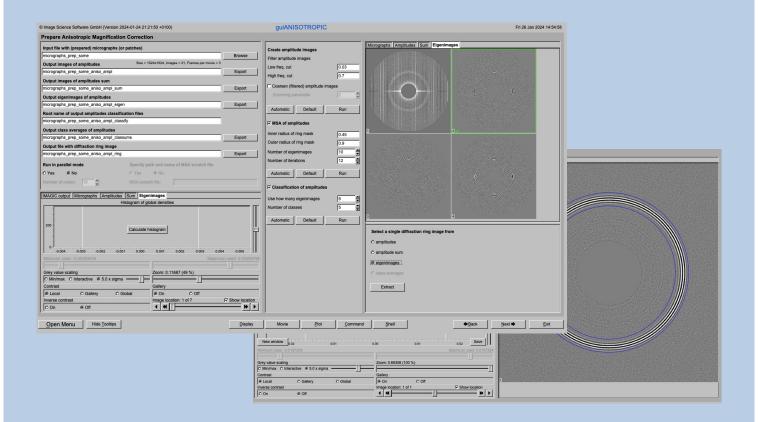


Correct
Anisotropic Magnification

#### A Brief Introduction

Version 30-Jan-2024 www.ImageScience.de © Michael Schatz (Image Science)

## The IMAGIC guiANISOTROPIC program



The **guiANISOTROPIC** program follows a work-flow from Import Micrographs to anisotropic magnification correction.

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

#### **CONTENT:**

➤ IMAGIC GUI programs

guiANISOTROPIC

> Import Micrographs

> Prepare Correction

> Correction

> Error hints

How to use IMAGIC GUI programs How to correct micrographs

How to send us feedback



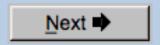
# **IMAGIC GUI Programs**

#### Workflow

The idea of **guiANISOTROPIC** is to guide you through a typical anisotropic magnification correction.

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

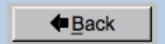
If **guiANISOTROPIC** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

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

IMAGIC-COMMAND : GUI-ANISOTROPIC

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 **guiANISOTROPIC**.

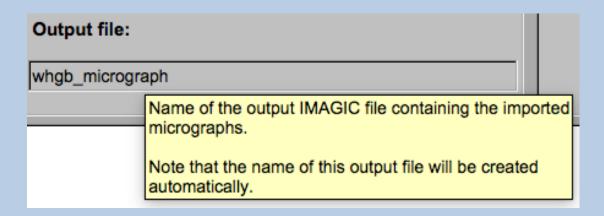
All output files will be stored in the working directory which you have specified on the start page.

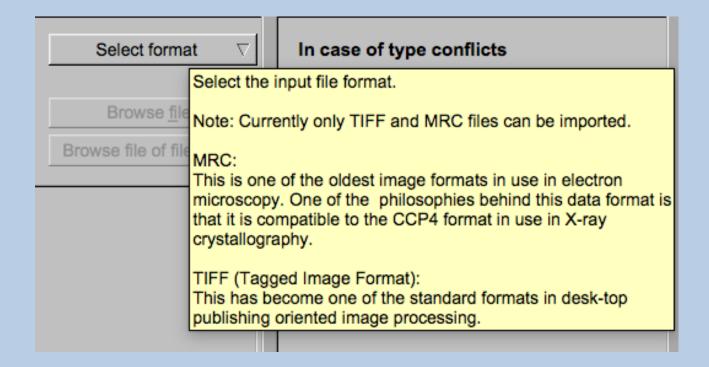
Input files can be chosen from other directories.



#### Help

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





## **Input Files**

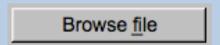
Usually the input files on each page are output file(s) from the previous page(s) and are suggested automatically.

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

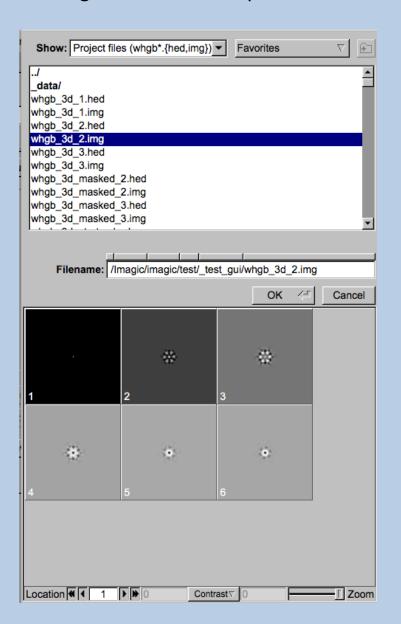
Input file with (raw) micrographs	Browse file
my_micrographs	

#### Input File Chooser

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

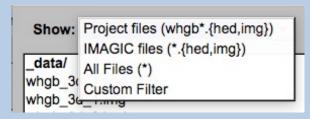


Pressing this button will open the IMAGIC file chooser:



Choose the wanted file by clicking its name

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



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



Note that you can store your directory in "Favorites".



