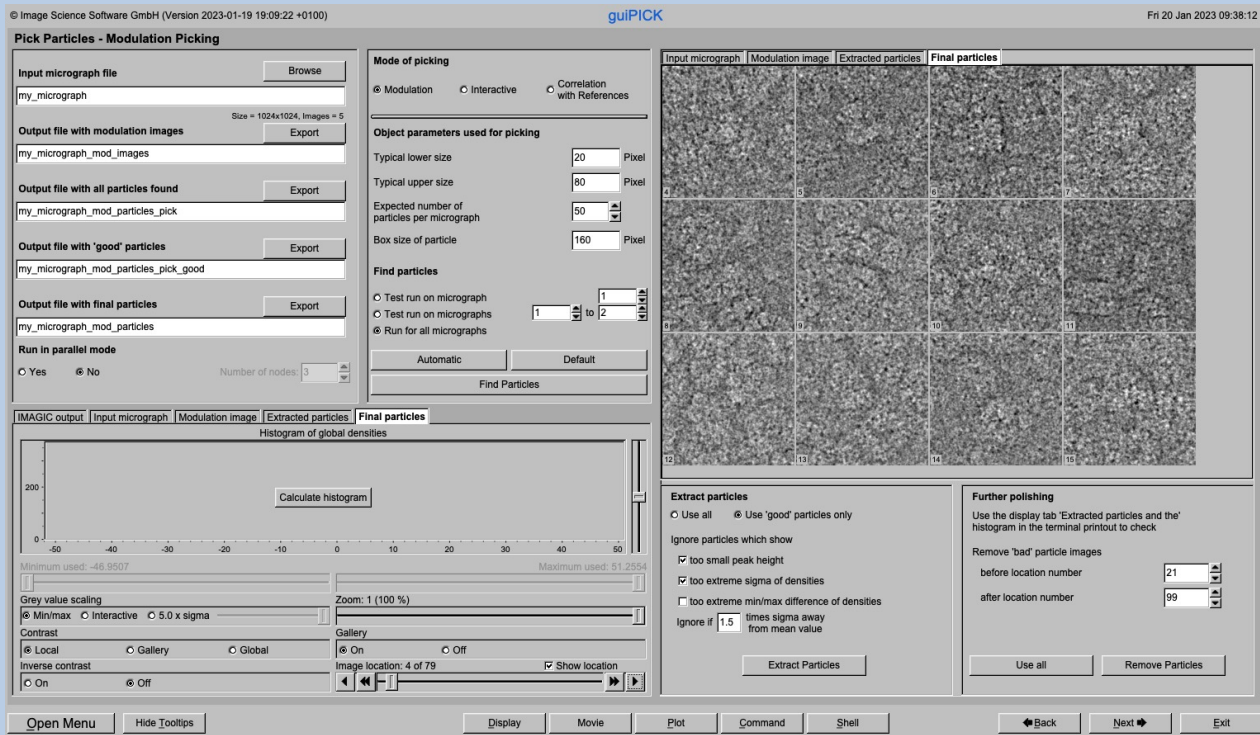


A Brief Introduction

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



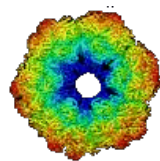
The **guiPICK** program follows a work-flow from Import Micrographs to various pages to search and extract particles from input micrographs.

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

CONTENT:

- IMAGIC GUI programs
 - **guiPICK**
 - > Import Micrographs
 - > Prepare Micrographs
 - > Pick Particles: Modulation Search
 - > Pick Particles: Interactive Search
 - > Pick Particles: Get References from Modulation Search Search
 - > Pick Particles: Prepare Correlation Search References
 - > Pick Particles: Correlation Search
 - Error hints
- How to use IMAGIC GUI programs
How to search and extract particles
- How to send us feedback





IMAGIC

GUI Programs

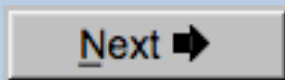


Workflow

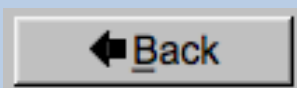
The idea of **guiPICK** is to guide you through a typical camera/detector correction measurement or camera .

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

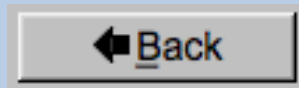
If **guiPICK** is called from the programs list, by using an icon or in a command line the working directory will be your default system directory.

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

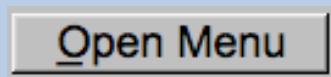
```
IMAGIC-COMMAND : guiPICK
```

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 **guiPICK**.

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

Input files can be chosen from other directories.



Help

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

Output file:

whgb_micrograph

Name of the output IMAGIC file containing the imported micrographs.

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

Select format ▼

In case of type conflicts

Select the input file format.

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

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

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



Input Files

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

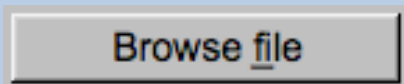
You can, of course, always use other input files names and even use other input directories.

Input file with (raw) micrographs	Browse file
my_micrographs	

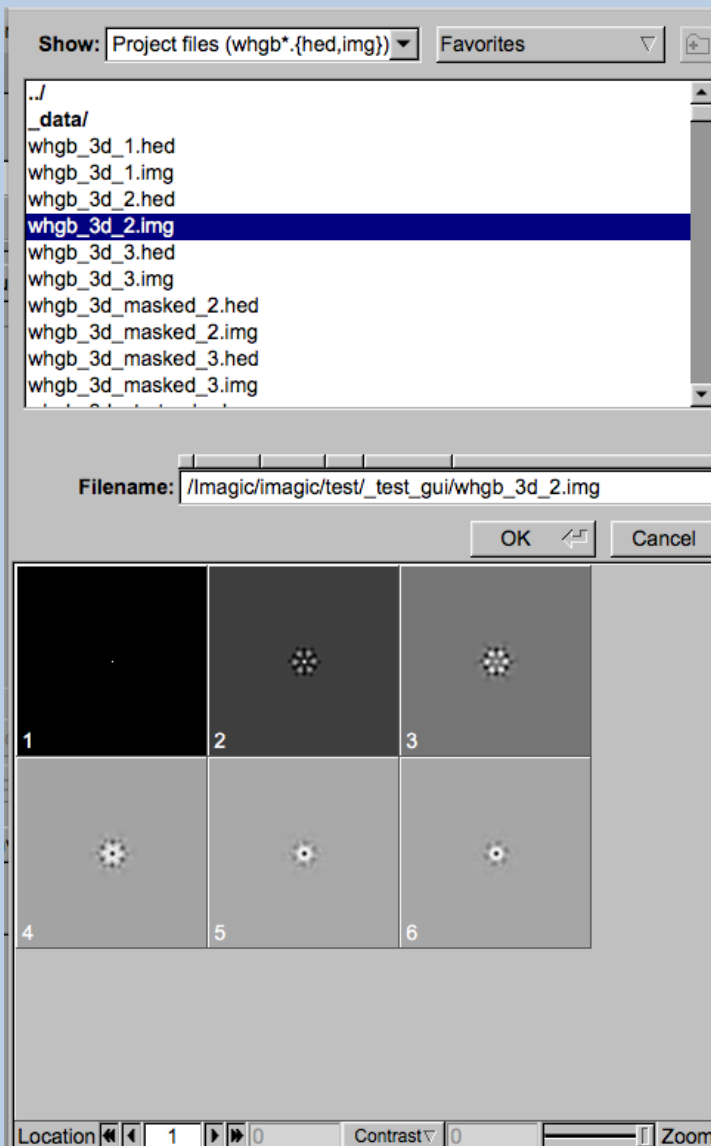


Input File Chooser

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

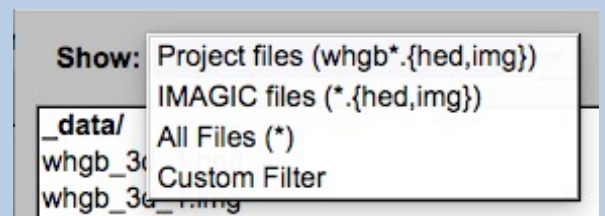


Pressing this button will open the IMAGIC file chooser:

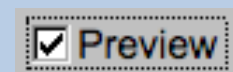


Choose the wanted file by clicking its name

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



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



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



Output Files

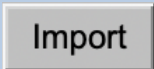
Usually the names of the output files are suggested but it is your choice, of course. On each page you can specify these output file names on the left hand side.

Output file	Export
my_micrographs	

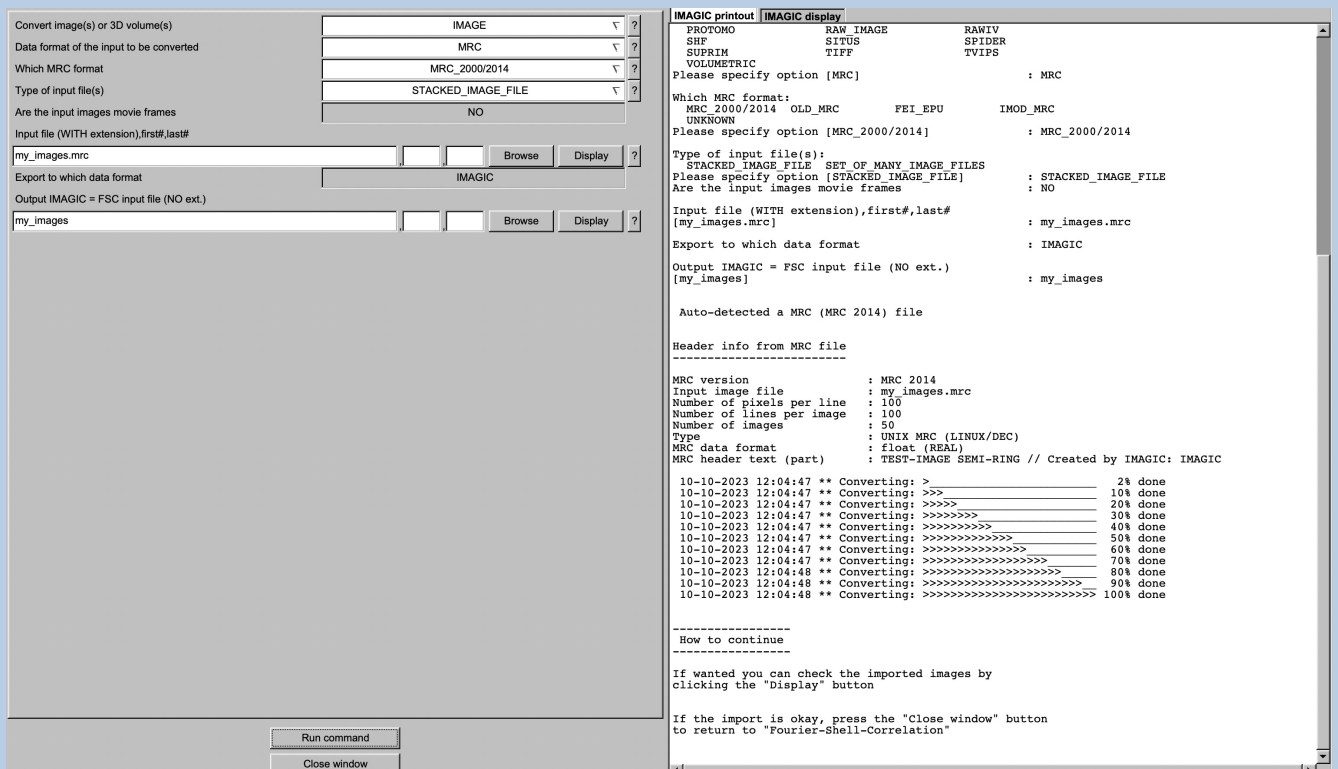


Import Buttons

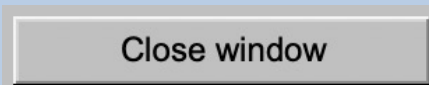
You do not want to use the “Import page” you can sometimes use an “Import” button to import the input images/3D volumes from any 3DEM format. The “Import” button which is located above the text field specifying the name of the related file.



