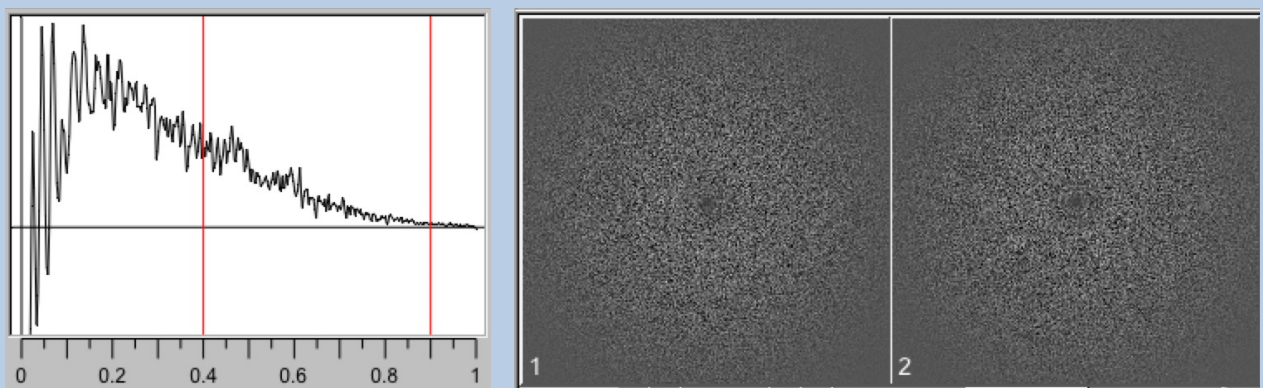




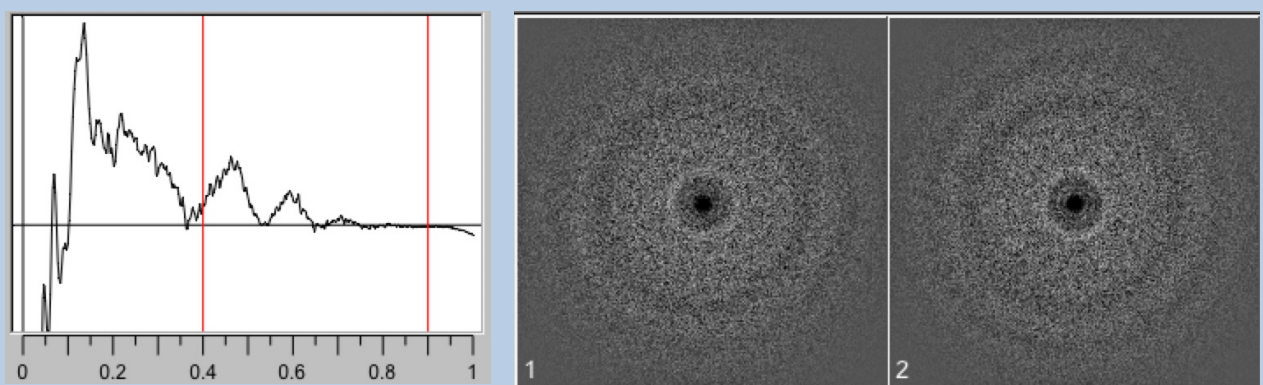
The second display row shows the created amplitude images. If the filter parameters are chosen correctly you should see Thon rings.

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:

**MSA classification of amplitudes**

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.).

