AutoAligner Manual V 6.0.0.

BITPLANE
SCIENTIFIC SOLUTIONS

1   Preface

This Manual provides a description of all menu entries, display modes, functions and parameters.

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AutoAligner Manual V 6.0.0

1.1   Getting Started

The software is delivered on a standard CD or downloaded from www.bitplane.com. The CD includes a folder containing the necessary manuals, or the manuals can be downloaded.

Minimum hardware/software requirements are:

- Windows NT 4.0, or a more recent version; Windows 2000 or XP; Mac OS X 10.3.9 or later
- CD-ROM
- Graphics card with 3D accelerator
- Network facilities for image import from the microscope
- 512 MB RAM (> 1 GB recommended)

Bitplane also recommends:

- A database for storing images (e.g., Image Access)

Installation

To install the software, please proceed as follows:

- Insert your AutoAligner CD-Rom in the computer.
- Follow the instructions on the screen.
- The installation is completed automatically.

For support please contact: support@bitplane.com

Licensing

To run the AutoAligner system you need the appropriate license. In case of any license problems, please refer to the support information on our website www.bitplane.com for detailed instructions.

Starting AutoAligner

AutoAligner can be started by one of the following methods:

- Double-click on the AutoAligner icon (we recommend copying the icon to the desktop).
- Drag the icon of an image or a file to the AutoAligner program icon.

The software opens with the main screen.

1.2   Terminology

Term	Description
Channel	A channel is a particular piece of information that has been recorded for all voxels. Most of the time in light microscopy, the different channels are intensities that have been measured at different wavelengths. For instance, a two-channel data set contains two intensities for each voxel, one intensity measured in channel 1 (e.g., green light) and one intensity in channel 2 (e.g., red light). Ideally, the intensity values present in different channels are independent of one another.
Coordinates	Any position in the data set can be described either in the voxel grid (e.g., voxel no. 5 in x-direction) or by a coordinate, which corresponds to a m). The distance relative to a defined origin in the real world (e.g. 2 latter) requires the correct calibration of the data set, i.e. that the voxel size is defined appropriately. Generally, in AutoAligner the origin of the data set is in the left bottom corner. If the data set was cropped from a larger data set, the bottom left corner may have minimum coordinates that are not equal to (0,0,0).
Pixels	A 2D image is composed of picture elements (pixels) which are all of the same size and shape, but do represent individual gray values. All pixels together form the visible image.
Slice/Layer	A slice or a layer is a 2D image, representing a cut through the sample object.
Image stack	The 3D reproduction of all the layers during processing.
Voxels	A volume data set is composed of volume elements called voxels. The voxels are the smallest units within the image about which we have distinct information in the form of a measured intensity. The dimensions of an individual voxel are specified in most image file formats supported by AutoAligner.
Data set	A stored 3D data set comprised of all layers.

2   Quick Start Tutorial

AutoAligner aligns 2D images of serial sections. In this Quick Start Tutorial you will learn the AutoAligner basic working steps. The demo data set are slices of a mouse embryo.

You will practice how to open the data set, review the slices, do all necessary pre-processing steps before AutoAligner does the automatic alignment of the layers.

The automatic alignment mode uses image features to find the optimal alignment between neighboring slices and then rotates and translates the slices to achieve the closest possible match. Once the alignment is

calculated and shows a satisfactory result, you can apply the alignment to the image stack and save the data set.
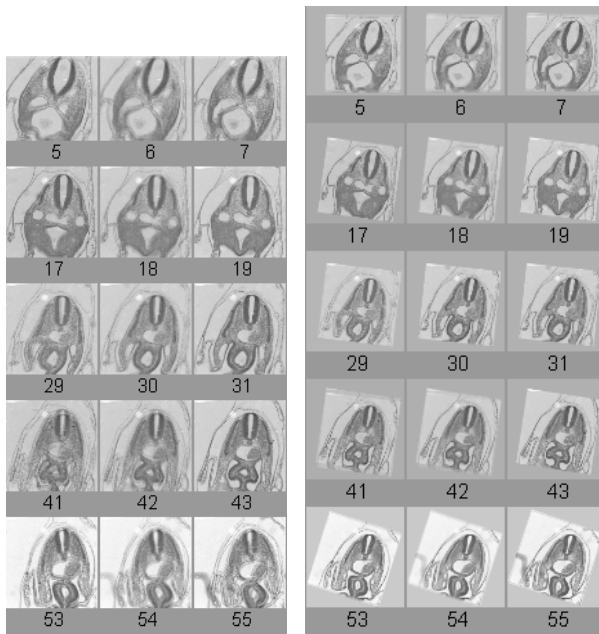

