

### **A Brief Introduction**

Version 15-Jan-2024 www.ImageScience.de © Michael Schatz (Image Science)

# The IMAGIC guiALIGN program

Image Science Software GmbH (Version 2023-10-06 14:45:30 +0200)	guiMSA		Fri 6 Oct 2023 17:20:0
MSA and Classification			
Input file with (prepared) images JUNK2D_NOISE Browse	file MSA mask	Input Images MSA eigenimages All classures Class at	verages after remove
Root name of MSA results files Size = 64x64, Images = 100 JUNK2D_NOISE_msa	Centred mask Circular		6. 7. 8
Root name of classification results files JUNK2D_NOISE_classify	Center X 33 Y 33 Radius 0.8		
Output file with MSA eigenimages JUNK2D_NOISE_eigen Expor		nnnn	$\cap \cap \cap$
Output file with all class averages JUNK2D_NOISE_classums Utput file with class averages after remove			22 23 24
JUNK2D_NOISE_classums_masked_good Expor		20 21 23 23	30 31 32
Run in parallel mode Specify path and name of MSA scratch file	Number of eigenimages     12       Number of classes     10	OOOOO	
O Yes 6 No     O Yes 6 No     Mumber of nodes:	Class Averages Fraction of worst class members to ignore	nnnn	nnn
MAGIC output Input images Eigenimages All classums Class averages after remove Histogram of global densities	Percentage of mask 40		
200 - Calculate histogram	Image: Finally remove bad class averages if           Image: Finally remove		
	50 iii sigma in densities is 1.0000 times sigma off average		
Minimum used: -41.5264 Maximum use Grey value scaling Zoom: 1 (100 %)	4:41.5002 		
OMIn/max O Interactive 64.2 x sigma Contrast 6 Local O Gallery O Global   6 On O Off	Automatic Default	00000	OOO
Inverse contrast Image location: 1 of 100 IF Show C On © Off	Run all           Re-run starting with	81 UT 102 103 103 103 103 103 103 103 103 103 103	2 190 7 197 197 197 198 199 199 199 199 199 199 199 199 199
Open Menu Hide Toolitips	Display Movie Plot Comman	nd Shell <b>♦</b> Back	K <u>N</u> ext ⊯ <u>E</u> xit

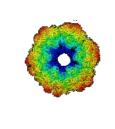
The **guiALIGN** aligns images (NOT 3D volumes) to single or a stack if references (multi-reference alignment). If wanted, the references can be selected from a stack of images or can be created from a 3D volume .

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

#### CONTENT:

IMAGIC GUI programs
 guiALIGN
 How to use IMAGIC GUI programs
 How to Import images to IMAGIC
 How to prepare the images (filter, masks...)
 How get the references
 How to multi—reference align the images
 How to send us feedback





## IMAGIC

# **GUI Programs**



IMAGIC guiALIGN - Hands-On

### Workflow

The idea of **guiALIGN** 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 **guiALIGN** 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 **guilALIGN** is called by an IMAGIC command in a terminal / command window

IMAGIC-COMMAND : gui-align-images

the working directory will be the directory used in this window.

If you want to change this directory use the "Back" button(s)

Eack

or the "Open Menu" button

Open Menu

to navigate to the "Start" page where you can specify the working directory of **guilALIGN**.

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_microgra	aph
	Name of the output IMAGIC file containing the imported micrographs.
	Note that the name of this output file will be created automatically.

Select forma	at $\nabla$	In case of type conflicts
	Select th	e input file format.
Browse <u>fi</u> le	Note: Cu	rrently only TIFF and MRC files can be imported.
Browse file of file	MRC: This is or microsco	ne of the oldest image formats in use in electron py. One of the philosophies behind this data format is compatible to the CCP4 format in use in X-ray graphy.
	This has	gged Image Format): become one of the standard formats in desk-top g 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

my\_images

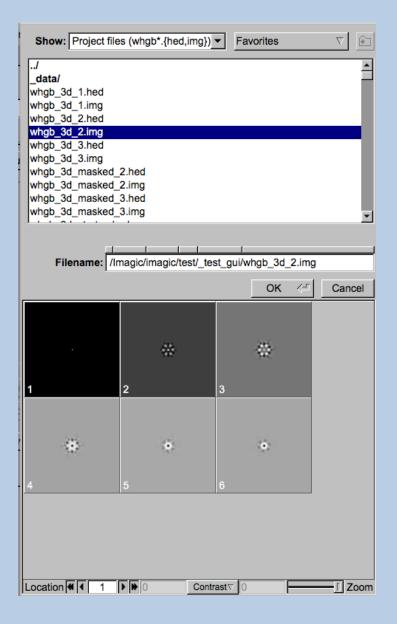


### Input File Chooser

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

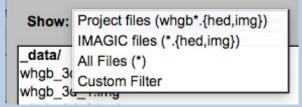
Browse file

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	
my_images	



### **Import Buttons**

You do not want to use the "Import page" you can use the "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.

