

# Map3-2D documentation

Version 1.0

## Introduction

This software is prepared to work with 5D images or, in other words, multi-channel time-lapse 3D stacks. The bit depth of these multi-dimensional images is set at the acquisition stage, but could also be modified in a pre-processing stage. In any case, the bit depth is the same for every image in the 5D stack.

Map3-2D allows the addition of *extra* channels. Those additional channels can have different bit depths (8 or 16 bit integer, 32 bit float). They can be added by the user (see Image Menu) or created by the software during certain processes like, for example, the creation of a projection.

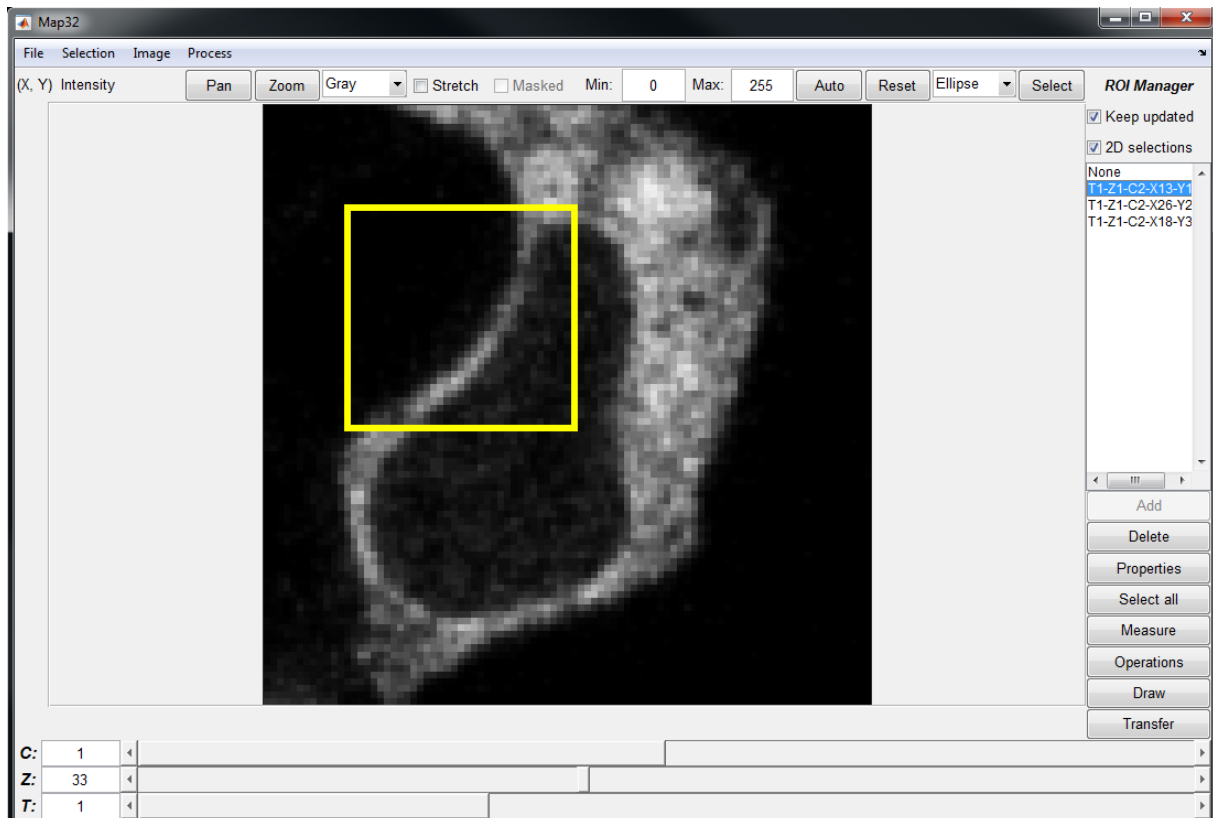
On top of the main and extra channels, one or two masks can be appended to the current image. These masks are binary 3D or 3D+time stacks that are used to select certain 3D regions of the (5D) image. Users can load these masks from a file or from a channel of the current image. Pixels (voxels) with zero values are considered *false* and the rest *true*.

