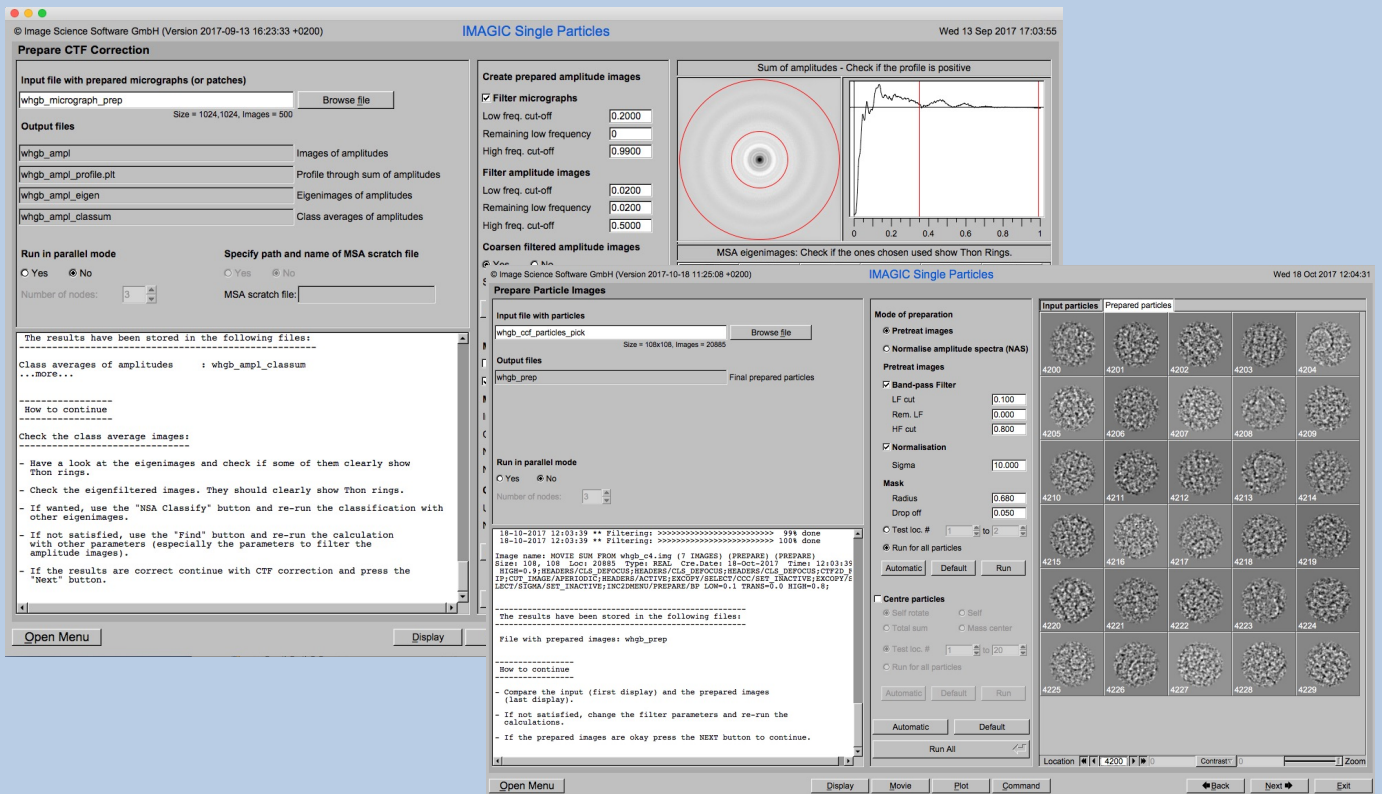




## A Brief Introduction

Version 10-Oct-2023  
[www.ImageScience.de](http://www.ImageScience.de)  
© Michael Schatz (Image Science)

# The GISP program



The **GISP** program follows the work-flow of a typical 4D alignment-by-Classification single particles analysis.

This is a brief hands-on on how to use this GUI oriented program.

FULL CRYO-EM DATA-SET ANALYSIS:

Refer to the manual: “Analysis of Wormhemoglobin - IMAGIC GISP“

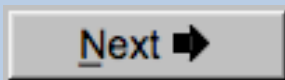


# Workflow

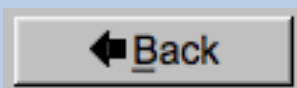
The idea of **GISP** is to guide you through a typical camera/detector correction measurement or camera .