Import

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

Data format dres input to be converted       MCC       Image: Strate in the converted       Strate in the converted<	Convert image(s) or 3D volume(s)	IMAGE T	AGIC printout IMAGIC display	
When MC Convert       MCC 2002014       If the Convert of the			SHF SITUS	
Wind Much Summal       MaxDOU_D/M       I SUC         Type of exp(1%)       STACKD_MAGE_FILE       I SUC         A the bip of images move frames       NO       I SUC         Much Much Garden       MAGGE       I SUC         Much Much Garden       MAGGE       I SUC         Much Much Garden       MAGGE       I SUC         Much Much Much Much Much Much Much Much				TVIPS
Are the topolomages movie frames       NO         Input field instance       NO         Input field NTH extension).first#.last#       : sy_images.nrc         Input field NTH extension).first#.la				: MRC
Read model       Image: I	Type of input file(s)	STACKED_IMAGE_FILE	ich MRC format:	
Impundee.nnc       Browse       Dapping       Type of ignuit file(s):       Type of ignuit file(s):         Begot wich data format       MAGC       MAGC       Type of ignuit file(s):       Type of ignuit file(s):       Type of ignuit file(s):         Myge MAGC = FSC input file(NO ext.)       Imput file(s):       Type of ignuit file(s):       Type of ignuit file(s):       Type of ignuit file(s):         Myge MAGC = FSC input file(NO ext.)       Imput file(s):       Type of ignuit file(s):       Type of ignuit file(s):       Type of ignuit file(s):         Myge Mage = TSC input file(s):       Type of ignuit file(s):       Type of ignuit file(s):       Type of ignuit file(s):       Type of ignuit file(s):         Myge Mage = TSC input file(s):       Type of ignuit file(s):	Are the input images movie frames	NO	UNKNOWN	IMOD_MRC
Export to which data format       : STACKED_INACK_FILE         Output MAGIC       FSC input file (ND ext.)       : STACKED_INACK_FILE         my_images       : STACKED_INACK_FILE       : stacked_INACK_FILE         Type file (MTH stachasics)       : stacked_INACK_FILE       : stacked_INACK_FILE         Nuto-detected a MRC (MRC 2014)       : stacked_INACK_FILE       : stacked_INACK_FILE         Nuto-detected a MRC (MRC 2014)       : stacked_INACK_FILE       : stacked_INACK_FILE         Nuto-detected a MRC (MRC 2014)       : stacked_INACK_FILE       : stacked_INACK_FILE         Nuto-detected a MRC (MRC 2014)       : stacked_INACK_FILE       : stacked_INACK_FILE         Nuto-detected a MRC (MRC 2014)       : stacked_INACK_FILE       : stacked_INACK_FILE         Nuto-detected a MRC (MRC 2014)       : stacked_INACK<	Input file (WITH extension),first#,last#		ease specify option [MRC_2000/2014]	: MRC_2000/2014
Export which data format       MAGC         my_mages       Browns       Dapper         my_mages       Imput file (NO ext.)         my_mages       Imput file (NO ext.)         my_mages       Imput file (NT text.sci.nics), first#, last#       : my_mages.mcc         res_int informat       Imput file (NT text.sci.nics), first#, last#       : my_mages.mcc         my_images       : my_images.mcc       : my_images.mcc         http://mages.mcl       : my_images       : my_images         http://mages.mcl       : my_images       : my_images         http://mages.mcl       : my_images.mcl       : my_images         http://mages.mcl       : my_images.mcl       : my_images         http://mages.mcl       : my_images.mcl       : my_images.mcl         mode       : mode       : my_images.mcl       : my_images.mcl         mode       : mode       : mode       : my_images.mcl         mode       : mode       : mode       : mages.mcl         mode       : mode       : mode       : mode         into-detected a NWC (NWC 2014)       : my_images.mcl       : mode         into-detected into from NWC file       : mode       : mode         into: compart into intomage <td: mode<="" td="">       : mode       : m</td:>	my_images.mrc	Browse Display	pe of input file(s):	FTIFC
Output MAGIC = FSC input file (WO ext.)         my_mapps         my_map	Export to which data format	IMAGIC	ease specify option [STACKED IMAGE FIL	E] : STACKED IMAGE FILE
Images	Output IMAGIC = FSC input file (NO ext.)			
Output THAGIC = FSC input file (NO ext.) [Ny_langes]       : my_images         Auto-detected a NRC (MRC 2014) file         Header info from NRC file         NRC version       : NRC 2014         Input image file       : NRC 2014         Subscr of pixels per line       : 00         NRC to rest in the input image file       : 01         NRC to rest in the input image file       : 01         NRC to rest in the input image file       : 01         NRC to rest in the input image file       : 01         NRC to rest inform NRC file       : 01         Into rest in the inport in the inp	my_images	Browse Display	put file (WITH extension),first#,last# y_images.mrc]	
Rn command       Rn command         Rn command       Rn command		· · · · · · · · · · · · · · · · · · ·	port to which data format	: IMAGIC
Reader info from NRC file         Input image file       : WIC 2014         Input image file       : WIC 2014         Wide of pixels per line       : 100         Number of pixels per line       : 00         Number of lange per line       : 00         NRC data format       : 100         NRC data format       : 100         NRC header text (part)       : Text-TAMGE SEMI-RING // Created by INAGIC: IMAGIC         10-10-2023 12:04:17       : Converting: >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>				
RC version       : MRC 2014         Input image file       : mV images.mcc         Input image file       : mV images.mcc         Number of lines per image       : 100         Number of images       : 50         Type       : UNIX MRC (LINUX/DEC)         Number of images       : 50         Type       : UNIX MRC (LINUX/DEC)         NRC data format       : 10-2023 12:04:47         10-10-2023 12:04:47       : Converting: >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>			uto-detected a MRC (MRC 2014) file	
Input image file       : my images.mc         Number of lines per ing : 100         Number of lines per ing : 101         Number of line				
10-10-2021 12:04:47 ** Converting: >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>			put image file         : my images.           mber of pixels per limage         : 100           mber of limes per image         : 50           pe         : UNIX MRC           C data format         : float (REF           C header text (part)         : TEST-IMAGE	LINUX/DEC) L) SEMI-RING // Created by IMAGIC: IMAGIC
Run command Tf wanted you can check the imported images by clicking the "Display" button If the import is okay, press the "Close window" button to return to "Fourier-Shell-Correlation"			D-10-2023 12:04:47 ** Converting: >>> D-10-2023 12:04:48 ** Converting: >>> D-10-2023 12:04:48 ** Converting: >>> D-10-2023 12:04:48 ** Converting: >>>> D-10-2023 12:04:48 ** Converting: >>>>	10% done           20% done           30% done           30% done           50% done           500% done           500% done           500% done           500% done           500% done
Kur command			wanted you can check the imported ima	ges by
Close window 4	Run	l command	the import is okay, press the "Close return to "Fourier-Shell-Correlation"	window" button
	Close	se window		