Together with the main graphical interface a small dialog, called 'Log...', shows the current operation running. This dialog also provides a *Recovery* button to recover halted software. Upon recovery, please, keep in mind that an update on the images might be necessary (for example, by adjusting properties in the 2D projections).

The next sections provide an overview of the graphical interface and a detailed description of the main menu.

## Main graphical interface

The visual appearance of the main graphical interface is shown here. It consists of the central image area with the menu bar and the toolbar on top, a sidebar on the right and 3 sliders at the bottom.



### Toolbar

From the left to the right, the toolbar provides information of the current pixel coordinates and its intensity value followed by two buttons for panning and zooming. A popup menu with the current color map or lookup table (LUT) of the current channel allows the user to freely change the LUT. As images could have a width/height proportion different from the image area in the window, the user can stretch it to fully fit in the window by activating the *Stretch* checkbox.

The *Masked* checkbox is enabled when the “Mask 1” is defined (see *Image* menu). When checked those parts of the image which are not part of the mask are display as background. The user can define the background value for each channel (see *Image* menu).

For display range control, it is possible to set the minimum and maximum value, to estimate the best display range without saturation (*Auto* button) or to reset the display to the full bit depth of the channel (*Reset* button). The *Auto* button combined with the [Ctrl] and/or [Shift] keys considers all the slices and/or all the frames in the estimation of the display range, respectively. Each channel has its own display range and has to be adjusted independently.

The last two controls of the toolbar are shortcuts to create 2D selections. The popup menu provides a whole set of possibilities: point, line, rectangle, ellipse, polygon and free-hand selections. The *Select* button is used to recreate the current selection. However, combined with the [Ctrl] key it removes the current selection. Only one selection can exist at a time (see *Selection* Menu).

## Sliders

By moving the sliders at the bottom of the window the user can navigate within the hyper-stack (channel/Z-slice/time frame). The numbers left to the sliders display (and allow for manual changes of) the current channel/Z-slice/time frame.

## Sidebar

The sidebar manages regions of interest (ROI) and is called ROI Manager.

The box in the middle lists all ROIs. ROIs can be created in two ways:

- adding a 2D selection with the *Add* button, or
- getting it from another image through the *Transfer* button

After a 2D ROI is transferred it can become 3D depending on the kind of connection between the images.

By clicking on the list box together with the [Ctrl] / [Shift] keys the user can select/deselect one or more ROIs. There are two checkboxes over the ROI list box: *Keep updated* and *2D selections*. The first one updates the ROIs when the user change the current image (channel or frame), while the second one is used to show 2D selections even when they were not created in the current slice.

The buttons below the list box are shortcuts to the *Selection* Menu (See *Selection* Menu).

## File Menu

### File->Open

The Map3-2D software is designed to work only with **“Tiff or BigTiff”** files. As ImageJ/FIJI is one of the most popular image processing software for scientific research, it is prepared to open hyper-stacks saved in the **“ImageJ Tiff”** format.

It is recommended to use ImageJ/FIJI to prepare the hyper-stack before opening in Map3-2D.

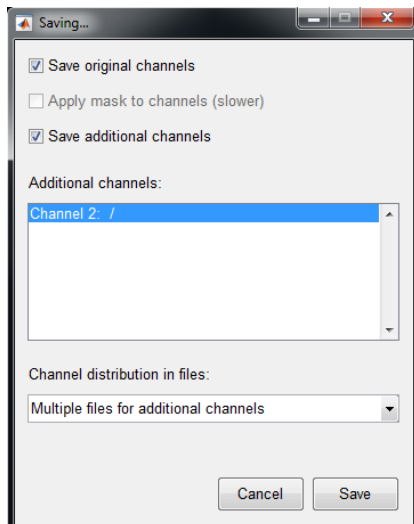
If a different folder than the default one is used to open the file, the software will ask if this should be considered the project folder (see File->Project folder).

Immediately after opening an image or stack, a dialog will pop up to adjust the voxel size. The software is prepared to automatically read it from files saved by ImageJ/FIJI (see Image->Voxel Size).