## **Output Files**

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

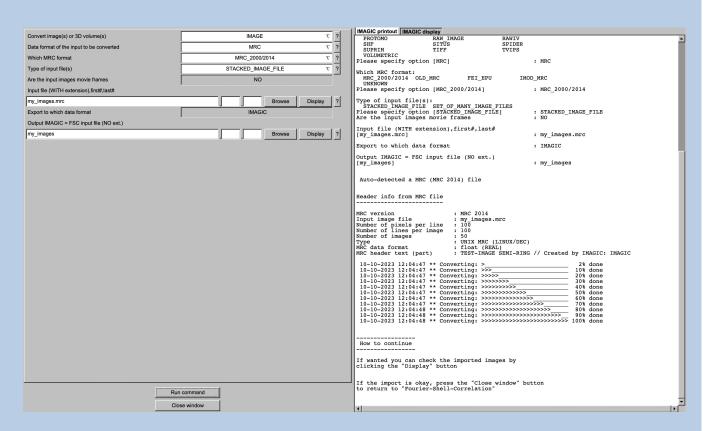


#### **Import Buttons**

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



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



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

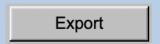


Refer to the guiEM2EM manual to get further help.

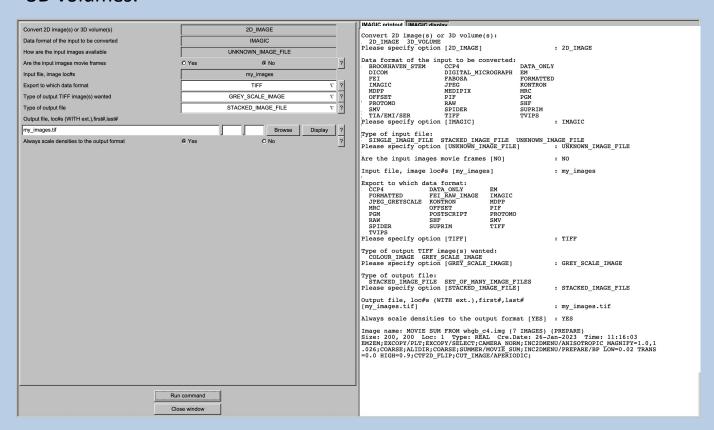


#### **Export Buttons**

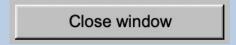
You can export output images/3D volumes to any 3DEM format. Click the "Export" button which is located above the text field specifying the name of the related file.



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



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



Refer to the guiEM2EM manual to get further help.



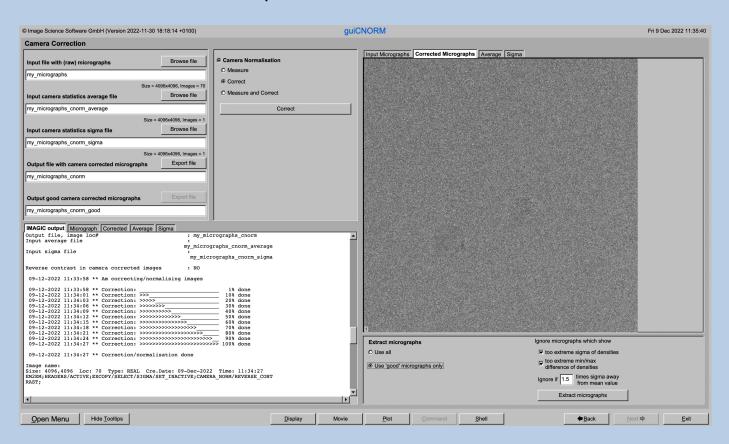
#### A Typical Page

A typical IMAGIC GUI program page has three columns.