**MSA**

Inner radius of ring mask

Outer radius of ring mask

Number of eigenimages  ▲▼

Number of iterations  ▲▼

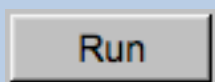
**Classification**

Use how many eigenimages  ▲▼

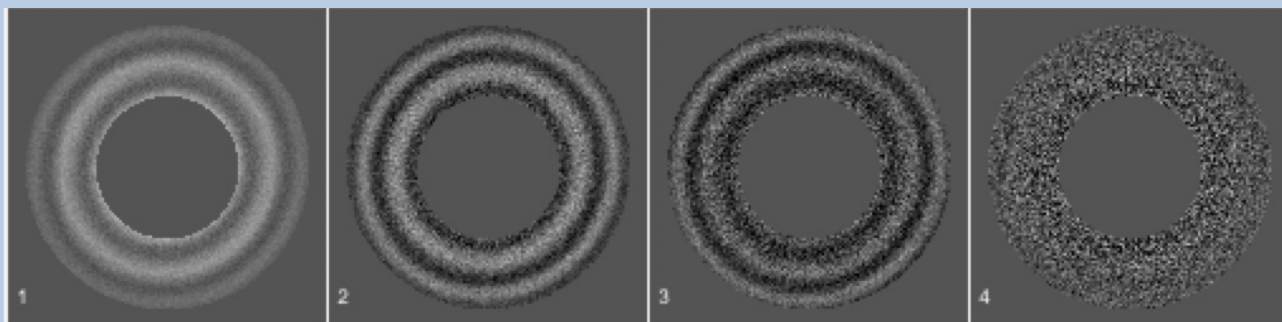
Number of classes  ▲▼



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

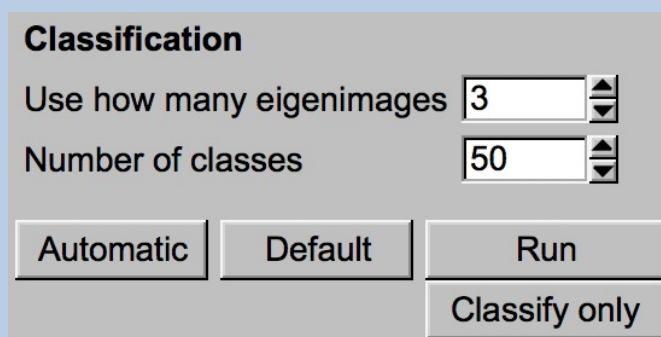


Check the MSA eigenimages and find out the range of MSA eigenimages showing Thon rings:

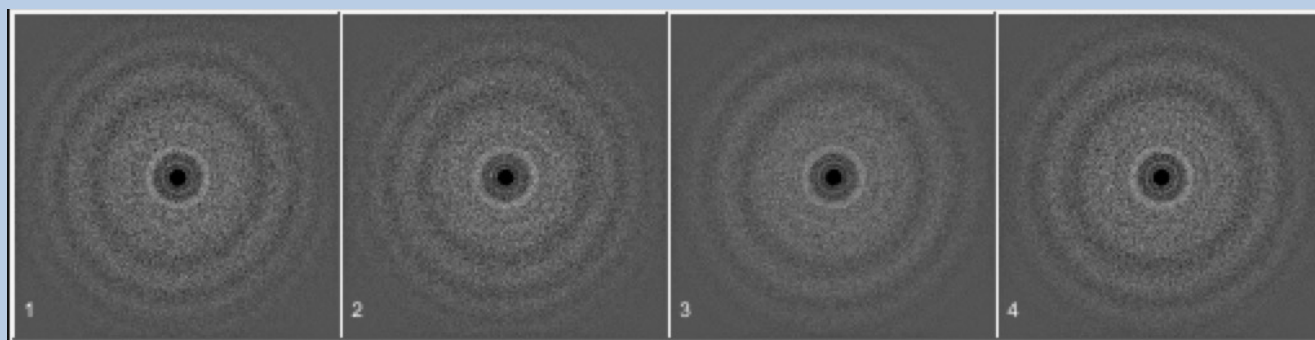


For classification only use the eigenimages showing Thon rings.

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

**CTF Correction**

Input file with amplitude class averages  
my\_micrograph\_prep\_amp\_classums  Size = 512x512, Images = 50

Input file with prepared micrographs/patches  
my\_micrograph\_prep

Root name of input amplitude classification files  
my\_micrograph\_prep\_amp\_classify

Output file with CTF half-half images  
my\_micrograph\_prep\_amp\_classums\_half\_half

Output file with CTF phase flipped micrographs/patches  
my\_micrograph\_prep\_flip

Output file with 'good' CTF phase flipped micrographs/patches  
my\_micrograph\_prep\_best

Run in parallel mode  
 Yes  No Number of nodes: 3

IMAGIC output | **IMAGIC display**

The results have been stored in the following files:

```
Header updated in input micrograph images: my_micrograph_prep
PLT file with defocus values found      : my_micrograph_prep_amp_classums_defocus
CTF half half images                   : my_micrograph_prep_amp_classums_half_half
```

Some useful next IMAGIC commands:

```
DISPLAY-IMAGE      Input: Half-half image. Check if the
                   Thon rings in both halves match
                   If not, redo CTF-CORRECT-IF-ALL with
                   other defocus range parameters or use
                   command EXCLUDE-IMAGE

EXTRACT-IMAGE      Input: Micrograph images. Remove all
                   micrographs, whose halves do not match

CTF_FLIP           Input: Micrograph images. Plip phases
```

The input amplitude file contains  
 amplitude class averages  
 prepared amplitudes

High-pass filter amplitude images  
Low freq. cut

Correlation area  
Inner radius   
Outer radius

Defocus search range  
Start value  Å  
End value  Å  
Step size  Å

Other parameters  
Astigmatism expected  Å  
Gen. envelope halfwidth   
 Test run on class  to

Check if the estimated Thon rings (lower right) and 'real' Thon rings (upper left part) fit. If necessary use other parameters and re-do CTF correction ("Run all").