### File->Save

A dialog will pop up allowing the user to decide whether to save the main and/or additional channels. If the mask 1 is defined, the second checkbox is enabled to allow the application of the mask on the main channels. In case of saving additional channels, the user can decide which channels to save, optionally, in independent files, bundled together in one file but separated from the main channels or bundled together with the main channels. The bit depth for each file is always adjusted to the largest bit depth of the channels saved.

Map3-2D uses only the **“BigTiff”** format to save images, which can be opened straightforward in ImageJ/FIJI (using the *Bioformats* library). Depending on the amount of data, the saving process can take several minutes.



## File->Project folder

This software follows a project approach, meaning the the user can determine the folder where to save the project. Every new map created will be, by default, saved in a subfolder of the project folder.

## Selection menu

### Selection -> Make selection

This option allows the user to make a 2D selection (point, line, rectangle, ellipse, polygon and free hand). The selection remains on the figure until either another selection is placed or it is removed or added to the RoiManager.

### Selection -> Clear selection

Remove the current selection. The same effect can be achieved with the toolbar button “Select” by pressing [ctrl] + *click*.

### Selection -> Repeat selection

The current selection is removed and the user can repeat another one. The same effect can be achieved with the toolbar button “Select”.

### Selection -> Add to ROI Manager

The current selection is added to the list of the ROI Manager. Upon addition it cannot be modified again.

### Selection -> Delete ROI

The currently selected ROI is deleted from the ROI Manager

### Selection -> ROI properties

A dialog pops up to change the name of the ROI in the ROI Manager list.

### Selection -> Select all ROIs

All the ROIs in the ROI Manager are selected or deselected.

### Selection -> Measure

Performs several measurements of the area selected by the ROI (mean, median, minimum, maximum, volume/area, centroid, etc.). Only visible 2D ROIs are considered while in the case of 3D

ROIs the measurements are performed on the whole stack. Different type of measurements can be achieved in combination with the keyboard:

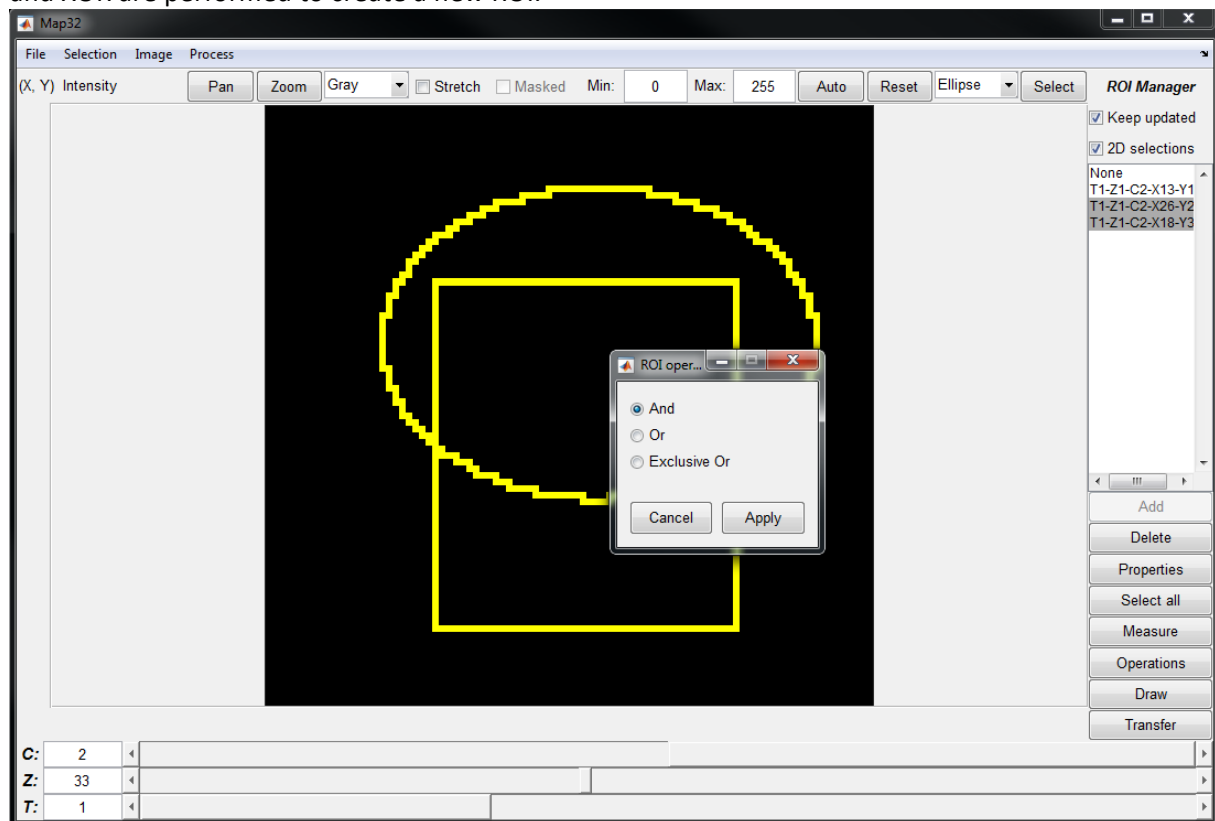
	2D ROIs	3D ROIs
Click	Only if visible in the current slice	Whole 3D stack
[Ctrl] + click	Only if visible in the current slice	Only what is visible in the current slice
[Shift] + click	Not measured	Measured
[Alt] + click	Measure on every frames	Measure on every frame