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

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

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



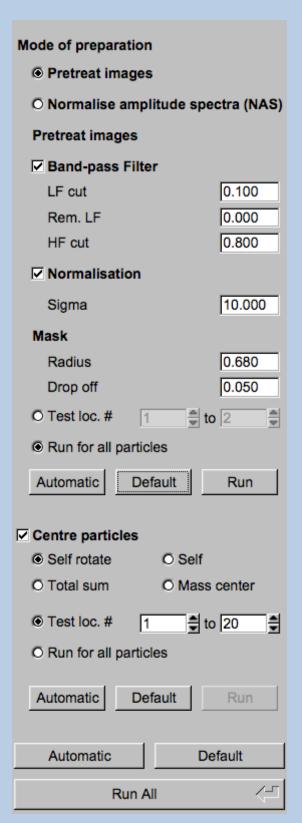


## A Typical Page - MPI Parallel

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

Run in parallel mode	Specify path and name of MSA scratch file
Yes	O Yes
Number of nodes: 3	MSA scratch file:

## A Typical Page - Program Parameters



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

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



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

■ 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.

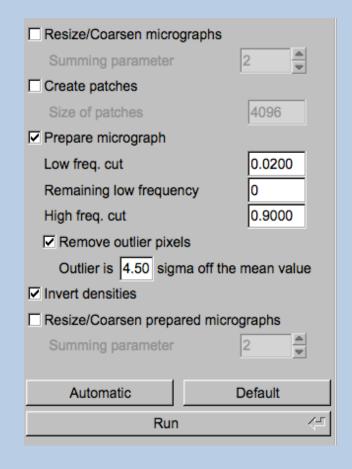
10.000

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



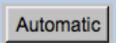


## A Typical Page - Automatic / Default

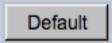


In the middle part of a typical **IMAGIC GUI program** page you will also find
"Automatic" and "Default buttons.

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



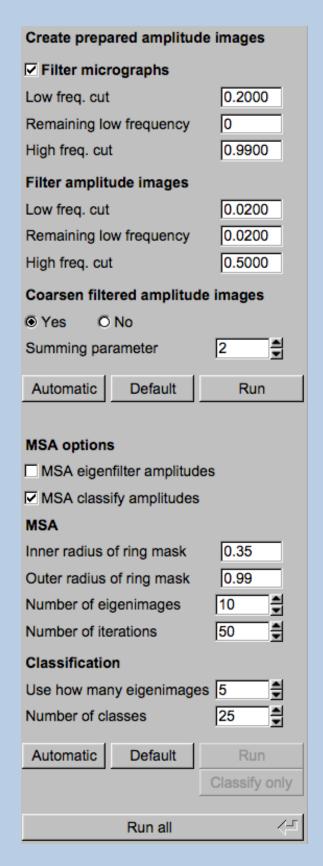
Pressing the "Default" button will fill in the values which you have used during the last "Run".



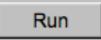
The values shown when entering a page are the default values (your last values given) if they are available. Else the automatic values are shown.



#### A Typical Page - Run buttons



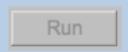
To run the calculations press the "Run" button.



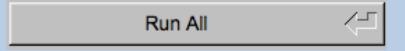
On a number of pages the calculations can be split. In this case you will find more than one single "Run" button.

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

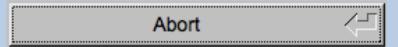
May be a certain "Run" button is not yet activated because it needs the results of calculations not yet done.



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



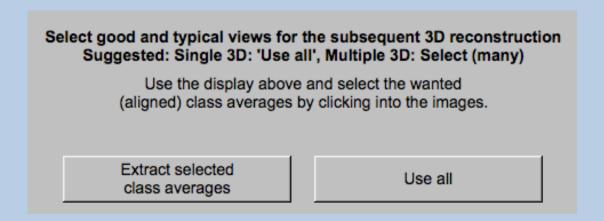
You can abort a running program by pressing the "Abort" button.