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



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



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



Export Buttons

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

Export

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

The screenshot displays the 'Export' dialog box on the left and the 'IMAGIC EM2EM' command window on the right. The dialog box contains the following fields and options:

- Convert 2D image(s) or 3D volume(s): 2D_IMAGE
- Data format of the input to be converted: IMAGIC
- How are the input images available: UNKNOWN_IMAGE_FILE
- Are the input images movie frames: Yes No
- Input file, image loc#s: my_images
- Export to which data format: TIFF
- Type of output TIFF image(s) wanted: GREY_SCALE_IMAGE
- Type of output file: STACKED_IMAGE_FILE
- Output file, loc#s (WITH ext.),first#,last#: my_images.tif
- Always scale densities to the output format: Yes No

The 'IMAGIC EM2EM' window shows the following command text:

```
Convert 2D image(s) or 3D volume(s):
 2D_IMAGE 3D_VOLUME
Please specify option [2D_IMAGE]          : 2D_IMAGE

Data format of the input to be converted:
BROOKHAVEN_STEM  CCP4          DATA_ONLY
DICOM             DIGITAL_MICROGRAPH EM
FEI              FABOSA       FORMATTED
IMAGIC           JPEG         KONTRON
MDPP             MEDIPIX      MRC
OFFSET           PIF          PGM
PROTOMO          RAW          SHF
SMV              SPIDER       SUPRIM
TIA/EMI/SER     TIFF         TVIPS
Please specify option [IMAGIC]           : IMAGIC

Type of input file:
SINGLE_IMAGE_FILE STACKED_IMAGE_FILE UNKNOWN_IMAGE_FILE
Please specify option [UNKNOWN_IMAGE_FILE] : UNKNOWN_IMAGE_FILE

Are the input images movie frames [NO]
Input file, image loc#s [my_images]      : my_images

Export to which data format:
CCP4          DATA_ONLY  EM
FORMATTED    FEI_RAW_IMAGE IMAGIC
JPEG_GREYSCALE KONTRON  MDPP
MRC          OFFSET     PIF
PGM          POSTSCRIPT  PROTOMO
RAW          SHF         SMV
SPIDER       SUPRIM      TIFF
TVIPS
Please specify option [TIFF]             : TIFF

Type of output TIFF image(s) wanted:
COLOUR_IMAGE GREY_SCALE_IMAGE
Please specify option [GREY_SCALE_IMAGE] : GREY_SCALE_IMAGE

Type of output file:
STACKED_IMAGE_FILE SET_OF_MANY_IMAGE_FILES
Please specify option [STACKED_IMAGE_FILE] : STACKED_IMAGE_FILE

Output file, loc#s (WITH ext.),first#,last#
[my_images.tif]    : my_images.tif

Always scale densities to the output format [YES] : YES

Image name: MOVIE SUM FROM whgb.c4.img (7 IMAGES) (PREPARE)
Size: 200, 200 Loc: 1 Type: REAL Cre.Date: 26-Jan-2023 Time: 11:16:03
EMEM;EXCOPY/PLT;EXCOPY/SELECT;CAMERA NORM;INCDMENU/ANISOTROPIC MAGNIFY=1.0,1
.026;COARSE;ALIDIR;COARSE;SUMMER/MOVIE SUM;INCDMENU/PREPARE/BP LOW=0.02 TRANS
=0.0 HIGH=0.9;CTF2D_FLIP;CUT_IMAGE/APERIODIC;
```

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

Close window

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



A Typical Page

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

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

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

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

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

Camera Correction

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

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

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

Output file with camera corrected micrographs
my_micrographs_cnorm

Output good camera corrected micrographs
my_micrographs_cnorm_good

Camera Normalisation

Measure
 Correct
 Measure and Correct

Input Micrographs | Corrected Micrographs | Average | Sigma

Extract micrographs
 Use all
 Use 'good' micrographs only

Ignore micrographs which show
 too extreme sigma of densities
 too extreme min/max difference of densities
Ignore if 1.5 times sigma away from mean value

IMAGIC output | Micrograph | Corrected | Average | Sigma

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

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



A Typical Page - MPI Parallel

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

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



A Typical Page - Program Parameters

Mode of preparation

Pretreat images

Normalise amplitude spectra (NAS)

Pretreat images

Band-pass Filter

LF cut

Rem. LF

HF cut

Normalisation

Sigma

Mask

Radius

Drop off

Test loc. # to

Run for all particles


Centre particles

Self rotate Self

Total sum Mass center

Test loc. # to

Run for all particles



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

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

Self rotate Self



Total sum Mass center

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

Band-pass Filter

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

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



A Typical Page - Automatic / Default

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

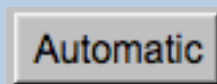
Resize/Coarsen prepared micrographs
Summing parameter 2

Automatic Default

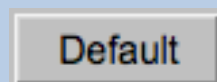
Run

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

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



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



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



A Typical Page - Run buttons

Create prepared amplitude images

Filter micrographs

Low freq. cut

Remaining low frequency

High freq. cut

Filter amplitude images

Low freq. cut

Remaining low frequency

High freq. cut

Coarsen filtered amplitude images

Yes No

Summing parameter

MSA options

MSA eigenfilter amplitudes

MSA classify amplitudes

MSA

Inner radius of ring mask

Outer radius of ring mask

Number of eigenimages

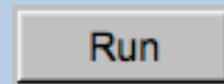
Number of iterations

Classification

Use how many eigenimages

Number of classes

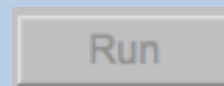
To run the calculations press the “Run” button.



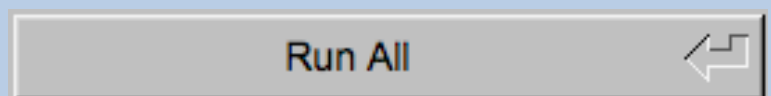
On a number of pages the calculations can be split. In this case you will find more than one single “Run” button.

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

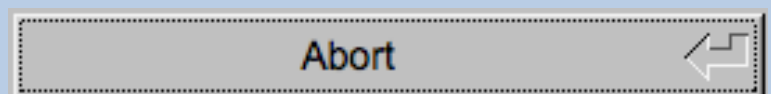
Maybe a certain “Run” button is not yet activated because it needs the results of calculations not yet done.



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

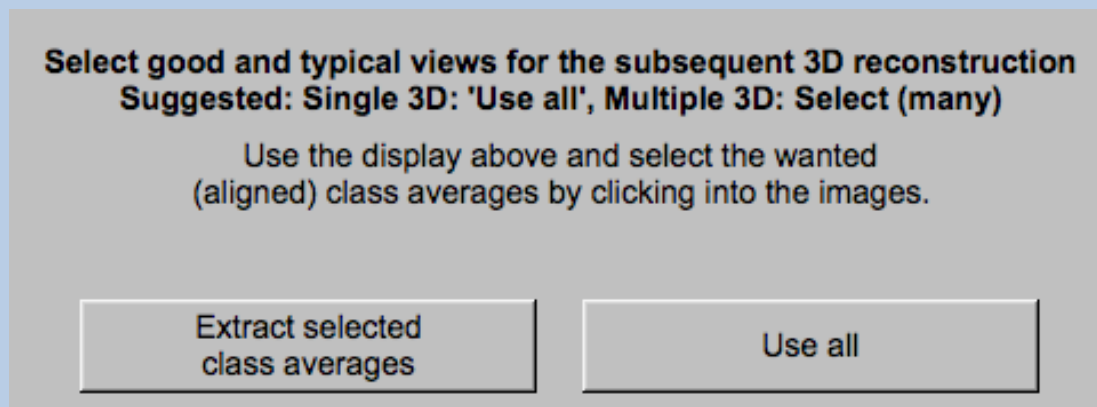


You can abort a running program by pressing the “Abort” button.



A Typical Page - Additional Tasks

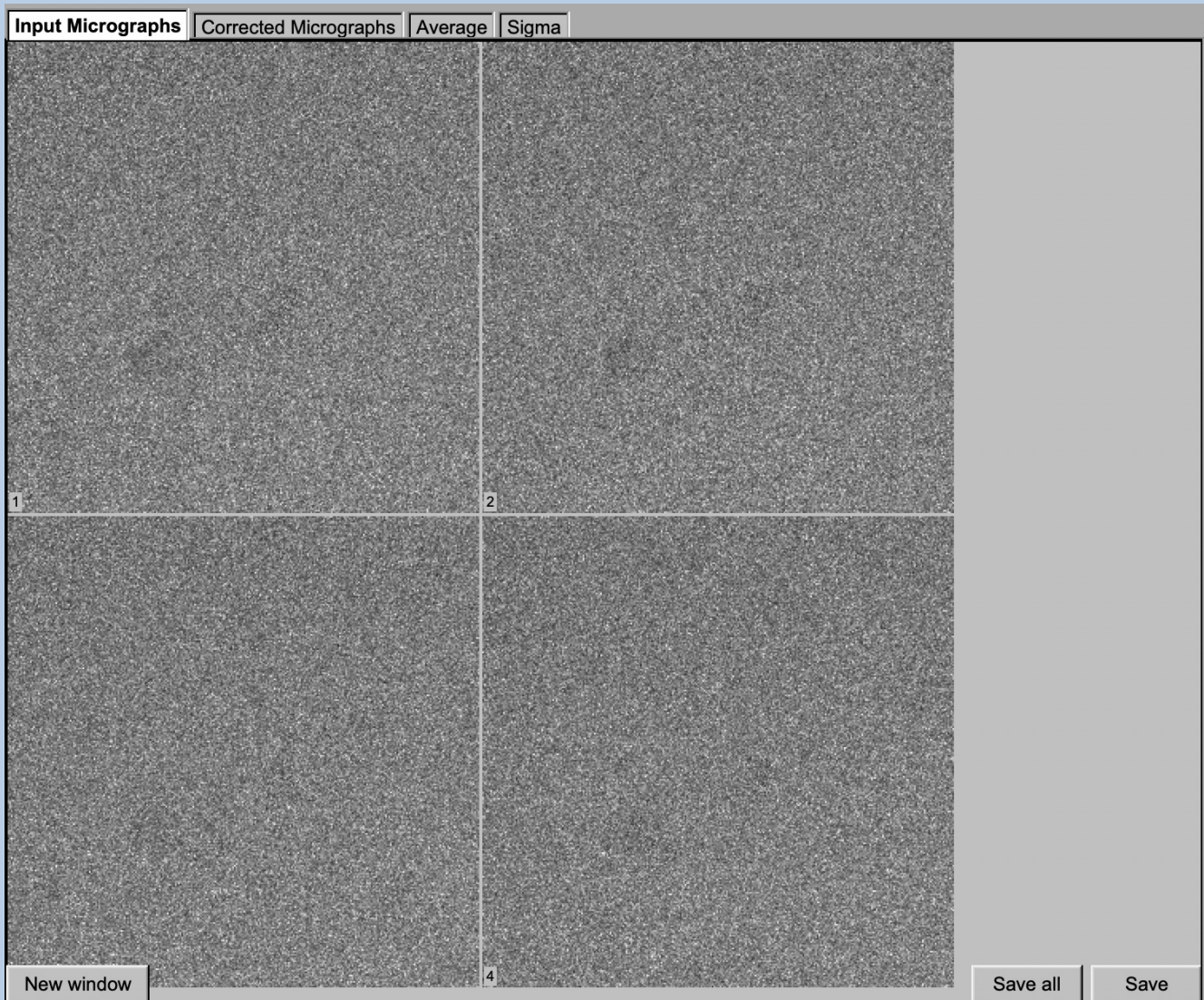
The main calculations on the page are done using the middle part of an typical **IMAGIC GUI program** page. But on a number of pages some additional calculations have to be done. Please follow the instructions given.