Fig.: Slices before (left) and after (right) automatic alignment.

The Tutorial is designed to be followed sequentially. It is a hands-on seminar, therefore open AutoAligner on the left hand side of the screen and adjust the online Tutorial on the right hand side of the screen and practice step-by-step.

If you have further questions please contact us via email: support@bitplane.com.
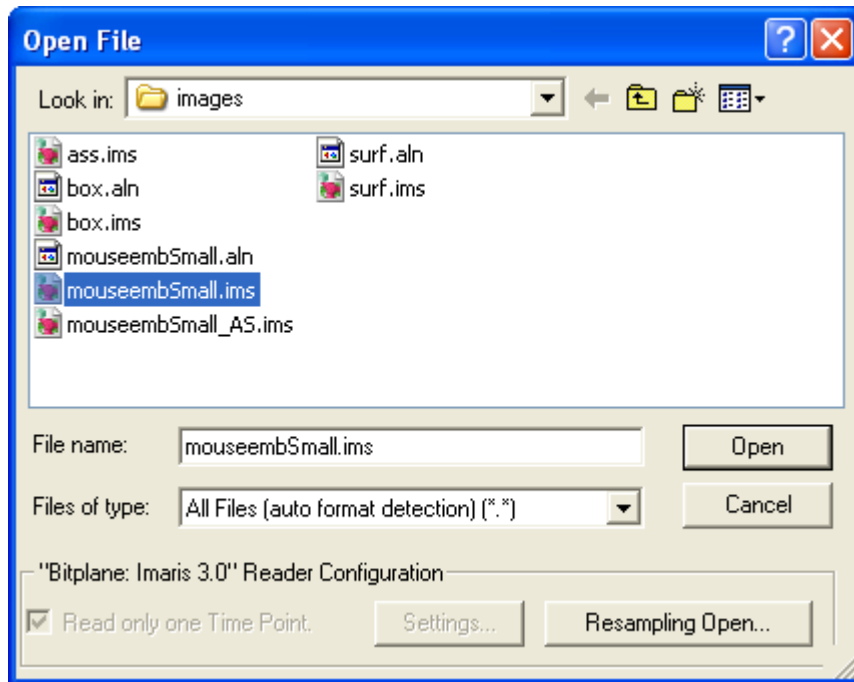

2.1   Open Data Set

PC: Double-click on the AutoAligner shortcut on the desktop of your computer to open the program.
Mac: In the folder Applications double-click on AutoAligner to open the program.