Note: The [Ctrl], [Shift] and [Alt] keys can be combined together.

If no ROI is selected, the measurements will be performed over the whole image/stack.

### Selection -> ROI operations

A dialog pops up and, when there are more than one selected ROI, logical operations like AND, OR and XOR are performed to create a new ROI.



### Selection -> Draw ROI

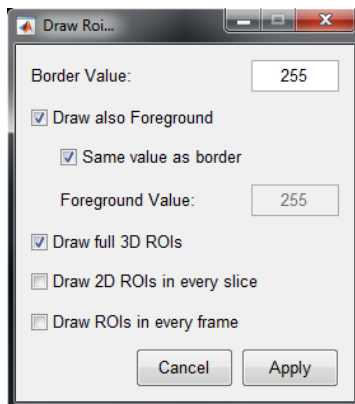
ROIs can also be drawn (or burned) into the current image. This operation **modifies permanently** the image and there is no undo operation. When the user selects "Draw ROI" the following dialog pops up.

The user must give a value to the border, but can decide whether to draw also on the inside of the ROI (*Draw also foreground* checkbox) and, in such a case, if it will have the same value or not. In the case of 3D ROIs, a 3D erosion is applied in order to determine the inside volume.

For the case of 3D ROIs, it is possible to decide whether to draw only the current slice or all the slices where the ROI appears.

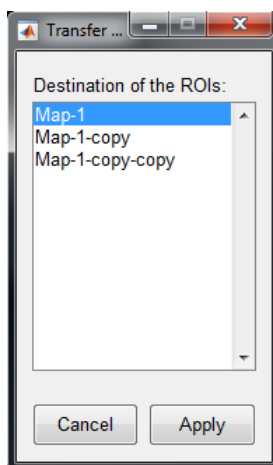
It is also possible to draw 2D ROIs (which come from selections) onto every slice (*Draw 2D ROIs on every slice* checkbox).

Finally, the last checkbox allows the user to draw the ROI on every frame.



### Selection -> Transfer

Each window can have child windows, like for example the 2D projections windows. It is possible to transfer the selected ROIs to either the child window or the parent window with this command. A dialog pops up to select the destination window(s) (multiple destinations can be selected at the same time by pressing [Ctrl] and/or [Shift] keys). If the transfer is done from a child window, the destination can only be the main window (i.e. the main graphical interface).



### Selection -> Load ROIs...

Previously saved ROIs are loaded. It cannot import files from other software.

### Selection -> Save ROIs

It saves current selected ROIs in a file. It cannot export the ROIs to be opened in another software.