#### A Typical Page - Additional Tasks

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



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



## A Typical Page - "Terminal Window"

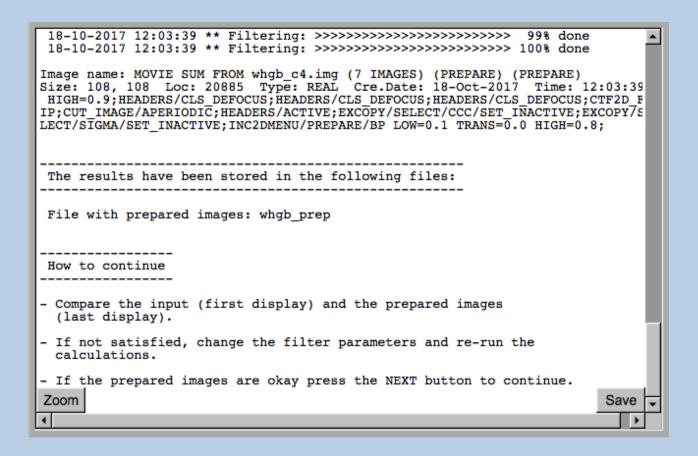
All print-out of an IMAGIC program started within the current **IMAGIC GUI program** page is shown in a kind of terminal window on the left hand side.

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

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

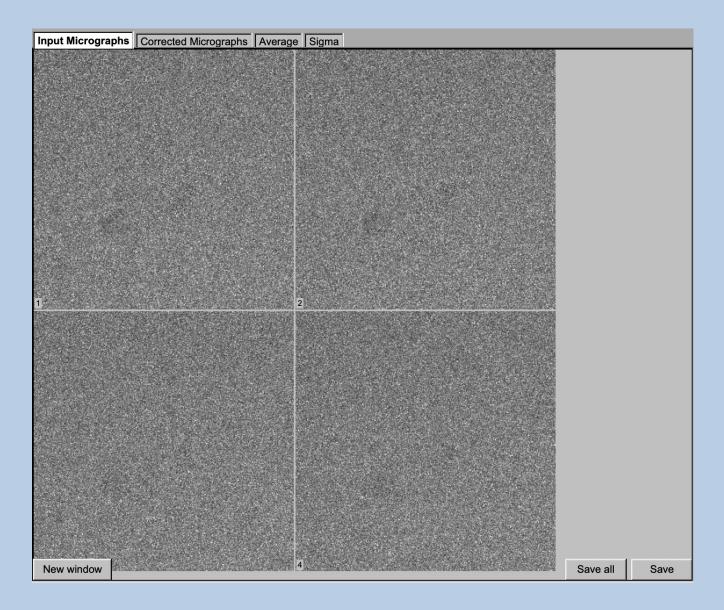
With the "Zoom" button you can open a separate larger window showing the print-out.

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





#### A Typical Page - Display



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

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

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

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



## A Typical Page - "Display Control" Tabs

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

Grey value scaling: Adjust the contrast

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

Interactive: Set the limits by giving numbers

Sigma: Use an amount of sigma to set the limits

Contrast How to calculate the grey value scaling

Local: Calculated in each image separately Global: Calculated using all image densities

(as displayed in the histogram)

Gallery: Calculated in the currently displayed images

Inverse contrast: Use one of the radio buttons

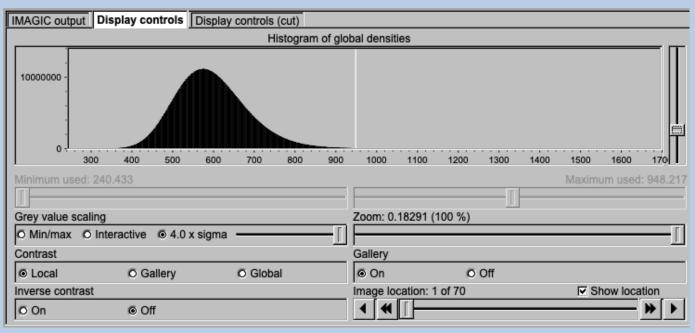
Zoom Enlarge the displayed images

Gallery On Display the images in a gallery

(may be you need another zoom to see

off Show only one image

Image Locations. Use the slider or the arrows to select image locations





## A Typical Page - "Plot Control" Tabs

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

Style, Colour, Grid: Adjust the curve line style, the colour and

add a grid if wanted

Horizontal,

vertical scaling: Set minimal and maximal horizontal or vertical limits

Plot title Set the text of the plot title

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

Use for all plots: Use the setting for all plots in a file independent of of

what is input in the PLT file

Reset:. Reset to the automatic values

Style	Colour	Grid
Select curve style	Select curve colour	Select curve grid
Horizontal scaling	Use for all plots	
1.00	32.00	Reset
Vertical scaling	Use for all plots	
-19.21	17.00	Reset
Plot title	☐ Use for all plots Reset	
Fourier Ring Information - 1/2-bit		
Text along horizontal axis	☐ Use for all plots Reset	
Text along horizontal axis Radius in Fourier space	Use for all plots	
	☐ Use for all plots  ☐ Use for all plots  Reset	