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

Close 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:

Convert 2D image(s) or 3D volume(s)	2D IMAGE	MAGIC printout IMAGIC display
Data format of the input to be converted	IMAGIC	Convert 2D image(s) or 3D volume(s):
	UNKNOWN IMAGE FILE	2D_IMAGE 3D_VOLUME Please specify option [2D_IMAGE] : 2D_IMAGE
How are the input images available		Data format of the input to be converted:
Are the input images movie frames		BROOKHAVEN_STEM CCP4 DATA_ONLY DICOM DIGITAL MICROGRAPH EM
Input file, image loc#s	my_images	FEI FABOSA FORMATTED
Export to which data format	TIFF 7 ?	IMAGIC JPEG KONTRON MDPP MEDIPIX MRC
Type of output TIFF image(s) wanted	GREY_SCALE_IMAGE	OFFSET PIF PGM PROTOMO RAW SHF
Type of output file	STACKED_IMAGE_FILE V ?	SMV SPIDER SUPRIM TIA/EMI/SER TIFF TVIPS
Output file, loc#s (WITH ext.),first#,last#		Please specify option [IMAGIC] : IMAGIC
my_images.tif	, Browse Display ?	Type of input file:
Always scale densities to the output format	Yes     O No     ?	SINGLE IMAGE_FILE STACKED_IMAGE_FILE UNKNOWN_IMAGE_FILE Please specify option [UNKNOWN_IMAGE_FILE] : UNKNOWN_IMAGE_FILE
		Are the input images movie frames [NO] : NO
		Input file, image loc#s [my_images] : my_images
		Export to which data format: CCP4 DATA_ONLY EM FORMATTED FEI RAW_INAGE INAGIC JPEG GREVSCALE KONTRON MDEP MRC OFFSET FIF MRC FORSCRTF FIF FORMO FOR FORSCRTF SNV SPIDER SUPRIM TIFF TVIPS
		Please specify option [TIFF] : TIFF
		Type of utput TIFF image(s) wanted: COLOR INAGE GREW SCALE IMAGE Please specify option [GREW_SCALE_IMAGE] : GREY_SCALE_IMAGE
		Type of output file: STACKED_IMAGE_FILE SET OF MANY_IMAGE FILES Please specify option [STACKED_IMAGE_FILE] : STACKED_IMAGE_FILE
		Output file, loc#s (WITH ext.),first#,last# [my_images.tif] : my_images.tif
		Always scale densities to the output format [YES] : YES
		<pre>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 EM22M; EXCOPY/PIT; EXCOPY/SIECT; CAMERA NORM, INC2DNEON/ANISOTROPIC MAGNIFY=1.0,1 .025;COARSE;ALIDIR; COARSE; SUMMER/MOVIE SUM; INC2DMENU/PREPARE/BP LOW=0.02 TRANS =0.0 HIGH=0.9;CTF2D_FLIP;CUT_IMAGE/APERIODIC;</pre>
	n command	

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

Close window

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



# **A Typical Page**

A typical IMAGIC GUI program page has three columns.