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



A Typical Page - Display



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

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

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

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



A Typical Page - “Display Control” Tabs

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

Grey value scaling: Adjust the contrast

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

Interactive: Set the limits by giving numbers

Sigma: Use an amount of sigma to set the limits

Contrast

How to calculate the grey value scaling

Local: Calculated in each image separately

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

Gallery: Calculated in the currently displayed images

Inverse contrast:

Use one of the radio buttons

Zoom

Enlarge the displayed images

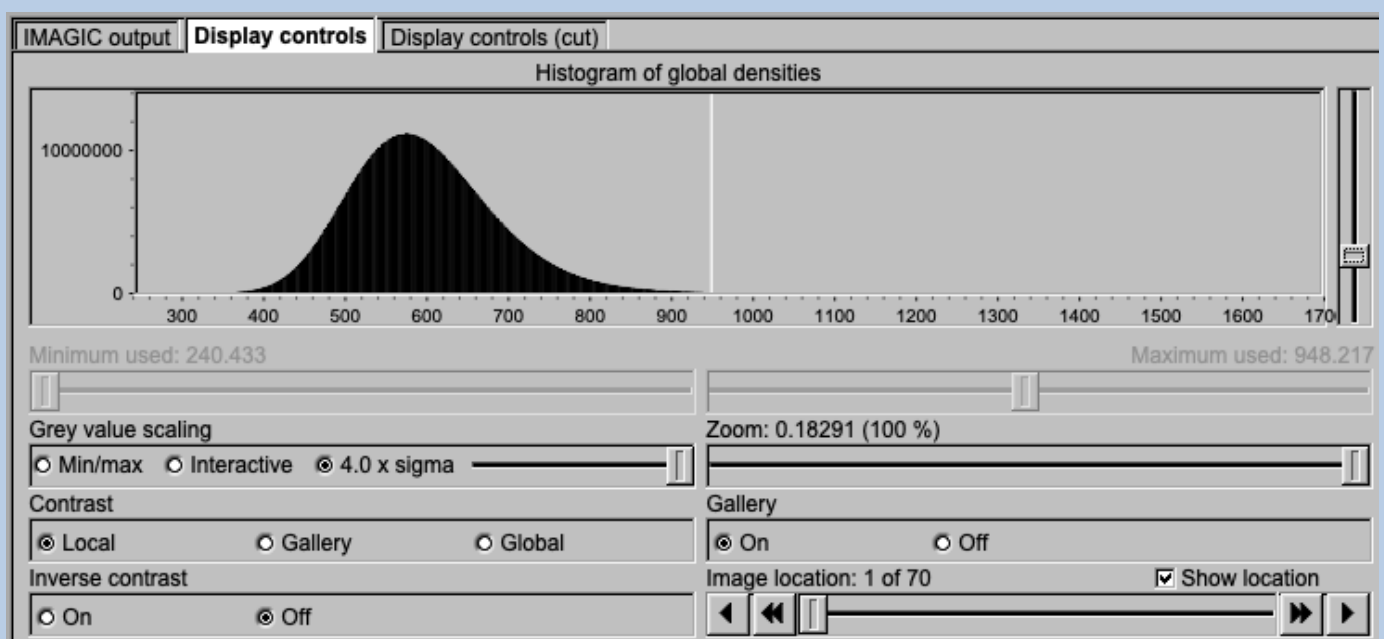
Gallery

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

Off Show only one image

Image Locations.

Use the slider or the arrows to select image locations



A Typical Page - “Plot Control” Tabs

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

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

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

Plot title Set the text of the plot title

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

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

Reset:. Reset to the automatic values

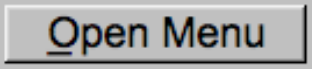
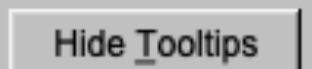
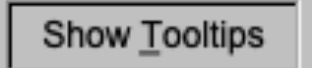
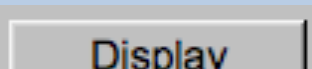
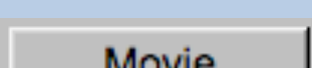
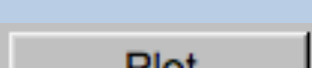
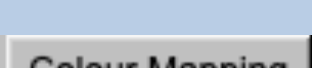

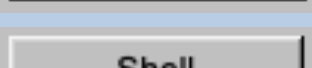
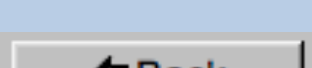
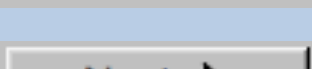
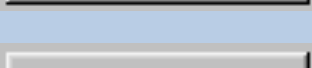
Style Select curve style ▾	Colour Select curve colour ▾	Grid Select curve grid ▾
Horizontal scaling 1.00	<input type="checkbox"/> Use for all plots 32.00	Reset
Vertical scaling -19.21	<input type="checkbox"/> Use for all plots 17.00	Reset
Plot title Fourier Ring Information - 1/2-bit	<input type="checkbox"/>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



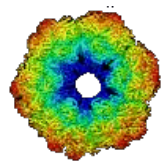
A Typical Page - The Toolbar

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

The toolbar buttons:

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



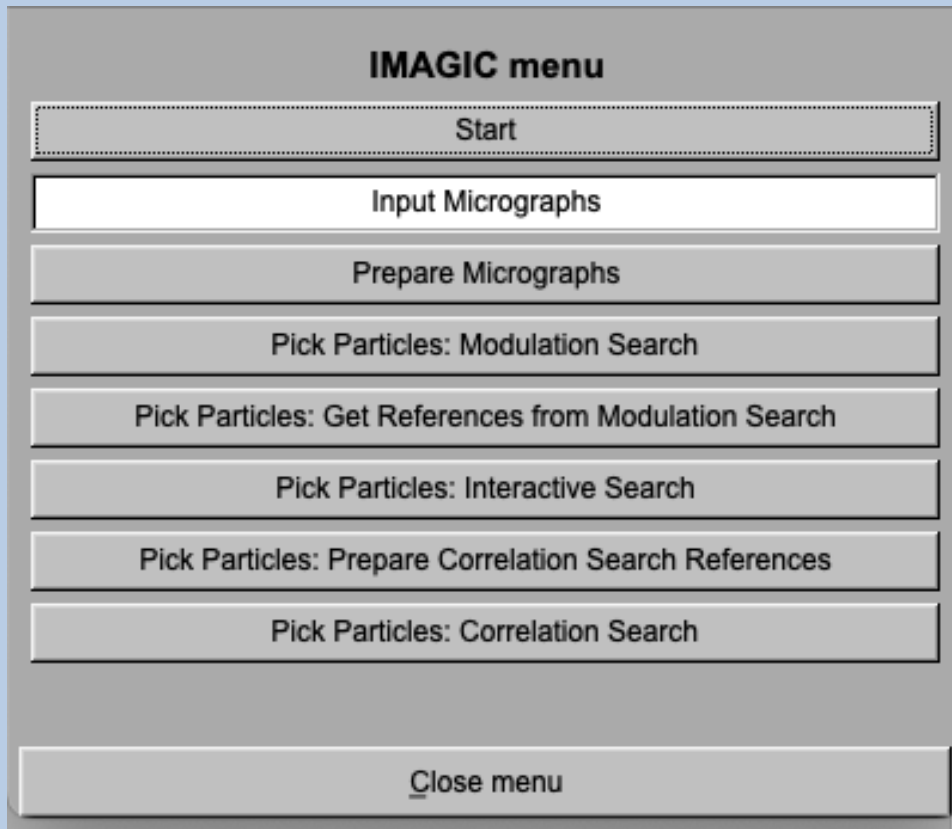


IMAGIC

guiPICK



The guiPICK Menu



PAGES:

guiPICK:

Import Micrographs:	Convert micrographs into IMAGIC format
Prepare Micrographs:	Pre-treat Micrographs
Modulation Search:	Modulation search and extract particles
Interactive Search:	Interactively search and extract particles
Get References from Modulation Search:	Select references from modulation search
Prepare Correlat. References:	Prepare references for correlation search
Correlation Search:	Correlation search and extract particles

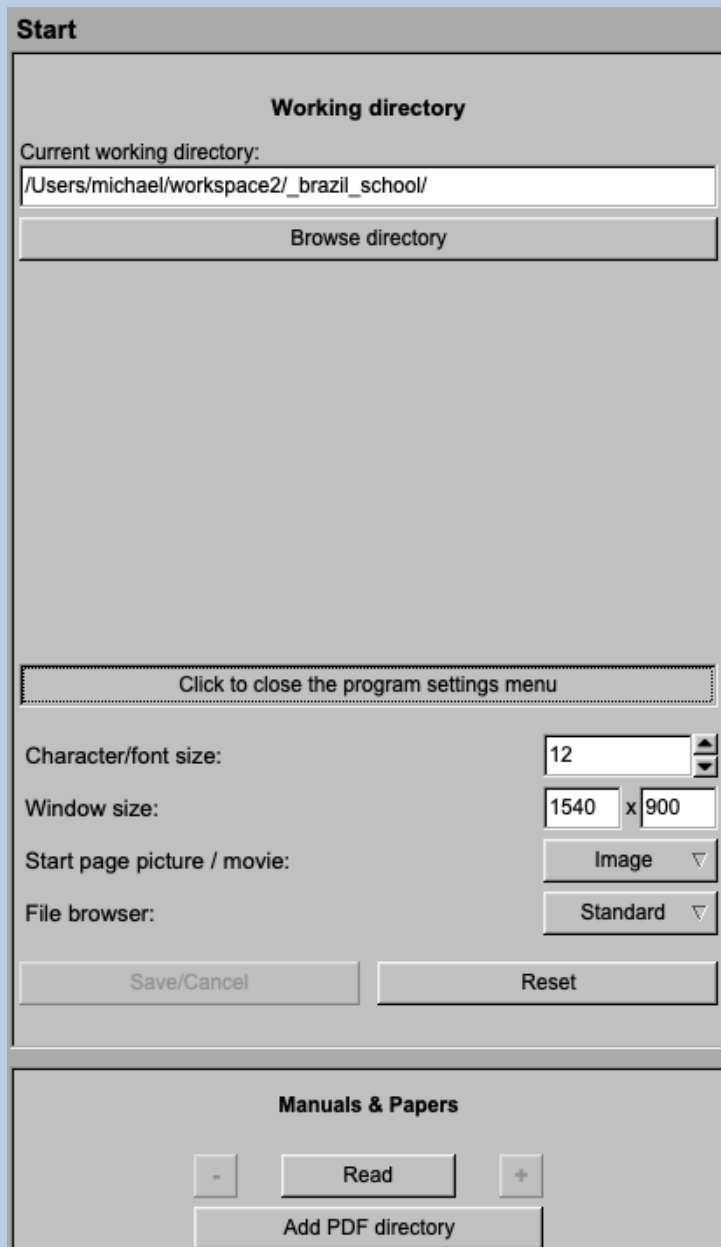
General:

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



Start Working

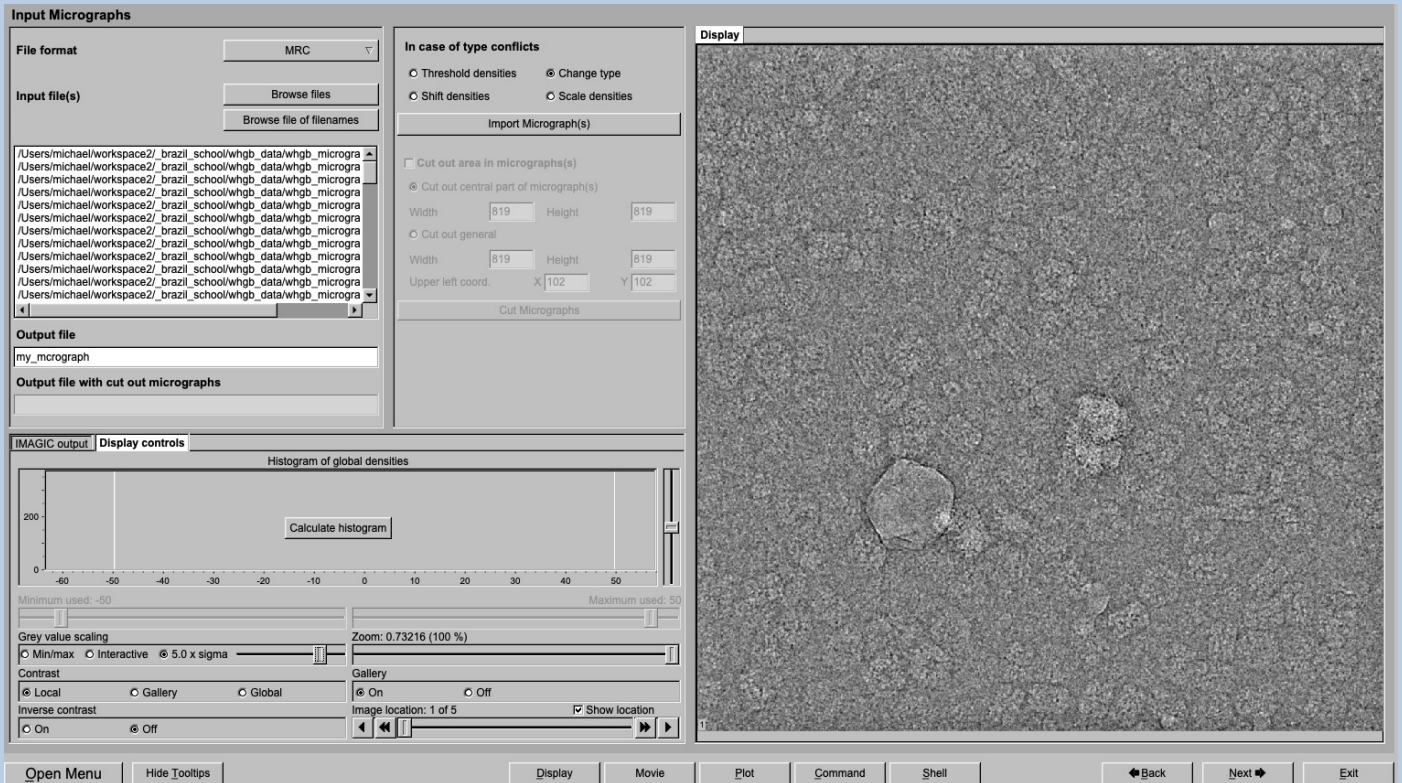
The page **guiPICK** starts with the “Import Micrograph” page.