## A Typical Page - The Toolbar

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

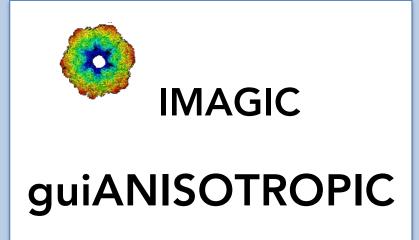
The toolbar buttons:

Exit

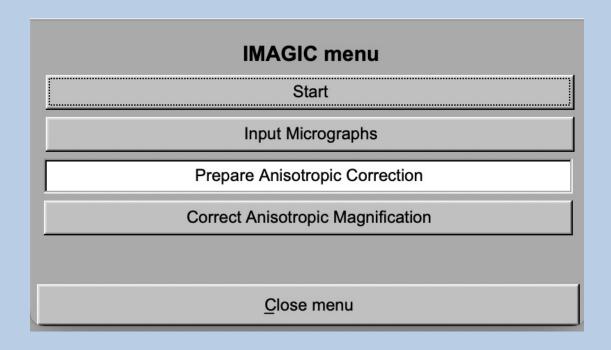
Open Menu	Open the MENU to navigate to each page wanted
Hide Tooltips Show Tooltips	Show or hide the context sensitive tooltips (the help text may sometimes disturb)
Display	Open a DISPLAY page to visualize IMAGIC images. Refer to <b>guiDISPLAY</b> .
Movie	Open a MOVIE page (display in an endless loop).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



Exit guiANISOTROPIC



#### The guiCTF Menu



PAGES:

guiANISOTROPIC:

Input Micrographs: Convert micrographs/images into

**IMAGIC** image format

Prepare Micrographs Pre-treat Micrographs

Prepare correction Prepare the anisotropic magnification correction

(find diffraction ring)

CTF Correction Find anisotropic magnification and correct

micrographs for it

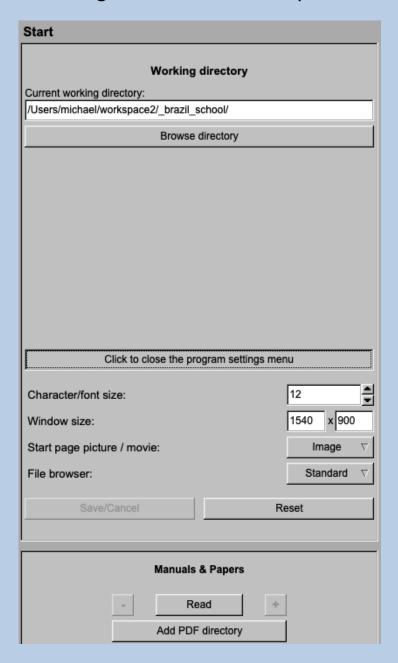
General:

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



## **Start Working**

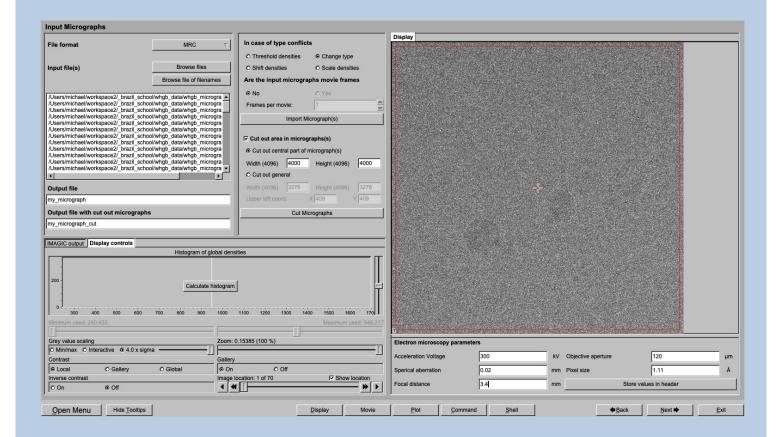
The page guiANISOTROPIC starts with the "Import Micrograph" page.

The workflow using the "Next" button will guide you through all **guiANISOTROPIC** 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 guilMPORT.



