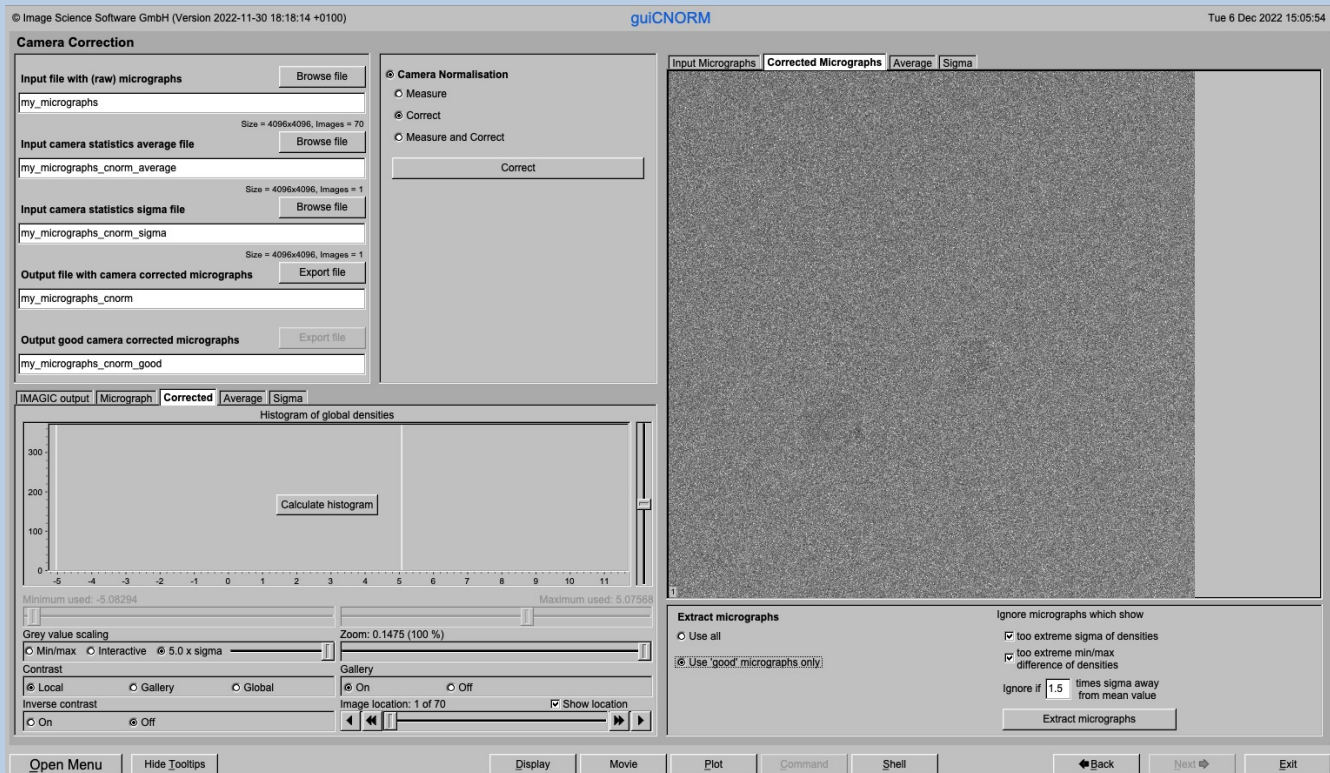




A Brief Introduction

Version 10-Oct-2023
www.ImageScience.de
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The IMAGIC guiCNORM program



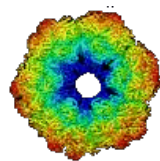
The **guiCNORM** program follows a work-flow from Import Micrographs to Camera/Detector Correction.

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

CONTENT:

- IMAGIC GUI programs How to use IMAGIC GUI programs
- **guiCNORM** How to perform camera correction
- Error hints How to send us feedback





IMAGIC

GUI Programs

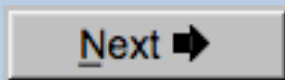


Workflow

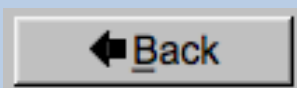
The idea of **guiCNORM** is to guide you through a typical camera/detector correction measurement or camera .

The workflow consists of several pages. Each page will perform a specific image processing step.

If the calculations are finished the results are shown and you can press the “Next” button to continue with the next page.



Of course, there is also a “Back” button. But be careful: when leaving a page the results shown on the page may get lost and when coming back you might have to do the calculations once more to get the results printed. The output files do not get lost, of course.



The Working Directory

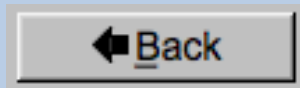
If **guiCNORM** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

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

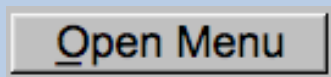
```
[IMAGIC-COMMAND : guiCNORM
```

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 **guiCNORM**.

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

Input files can be chosen from other directories.



Help

Move the cursor on (nearly) any item (questions, radio buttons, display windows...) shown on the pages and you will get context sensitive help.

Output file:

whgb_micrograph

Name of the output IMAGIC file containing the imported micrographs.

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

Select format ▼

In case of type conflicts

Select the input file format.

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

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

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



Input Files

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

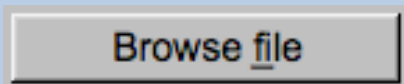
You can, of course, always use other input files names and even use other input directories.

Input file with (raw) micrographs	Browse file
my_micrographs	

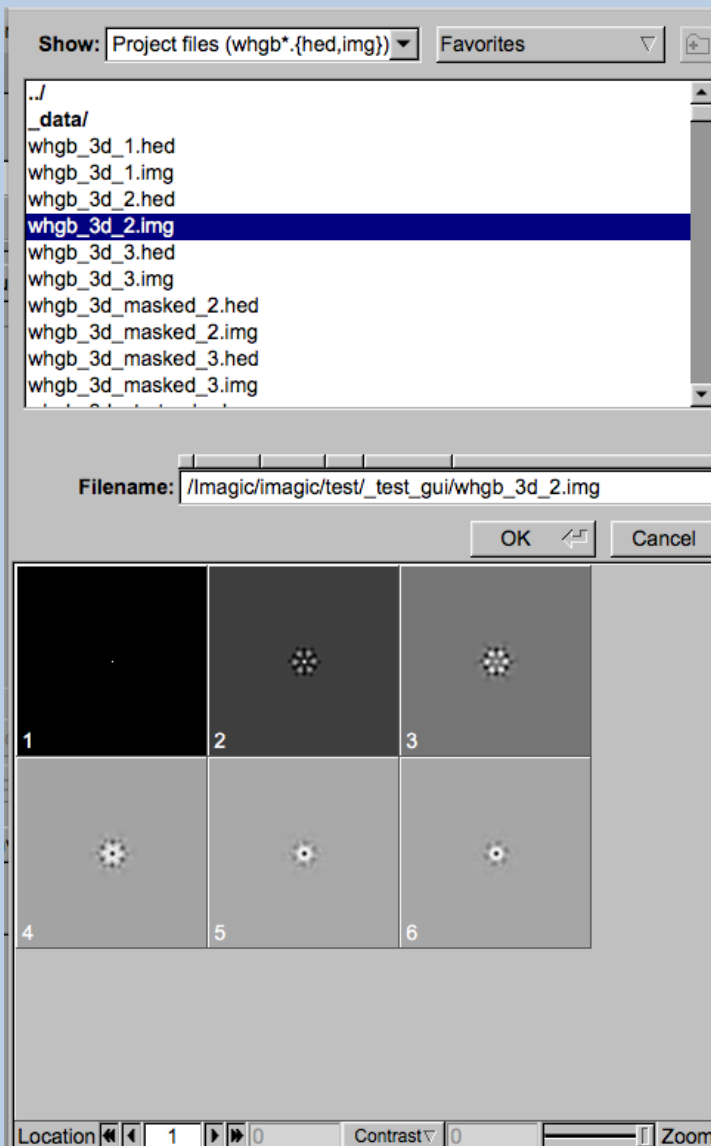


Input File Chooser

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

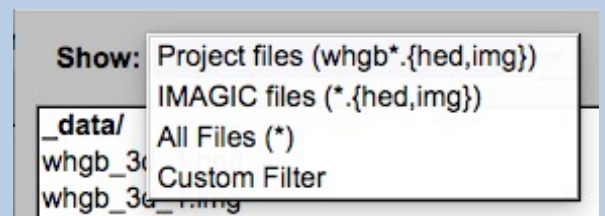


Pressing this button will open the IMAGIC file chooser:

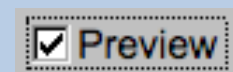


Choose the wanted file by clicking its name

You can use a pre-selection of the files shown:



If the images are in IMAGIC format you can get a pre- view of the images.



Note that you can store your directory in “Favorites”.



Output Files

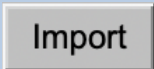
Usually the names of the output files are suggested but it is your choice, of course. On each page you can specify these output file names on the left hand side.

Output file	Export
my_micrographs	

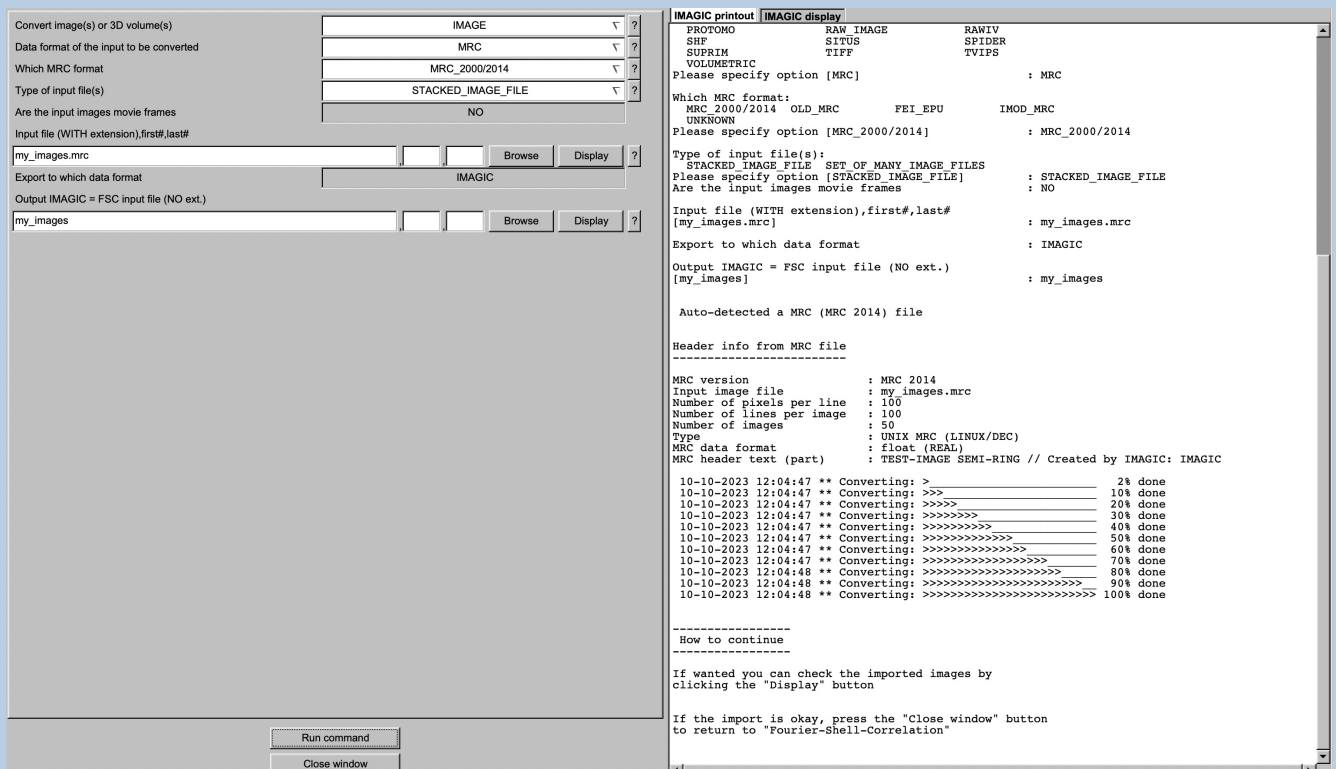


Import Buttons

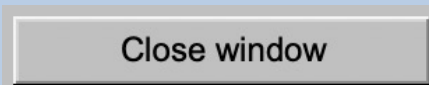
You do not want to use the “Import page” you can sometimes use an “Import” button to import the input images/3D volumes from any 3DEM format. The “Import” button which is located above the text field specifying the name of the related file.



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



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

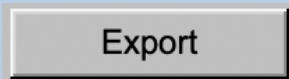


Refer to the **guiEM2EM** manual to get further help.




Export Buttons

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



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

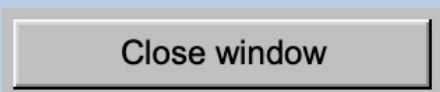
The screenshot shows the IMAGIC EM2EM software interface. On the left is a configuration panel with various options and dropdown menus. On the right is a terminal window displaying the command-line output of the software. The configuration panel includes fields for input file name, output format, and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

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 printout IMAGIC display
Convert 2D image(s) or 3D volume(s):
2D_IMAGE 3D_VOLUME : 2D_IMAGE
Please specify option [2D_IMAGE]
Data format of the input to be converted:
BROOKHAVEN_STEM CCP4 DATA_ONLY
DICOM DIGITAL_MICROGRAPH EM
FEI FABOSA FORMATTED
IMAGIC JPEG KONTRON
MDPP MEDIPIX MRC
OFFSET PIF PGM
PROTOMO RAW SHF
SMV SPIDER SUPRIM
TIA/EMI/SER TIFF TVIPS
Please specify option [IMAGIC]
Type of input file:
SINGLE_IMAGE_FILE STACKED_IMAGE_FILE UNKNOWN_IMAGE_FILE
Please specify option [UNKNOWN_IMAGE_FILE]
Are the input images movie frames [NO]
Input file, image loc#s [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]
Type of output TIFF image(s) wanted:
COLOUR_IMAGE GREY_SCALE_IMAGE
Please specify option [GREY_SCALE_IMAGE]
Type of output file:
STACKED_IMAGE_FILE SET_OF_MANY_IMAGE_FILES
Please specify option [STACKED_IMAGE_FILE]
Output file, loc#s (WITH ext.),first#,last# [my_images.tif]
Always scale densities to the output format [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;

Run command
Close window

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



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



A Typical Page

A typical **IMAGIC GUI program** page has three columns.

The left part contains the file information and a kind of terminal window showing the print-out of the currently running IMAGIC program(s). In additional tabs you can find the control windows to adjust the displays on the left hand side.