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

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



The “Import Micrographs” Page



DESCRIPTION:

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

The page can be skipped if your input images are already stored in IMAGIC format and if all electron microscopy parameters are already stored in the input headers.

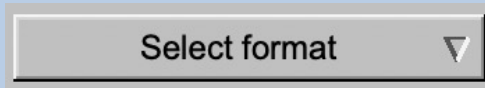
If wanted you can cut-out parts of the input images. Not suggested for CTF correction.

Also refer to program **guiIMPORT**.



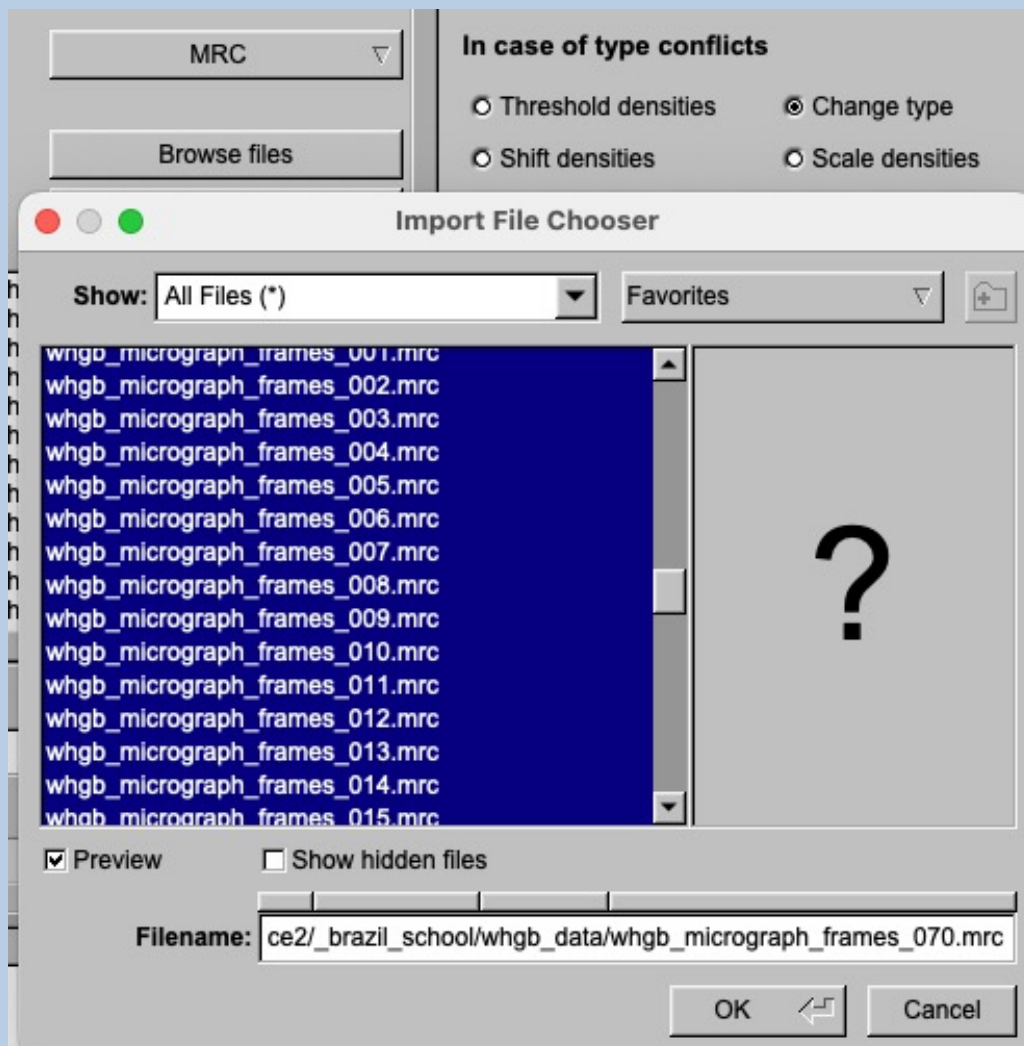
IMPORT MICROGRAPHS:

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

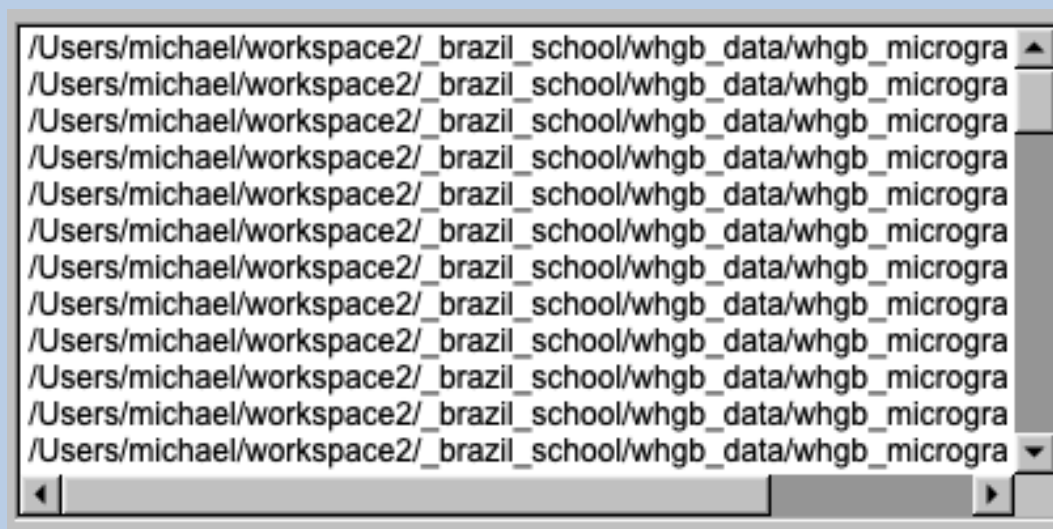


and choose one of the formats in the listing.

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



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



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

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

Format MRC, for example:



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

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

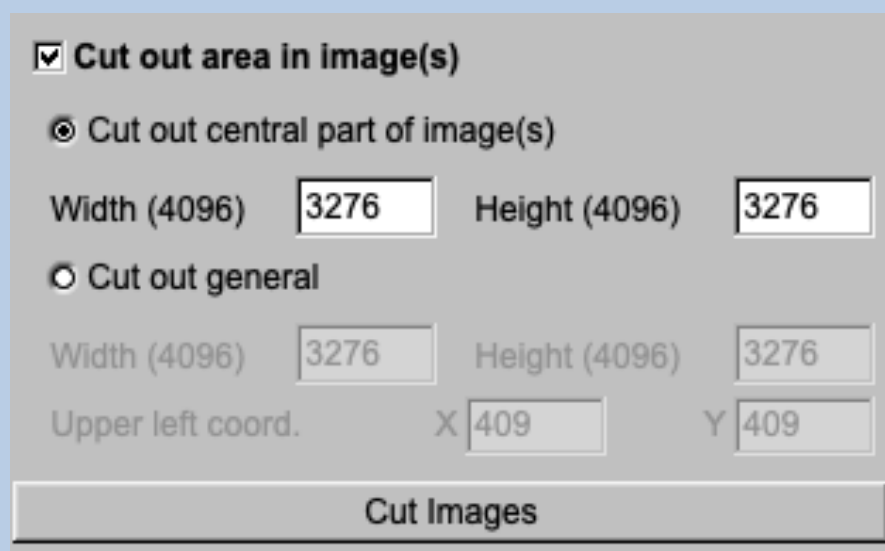


CUT MICROGRAPHS / IMAGES

(not suggested in **guiPICK**):

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

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



Cut out area in image(s)

Cut out central part of image(s)

Width (4096) Height (4096)

Cut out general

Width (4096) Height (4096)

Upper left coord. X Y

Cut Images

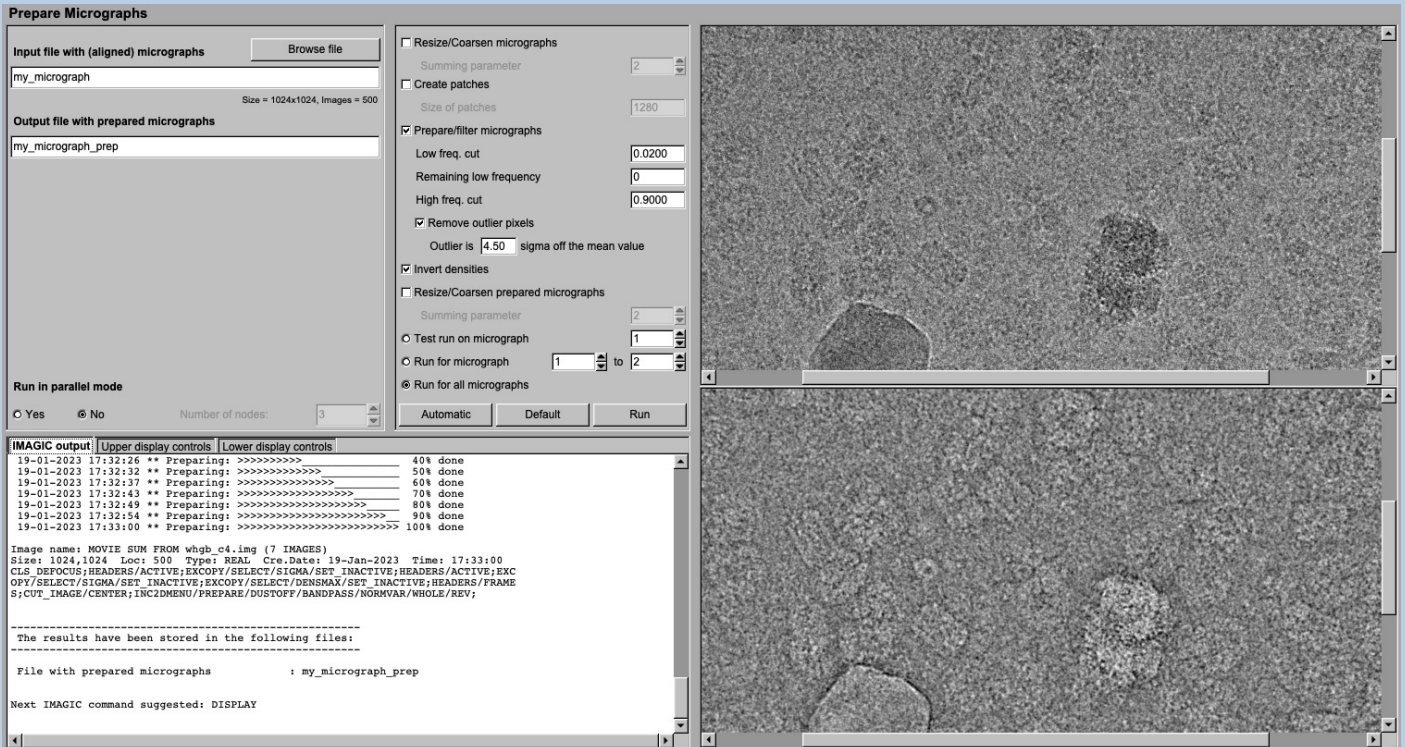
The chosen part is shown in the display window. You can cut-out a central part or any part wanted. The cut-out part is the same in all images, of course.

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

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



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

Resize/Coarsen micrographs

Summing parameter

or create patches

Create patches

Size of patches

to speed up the CTF calculations.