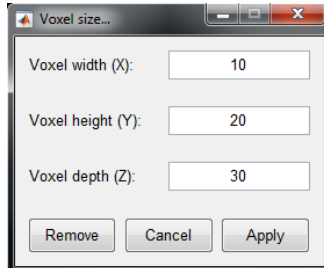
### Selection -> ROI color

A dialog pops up to change the color used by the software to show the ROIs. It is defined as an RGB color.

## Image menu

### Image -> Voxel size...

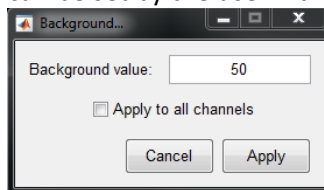
This option allows the user to change the size of the voxels. A correct voxel size is crucial for the 2D projection. This dialog can also be used to remove the current voxel size, i.e. setting all values to 1.



This dialog also pops up every time a new image is opened. The software tries to determine the voxel size from the metafile in the Tiff figure, but it only works with Tiff files saved with the ImageJ/FIJI software.

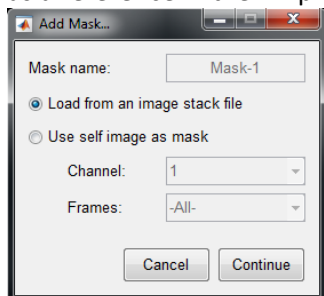
### Image -> Channel background value...

When masks are used, the unmasked areas are shown as background. The value of the background can be set by the user individually for every channel.



### Image -> Add Mask 1 (object/surface) / Add Mask 2 (object/surface)

These two commands open a dialog to define the first or second mask. The first mask is used to define the region of the object/surface to be analyzed, while the second one can optionally be used as a reference in the 2D projections.



Two options are available to define a mask:

- *Load from an image stack file, or*
- *Use self image as mask.*

In the first case only the first channel of the loaded hyper-stack will be considered, while in the other option the user can decide which channel and frame is going to be employed.

It is important to emphasize that the software considers *zero* values as unmasked region and everything else as masked.

### Image -> Add extra channel

An additional channel is added to the image. The bit depth is the same as the main channels.

### Image -> Remove current channel

It removes the current channel. At least one main channel must remain.