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

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

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

Camera Correction

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

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

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

Output file with camera corrected micrographs
my_micrographs_cnorm

Output good camera corrected micrographs
my_micrographs_cnorm_good

Camera Normalisation

Measure
 Correct
 Measure and Correct

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

Extract micrographs
 Use all
 Use 'good' micrographs only

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

IMAGIC output | Micrograph | Corrected | Average | Sigma

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

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



A Typical Page - MPI Parallel

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

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



A Typical Page - Program Parameters

Mode of preparation

Pretreat images

Normalise amplitude spectra (NAS)

Pretreat images

Band-pass Filter

LF cut

Rem. LF

HF cut

Normalisation

Sigma

Mask

Radius

Drop off

Test loc. # to

Run for all particles


Centre particles

Self rotate Self

Total sum Mass center

Test loc. # to

Run for all particles



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

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

Self rotate Self



Total sum Mass center

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

Band-pass Filter

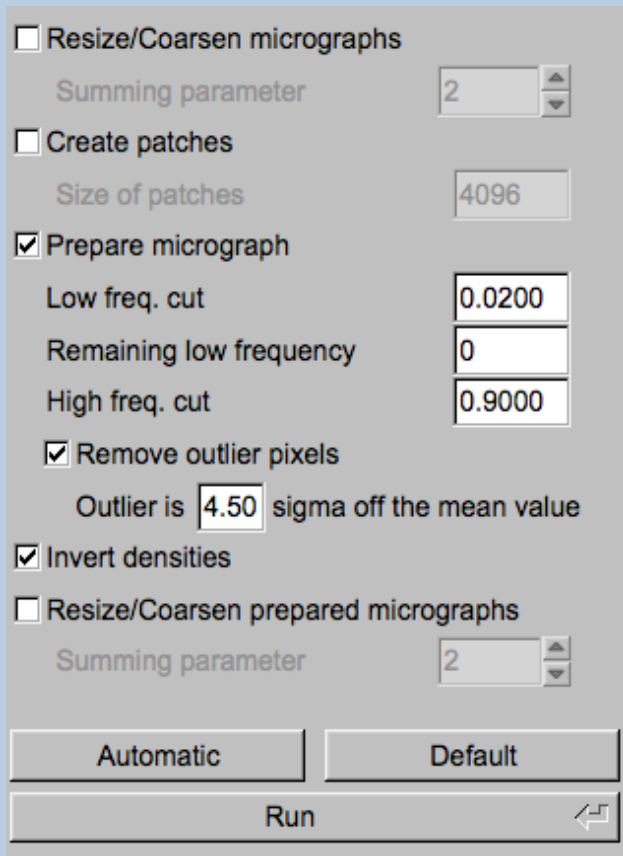
In text fields you can type in the wanted value. If the needed value is a number you can also move the cursor into this field, press the mouse key and keep it pressed and move the cursor to change the value.

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



A Typical Page - Automatic / Default



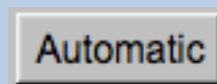
The screenshot shows a GUI window with the following elements:

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

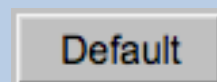
At the bottom, there are three buttons: "Automatic", "Default", and "Run".

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

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



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



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



A Typical Page - Run buttons

Create prepared amplitude images

Filter micrographs

Low freq. cut

Remaining low frequency

High freq. cut

Filter amplitude images

Low freq. cut

Remaining low frequency

High freq. cut

Coarsen filtered amplitude images

Yes No

Summing parameter

MSA options

MSA eigenfilter amplitudes

MSA classify amplitudes

MSA

Inner radius of ring mask

Outer radius of ring mask

Number of eigenimages

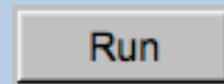
Number of iterations

Classification

Use how many eigenimages

Number of classes

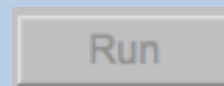
To run the calculations press the “Run” button.



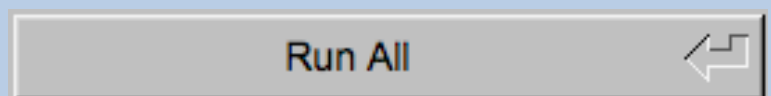
On a number of pages the calculations can be split. In this case you will find more than one single “Run” button.

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

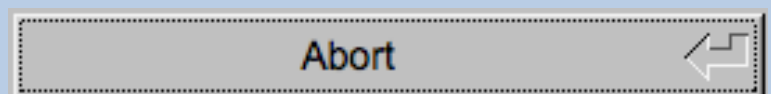
Maybe a certain “Run” button is not yet activated because it needs the results of calculations not yet done.



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

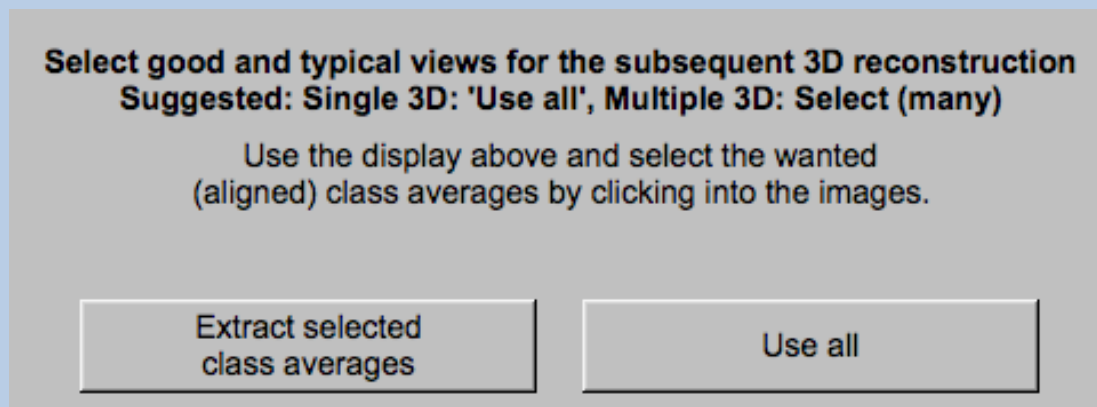


You can abort a running program by pressing the “Abort” button.