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

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

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

© Image Science Software GmbH (Version 2022-11-30 18:18:14 +0100)	guiC	NORM	Fri 9 Dec 2022 11:35:40
Camera Correction			
Input file with (raw) micrographs Try_micrographs Try_micrographs Size = 4096x4098, Images = 70 Input camera statistics average file Try_micrographs_cnorm_average Size = 4096x4096, Images = 1 Input camera statistics sigma file Try_micrographs_cnorm_sigma Size = 4096x4096, Images = 1 Output file with camera corrected micrographs Export file Try_micrographs_cnorm Output good camera corrected micrographs Export file Try_micrographs_cnorm Try_micrographs_cnorm Try_micrographs_cnorm Size = 4096x4096, Images = 1 Size = 4096x4096, Images =	Camera Normalisation     O Measure     Correct     O Measure and Correct     Correct	Input Micrographs Corrected Micrographs Average Sg	me
Image couput         Mcrograph         Corrected         Average         Sigma           Output file, image loc#         imy_mic         imy_mic         imy_mic           Input syma file         imy_mic         imy_mic         imy_mic	rrographs_enorm 🔺		
09-12-2022         11:31:58         ** Correction:           09-12-2022         11:34:03         ** Correction:         >>>>>>>>>>>>>>>>>>>>>>>>>>>>	2 Time: 11:34:27	T Extract micrographs O Use all (© Use 'good' micrographs only)	Ignore micrographs which show IF too extreme signa of densities IF too extreme min/max difference of densities
ENZEM, HEADERS/ACTIVE; EXCOPY/SELECT/SIGNA/SET_INACTIVE; CAMER RAST; 4 Open Menu Hide Toollips	Display Movie	Plot Command Shell	Ignore if 1.5 from mean value Extract micrographs   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
Yes O No	O Yes   No
Number of nodes: 3	MSA scratch file:



# **A Typical Page - Program Parameters**

O Normalise amplitude spectr	a (NAS)
Pretreat images	
Band-pass Filter	
LF cut	.100
Rem. LF	.000
HF cut 0	.800
Normalisation	
Sigma 1	0.000
Mask	
Radius 0	.680
Drop off 0	.050
O Test loc. # 1 € to 2	<u>A</u>
Run for all particles	
Automatic Default	Run
Centre particles	
Self rotate     O Self	
O Total sum O Mass ce	nter
Test loc. #     1	0
O Run for all particles	
Automatic Default	Run
Automatic Defa	ult
Run All	(L

Mode of preparation

Pretreat images

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	O Self
O Total sum	O 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

Resize/Coarsen microg	raphs
Summing parameter	2
Create patches	
Size of patches	4096
Prepare micrograph	
Low freq. cut	0.0200
Remaining low frequence	су О
High freq. cut	0.9000
Remove outlier pixels	;
Outlier is 4.50 sigma	a off the mean value
Invert densities	
Resize/Coarsen prepare	ed micrographs
Summing parameter	2
Automatic	Default
Run	با ج

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.

Automatic

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

Default

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 amplitud	e images
Filter micrographs	
Low freq. cut	0.2000
Remaining low frequency	0
High freq. cut	0.9900
Filter amplitude images	
Low freq. cut	0.0200
Remaining low frequency	0.0200
High freq. cut	0.5000
Coarsen filtered amplitud	e images
Yes O No	
Summing parameter	2
Automatic Default	Run
MSA options ☐ MSA eigenfilter amplitude ☑ MSA classify amplitudes MSA	es 0.35
Inner radius of ring mask	0.99
Outer radius of ring mask Number of eigenimages	10
Number of iterations	50
Classification	
Use how many eigenimage	s 5 🚔
Number of classes	25
Automatic Default	Run
	Classify only
Run all	4

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.

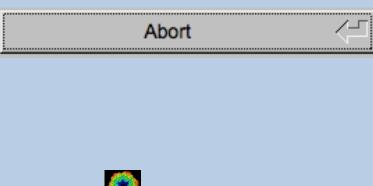
Pressing the "Run All" button starts all calculations currently activated on the page.

Run All

Run

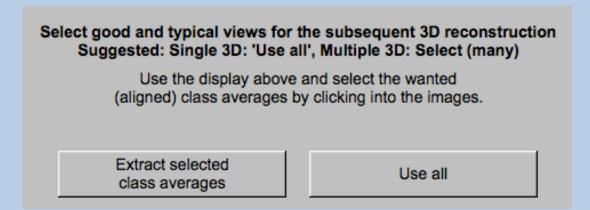
1	-
/-	

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.