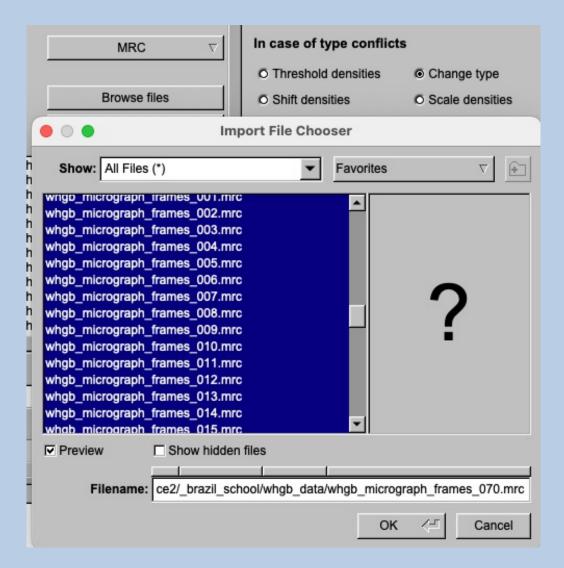
#### **IMPORT MICROGRAPHS:**

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



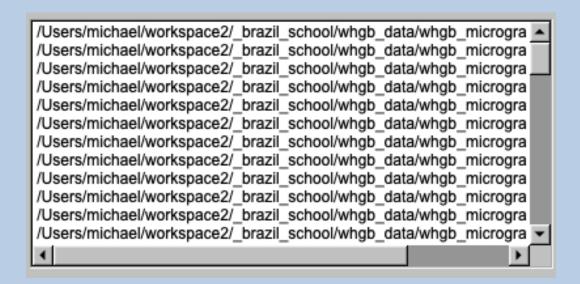
and choose one of the formats in the listing.

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





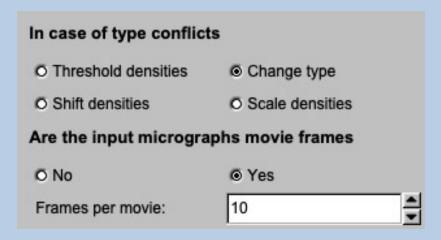
If wanted you can edit the list of files. But be careful there is no automatic control of file names in this list.



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

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

#### Format MRC, for example:



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

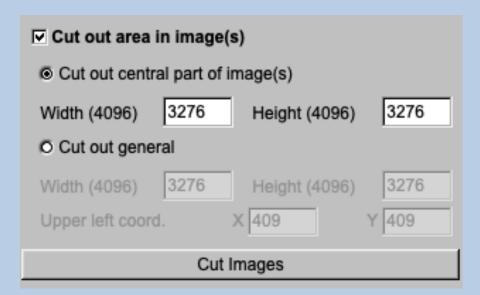
The imported images are shown in the display tab on the right-hand side. See chapter "A Typical Page - Display control tabs".



## CUT MICROGRAPHS / IMAGES (not suggested in **guiANISOTROPIC**):

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

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



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

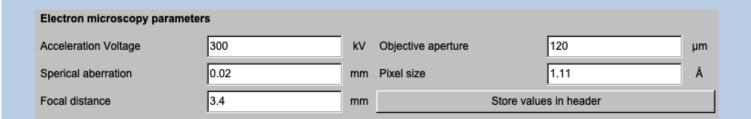
The name of the output file containing the cut-out images is suggested on the left-hand side. As usual you can change this name, of course.

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



#### SPECIFY THE ELECTRON MICROSCOPY PARAMETERS):

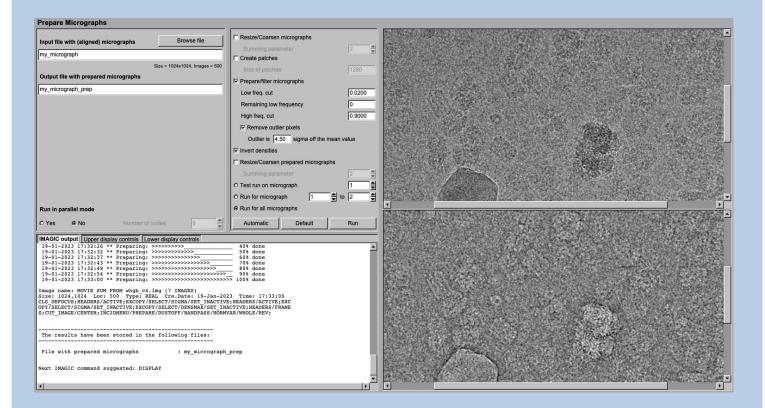
If not yet stored in the input headers you have to specify the the parameters of the electron microscope the micrographs were imaged with.