A Typical Page - Additional Tasks

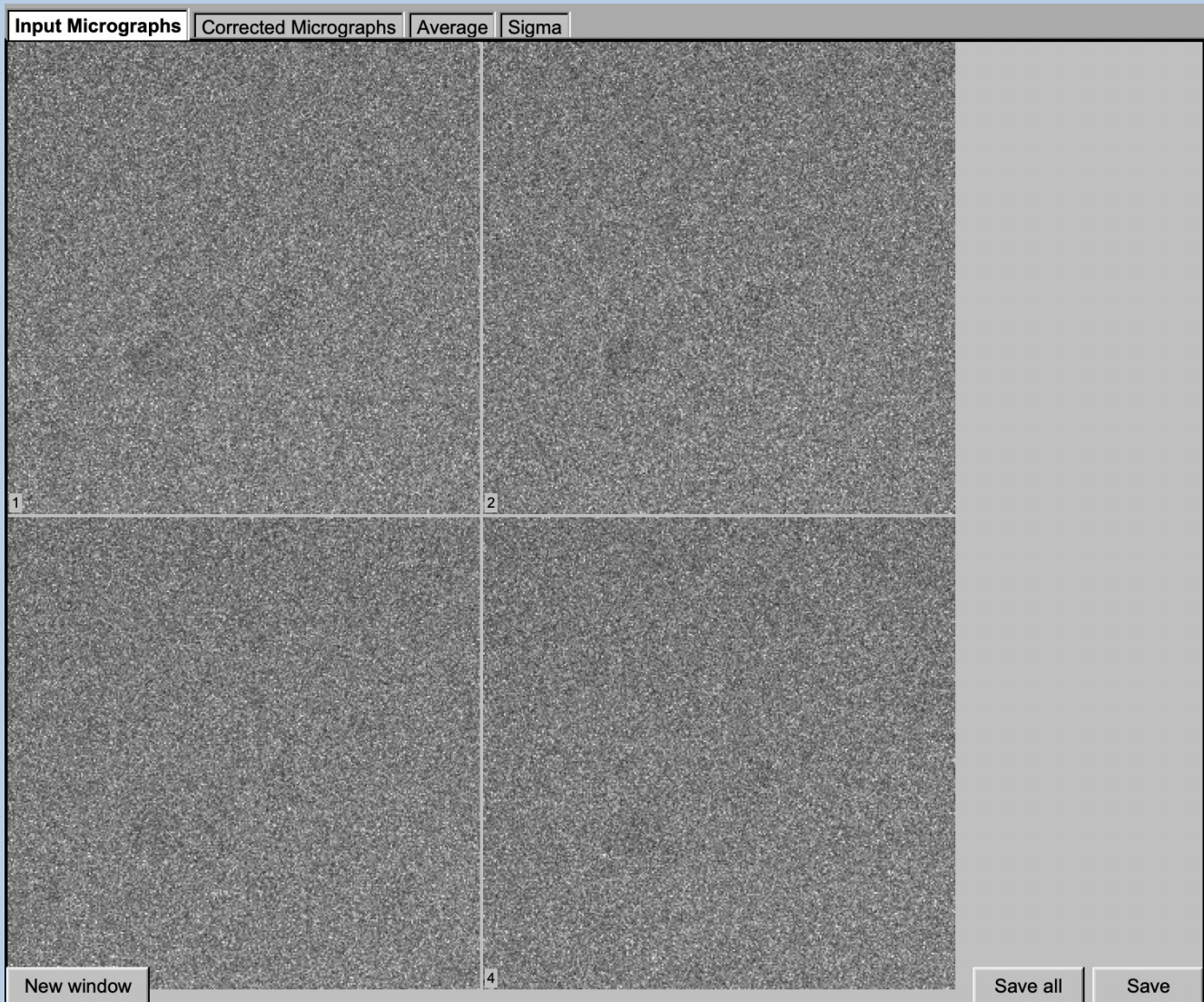
The main calculations on the page are done using the middle part of an typical **IMAGIC GUI program** page. But on a number of pages some additional calculations have to be done. Please follow the instructions given.



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



A Typical Page - Display



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

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

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

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



A Typical Page - “Display Control” Tabs

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

Grey value scaling: Adjust the contrast

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

Interactive: Set the limits by giving numbers

Sigma: Use an amount of sigma to set the limits

Contrast

How to calculate the grey value scaling

Local: Calculated in each image separately

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

Gallery: Calculated in the currently displayed images

Inverse contrast:

Use one of the radio buttons

Zoom

Enlarge the displayed images

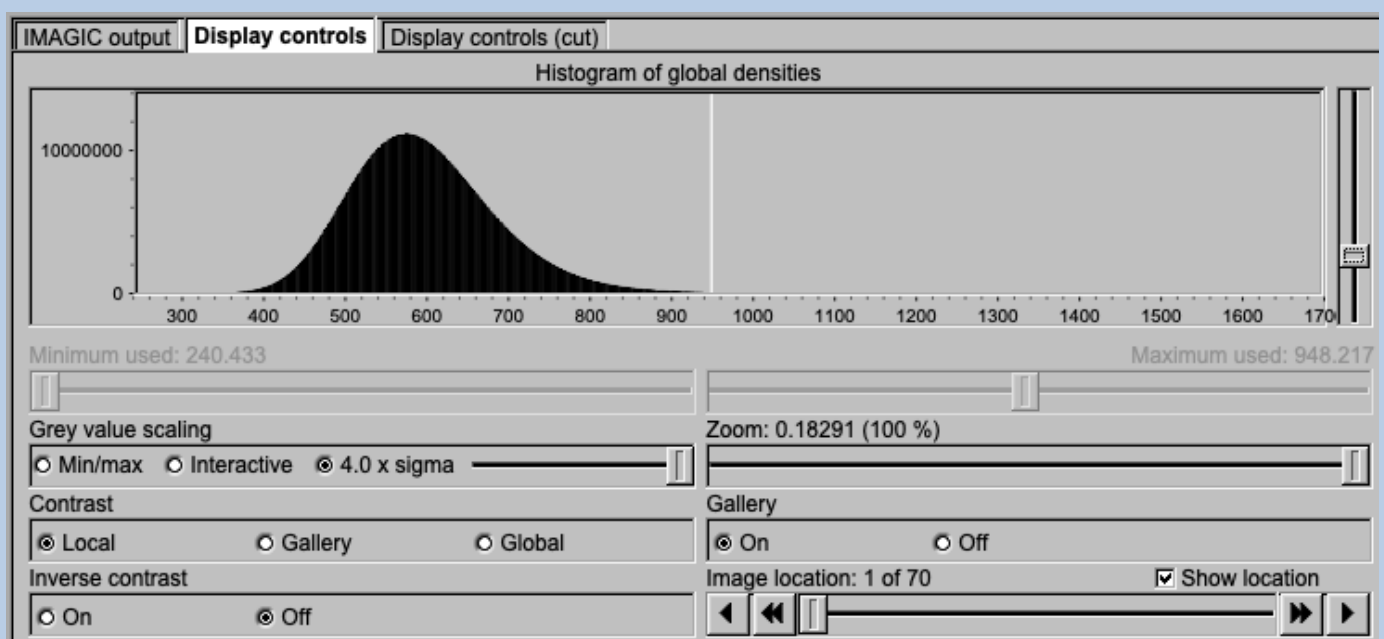
Gallery

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

Off Show only one image

Image Locations.

Use the slider or the arrows to select image locations



A Typical Page - "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

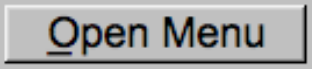
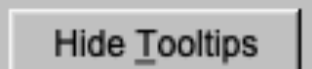
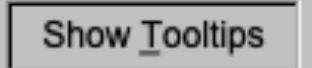
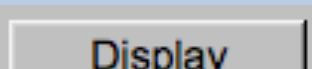
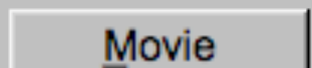
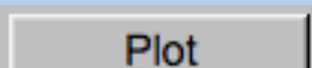
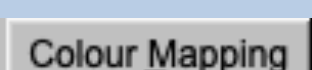
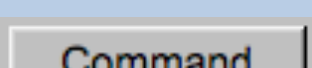
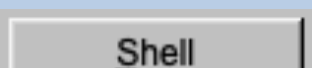
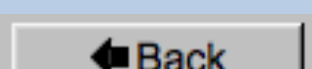
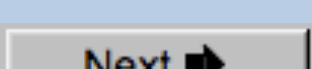
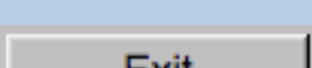
Style Select curve style ▾	Colour Select curve colour ▾	Grid Select curve grid ▾
Horizontal scaling 1.00	<input type="checkbox"/> Use for all plots 32.00	Reset
Vertical scaling -19.21	<input type="checkbox"/> Use for all plots 17.00	Reset
Plot title Fourier Ring Information - 1/2-bit	<input type="checkbox"/>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



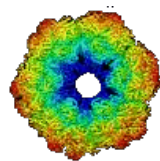
A Typical Page - The Toolbar

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

The toolbar buttons:

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



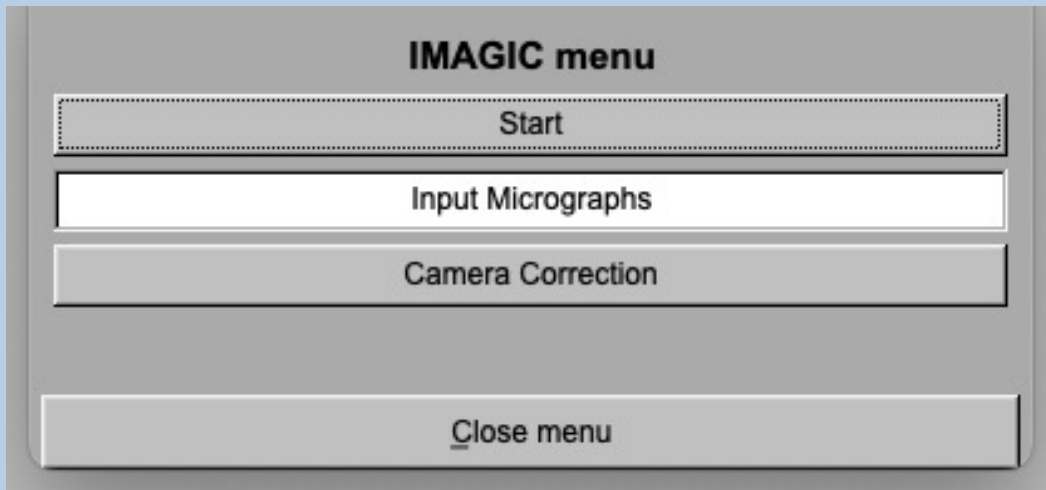


IMAGIC

guiCNORM



The guiCNORM Menu



PAGES:

guiCNORM:

Import Micrographs:	Convert micrographs/images into IMAGIC image format
Camera Correction:	Measure and/or correct for camera statistics

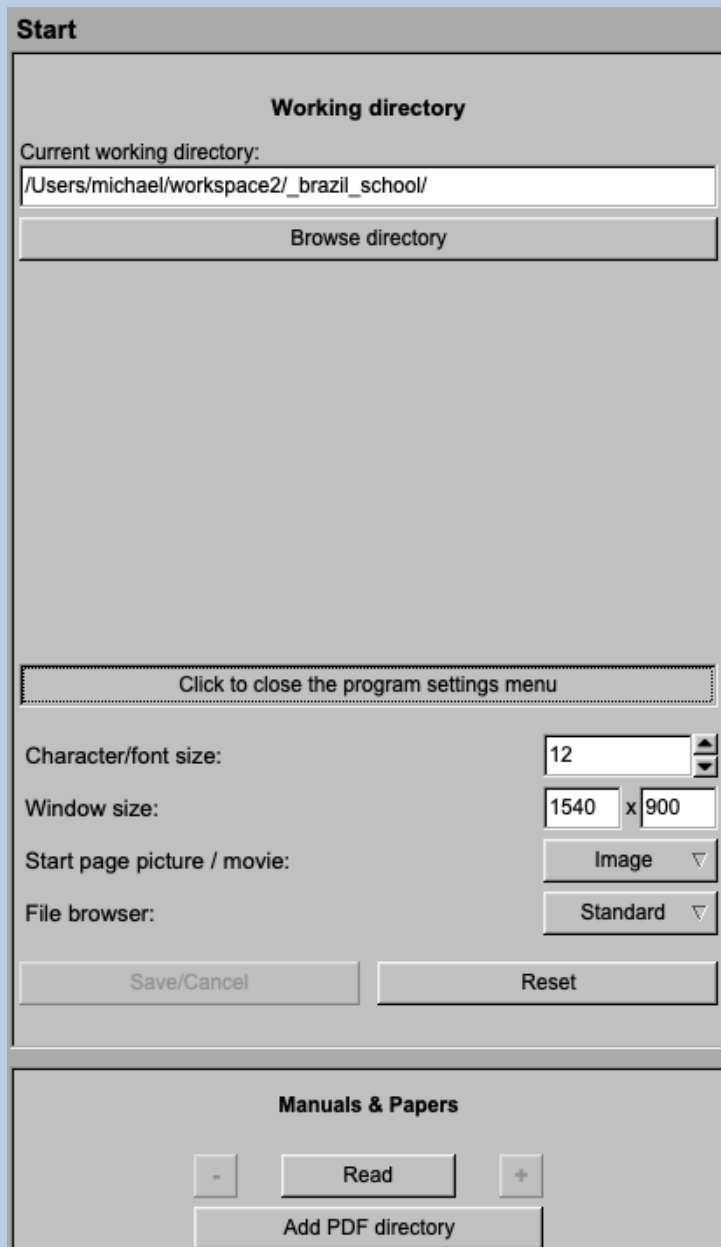
General:

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



Start Working

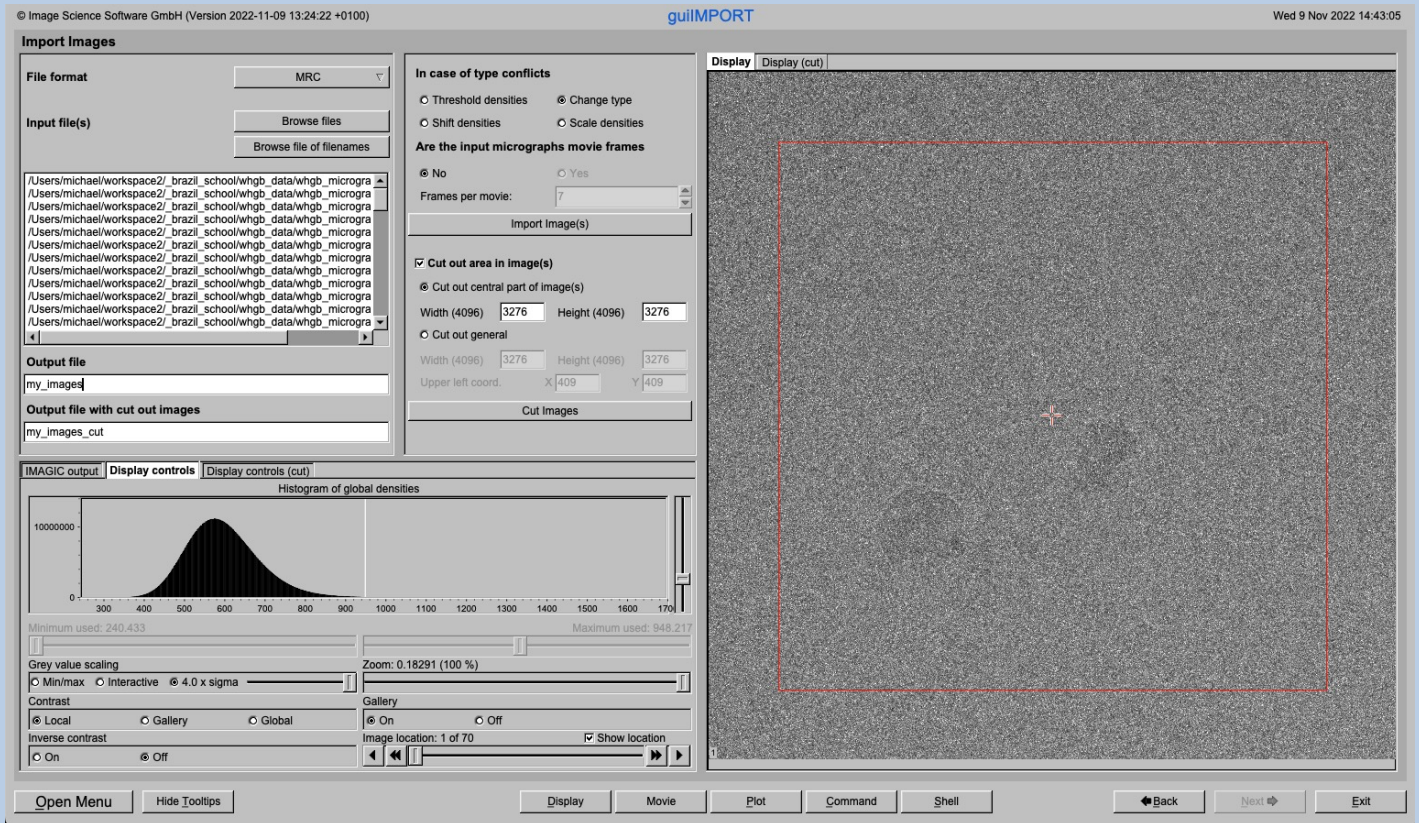
The page **guiCNORM** starts with the “Import Micrograph” page.