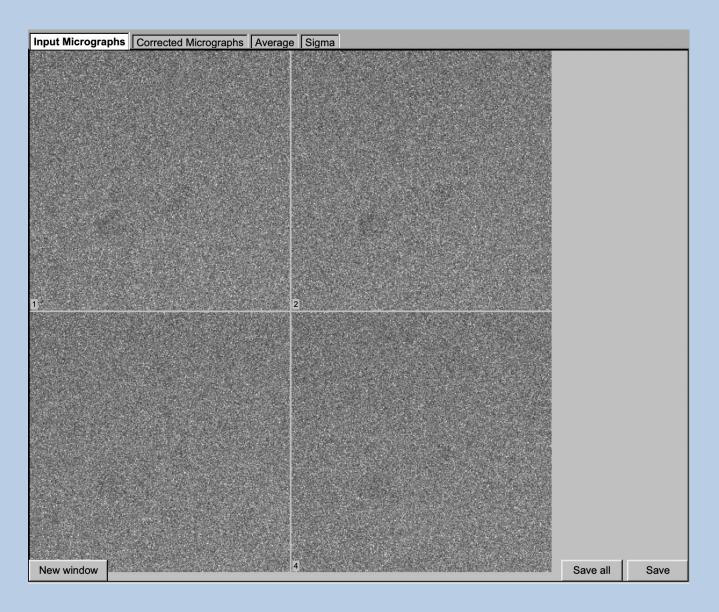
```
99% done
                                                                                  ٠
 Image name: MOVIE SUM FROM whgb_c4.img (7 IMAGES) (PREPARE) (PREPARE)
Size: 108, 108 Loc: 20885 Type: REAL Cre.Date: 18-Oct-2017 Time: 12:03:39
HIGH=0.9; HEADERS/CLS DEFOCUS; HEADERS/CLS DEFOCUS; HEADERS/CLS DEFOCUS; CTF2D F
IP; CUT_IMAGE/APERIODIC; HEADERS/ACTIVE; EXCOPY/SELECT/CCC/SET_INACTIVE; EXCOPY/S
LECT/SIGMA/SET INACTIVE;INC2DMENU/PREPARE/BP LOW=0.1 TRANS=0.0 HIGH=0.8;
The results have been stored in the following files:
 File with prepared images: whgb prep
How to continue
 Compare the input (first display) and the prepared images
  (last display).

    If not satisfied, change the filter parameters and re-run the

  calculations.
- If the prepared images are okay press the NEXT button to continue.
Zoom
                                                                            Save
```



# 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 cal	culate 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	e 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 slid	der or the arrows to select image locations

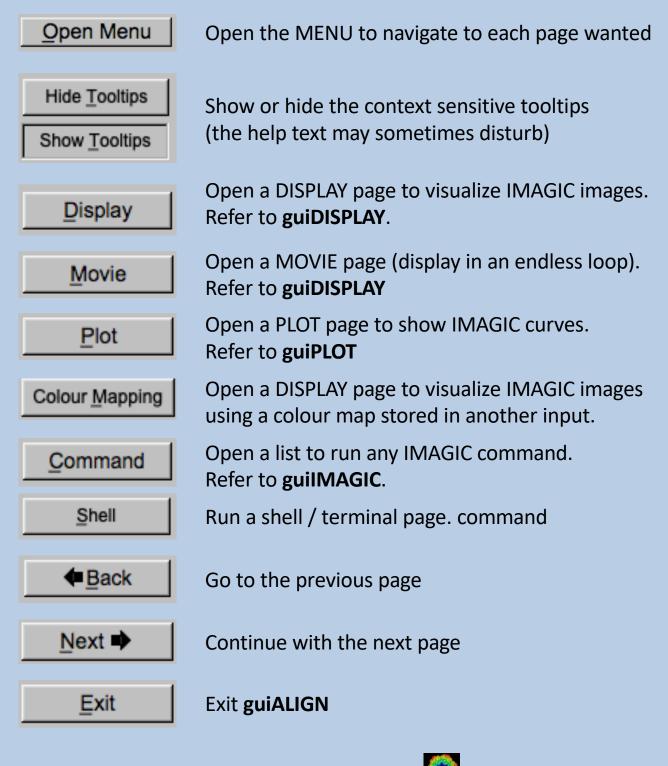
IMAGIC output	t Display co	ontrols	Display o	controls	(cut)									
	Histogram of global densities													
10000000 -														
	300 400	500	600	700	800	900	1000	1100	1200	1300	1400	1500	1600	170
Minimum use	d: 240.433											Maxim	um used:	948.217
										[				
Grey value se	aling						Zoom: 0	.18291 (1	100 %)		-			
O Min/max	O Interactive	⊚ 4.0 x	sigma			<u> </u>								[]
Contrast							Gallery							
Local	Ø G	allery		O Globa	al		© On		ō	Off				
Inverse contr	ast						Image lo	cation: 1	of 70			I SI	now loca	tion
© On	۵ 0	ff					•							₩ ►