Imposing a band-pass filter is always suggested:

Prepare/filter micrographs

Low freq. cut

Remaining low frequency

High freq. cut

Remove outlier pixels

Outlier is sigma off the mean value

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

Invert densities

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

Resize/Coarsen prepared micrographs

Summing parameter



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

Input file with (aligned) micrographs Browse file

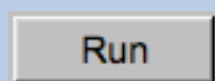
my_micrograph

Size = 1024x1024, Images = 5

Output file with prepared micrographs

my_micrograph_prep

Start the calculations by clicking the “Run” button:



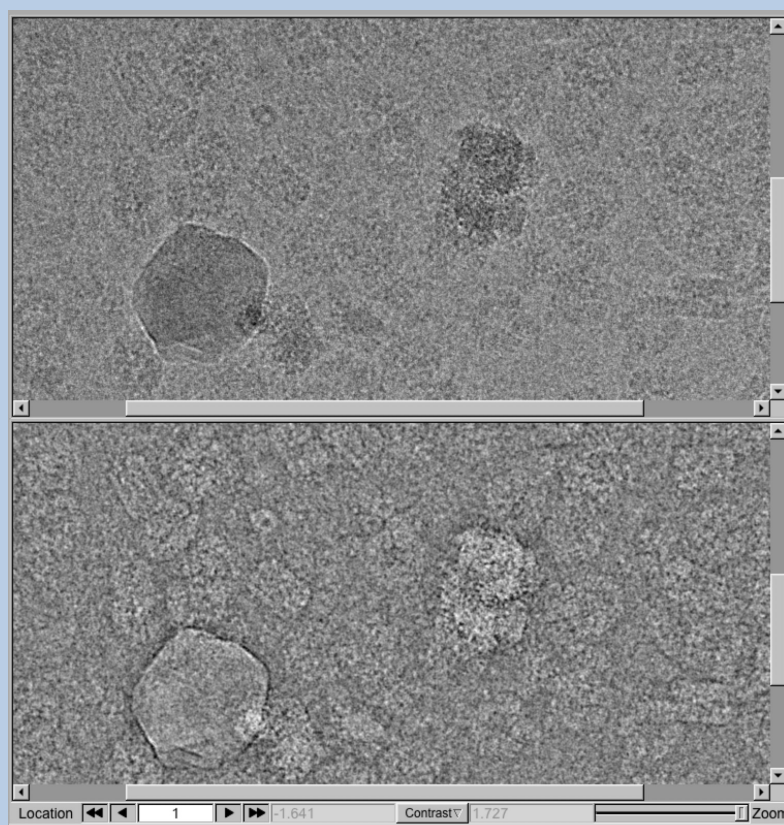
Play around with the parameters running movie alignment on a single or a small range of micrographs.

Test run on micrograph

Test run on micrographs 1 to 2

Run for all micrographs

Check the pre-treated micrographs:



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

Test run on micrograph

Test run on micrographs

Run for all micrographs

1 to 2



The “Pick Particle - Modulation Search” Page

Pick Particles - Modulation Picking

Input micrograph file: Size = 1024x1024, Images = 5

Output file with modulation images:

Output file with all particles found:

Output file with 'good' particles:

Output file with final particles:

Run in parallel mode: Yes No Number of nodes:

Mode of picking: Modulation Interactive Correlation with References

Object parameters used for picking: Typical lower size: Pixel; Typical upper size: Pixel; Expected number of particles per micrograph: ; Box size of particle: Pixel

Find particles: Test run on micrograph; Test run on micrographs; Run for all micrographs; Range: to

Buttons: Automatic, Default, Find Particles

Grid of 15 images: Input micrograph, Modulation image, Extracted particles, Final particles

Extract particles: Use all Use 'good' particles only; Ignore particles which show: too small peak height; too extreme sigma of densities; too extreme min/max difference of densities; Ignore if: times sigma away from mean value

Further polishing: Use the display tab 'Extracted particles and the histogram in the terminal printout to check'; Remove 'bad' particle images: before location number: ; after location number: ; Buttons: Use all, Remove Particles

IMAGIC output: Input micrograph Modulation image Extracted particles Final particles

Histogram of global densities: Minimum used: -46.9507; Maximum used: 51.2554

Grey value scaling: Min/max Interactive 5.0 x sigma

Contrast: Local Gallery Global; Inverse contrast: On Off; Zoom: 1 (100 %); Gallery: On Off; Image location: 4 of 79; Show location

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

DESCRIPTION:

Find particles by modulation search. Extract (cut-out) particle images.



Mode of picking is modulation search:

Mode of picking

Modulation Interactive Correlation with References

But, if course, you can choose an other mode of picking which will lead you to another picking page.

In modulation search, specify some parameters. Move the cursor over any input box to get help.

Object parameters used for picking

Typical lower size	<input type="text" value="20"/>	Pixel
Typical upper size	<input type="text" value="80"/>	Pixel
Expected number of particles per micrograph	<input type="text" value="50"/>	<input type="button" value="▲"/> <input type="button" value="▼"/>
Box size of particle	<input type="text" value="160"/>	Pixel

First search particles in a single or in a small number of micrographs to check the parameters:

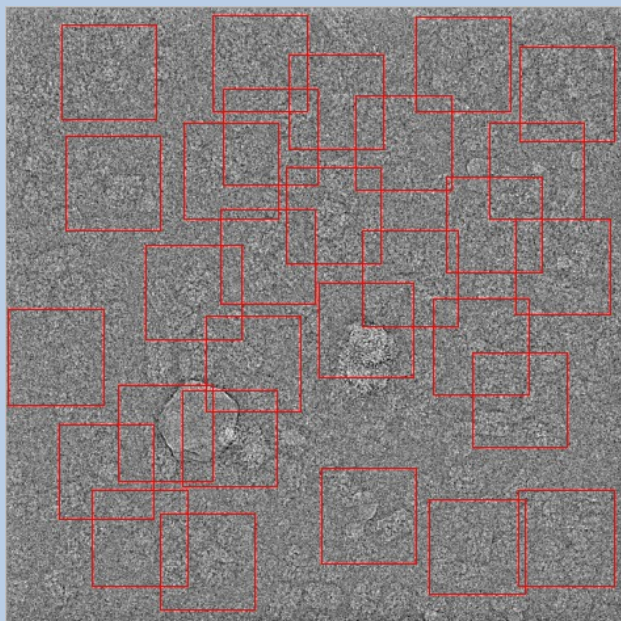
Test run on micrograph
 Test run on micrographs to
 Run for all micrographs

Click the “Find Particles” button to start the modulation search:

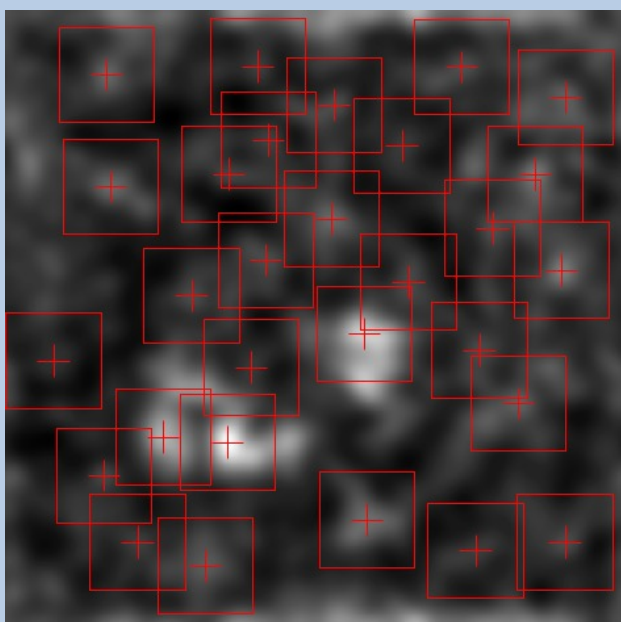
Play around with the search parameters and compare the results.



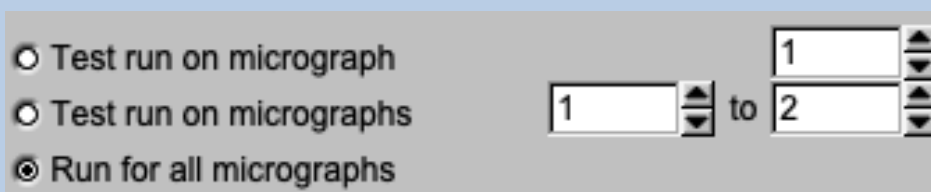
Check the particles found in the displayed micrographs:



Also have a look at the modulation images:



Having found the best search parameters run the modulation search for all micrographs.



Now the particles can be extracted. Particles found using modulation search usually contain a lot of junk (ice, carbon foil, clumped particles). In a first approach, they can be sorted out by looking at the statistics of the picked particles:

Extract particles

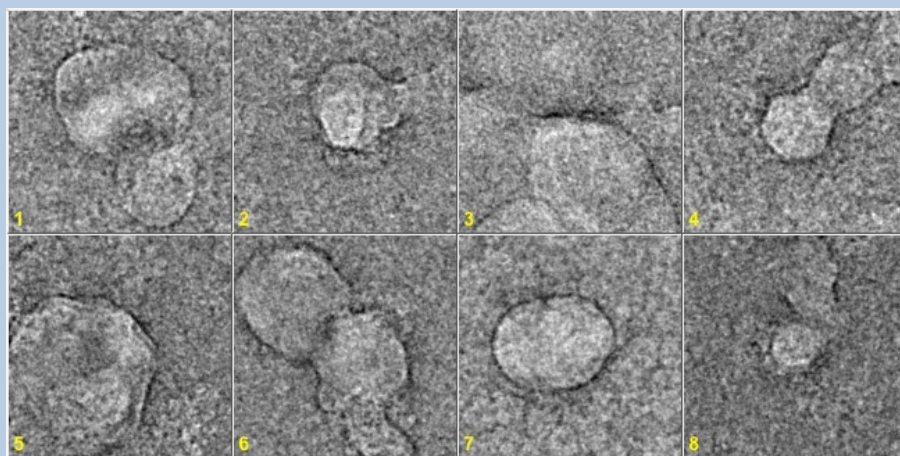
Use all Use 'good' particles only

Ignore particles which show

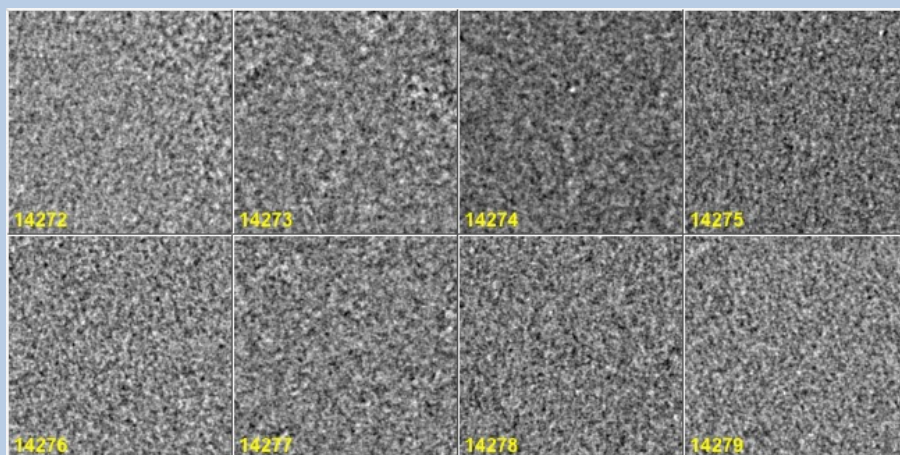
- too small peak height
- too extreme sigma of densities
- too extreme min/max difference of densities

Ignore if times sigma away from mean value

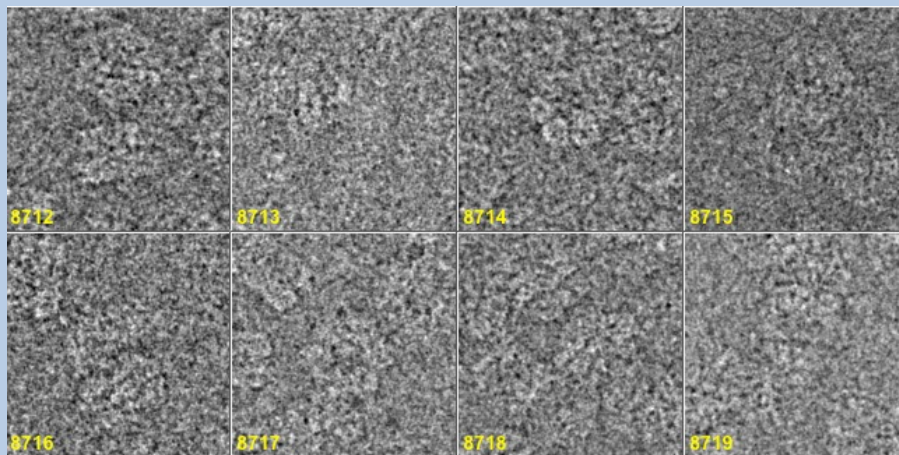
Check the extracted particles displayed on the right-hand side. You can see that the first displayed images show ice blobs etc.



whereas the last images usually contain noise:



The particle images are in between:



Check the extracted particles and write down the location numbers where the good particle images start and where they end.