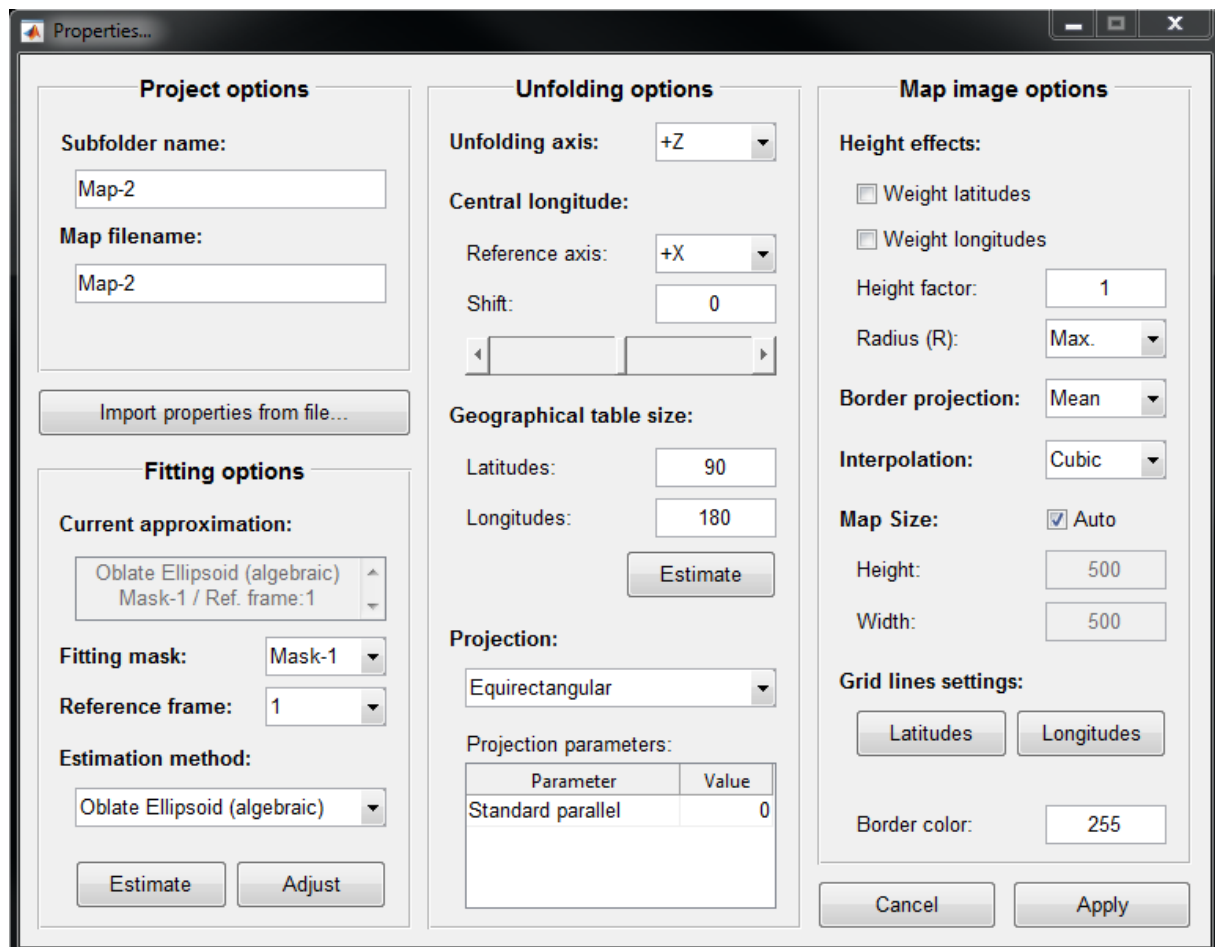
### Image -> Remove all extra channels

It removes all additional channels.

## 2D projections

As soon as you click on *Process -> 2D projection* at the menu bar, a new image window is displayed and a dialog pops up in order to set the properties of the projection.

### Properties dialog



**Project options**

Subfolder name:

Map filename:

**Fitting options**

Current approximation:

Fitting mask:

Reference frame:

Estimation method:

**Unfolding options**

Unfolding axis:

Central longitude:

Reference axis:

Shift:

**Geographical table size:**

Latitudes:

Longitudes:

**Projection:**

Projection parameters:

Parameter	Value
Standard parallel	0

**Map image options**

**Height effects:**

Weight latitudes

Weight longitudes

Height factor:

Radius (R):

Border projection:

Interpolation:

Map Size:  Auto

Height:

Width:

**Grid lines settings:**

Border color:

### Subfolder name

This is a subfolder of the project folder and it is intended to be the place for saving the 2D projections (one or more). The chosen properties will also be saved automatically in this folder with the file extension “.emp”.

### Map filename

This is the name for the projection, which will be used as the window title and also as filename for properties “.emp”.

### Import properties from file

This button is used to import the properties from a previously saved properties “.emp” file.



### Current approximation

In this box the software shows the current approximation of the reference ellipsoid that is used for the 2D unfolding. The mask employed as well as the reference frame are written in the second line.

### Fitting mask

If the Mask 2 is defined then the user can decide whether to use Mask 1 or Mask 2 for the estimation of the reference ellipsoid.

### Reference frame

If the chosen mask contains more than one frame, then the user can decide which frame will be used for the estimation of the reference ellipsoid. It is also possible to select all of them. In such a case, a time maximum projection will be done. No changes will be applied until the button *Estimate* is pressed.

### Estimation method

The user can select here if a sphere, oblate ellipsoid or tri-axial ellipsoid for the estimation is applied. It is recommended to use, for example, the ImageJ/FIJI 3D Viewer plugin to get an idea of the shape of the sample/mask. No changes will be executed until the button *Estimate* is pressed.

### Estimate button

By clicking on this button the reference ellipsoid will be re-estimated based on the *Fitting mask*, *Reference frame* and *Estimation method*. The previous parameters of the reference ellipsoid will be deleted and the only way to recover them is by pressing the *Cancel* button.