Extract good micrographs/patches  
Mark either 'good' or 'bad' half-half images by clicking into the image which you want to select.

The selection contains  bad micrographs/patches  good micrographs/patches

## 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)

**Input file with amplitude class averages**

Size = 512x512, Images = 50

**Root name of input amplitude classification files**

or from the amplitude images

**Input file with (CTF prepared) amplitudes**

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

**Input file with prepared micrographs/patches**

Size = 1024x1024, Images = 500

You can adjust some CTF search parameters:

**Correlation area**

Inner radius

Outer radius

**Defocus search range**

Start radius  Å

End radius  Å

Step size  Å

**Other parameters**

Astigmatism expected  Å


Generic envelope halfwidth



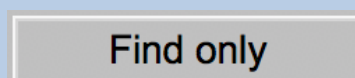
Important parameters are the following:

- 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:

Test run on class    to   

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

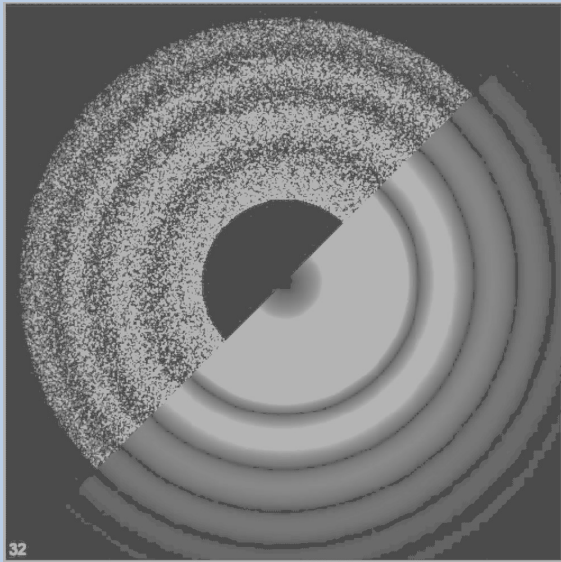


Check the defocus values found in the IMAGIC output on the left-hand side.

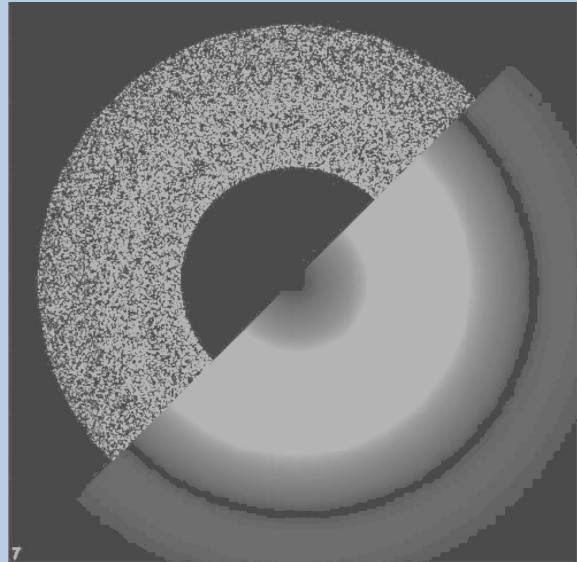
Also have a look at the half-half images in the display on the right-hand side. Each image will contain a) in the left half the input amplitude image, and b) in the right half the “estimated” CTF.

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





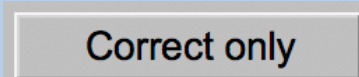
Good fit



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.

### Extract good micrographs

Mark either 'good' or 'bad' half-half images by clicking into the image which you want to select.

The selection contains

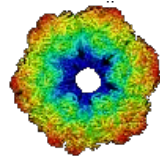
- bad micrographs
- good micrographs

Extract good micrographs



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





**IMAGIC**

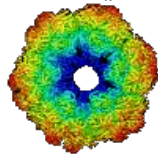
**guiCTF**

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](mailto:imagic@ImageScience.de)

THANK YOU VERY MUCH.



# Image Science

[www.ImageScience.de](http://www.ImageScience.de)  
[imagic@ImageScience.de](mailto:imagic@ImageScience.de)