# A Typical Page - The Toolbar

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

The toolbar buttons:







IMAGIC guiALIGN - Hands-On

# The guiALIGN Menu

#### IMAGIC menu

Start

Input Images

Prepare Images

Multi-Reference Alignment

Close menu

PAGES:

StartPage to adjust guiALIGN program parametersImport Images...Import or specify the input.Prepare Images...Pre-treatment: Mask, filter, normalise<br/>variance, resize, summing ...Multi-Reference<br/>AlignmentMulti-reference alignment of images.<br/>If wanted references can be selected from a<br/>stack of images or from (a) 3D volume(s).



### The "Start" Page

This page is not part of the **guiALIGN** workflow and can only be reached using the "Back" or the "Open Menu" button(s).

Working	lirectory			
Working directory Current working directory:				
/Users/michael/workspace2/_brazil_so	chool/			
Browse d	irectory			
Click to close the prov	aram settings n	enu		
Click to close the pro-	gram settings n	nenu		
Click to close the pro	gram settings n	12		
F	gram settings n			
Character/font size:	gram settings n	12		
Character/font size: Window size:	gram settings n	12 × 900		
Character/font size: Window size: Start page picture / movie: File browser:	gram settings n	12 ▼ 1540 x 900 Image ∇ Standard ⊽		
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Character/font size: Window size: Start page picture / movie: File browser:		12 ▼ 1540 x 900 Image ∇ Standard ⊽		
Character/font size: Window size: Start page picture / movie: File browser: Save/Cancel	k Papers	12 ▼ 1540 x 900 Image ∇ Standard ⊽		

On this page you can set some program parameters:

- a) the working directory
- b) the size of the **guiALIGN** program windows and/or text (a re-start is needed)
- c) the type of file browser



### **Start Working**

guiALIGN starts with the "Import" page.

The workflow using the "Next" button will guide you through all **guiALIGN** pages.

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



# The "Import" Page

Input Images				
File format	TIFF	v In case of type conflicts	Display	
		O Threshold densities  Change type		and the other sectors
Input file(s)	Browse files	O Shift densities O Scale densities		
input me(s)			and the second se	a sector
	Browse file of filename	s Import Image(s)		and the second sec
/Users/michael/Workspace/marin_virus1/	test/my_image_1.tif	─────────────────────────────────────	E States	15 A
/Users/michael/Workspace/marin_virus1/	test/my_image_2.tif	© Cut out central part of image(s)		14 A 14
		Width (400) 320 Height (400) 320		A CONTRACTOR OF
		O Cut out general		
		Width (400) 320 Height (400) 320		
		Upper left coord. X 40 Y 40		
I		Cut Images	Strange Con	
Output file				
my_images		—		
Output file with cut out images			1	2
my_images_cut		-		
-				
IMAGIC output Display controls				
	Histogram of glo	al densities		
400 :				
	. بينغالغانسين			
200 -	يتبالا المقربين	••••••••••••••••••••••••••••••••••••••		
	المتحديد			
-30000 -20000	-10000	10000 20000 30000		
Minimum used: -25509.6		Maximum used: 17559.8		
Grey value scaling		Zoom: 0.84622 (92 %)		
O Min/max O Interactive				
Contrast		Gallery		
Local     O Gallery		© On O Off		
Inverse contrast		mage location: 1 of 2 Show location		
O On Off				

#### **DESCRIPTION:**

Convert import image 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.

Also refer to program guilMPORT.



#### **IMPORT**:

Specify the file format in which your input images/3D volumes/spectra are stored. Click the "Select format" button

Select format	$\nabla$
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and choose one of the formats in the listing.

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

Input Images		
File format	TIFF V	In case of type conflicts © Threshold densities  © Change type
Input file(s)	Browse files	O Shift densities O Scale densities
		mport File Chooser
	Show: *.tif	▼ Favorites ∇
	/ my_image_1.tif	MM
	my_image_2.tif	
Output file		
Output file with cut out images		
	Preview Show hidd	en files
	Filename: /Users/micha	ael/Workspace/marin_virus1/test/my_image_2.tif
IMAGIC output Display controls		OK <



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

/Users/michael/Workspace/marin_virus1/test/my_image_1.tif /Users/michael/Workspace/marin_virus1/test/my_image_2.tif

Next, you need to specify the name of the output file which is the IMAGIC image file which will contain the imported images/3D volumes/spectra.