### Adjust button

As the estimation of the reference ellipsoid is based on an algebraic approximation, it is often not the best fit. Apart from that, some samples are not exactly a sphere or symmetrical ellipsoid. Due to these reasons, the user can manually adjust the ellipsoid by pressing this button. A new *Fitting adjustment* dialog will pop up and will be described later (see *Fitting adjustment dialog*).

### Unfolding axis

The most common maps of the Earth are based on the unfolding around the rotation axis (north-south axis). With this popup menu, Map3-2D allows the user to select the X, Y or Z unfolding axis. This axis is directly related with the latitudes, which are on the  $[-90^\circ, 90^\circ]$  range, corresponding to the negative and positive semi-axis respectively. There are also “flipped” versions of the axes in which case the negative latitudes are on the positive semi-axis and the positive latitudes on the negative semi-axis.

In order to have a better visualization of the axes directions it is recommended to go to the *Fitting adjustment* dialog by clicking on the *Adjust* button.

### Central longitude

The central longitude of the 2D projection is equivalent to the Greenwich meridian in the common maps of the Earth. After selecting the unfolding axis, the central meridian can be chosen in the *Reference axis* popup menu as any of the other four semi-axes.

The resulting longitude values will be in the range  $[-180^\circ, 180^\circ]$  and the direction of the non-used axis (neither as unfolding axis nor as central longitude) determines the direction of the increments in the longitudes.

The central longitude can be shifted to an exact value by using the *Shift* slider or edit control.

### Geographical table size

For the 2D projections the Map3-2D software uses an intermediate table of latitudes and longitudes called *Geographical table*. This table establishes the resolution in latitudes and longitudes for the map. The resolution might not be linear since it depends on the projections.

The *Estimate* button finds the best size based on the ellipsoid semi-axes and the voxel size. However, this value should be manually adjusted to get better looking maps.

### Projection

The user can select among the available projections in the popup menu (Equirectangular, Mercator, Braun, Miller, Lambert-Cylindrical, Sinusoidal, Mollweide, Wagner-VI and Bonne). Some of the projections include parameters that can be adjusted in the table below.

More information about these projections can be found, for example, on Wikipedia.org (*List of map projections*).

### Height effects

Map3-2D software allows also the weighting of the latitudes and/or longitudes map positions by the height (or elevation) with respect to the reference ellipsoid. A radius value is added to the weights in order to avoid negative values. This radius can be the local ellipsoid radius for the current (latitude, longitude) or the maximum radius for the whole ellipsoid.

A *height factor* can also be applied to increase or reduce the height influence.

### Border projection

If the mask is thick, more than one pixel will probably have the same latitude and longitude.

Consequently they will overlap in the geographical table and hence in the resulting map. With the *Border projection* popup menu it is possible to choose between mean, maximum, minimum or sum operations to deal with the overlapping pixels.

Unfortunately this effect is not only caused by the surface thickness but also by the sampling of the geographical table. This is another good reason to manually adjust the geographical table size, if needed.

### Interpolation

During the unfolding there are parts of the map that might not contain any value. This effect appears, for example, on the pole regions for the cylindrical projections or when there are gaps in the geographical table where there are no values. These values can be interpolated with different interpolation methods: *nearest*, *linear*, *natural*, and *cubic*.

### Map size

The map size (height and width) is controllable by the user. By clicking on the *Auto* checkbox, the size of the map will be the same as the geographical table size.

### Grid lines settings

The resulting 2D projection contains a first additional channel, called *grid* channel, with the meridian parallel lines, as in the case of the Earth maps. The number of lines as well as their individual colors (intensity values) can be adjusted with the *Latitudes* and *Longitudes* buttons. These buttons will open a window with a two-columns table where the first column contains the latitudes/longitudes lines and the second the corresponding intensity values:

Latitude lines...			Longitude lines...		
Options			Options		
	Latitude	Color Value		Longitude	Color Value
1	-75	100	1	-180	100
2	-60	110	2	-165	104.3478
3	-45	120	3	-150	108.6957
4	-30	130	4	-135	113.0435
5	-15	140	5	-120	117.3913
6	0	150	6	-105	121.7391
7	15	160	7	-90	126.0870
8	30	170	8	-75	130.4348
9	45	180	9	-60	134.7826
10	60	190	10	-45	139.1304
11	75	200	11	-30	143.4783
			12	-15	147.8261
			13	0	152.1739
			14	15	156.5217
			15	30	160.8696
			16	45	165.2174
			17	60	169.5652
			18	75	173.9130
			19	90	178.2609
			20	105	182.6087

In the *Options* menu there are commands to add/remove lines and to reset the table for the default values.