Also have a look at the histogram printed in the terminal window to get an idea where to find this range of good particle images

```

Histogram of CCCs from whgb_mod_particles_pick
14279      0      0  3.43E-02
14277      2      2  5.01E-02
14258     21     19  5.81E-02
14200     79     58  6.60E-02  *
14081    198    119  7.39E-02  ***
13887    392    194  8.18E-02  *****
13596    683    291  8.98E-02  *****
13089   1190    507  9.77E-02  *****
12326   1953    763  1.06E-01  *****
11215   3064   1111  1.14E-01  *****
9777    4502   1438  1.21E-01  *****
8112    6167   1665  1.29E-01  *****
6502    7777   1610  1.37E-01  *****
5136    9143   1366  1.45E-01  *****
3984   10295   1152  1.53E-01  *****
3117   11162    867  1.61E-01  *****
2460   11819    657  1.69E-01  *****
1998   12281    462  1.77E-01  *****
1640   12639    358  1.85E-01  *****
1360   12919    280  1.93E-01  *****
1137   13142    223  2.01E-01  *****
965    13314    172  2.09E-01  *****
842    13437    123  2.17E-01  ***
706    13573    136  2.25E-01  ***
602    13677    104  2.32E-01  **
518    13761     84  2.40E-01  **
449    13830     69  2.48E-01  **
375    13904     74  2.56E-01  **
316    13963     59  2.64E-01  *
271    14008     45  2.72E-01  *
218    14061     53  2.80E-01  *
188    14091     30  2.88E-01  *
147    14132     41  2.96E-01  *
118    14161     29  3.04E-01  *
102    14177     16  3.12E-01  *
0      14279    102  3.28E-01  **

Low values binned at lower edge : 0
High values binned at higher edge: 97
Meaning of columns: # remaining, # accumulated, # in this bin, bin value
    
```



Specify the first and last image number (location of the 'good' particles images):

Further polishing
Use the display tab 'Extracted particles and the histogram in the terminal printout to check

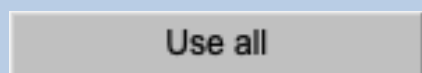
Remove 'bad' particle images

before location number	<input type="text" value="1570"/>	<input type="button" value="▲"/> <input type="button" value="▼"/>
after location number	<input type="text" value="12854"/>	<input type="button" value="▲"/> <input type="button" value="▼"/>

Extract the final 'good' particle images by clicking the "Remove Particles" button:



Of course, you don't have to remove particles. In this case (usually not suggested) click the "Use all" button.



The “Pick Particle - Get References from Modulation picked Particles” Page

DESCRIPTION:

Find references for correlation picking using MSA class averages of the modulation picked particle images.



As usual you have to specify the input file. This file is expected to contain the (final best) modulation picked particles:

Input file with modulation picked particles	
<input type="text" value="my_micrographs_prep_mod_particles"/>	<input type="button" value="Browse"/>

Use the browse button or enter the name into the text field.

You are also requested to specify the names of the output files. Note that depending on the options chosen the number of output files can change:

Centred particles	
<input type="text" value="my_micrographs_prep_mod_particles_cent"/>	<input type="button" value="Export"/>
Eigenimages (of the centred particles)	
<input type="text" value="my_micrographs_prep_mod_particles_cent_eigen"/>	<input type="button" value="Export"/>
Class averages (of the centred particles)	
<input type="text" value="my_micrographs_prep_mod_particles_cent_classsums"/>	<input type="button" value="Export"/>
'Good' class averages (of the centred particles)	
<input type="text" value="my_micrographs_prep_mod_particles_cent_classsums_good"/>	<input type="button" value="Export"/>
Final references	
<input type="text" value="my_micrographs_prep_mod_particles_cent_cls_ref"/>	<input type="button" value="Export"/>

Enter the names into the text fields.

As usual you also find the “Export” buttons to export the resulting images to any 3DEM image format.



Modulation picked particles are usually not well centred. So, centring is the first processing suggested:

Centre particles

Self rotate Self

Total sum Mass center

Options are:

Self rotate: Each image is rotated over 180 degrees and translationally aligned to this 180 degrees rotated version. The centring is over half the shift required for the full alignment.

Self: Each image is iteratively centred relative to rotationally symmetrized version of itself so as to obtain a good centring of relatively globular particles.

Total sum: The input image is iteratively centred relative to a rotationally symmetrized version of the total sum created from of all input images.

Mass centre: Straightforward shifting the centre of mass of the input image to the logical centre of the output image (half+1 in both vertical and horizontal directions).

Play around with the options using a some images only to find out the best centring.

Test loc. # to

Usually the suggested is option is self.

The centred particles need less image sizes so you can reduce the box size:

Reduce box size to

Having found the best option and box parameter you can centre all images:

Test loc. # to

Run for all locations



Usually the centred particle images are still not good references but can be used for MSA and classification to get (better) class averages:

MSA classification

MSA

Number of eigenimages

Number of iterations

Classification

Number of classes

Class averages

Fraction of worst class members to ignore

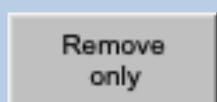
Remove bad class averages if

- too few members
 - Minimal number
- too bad overall quality
- sigma in densities is times sigma off the mean value

After the first MSA and Classification you can re-run “Classify” to change the number of classes without re-calculating the more time-consuming MSA:

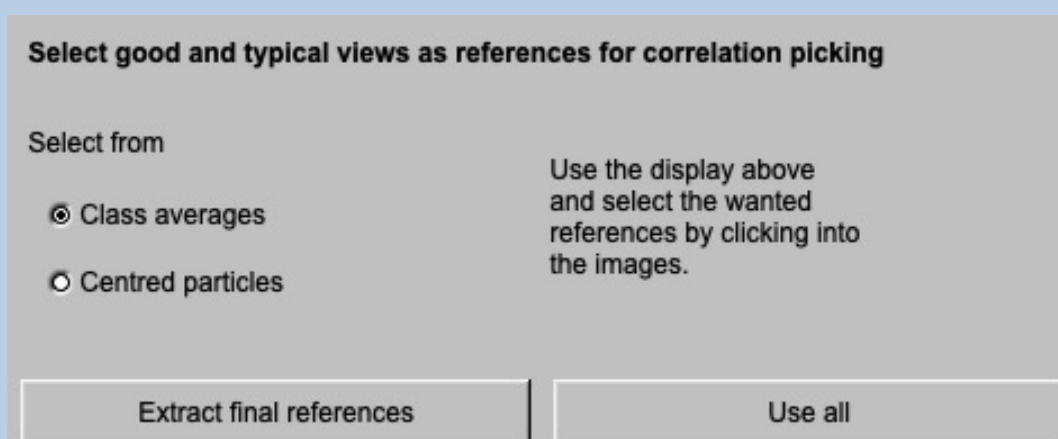


You can also re-run “Remove” with other options and parameters without re-calculating the more time-consuming MSA and classification :



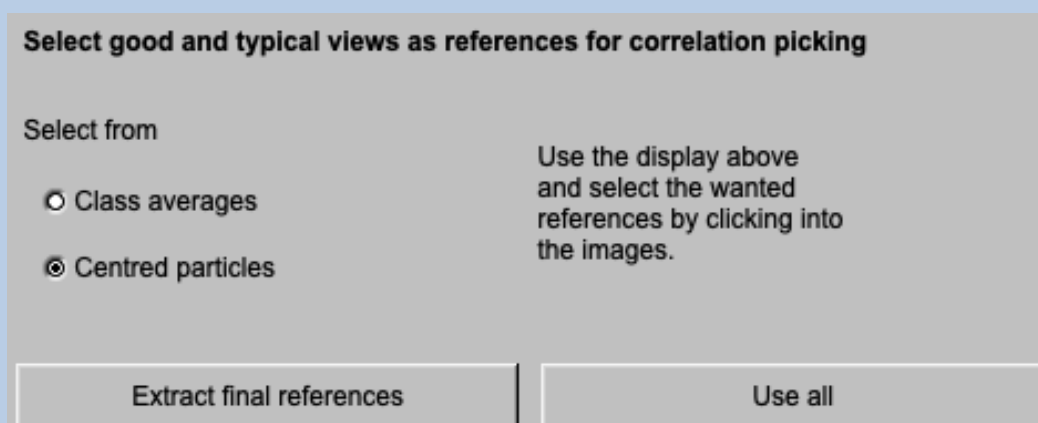
Refer to **guiMSA** to get additional help on MSA and classification.

Check the displayed class averages and select good and typical particle views by clicking into the related image (a second click will de-select the image). Finally extract the selected references clicking the “Extract final references” button:



You can also use “Use all” button to use all class averages as references. This is usually never suggested but it is your choice, of course

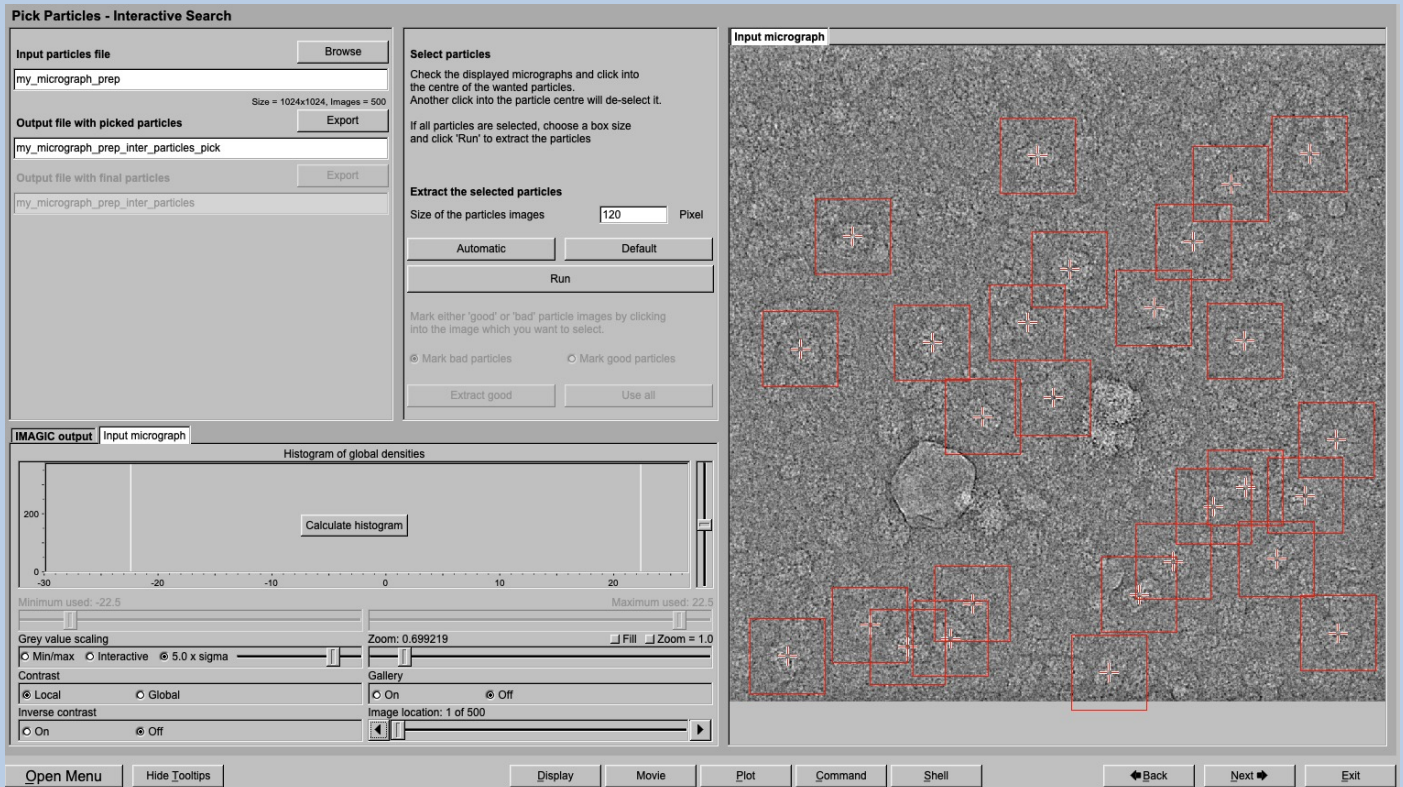
If the class averages do not show good and typical particle views you can alternatively select from the centred particle images. Like “Use all” this option is usually never suggested.



The “Next” button leads to the “Prepare Correlation References” Page”.



The “Pick Particle - Interactive Search” Page



DESCRIPTION:

Find particles by interactive picking. Extract selected particle images.

You may want to pick your particles interactively or interactively select reference particles for correlation picking. Both is not suggested but it is your choice, of course.