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

Format TIFF, for example:

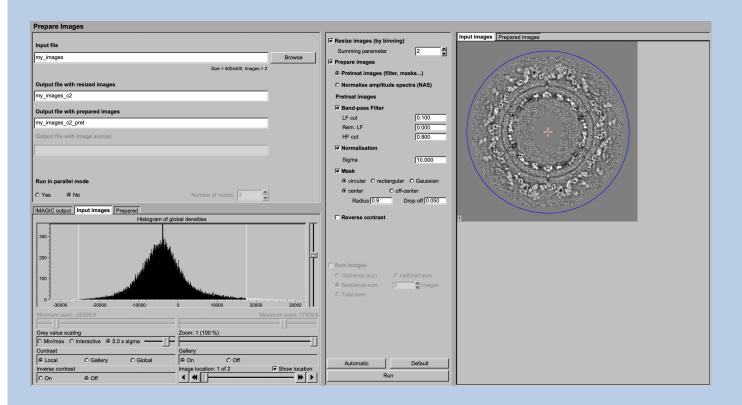


Having specified every information needed click the "Import" button to start the import of the images.

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



# The "Prepare Images" Page



#### **DESCRIPTION:**

It can be helpful to pre-treat the input images by imposing a band-pass filter, normalise the variance, impose a mask and...

#### NOTE:

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



#### You can resize the images by binning

Resize images (by binning)		
Summing parameter	2	

You can pre-treat the images. Options are

Prepare images	
Pretreat images (filter, mas	sks)
O Normalise amplitude spec	tra (NAS)
Pretreat images	
Band-pass Filter	
LF cut	0.100
Rem. LF	0.000
HF cut	0.800
Normalisation	
Sigma	10.000
Mask	
o circular O rectangular	O Gaussian
center     O off-ce	nter
Radius 0.800 Dr	op off 0.050
Reverse contrast	

#### Options are band-pass filtering

Band-pass Filter	
LF cut	0.100
Rem. LF	0.000
HF cut	0.800



#### normalise the variance in each image

	Normalisation	
	Sigma	10.000
mp	osing a mask	
	Mask	
	<ul> <li>circular</li> </ul>	O rectangular
	<ul> <li>center</li> </ul>	O off-center
	Radius 0.800	Drop off 0.050

if wanted you can also reverse the contrast.

Reverse contrast

Instead of using these pre-treatment options you can apply a NAS filter which means that the amplitude spectra of the images are normalised:

Prepare images		
O Pretreat images (filter, masks)		
Normalise amplitude spectra (NAS)		
☐ Mask		
	O rectangular	
<ul> <li>center</li> </ul>	O off-center	
Radius 0.800	Drop off 0.050	
Reverse contrast		
NAS Filter		
LF cut	0.050	
Rem. LF	0.200	
HF cut	0.300	

Imposing a mask and reversing the contrast are also options here.





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

Input file		
my_images		Browse
	Size = 400x400, Images = 2	
Output file with resized images		
my_images_c2		
Output file with prepared images		
my_images_c2_pret		

Start the calculations by clicking the "Run" button:

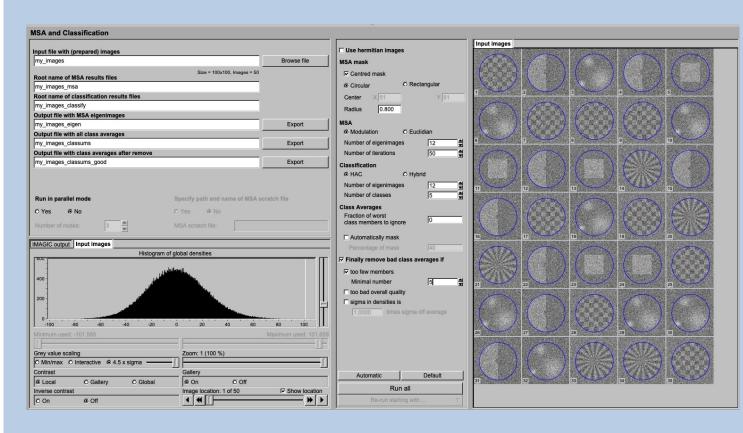


You can also create various image sums which you may need for the subsequent calculations of the global and local information content in your images.

Sum images		
Odd-even sum	O Half-half sum	
O Sequence sum	2 dimages	
O Total sum		
Output file with image sum(s)		
my_images_c2_pret_odd_even_sur	m	



# The "Multi-Reference Alignment" Page



#### **DESCRIPTION:**

The **guiALIGN** aligns images to a stack of references (multi-reference alignment ).

If wanted references can be selected from a stack of images or can be generated as forward projections from (a) 3D volume(s).

FULL IMAGE DATA-SET ANALYSIS:

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



To align the particle images you need references. In principle, there are three options to get these references:

#### GET REFERENCES FROM A 3D VOLUME:

The first option is to generate the references from a 3D volume which is related to the images to be aligned.

Choice of references			
Get from 3D volume			
O Select from images			
O Get from file			
Input 3D volume file my_3d	Size = 64x64x64, Volumes = 5	Import	Browse
Output file with generated references			
my_3d_ref		Export	

The advantage is that you can get all possible directions (all possible "views") and that the references are perfectly (3-D) aligned to each other. So, when using this option do NOT centre or align these references any more.