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

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



The “Import Micrographs” Page



DESCRIPTION:

Convert import micrograph files using any 3D-EM format (or TIFF) into a single (stacked) IMAGIC image file.

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

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

Also refer to program **guiIMPORT**.



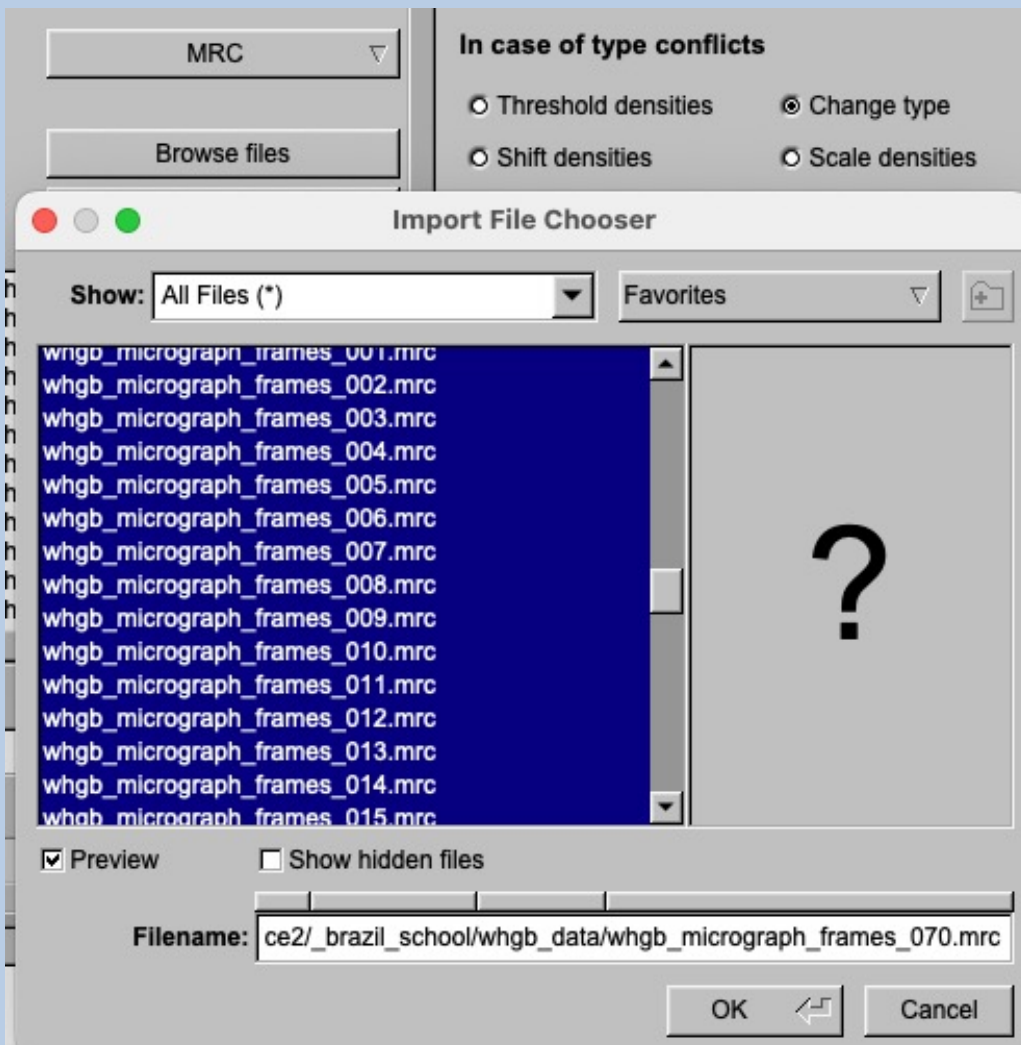
IMPORT MICROGRAPHS:

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

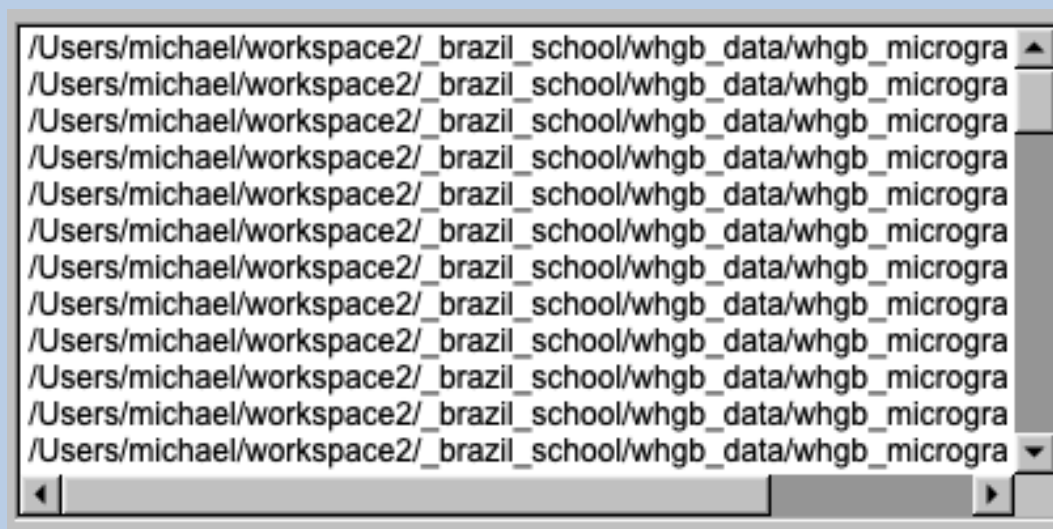


and choose one of the formats in the listing.

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.



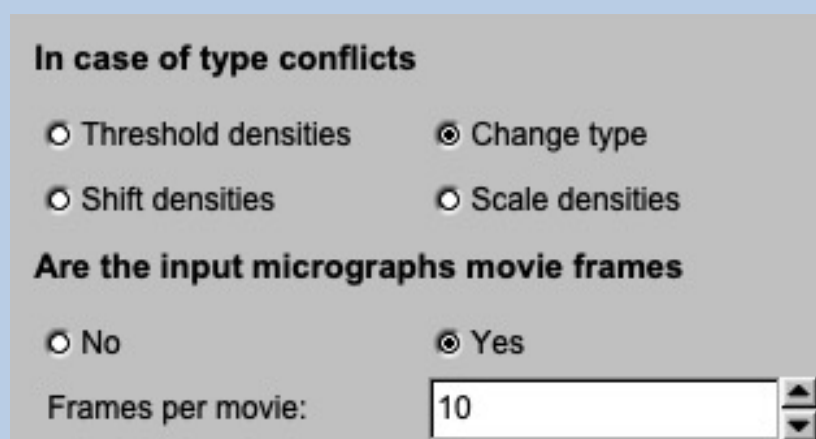
If wanted you can edit the list of files. But be careful there is no automatic control of file names in this list.