Apart from the intensity of the latitude and longitude lines (or parallels and meridians), there is a line for the border of the map whose intensity values can be adjusted with the *Border color* edit box.

### Apply

By pressing *Apply* the map will be created or updated.

### Cancel

The *Cancel* button will also close the map image window if the map was not created before. It will not update the map image.

## Fitting adjustment dialog

The Fitting adjustment dialog is a graphical interface to visually adjust the reference ellipsoid position and rotation, as well as each individual semi-axis length. It shows three images, each of them corresponding to the maximum intensity projection of the mask in the X, Y and Z directions. Every axis has its own color and the position of the letter (x, y or z) indicates the positive semi-axes. For the third axis there is a circle with a cross, denoting that this axis increases its values towards behind the screen.

### Manual fitting

The controls on top of the left panel (*Manual fitting*) to estimate the reference ellipsoid are the same as in the Properties dialog. It is also possible to write the exact value for the *Ellipsoid center*, *Ellipsoid orientation*, as well as *Positive* and *Negative semi-axes*.

Three vectors representing the axes of the real ellipsoid define the orientation, the perpendicularity between them must be kept.

Upon changes, the *Update* button must be pressed to actually apply the changes.

**It is strongly recommended not to change these parameters here but instead using the bottom right panel, as described below.**

### Orientation

This button transforms the bottom right panel to adjust the axes direction. The user can decide around which axis to rotate in the *Rotation around* popup menu, and then write the *Angle* or adjust it with the slider.

### Semi-axes

This button transforms the bottom right panel to adjust the semi-axes length. The user can decide which *Semi-axis* to modify with a popup menu, and then write the *Length* or adjust it with the slider. There is also a checkbox to adjust simultaneously the positive and negative semi-axes in case of a symmetrical ellipsoid.

### Center

This button transforms the bottom right panel to adjust the position of the ellipsoid. The user can decide which *Axis* to adjust with a popup menu, and then write the *Displacement* or adjust it with the slider.

### Confirm

The current adjustment will become permanent, allowing the user to proceed to the next adjustment. There is no order for the adjustments, but each step must be confirmed.

### Restore

In case the user is not satisfied with the new adjustments, it is possible to restore the ellipsoid to any of the previous stages.

### Reset

The current adjustments are reset to the current ellipsoid parameters.

### Apply

By pressing *Apply* the reference ellipsoid will be updated in the *Properties* dialog.

### Cancel

The *Cancel* button will not update the reference ellipsoid in the *Properties* dialog.

## The 2D projection window

Once the 2D projection is created, the result is displayed in a window that is very similar to the main graphical interface. The image has as many main channels as the original one plus 2 additional channels: the *grid* and the *height* channels. The first one reproduces the parallel and meridian lines as in Earth maps, while the second is like a cartographic relief depiction where the baseline is determined by the reference ellipsoid.

There are only a few differences in the menu bar, some commands were removed and some others were added, as described below:

### **File -> Duplicate Map**

This command duplicates the map in a new window. It is very useful to compare different maps of the same object.

### **Map -> Transfer Grid**

This command transfers the 2D grid channel to an additional channel in the original stack image. Consequently the 2D image with the grid is transformed into a 3D stack.

### **Map -> Transfer Heights**

This command also creates an additional channel in the original stack image, where the values of mask 1 now contain real values (float) representing the shortest distance to the reference ellipsoid. These values are actually the height with respect to the reference ellipsoid.

### **Map -> Transfer Ellipsoid with Heights**

This command creates an additional channel in the original stack image, which contains the reference ellipsoid, whose pixel (voxel) intensities are the average height for the corresponding latitudes and longitudes.

### **Map -> Properties**

By clicking this command the user can modify the properties of the current 2D projection (see *Properties* dialog)