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.



# Start Working

You always have to begin with the “Start” page where you have to specify some important project parameters which are needed to use the GISP pages.

As already mentioned, the idea of “IMAGIC Single Particles GUI” is to guide you through a typical single particles analysis. So the usual way is to use **GISP** page by page.

For whatever reason you may want to use a single page only. This is possible but make sure that all results and header information are available. But note: also in this case you first have to specify the parameters on the “Start” page.



# The Working Directory

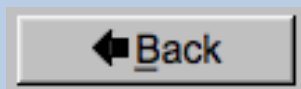
If **GISP** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

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

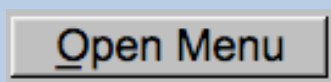
```
IMAGIC-COMMAND : gisp
```

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 **GISP**.

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.



# Input Files

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

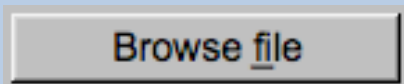
If you do not follow the workflow using page by page (not suggested) you can, of course, also use other input files and even use other input directories. But be careful: make sure that the headers of these input images contain all the information needed.

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

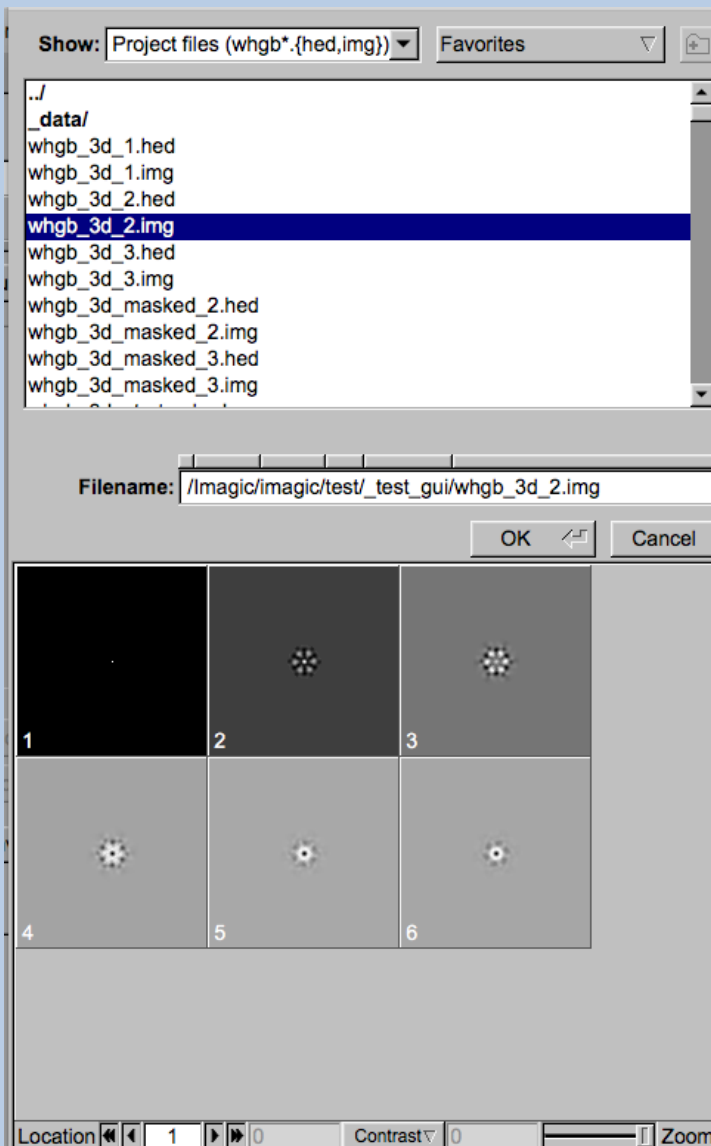


# Input File Chooser

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

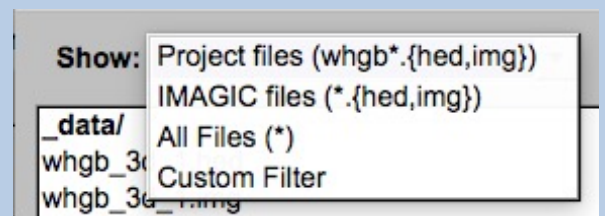


Pressing this button will open the IMAGIC file chooser:

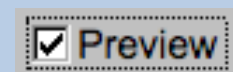


Choose the wanted file by clicking its name

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

The names of the output file(s) created on the various pages are always created automatically. You can not specify any output file name.