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

Store values in header
------------------------

## The "Prepare Micrograph" Page



#### **DESCRIPTION:**

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

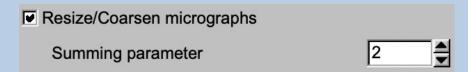
#### NOTE:

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



#### Prepare the micrograph images for CTF determination

You can resize the micrograph images

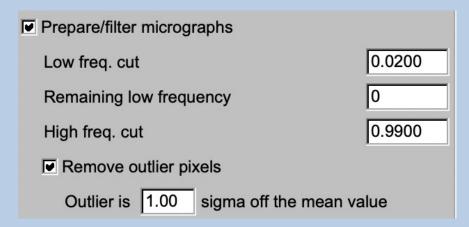


#### or create patches



to speed up the CTF calculations.

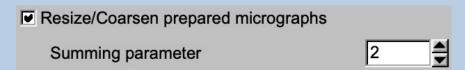
Imposing a band-pass filter is always suggested:



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

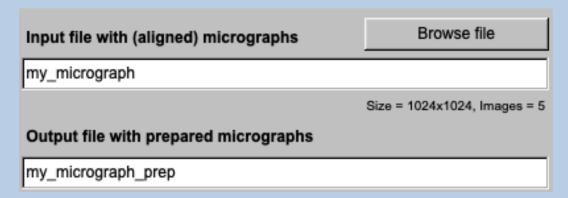
■ Invert densities

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

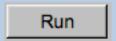




As usual, specify the names of the input and the output files:



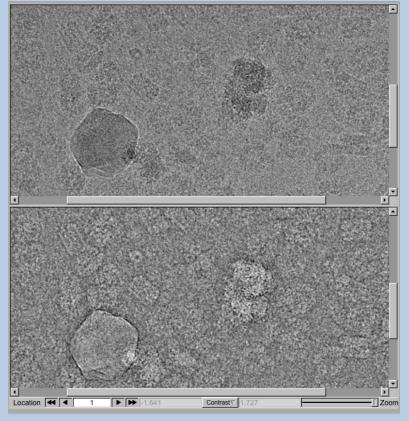
Start the calculations by clicking the "Run" button:



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



Check the pre-treated micrographs:

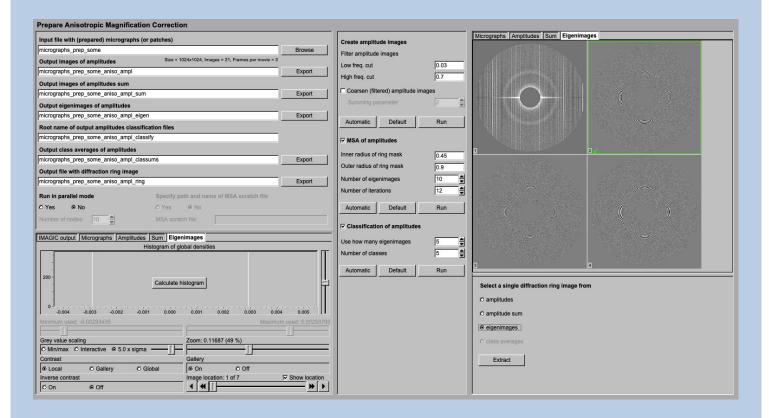




If all parameters are adjusted and the pre-treated micrographs look okay run the calculations for all micrographs:



## The "Prepare Anisotropic Magnification Correction" Page

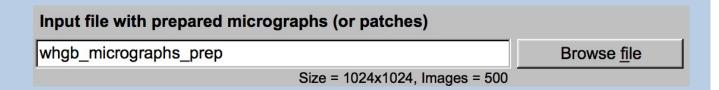


#### **DESCRIPTION:**

Create amplitude images of the input micrographs, the amplitude sum, the amplitude MSA eigenimages and/or the amplitude class averages. Finally select the image with the best visible diffraction ring to be used on the next page to correct for the anisotropic magnification.



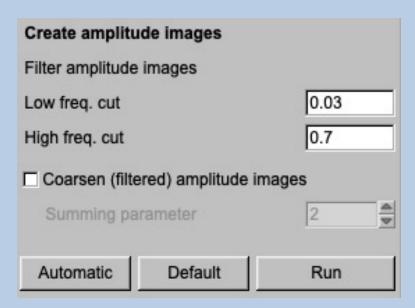
Input are the prepared micrograph images which file name you have to specify



As usual, also specify the output file names.