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).

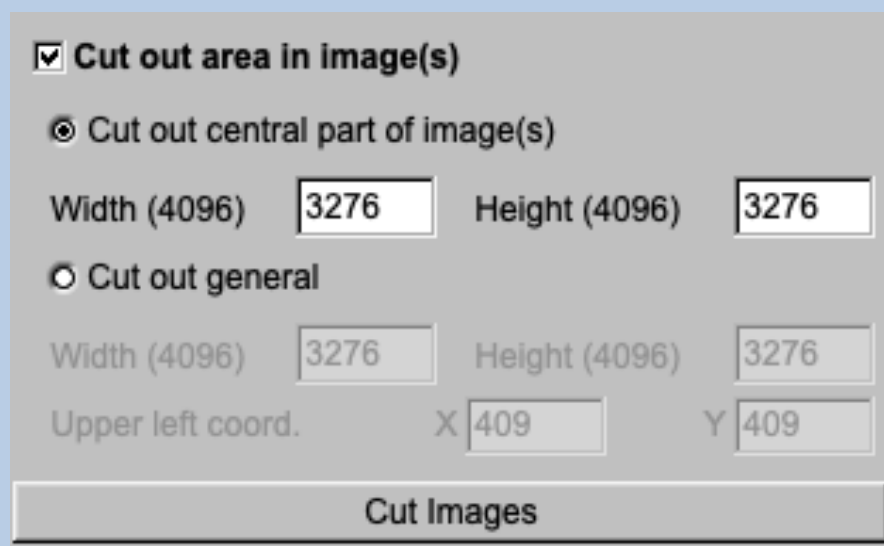
The imported images are shown in the display tab on the right-hand side. See chapter “A Typical Page - Display control tabs”.



CUT MICROGRAPHS / IMAGES:

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

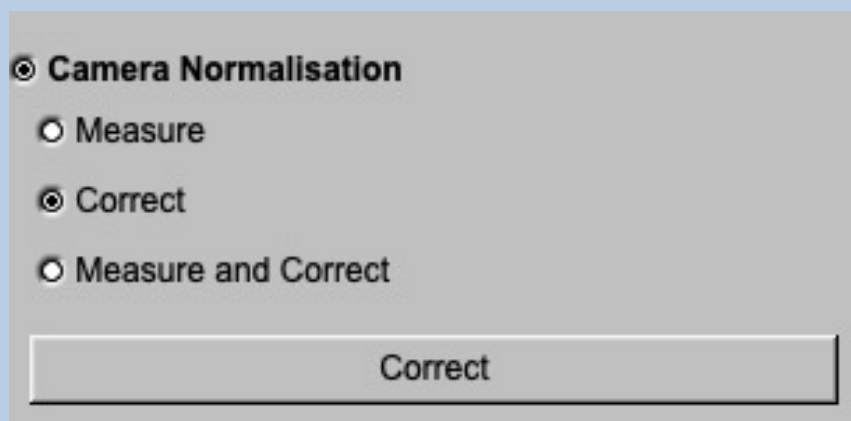
The chosen part is shown in the display window. You can cut-out a central part or any part wanted. The cut-out part is the same in all images, of course.

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.



Choose one of the options



The image shows a software dialog box with a light gray background. At the top left, there is a bold label "Camera Normalisation" preceded by a radio button that is currently selected. Below this label are three more radio buttons, each followed by a text label: "Measure", "Correct", and "Measure and Correct". The "Correct" option is also selected. At the bottom of the dialog box is a single button labeled "Correct".

Measure: Measure the camera statistics and create the camera statistics images needed to camera correct micrographs images taken with this camera/detector.

Correct: The camera statistics images are already available. Camera correct the input images using these camera statistics images.

Measure and Correct: Do both, measure the camera statistics and correct for it.



MEASURE:

Measure the camera statistics and create the camera statistics images needed to camera correct micrographs images taken with this camera / detector.

Mode

Measure
 Correct
 Measure and Correct

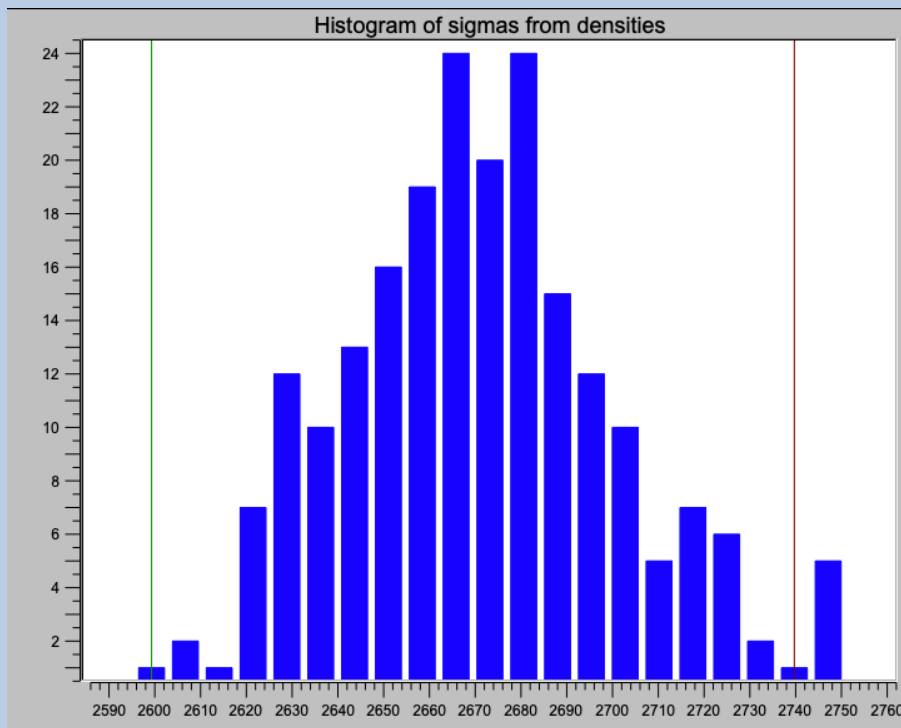
Micrograph Statistics

Calculate Statistics Use from header

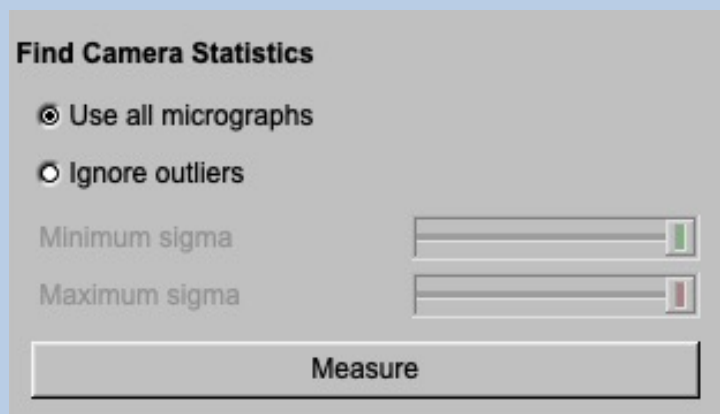
Note that you usually need a huge number of input images to get a good statistics and a good subsequent camera correction.

First the statistics of the input image densities is needed. Normally you will use the “Calculate Statistics” button to calculate this statistics.

The histogram of sigma values is shown on the right-hand side. For a “good” dataset this histogram usually has a Gaussian like shape.



If the shape looks correct you can “Use all micrographs”.