Note:

On each page the names of the output files are printed on the left-hand side of the page.

**Output file:**

whgb\_micrograph





# 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.

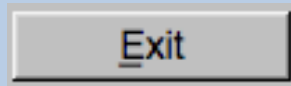
**MRC:**  
This is one of the oldest image formats in use in electron microscopy. One of the philosophies behind this data format is that it is compatible to the CCP4 format in use in X-ray crystallography.

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



# Exit GISP

Click the “Exit” button to leave the **GISP** program.



# A Typical Page

A typical **GISP** 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.

The screenshot shows the **guiCNORM** software interface. The title bar indicates the version (2022-11-30 18:18:14 +0100) and the date (Fri 9 Dec 2022 11:35:40). The interface is divided into several sections:

- Camera Correction:** Contains input fields for raw micrographs, average file, and sigma file, along with buttons for "Browse file" and "Export file".
- Camera Normalisation:** Includes radio buttons for "Measure", "Correct", and "Measure and Correct", and a "Correct" button.
- IMAGIC output:** A terminal window showing the progress of the correction process, including timestamps and completion percentages for various steps.
- Image Display:** A large area showing the input and corrected micrographs, with tabs for "Input Micrographs", "Corrected Micrographs", "Average", and "Sigma".
- Extract micrographs:** A section with radio buttons for "Use all" and "Use 'good' micrographs only", and checkboxes for "too extreme sigma of densities" and "too extreme min/max difference of densities".

The terminal window output shows the following progress:

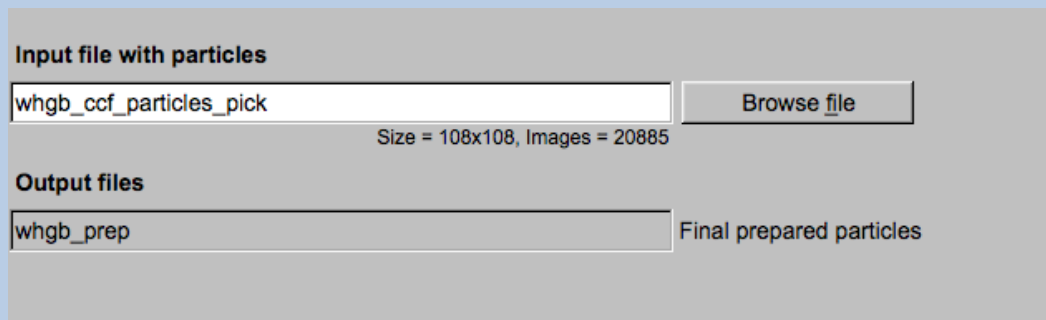
```
Reverse contrast in camera corrected images : NO
09-12-2022 11:33:58 ** Am correcting/normalising images
09-12-2022 11:33:58 ** Correction: _____ 1% done
09-12-2022 11:34:01 ** Correction: >>>>> 10% done
09-12-2022 11:34:03 ** Correction: >>>>>> 20% done
09-12-2022 11:34:06 ** Correction: >>>>>>>> 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
```



# A Typical Page - File Information

The left part of a typical **GISP** page” shows/asks for the input file(s) needed. It also prints the file names of the output file(s) which will be created during the calculations.

The input file can be used as suggested or can be specified by either typing the file name into the text field or by using the “Browse file” button (see: “IMAGIC File Chooser”).




The screenshot shows a software interface with a light gray background. At the top left, the text "Input file with particles" is displayed in a bold font. Below this, there is a text input field containing the filename "whgb\_ccf\_particles\_pick". To the right of the input field is a button labeled "Browse file". Below the input field, the text "Size = 108x108, Images = 20885" is displayed. Below the "Input file with particles" section, the text "Output files" is displayed in a bold font. Below this, there is a text input field containing the filename "whgb\_prep". To the right of the input field, the text "Final prepared particles" is displayed.