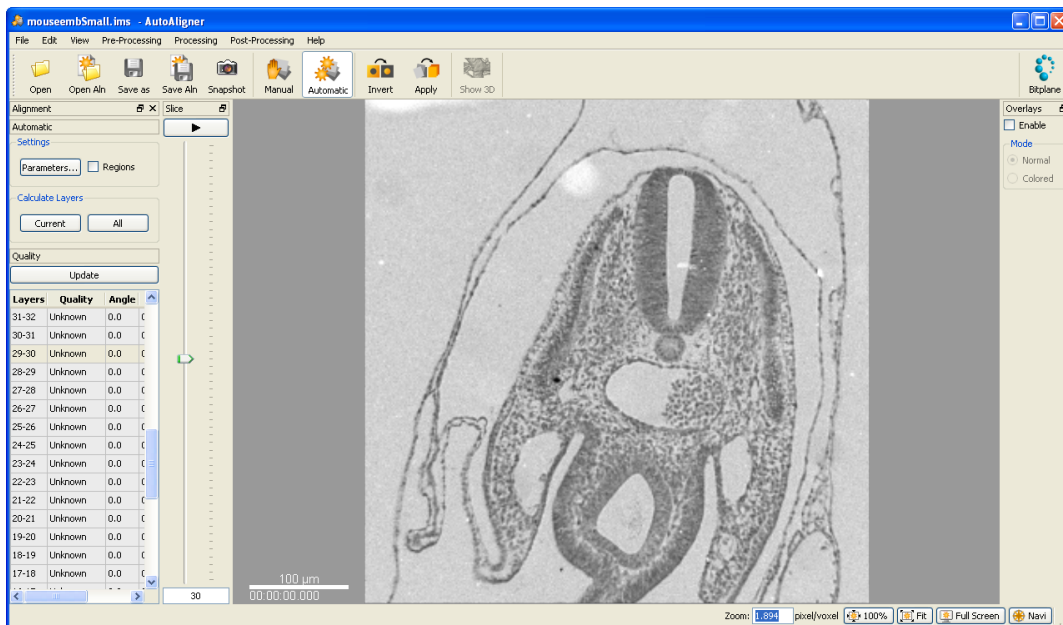In the main tool bar of AutoAligner click on Open. The Open File selection window displays on screen.

This is the Open File dialog on a PC.

Select the data set mouseembSmall.ims and click on Open.

PC file path \ProgramFiles\Bitplane\AutoAligner\images\mouseembSmall.ims
Mac file path /Applications/autoaligner.app/Contents/SharedSupport/images/mouseembSmall.ims

A single layer is displayed in the viewing area.

2.2   View Slices

The slice concept in AutoAligner is very intuitive to understand. Whenever you load a data set with several slices automatically the slice window is displayed at the left hand side of the viewing area.
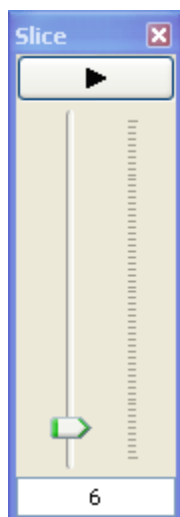
Fig. Slice Panel, slice 6 displays in the viewing area.

Play Button

On top of the Slice window you find the button **Play** (arrow to the right). Click on the button **Play/Pause** to see a continuous display through all the slices in the stack. To pause re-click on the button.

Slice Slider

To inspect any slice in the data set use the slider. Each bar represents one slice of the data set. In the example the slider is at slice 6.

Slice Number Field

Additionally, the slice number of the actual image is displayed at the bottom of the time slider in the slice number field. To change slices enter the desired slice number in the number field at the bottom of the slider.

Slice Panel Overview

▶	Play/Pause through All Slices
Click Slider + Drag Slider	Go to Previous/Next Slice
Click Slider (Select) + Right Arrow on Keyboard	Go to Next Slice
Click Slider (Select) + Left Arrow on Keyboard	Go to Previous Slice
Click Slider (Select) + Home-key on Keyboard	Go to First Slice
Click Slider (Select) + PageDown-key on Keyboard	Go to First Slice
Click Slider (Select) + End-key on Keyboard	Go to Last Slice
Click Slider (Select) + PageUp-key on Keyboard	Go to Last Slice
Enter Number in Slice Number Field	Go to Slice with respective Number

2.3   Check Neighboring Slices

To check the neighboring slices AutoAligner provides several viewing aids.
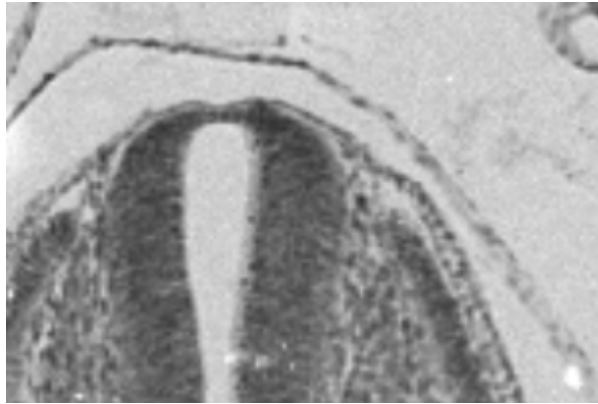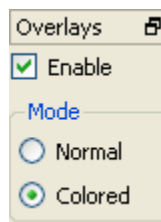

Fig.: Current slice.