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

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



Now it is necessary to check if the filter parameters were chosen correctly. Therefore, all pre-treated amplitudes will be averaged and displayed on the right-hand side.

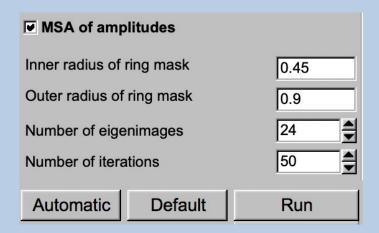


If the diffraction ring is clearly visible in one of the amplitude image or in the amplitude sum you can select and extract this image (on the right hand side of this page) to be used on the next page to find the anisotropic magnification parameters. In this case do not use options MSA and classification.

But very often MSA and/or classification is used to detect the diffraction ring:

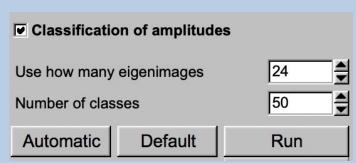
#### **■** MSA of amplitudes

Certain radial areas are not of interest and should not be taken into consideration for MSA. Check if the ring mask correctly masks out the unwanted inner and outer parts. Start with the automatically given MSA parameters. You can later adjust the parameters when checking the eigenimages.



In contrast to the individual amplitude images one of the MSA eigenimages can better show the diffraction ring. If this is the case you can select and extract this eigenimage (on the right hand side of this page) to be used on the next page to find the anisotropic magnification parameters. In this case do not use option classification.

If no eigenimage shows the diffraction ring you can create the amplitude class averages. Hopefully, one of the class averages shows the diffraction ring.





Use the option on the right hand side to select the image with the best diffraction ring by clicking into its image in the display tab on the right hand side. Not that in option "amplitude sum" is nothing to click because it is only one image.



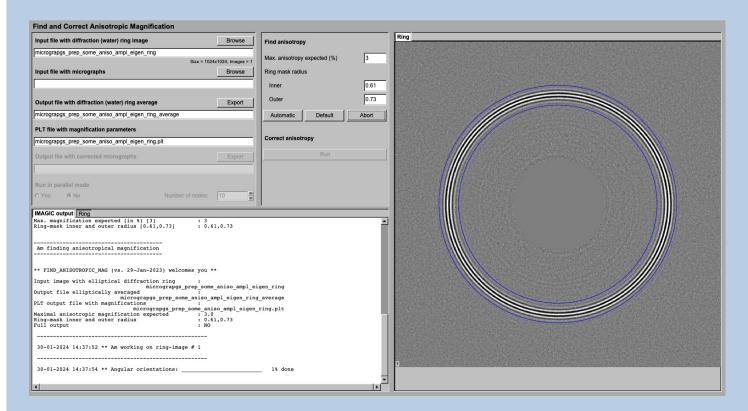
Click the "Extract" button to extract and store this image which will be used on the next page to find the anisotropic magnification parameters.

Extract

Click the "Next" button to continue with the next page to find and correct for the anisotropic magnification.



## The "Correct Anisotropic Magnification" Page



#### **DESCRIPTION:**

Find the anisotropic magnification parameters using an image with the diffraction ring and correct the input micrographs.



#### FIND ANISOTROPY:

As usual, specify the input file with the diffraction ring as well as the output file names on the left hand side.

Parameters which can be specified are the maximal anisotropy you expect



and the ring mask within which the diffraction ring is located



Click the "Run" button to start the calculation.

Check the magnification parameters found in the "MAGIC output" tab on the left hand side of the page. The display tab the right hand side shows a ring average which is the best fit elliptically averaged version of the ring image as a feedback to assess the quality of the best re-magnification parameters found.

#### **CORRECT ANISOTROPY:**

Having found the correct magnification parameters one specify the input micrographs and correct them for the anisotropic magnification found (and stored in the PLT file which now is an input file.

As usual, do not forget the specify the name of the output file to hold the corrected micrographs and start the calculations using the "Run" button.





Not (yet) possible

The following options are not (yet) possible:

- > Run in batch mode.
- > Store output files and results of different pages in different sub-directories of the working directory.





Feedback / Error hints

We intensively tested the **guiANISOTROPIC** 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**

www.lmageScience.de imagic@ImageScience.de