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

Input particles file	<input type="button" value="Browse"/>
<input type="text" value="my_micrograph_prep"/>	
Size = 1024x1024, Images = 500	
Output file with picked particles	<input type="button" value="Export"/>
<input type="text" value="my_micrograph_prep_inter_particles_pick"/>	
Output file with final particles	<input type="button" value="Export"/>
<input type="text" value="my_micrograph_prep_inter_particles"/>	

Specify the image size of the cut-out particle images. This box size is shown in red when you are picking particles so that you can check if the output image size is large enough.

Extract the selected particles		
Size of the particles images	<input type="text" value="120"/>	Pixel

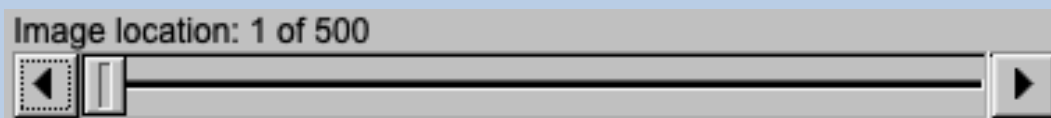
Now you can start picking particles by clicking into the centre of the wanted objects:



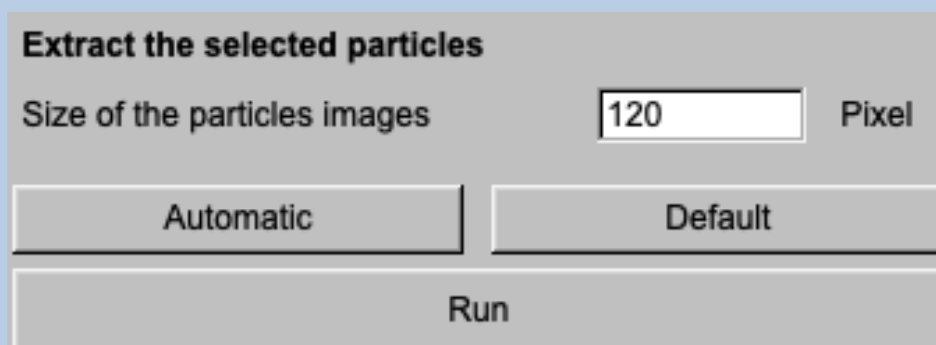
Clicking a particle a second time will remove it from the list.



Click particles for all micrographs wanted. Use the “Input micrograph” display control on the left hand side to navigate from micrographs to micrograph (image locations)

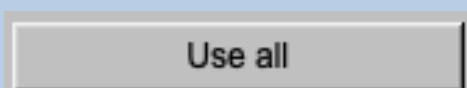


Having finished picking you can click the “Run” button to extract the selected particles. If wanted you can adjust the box size (= size of the output images)

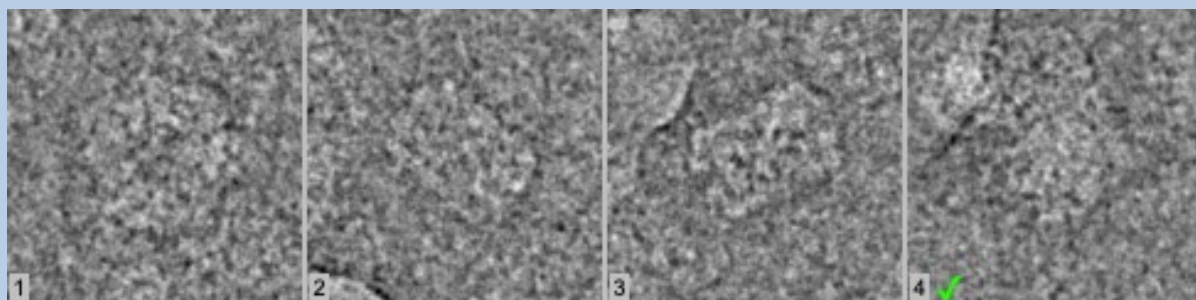


The extracted particle images are shown on the right hand side. Remember that you can use “Zoom” in the display control on the left hand side to enhance or reduce the number of images shown in this gallery.

If all extracted particle images are okay press the “Use all” button to get the “final particles”.



If wanted you can check the list and either mark the “good” or the “bad” particle images by clicking into the related image on the display on the right hand side.



Specify if you marked the “bad” or the “good” images and click the “Extract good” button to either select the “good” particles or remove the “bad” particles to get the “final particles”.

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

Mark bad particles Mark good particles

Note that you can use the “Export” button to open an additional EM2EM window to export the chosen output file to a file in any 3DEM format:

Output file with final particles

my_micrograph_prep_inter_particles

The “Next” button leads to the “Prepare Correlation References” Page”.



The “Pick Particle - Prepare Correlation References” Page

The screenshot displays the 'Pick Particles - Prepare Correlation References' window. On the left, there are sections for 'Choice of references' (radio buttons for 'References from modulation picking', 'Other image file', and 'Pick from micrograph(s)'), 'Input references file' (with a 'Browse file' button), and several 'Output file with...' sections for 'centred references', 'normalized references', 'aligned references', 'rot. symm. references', and 'final prepared references', each with an 'Export' button. Below these is a 'Run in parallel mode' section with 'Yes' and 'No' radio buttons and a 'Number of nodes' dropdown set to 3. The 'Mask reference' section includes 'Radius' (0.800) and 'Drop off' (0.050) input fields, and checkboxes for 'Centre references' (with 'Self rotate' and 'Self' radio buttons, and 'Total sum' and 'Mass centre' radio buttons), 'Normalise reference densities', 'Create mirror references', 'Sequentially align references' (with 'Also align mirrored references' checkbox), and 'Symmetrise references rotationally'. 'Automatic' and 'Default' buttons are also present. On the right, a gallery of 9 circular images is shown, with tabs for 'Input', 'Masked', 'Centred', 'Normalized', 'Aligned', 'Symmetrized', and 'Final'. The 'Final' tab is active, showing the processed images. At the bottom, a 'Histogram of global densities' plot is visible, along with 'Grey value scaling' and 'Contrast' controls.

DESCRIPTION:

Prepare the selected / picked images to be used as references in the subsequent correlation search.



Before correlation particles search one usually has to prepare the references.

As usual first specify the input file which contains the images which are to be prepared as references in the subsequent correlation search. These images be the best images from modulation picking

Choice of references

References from modulation picking
 Other image file
 Pick from micrograph(s)

Input references file

my_micrographs_prep_mod_particles_cent_cls_ref

or any other images you have selected / picked:

Choice of references

References from modulation picking
 Other image file
 Pick from micrograph(s)

Input references file

my_micrographs_particles_pick

At the edges of the images there are features which are not related to the particles. This information is “removed” by applying a circular mask:

Mask reference

Radius
Drop off

The reference particles have to be well centred. Play around with the various options to get good results.



The reference particles have to be well centred. Play around with the various options to get good results:

Centre references

Self rotate Self

Total sum Mass centre

It is also suggested to normalise the variance in the reference images:

Normalise reference densities

You can also create mirror versions of the reference images.

Create mirror references

The number of references is small, it can be good idea to sequentially align them:

Sequentially align references

If mirror versions are requested to have to choice to align or to align them:

Create mirror references

Sequentially align references

Also align mirrored references

When doing the correlation search for the first time you will usually not create mirror versions but rotationally average the references especially in the case particles show all possible rotational orientations on the micrograph (note that in this case mirrors do not make sense):

Create mirror references

Sequentially align references

Also align mirrored references

Symmetrise references rotationally



The “Pick Particle - Correlation Picking” Page

Pick Particles - Correlation Picking

Input micrograph file
my_micrographs_prep
Size = 1024x1024, Images = 500

Input references file
my_mod_cls_ref
Size = 108x108, Images = 9

Output file with resized micrograph
my_micrographs_prep_coarse
Export

Output file with resized references
my_mod_cls_ref_coarse
Export

Output file with filtered images
my_micrographs_prep_coarse_lp
Export

Output file with filtered references
my_mod_cls_ref_coarse_lp
Export

Output file with correlation images
my_micrographs_prep_coarse_lp_ccf_images
Export

Output file with 'good' particles found
my_micrographs_prep_ccf_particles_pick_good
Export

Output file with final particles
my_micrographs_prep_ccf_particles_best
Export

Run in parallel mode
 Yes No
Number of nodes: 5

IMAGIC output | Micrograph | References | Resized images | Resized refs | Filtered images | Filtered refs | particles

Histogram of Correlations (extracted particles)

```
** HEADERS (vs. 6-Sep-2022) welcomes you **
Headers options available           : HISTOGRAM
Histograms to be created from which images : ALL_IMAGES
Histogram options available         : CCC

Which correlation coefficients to be used:
  2D_CCC  3D_CCC
Please specify option [2D_CCC]       : *
```

The results have been stored in the following files:

Header updated in input particles images : my_micrographs_prep_ccf_particles_pick
Output file with "good" particles images : my_micrographs_prep_ccf_particles_pick_good

Some useful next IMAGIC commands:

```
=====
DISPLAY-IMAGE  Input: Good particles image file. Check
                if particles look okay. If not, re-do
                SELECT-IT-ALL with other parameters
```

Resize micrograph/references
Summing parameter: 2

Low-pass filter micrograph/references
High frequency cut: 0.3

Find particles

Rotational symmetry of references

References are rotationally symmetric
 References are not rot. symmetric

Number of rotational orientation for search: 33

Pick parameters (in input micrographs)

Minimal distance between particle peaks: 80
Minimal distance of particles from edge: 80
Expected number of particles per micrograph: 100
Box size of particles: 108

Run options

Test run on micrograph: 1
 ... micrographs: 1 to 2
 Run for all micrographs

Automatic Default
Find particles

Micrographs / Correlation Images | References | Particles

Input micrographs | Correlation images

Extract particles

Use all Use 'good' particles only

Ignore particles which show

too small peak height
 too extreme sigma of densities
 too extreme min/max difference of densities

Ignore if 1.5 times sigma away from mean value

Extract Particles

Further polishing

Use display tab 'Extracted particles' to check

Remove 'bad' particle images

before location number: 506
after location number: 27500

Use all Remove Particles

DESCRIPTION:

Find particles using correlation search and extract/cut-out particle images.



Specify the name of the (prepared) input micrograph file and the name of the file with the prepared references. As usual, output file names are suggested but you can always change names :

Input micrograph file	<input type="text" value="my_micrographs_prep"/>	<input type="button" value="Browse"/>
		Size = 1024x1024, Images = 500
Input references file	<input type="text" value="my_mod_cls_ref"/>	<input type="button" value="Browse"/>
		Size = 108x108, Images = 9
Output file with filtered images	<input type="text" value="my_micrographs_prep_coarse_lp"/>	<input type="button" value="Export"/>
Output file with filtered references	<input type="text" value="my_mod_cls_ref_coarse_lp"/>	<input type="button" value="Export"/>
Output file with correlation images	<input type="text" value="my_micrographs_prep_coarse_lp_ccf_images"/>	<input type="button" value="Export"/>

To speed up the calculations the micrographs can be resized (only used during the correlation search):

Resize micrograph/references

Summing parameter

It is very important to strongly low-pass filter the references to avoid overfitting (correlation of noise):

Low-pass filter micrograph/references

High frequency cut

If your input references are rotationally averaged, click the related button:

Rotational symmetry of references

References are rotationally symmetric

References are not rot. symmetric



If the references are not rotationally averaged rotated versions of the references will be created during particle search:

Rotational symmetry of references

References are rotationally symmetric

References are not rot. symmetric

Number of rotational orientation for search

When re-searching particles at a later stage of a “real science analysis” with better references you would go for this option. This option is very time-consuming.