# 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 **GISP** 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

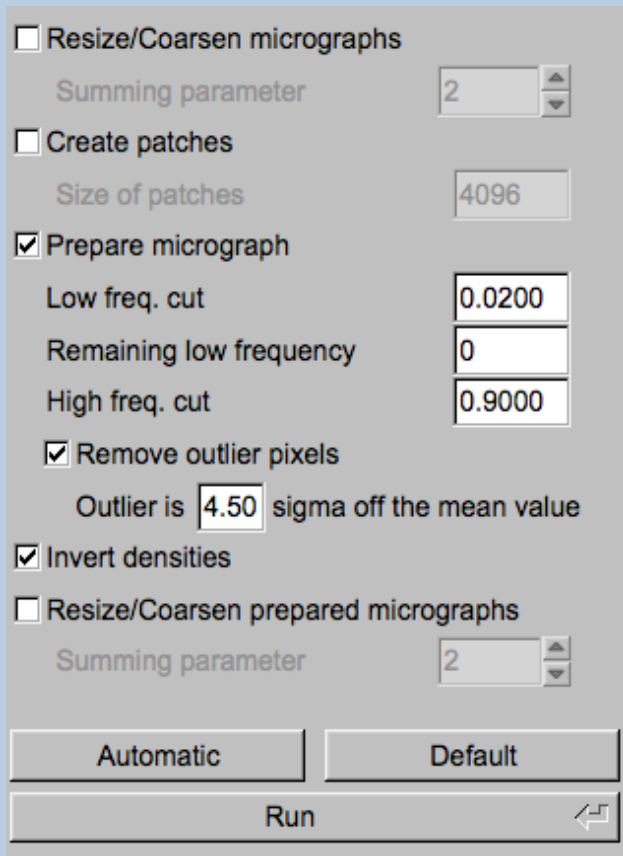
In text fields you can type in the wanted value. If the needed value is a number you can also move the cursor into this field, press the mouse key and keep it pressed and move the cursor to change the value.

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



# A Typical Page - Automatic / Default



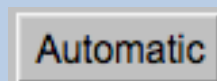
The screenshot shows a control panel with the following elements:

- 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

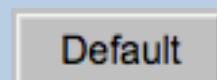
At the bottom, there are three buttons: "Automatic", "Default", and "Run".

In the middle part of a typical **GISP** 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

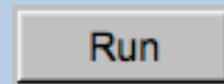
Number of iterations

**Classification**

Use how many eigenimages

Number of classes

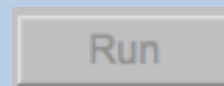
To run the calculations press the “Run” button.



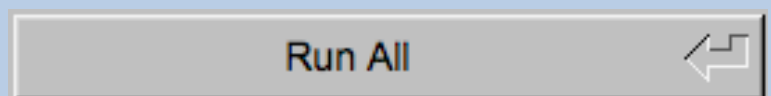
On a number of pages the calculations can be split. In this case you will find more than one single “Run” button.

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

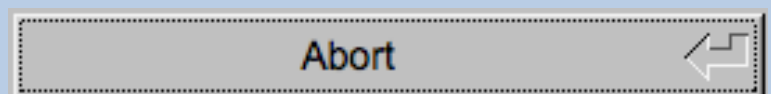
Maybe a certain “Run” button is not yet activated because it needs the results of calculations not yet done.



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



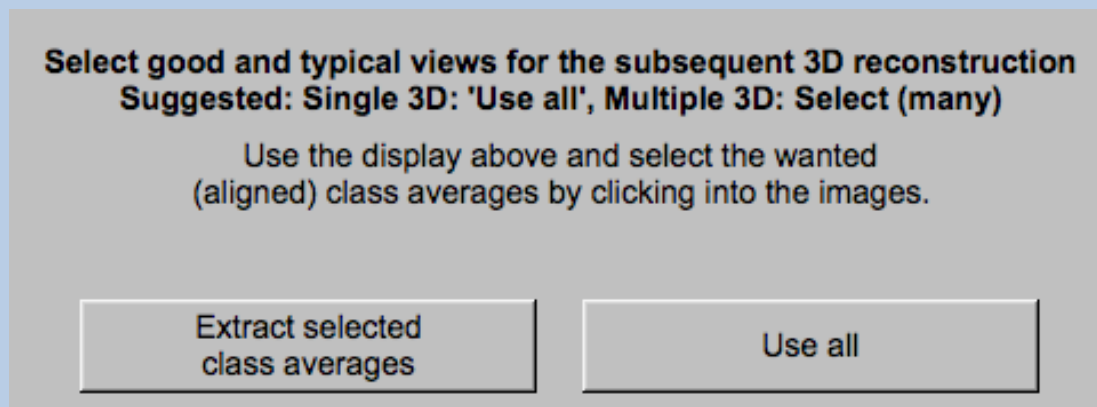
You can abort a running program by pressing the “Abort” button.