Overlays Panel

The parameters are grouped in the Overlays Panel on the right hand side of the screen.

In the **Overlays Panel** check the box **Enable** and select as **Mode Colored**.
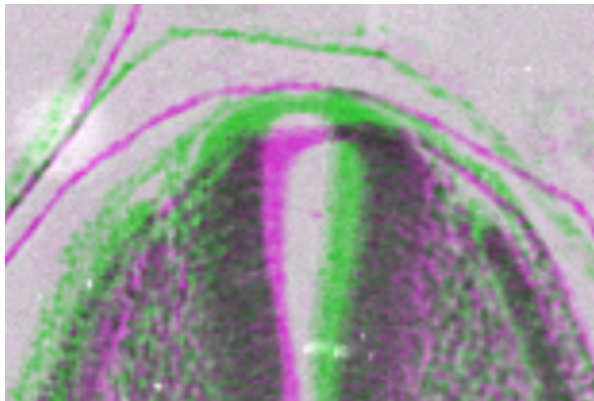
Fig.: The current (green) and the preceding (pink) layer are shown in different colors.

You can select the colors in the "Preferences" dialog (Menu Edit - Preferences... - Alignment Display) .

> Note: Another way to easily compare layers is flipping between two layers by means of the Slice panel. Overlays must be disabled. Then click into the slider of the Slice panel to activate it. You can now flip between the layers with the up and down arrow keys of your keyboard.

See also:
Quick Start Tutorials - View Slices (Slice panel)

Menu Edit - Preferences... - <u>Alignment Display</u> (to change the color definition of the Overlays Mode)

2.4   Pre-Processing

In the Pre-Processing the loaded data set or images are manually prepared for the following alignment. The Pre-Processing has three steps:
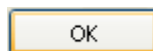
Invert

The structures you want to align should be the brightest in your image. If required, the layers can be turned back to normal after the alignment has been completed and applied, but before saving the data set.
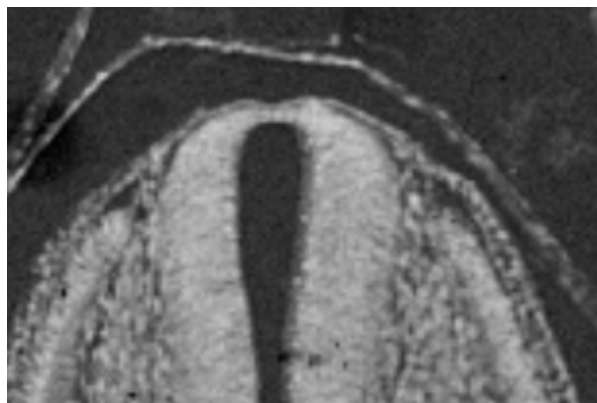
- Open the menu **Pre-Processing** and select **Invert**. (For details please refer to Menu Pre-Processing - <u>Invert</u>)
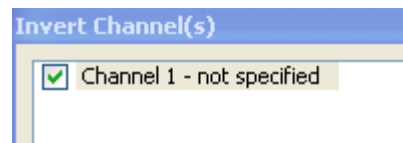
- In the **Invert Channels(s)** window check the box **Channel 1-not specified**.

- Click on the button **Ok**.

All layers are inverted.

Find Background

In order to perform the automatic alignment process, all images are embedded in a larger field. This field, which is called the background, should have a gray value that is similar to a mean gray value along the borders of all images to be aligned.

There are three ways to activate the automatic function:

- Open the menu Pre-Processing and select Find Background. (For details please refer to Menu Pre-Processing - <u>Find Background</u>)

The mean gray value is determined automatically along the border of all images in the data set. If the values of the single layers do not differ significantly, the resulting value is filled automatically in the respective field of the Parameters window.