As usual, specify the input file containing the 3D volume(s) to be used the generate the references as well as the name of the references output file.

Please also specify the number of references/forward projections wanted and the threshold value to be used for the forward projections. I wanted, you can also generate mirror references.

Number of references	10
Threshold	0.01
Also use mirror views	



There are three ways to calculate the forward projections:

The projection direction to create the references are chosen ad random:

Mode of projection

Random

- O Asymmetric triangle
- O Tomography

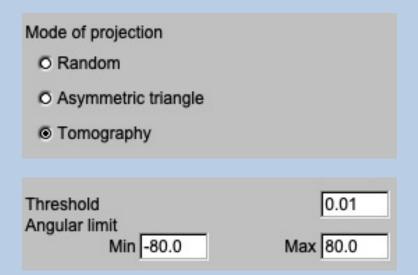
The references can re-present all typical views within the asymmetric triangle )in case the object has a certain 3D point-group symmetry):

Mode of projection

O Random

- Asymmetric triangle
- O Tomography

In certain cases you may want forward projection in an angular range around the equator of the object:



Click the "Generate references" button to generate the references:

Generate references



Check the references in the display tab on the right hand side. If needed, change the parameters and re-generate the references.

If the references are alright click the "Continue with alignment" button

Continue with alignment

and the page will skip to the "Get from file" option to run the alignment.

#### SELECT REFERENCES FROM A STACK OF IMAGES:

The second option is to select the references from a stack of images (usual "typical" and noise-free images):

	Import	Browse
Size = 100x100, Images = 50		
	Size = 100x100, Images = 50	

As usual, specify the input file containing the 3D volume(s) to be used the generate the references as well as the name of the references output file.

Now check the images in the display tab on the right hand side and select the wanted references (all typical views with good contrast, if possible) by clicking into the image. A second click will de-select it.

Having chosen all wanted images click the "Generate references" button to extract them and generate the references image file:

Generate references



Check the references in the display tab on the right hand side. If needed, select or de-select images and re-generate the references.

If the references are alright click the "Continue with alignment" button and the page will skip to the "Get from file" option.

Continue with alignment

and the page will skip to the "Get from file" option to run the alignment.

NOTE: This option bears the risk of reference bias!

GET REFERENCES FROM FILE:

If your reference images are already stored in an IMAGIC image file use the "Get from file" option to run the the multi-reference alignment

#### **Choice of references**

- O Get from 3D volume
- O Select from images
- Get from file

As usual, specify the input file containing the images to be aligned, the "original" images and the references. Also give the name of the output file to contain the aligned images.

Input images to be aligned		
my_images	Import	Browse
Size = 100x100, Images = 50	)	
my_images	Import	Browse
Size = 100x100, Images = 50	)	
my_ref	Import	Browse
Size = 100x100, Images = 5 Output file with aligned images	5	
my_images_ali	Export	

Note:

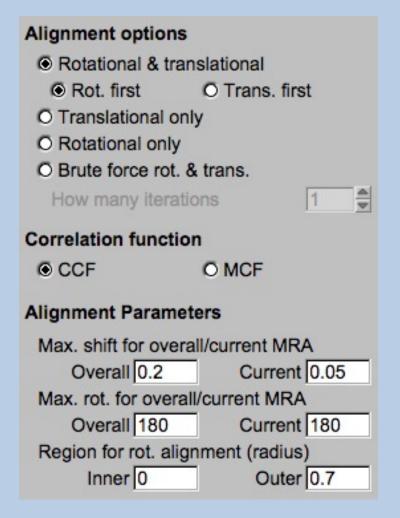
In case the input images are already moved (in a previous **IMAGIC** alignment, for example) the "original" non-moved images will be used for an "equivalent move" to avoid multiple interpolation artefacts.

If your references were generated from multiple 3D volumes it can be a good idea to align each particle image either to all references or to its own 3D volume references only. It is you choice:

#### Reference option

- Align to all references
- O Align to own 3D references only

There is a number of parameters which you can specify to adjust the alignment::



You can get detailed help by moving the cursor over the related boxes.



You can get help by moving the cursor over the related boxes.

Do not forget to strongly suppress the high frequencies during alignments to avoid bias and over-fitting:

Filter refere	ences		
• Low-pass	filter		
HF cut	0.7		
Filter refere	nces		
O Low-pass	filter		
HF cut	0.7		
O Band-pass filter			
LF cut	0.10	HF cut	0.10
Out-off high frequencies			
HF cut off	0.7	Drop off	0.1

Run the alignment and check the printout in the terminal window as well as the aligned images in the display on the right-hand side.

If necessary, change parameters and re-run the alignment,





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.





We intensively tested the **guiALIGN** 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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