Play around with the pick parameters, Run the search for only a limited number of micrographs to test how the given parameters influence the search:

Pick parameters (in input micrographs)

Minimal distance between particle peaks

Minimal distance of particles from edge

Expected number of particles per micrograph

Box size of particles

Run options

Test run on micrograph

... micrographs to

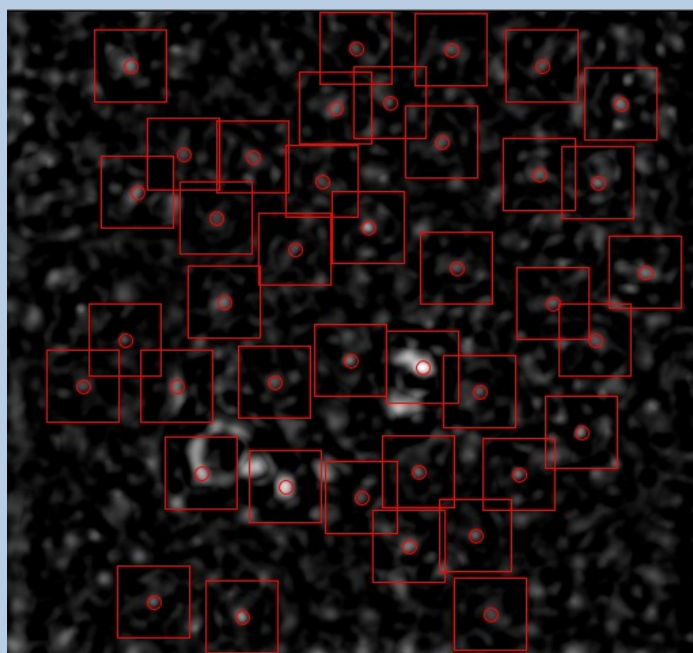
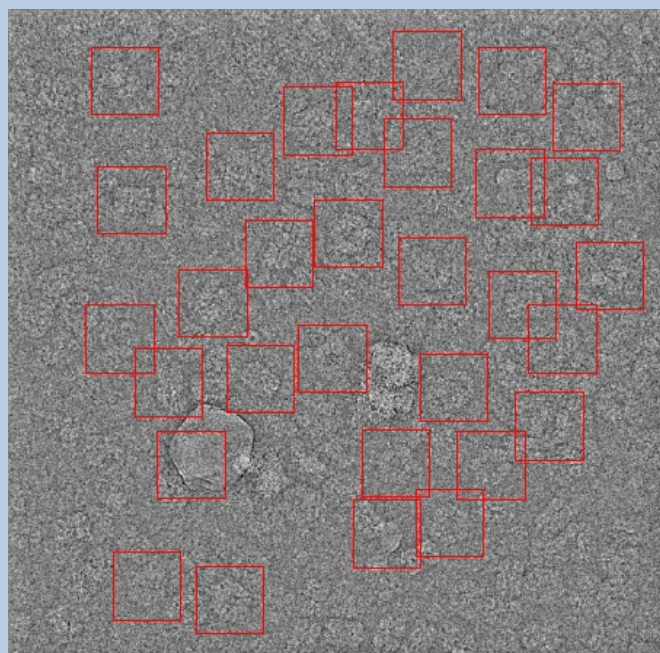
Run for all micrographs

References are not rot. symmetric

NOTE: The particles are not yet extracted



Check the particles found in the displayed micrographs and the peaks in the displayed correlation images:



Check Finally, search particles in all micrographs.

Now the particles can be extracted. Like in Modulation Picking, the images contain a lot of picked junk (ice, carbon foil, clumped particles). In a first approach, they can be sorted out by looking at the statistics of the picked particles using the button “Extract ‘good’ particles only”:

Extract particles

Use all Use 'good' particles only

Ignore particles which show

too small peak height

too extreme sigma of densities

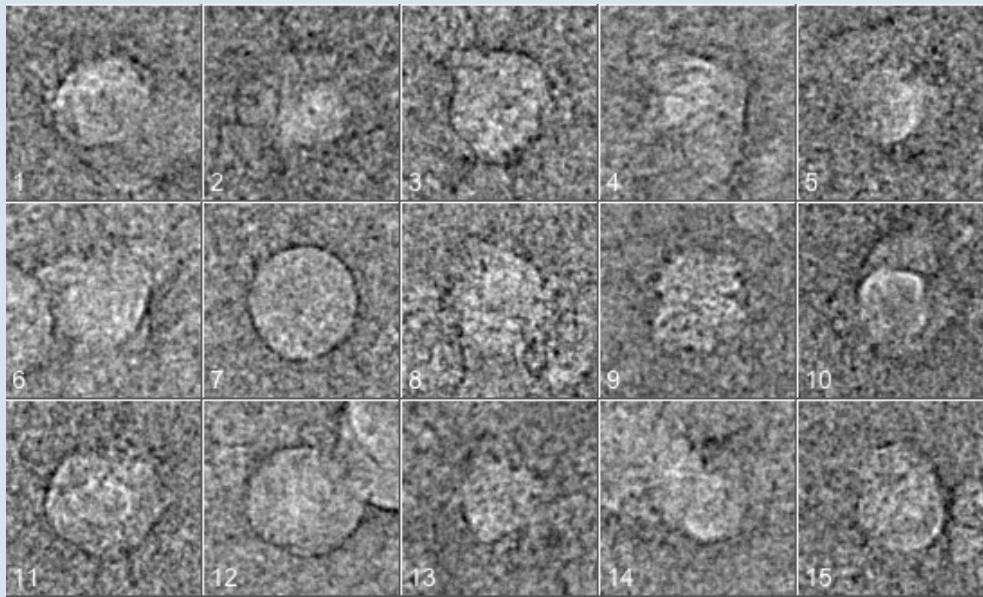
too extreme min/max difference of densities

Ignore if times sigma away from mean value

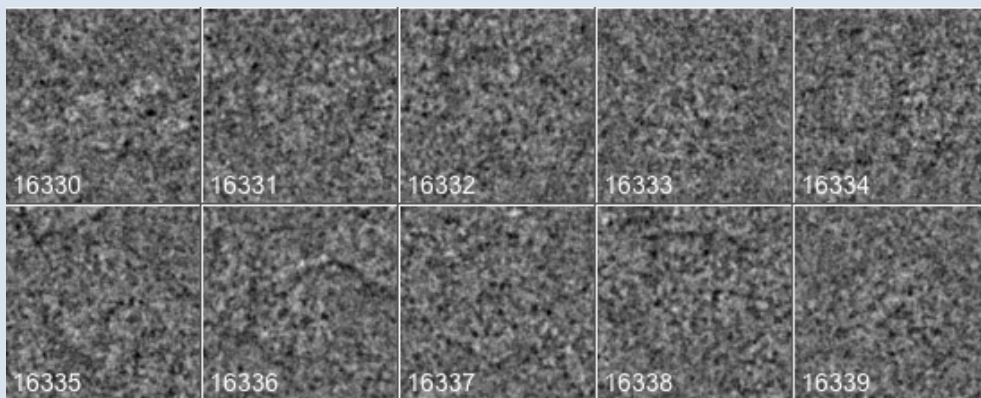
Check the extracted particles displayed on the right-hand side.



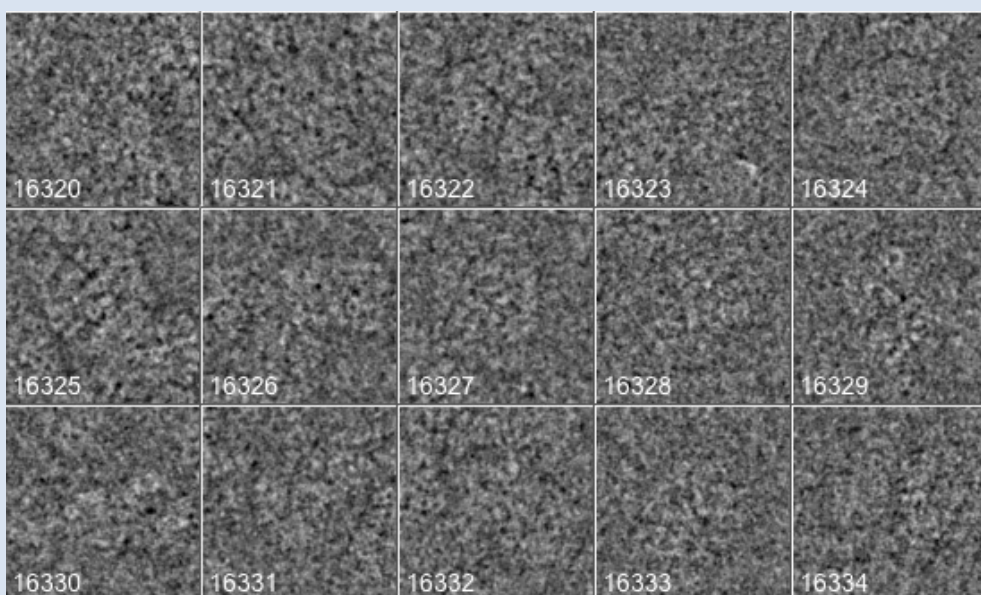
You can see that the first displayed images contain ice blobs etc.



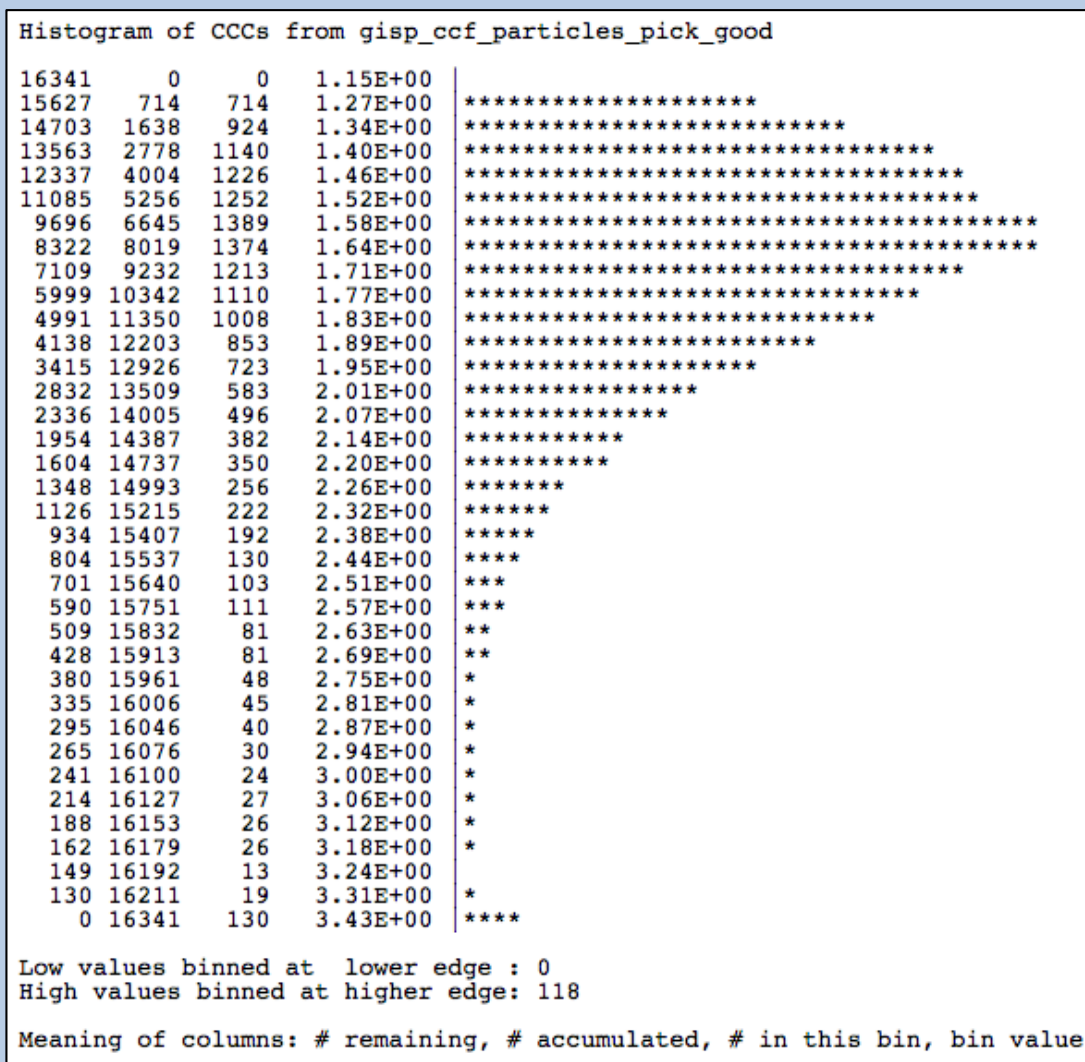
whereas the last images usually contain noise:



The particle images are in between:



Check the extracted particles and write down the location numbers where the good particle images start and where they end. Also have a look at the histogram printed in the terminal window to get an idea where to find this range of good particle images:



Finally extract the best particles using the “Remove Particles” button:

Further polishing

Use display tab 'Extracted particles' to check

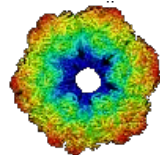
Remove 'bad' particle images

before location number ▲▼

after location number ▲▼

Remove Particles





IMAGIC

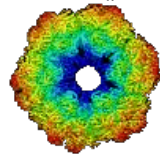
guiPICK

Not (yet) possible

The following options are not (yet) possible:

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





IMAGIC

guiPICK

Feedback / Error hints

We intensively tested the **guiPICK** 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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