Please note: If the values differ too much, you must define the background value manually. Please refer to chapter Menu Processing - <u>Auto Alignment Parameters</u> for further details.
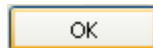
Normalize

In some cases images differ visibly in the average gray values.

- Open the menu Pre-Processing and select Normalize. (For details please refer to Menu Pre-Processing - Normalize)
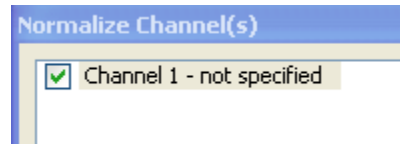
- In the **Normalize Channels(s)** window check the box **Channel 1-not specified**.

- Click on the button Ok.

The function is executed automatically and applied to the image stack.

See also:
Menu Pre-Processing - Find Background
Menu Pre-Processing - Invert
Menu Pre-Processing - Normalize
Menu Processing - Auto Alignment Parameters

2.5   Align Slices

After finishing the preparation of the image layers in the pre-processing stage, the next step is the alignment. Alignment does not take place on the original data set but on a copy of the image stack stored in the memory. The original data remains unchanged until the result of the alignment is applied to it.

Automatic Alignment

Basically, whenever possible you should perform an Automatic Alignment (Menu View - Automatic). An automatic alignment run may take a while, depending on the size of the data set.

In the **Main Toolbar** click on the Icon **Automatic** to activate the automatic alignment mode.

Adjust Region

Define a region of interest that covers the main structures needed for a proper alignment.
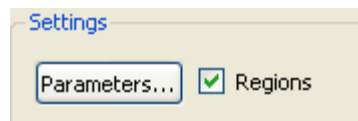
In the **Automatic Adjustments Panel** in the section **Setting** check the box **Regions**.
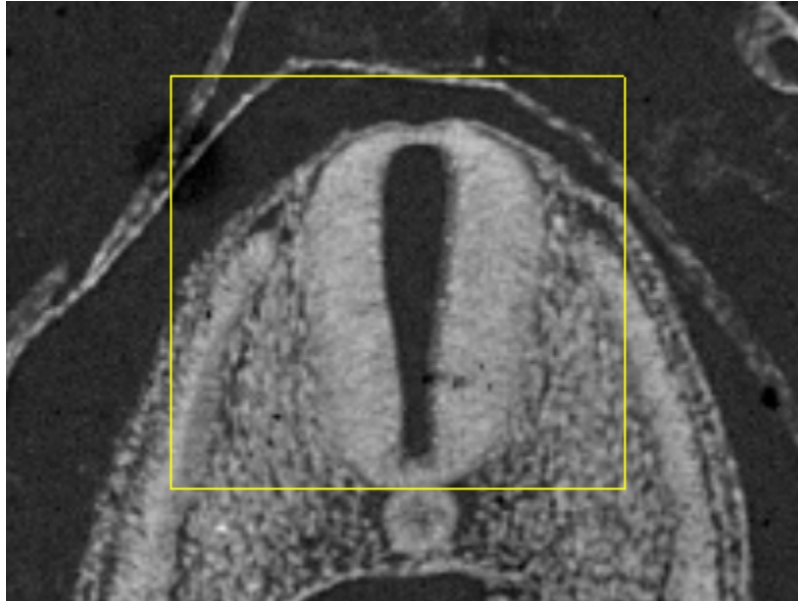
An adjustable, yellow box displays in the viewing area. Adjust the size and position of the box.

Size
- Point with the mouse on the region's contour and the cursor changes into a cross sign.
- Press the left mouse button and move the mouse to change the shape of the region.

Position
- Point with the mouse into the center of the region and the cursor changes into a cross with arrows at the ends.
- Press the left mouse button and move the mouse to shift the region on the image.