# 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.

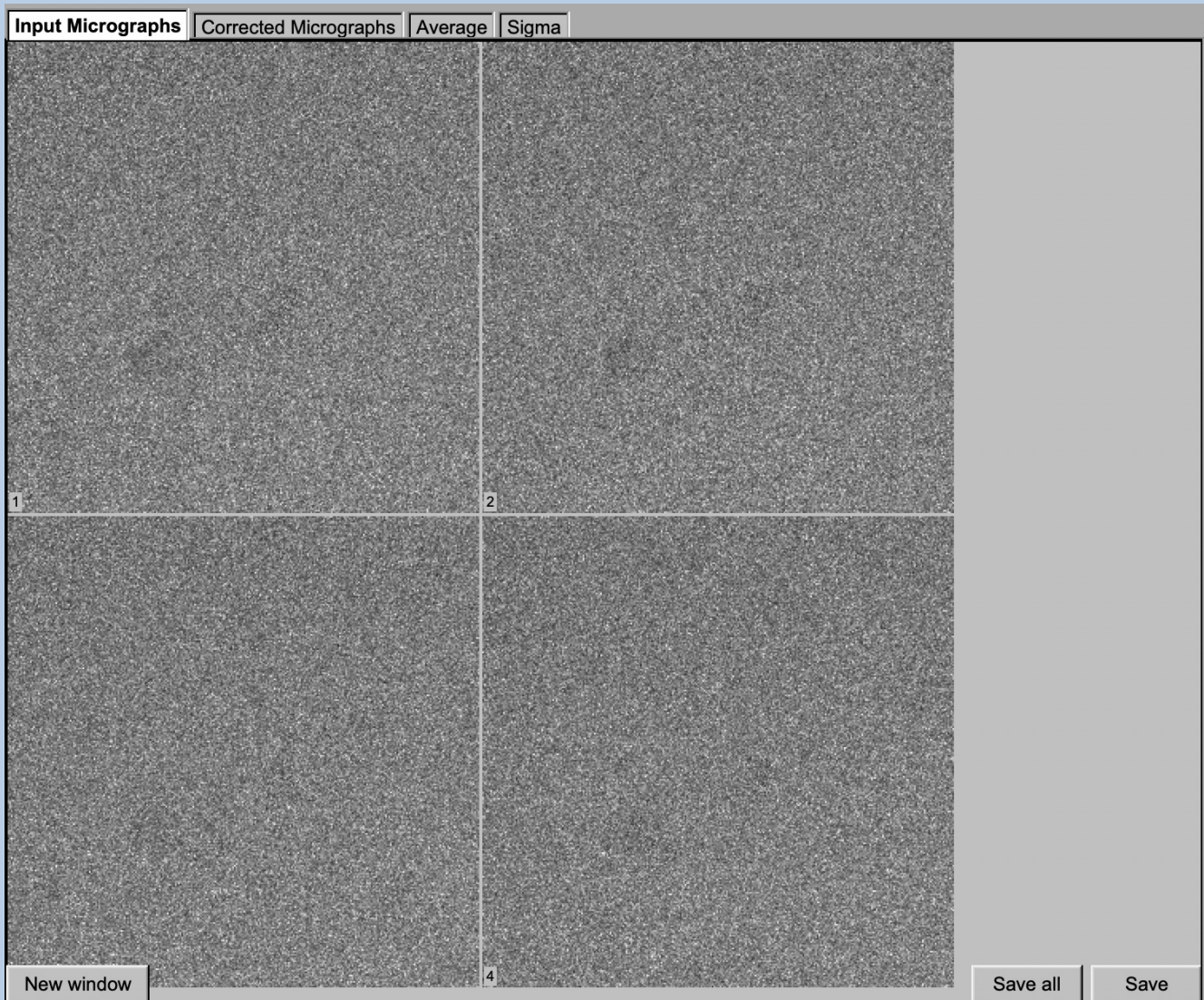


Note that the new output images are usually shown in a new display tab.