Find Camera Statistics

Use all micrographs

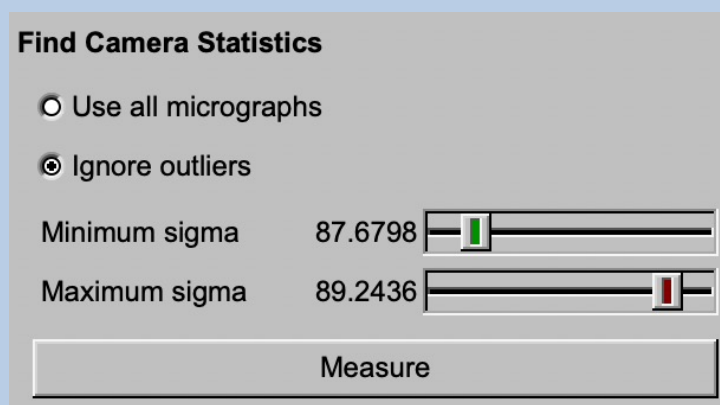
Ignore outliers

Minimum sigma

Maximum sigma

Measure

If wanted you can, of course, remove “outliers”.



Find Camera Statistics

Use all micrographs

Ignore outliers

Minimum sigma 87.6798

Maximum sigma 89.2436

Measure

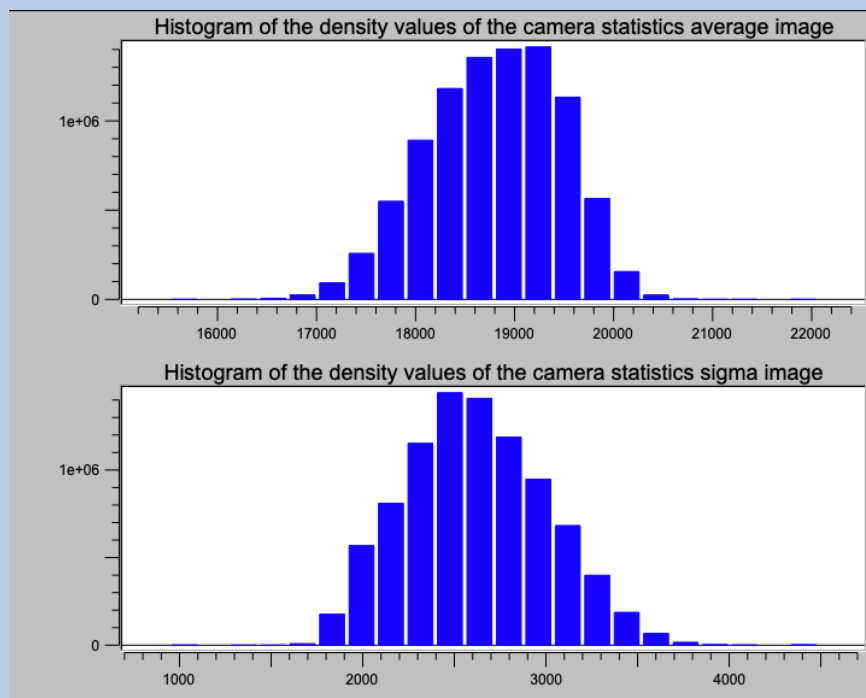
Use the up and down arrows to define a minimum and a maximum value for sigma (do NOT type the values). Only micrographs corresponding to the histogram part between the red lines will be used for the camera correction. The red vertical lines in the histogram will help you to check the chosen values.

Do not forget to specify the names of the output files on the left hand side of the page,

Press the “Measure” button to start the calculations.

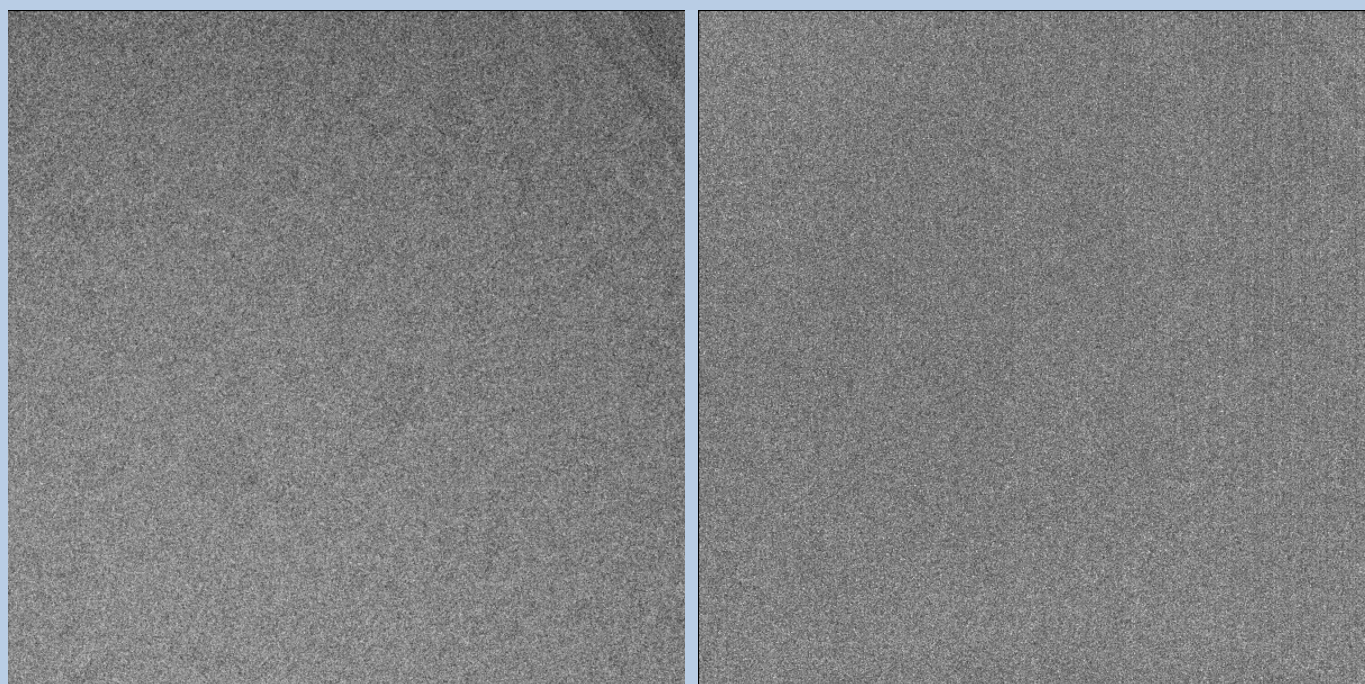
The measured camera statistics is shown in two histograms using the sigma of the densities in the camera statistics average and in the camera statistics sigma image, respectively.





The histograms usually have a Gaussian like shape. In case of camera errors (blind or dark pixels, for example) these can easily be seen as vertical lines.

The camera statistics average and sigma images are also displayed in tabs on the right hand side of the page. Always use a zoom factor of 1 (refer to chapter "A Typical Page - Display control" tabs) to make sure that you can check single pixels.



CORRECT:

Once having the camera statistics average and sigma images available you can correct all images/micrographs taken with this camera/detector (also the ones not used for getting the statistics).

Camera Normalisation

Measure

Correct

Measure and Correct

Correct

Of course, you need to 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

and, as usual, the output file name

Output file with camera corrected micrographs Export file

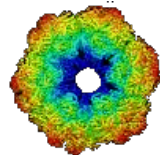
my_micrographs_cnorm

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

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

Note that the camera corrected images can be converted to any 3DEM format by clicking the "Export file" button which opens a separate "EM2EM" page.





IMAGIC

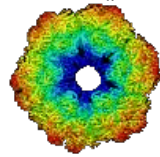
guiCNORM

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

guiCNORM

[Feedback / Error hints](#)

We intensively tested the **guiCNORM** 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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