# A Typical Page - Display



In the right part of a typical **GISP** 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 **GISP** 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

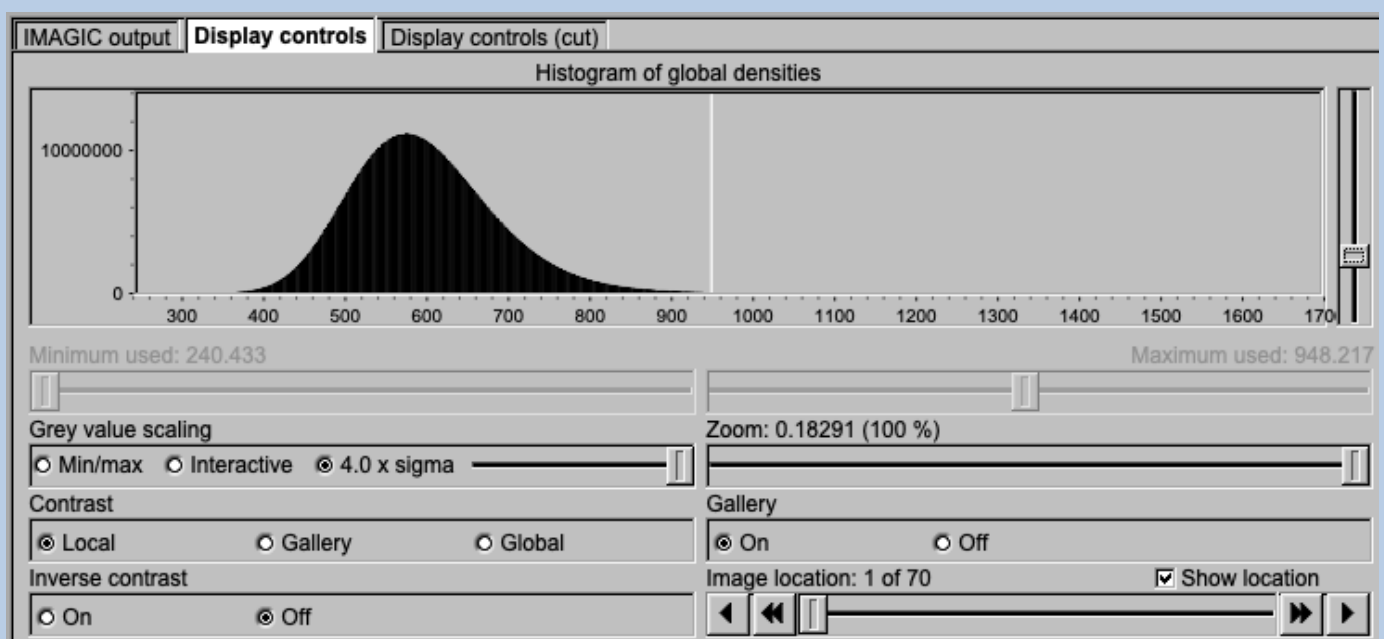
Gallery

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

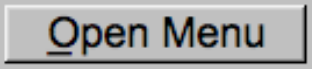
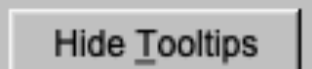
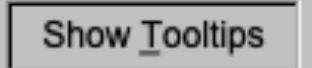
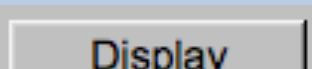
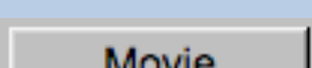
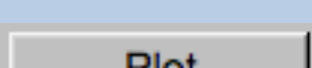
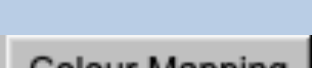

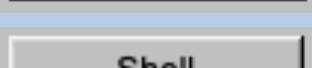
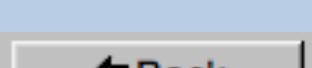
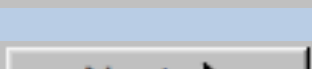
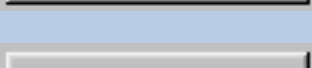




# A Typical Page - The Toolbar

There is a toolbar at the bottom of each **GISP** 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>GISP</b>



# All GISP Workflow Pages

## IMAGIC menu

Start

Import Micrographs

Project

Camera Correction

Movie Alignment

Prepare Micrographs

Prepare CTF Correction

CTF Correction

Pick Particles: Modulation Search

Pick Particles: Get References from Modulation Search

Pick Particles: Prepare Correlation Search References

Pick Particles: Correlation Search

Prepare Particle Images

First MSA and Classification

3D Reconstruction: Extract Class Averages

3D Reconstruction: Random Start-Up

3D Reconstruction: Refinement using Anchor Set(s)

MSA and Classification

Multi-Reference Alignment

MSA and Classification of Aligned Particles

Fourier Shell Correlation

End

Close menu



# The “Start” Page

**Start**

**Working directory**

Current working directory:  
/Imagic/imagick/test/

Browse directory

**Project**

Name:  
Brasilienschule

(Short) Prefix to be used for all file names:  
whgb

Remove project    Select project

Save as modified project    Save as new project

Before doing any calculation you have to define some project parameters:

- a) the working directory
- b) the project name (for your information only)
- c) the prefix to be used to create automatic file names

If wanted you can also change some program settings:

Click to close the program settings menu

Character/font size: 14

Window size: 1540 x 900

Start page picture / movie: Image

File browser: Standard

Save/Cancel    Reset

You can also get some manuals and papers:

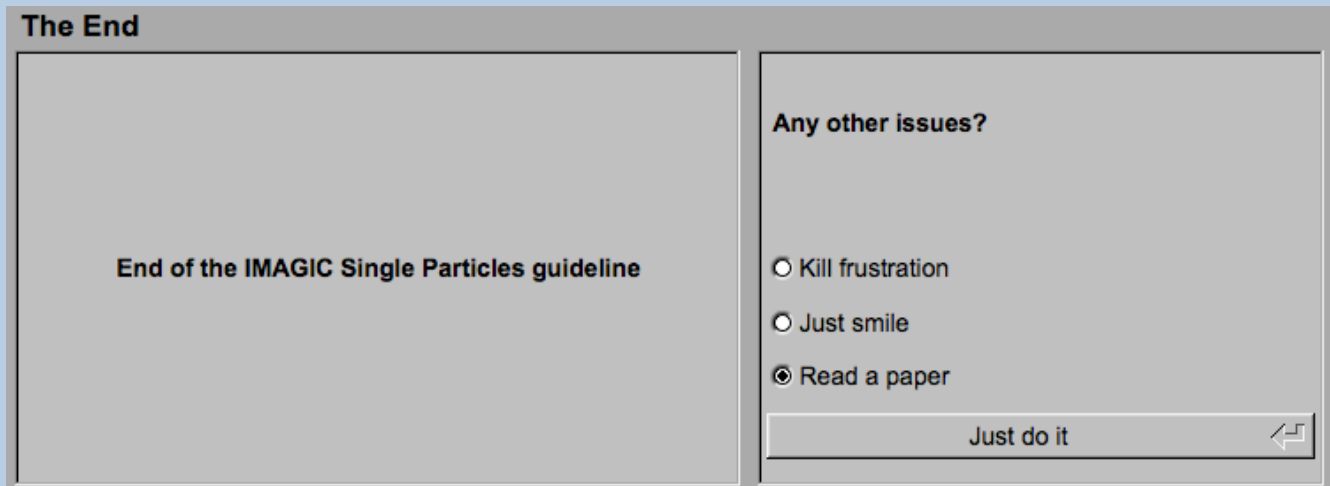
**Manuals & Papers**

Read





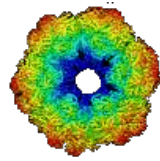
# The “End” Page



The last page of the “GISP - IMAGIC Single Particles GUI” workflow.

You can read some “Single Particles ” publications as well as some IMAGIC and/or **GISP** introductions. Also a single particles workflow is available.





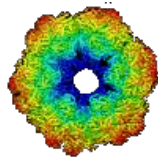
# GISP IMAGIC

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.





# GISP IMAGIC

Feedback / Error hints

We intensively tested the **GISP** program and tried to find all possible errors and inconsistencies. But the **GISP** workflow 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)