Start Alignment
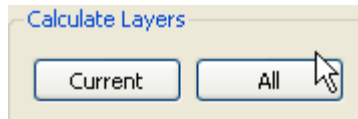
In the **Automatic Adjustments Panel** in the section **Calcualate Layers** check the box **All** to start the alignment of the whole stack.

Manual Alignment

If necessary a correction of single layers by Manual Alignment can follow (Menu View - Manual). Manual alignment is only necessary in exceptional cases or for corrections and fine-tuning of the automatic alignment result.

See also:
Menu View - Manual
Menu View - Automatic

2.6 Apply Alignment

Once the alignment is calculated and shows a satisfactory result, it can be applied to the image stack. This causes the single layers to be shifted and rotated and embedded into a larger image stack. Therefore no original data will be lost, but the resulting data set can be much larger than the original one.

Save Alignment

We recommend saving the alignment first before pressing Apply Alignment. Before applying the alignment the information about rotation and shifting is only administrated in an internal data format, which is shown in the Quality field in the left panel of the main screen. It may be saved at any time with Save Alignment (even before Apply Alignment).

- Open the menu **File** and select **Save Alignment**.

The Save As box is displayed. Select the directory and enter the name for the alignment to be saved or confirm the suggestion. We strongly recommend to save the alignment file as *.aln. The settings for Region and Background are saved together with the alignment. Click Ok and the alignment is saved.

Apply Alignment

Apply Alignment virtually overlays all layers and then generates an enlarged image stack in a size which covers entirely all moved layers. The area outside the original layers is then filled with the defined background gray value.

In the **Main Toolbar** click on the Icon **Apply** to apply the alignment. The layers are shown in a shifted and rotated version in the viewer. After Apply Alignment further (manual or automatic) alignment is no longer possible, because the original data was interpolated by applying the alignment. Only Post-Processing operations can be performed now on this data set.

Save Results

In AutoAligner there are three different types of saves that can be performed:

A. Save complete aligned data sets with Save as... in menu File (Menu File - Save as ...).
B. Save sample layer combinations as TIFF-Files with the Snapshot button (Menu File - Snapshot).
C. Save an alignment (relative shift and rotation of layers) with Save Alignment in menu File (Menu File - Save Alignment).

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3   Menu File

Open...	Ctrl + O
Revert to File	Ctrl + R
Save as...	Ctrl + S
Open Alignment	
Reset Alignment	
Save Alignment	
Snapshot...	Ctrl + T
Exit	Ctrl + Q

3.1   Open

Data sets can be loaded from various file formats.

Image File Series

If the data set consists of a whole series of images, each stored as individual file, select only one file to open and the system will automatically load the rest of the images that belong to the data set.

- In the menu select File – Open.
- Select file type from the Files of Type pull-down menu.
- Select a file name from the list and click Open or double-click on the requested file entry.

The file is loaded.

Supported File Formats

The following file formats are supported by AutoAligner:

- Bitplane: Imaris 5.5 (*.ims)
- Bitplane: Imaris 3.0 (*.ims)
- Bitplane: Imaris 2.7 (Classic) (*.ims)
- Image Cytometry Standard: ICS (*.ics; *.ids)
- Zeiss: LSM510 (*.lsm)
- Zeiss: LSM410, LSM310 (*.ics; *.ids)
- Biorad: MRC 1024, 600 (series) (*.pic)
- Applied Precision: DeltaVision (*.r3d; *.dv)
- Molecular Devices: MetaMorph STK (*.stk)
- Olympus: FluoView TIFF (*.tif; *.tiff)
- Olympus: cell^.R 1.1/standard (*.tif; *.tiff)
- Olympus: OIF (*.oif)
- PerkinElmer: Temporal (*.tif; *.tiff; *.htm; *.html; *.tim; *.zpo; *.cvs)
- PerkinElmer: UltraView (*.tim; *.zpo)
- Open Microscopy Environment XML (*.ome)
- Open Microscopy Environment XML: (*.tif; *.tiff)
- Zeiss: AxioVision (*.zvi)
- Till: TILLvisiON (*.rbinf)
- Leica: TCS-NT (*.tif; *.tiff)
- Leica: Series (*.tif; *.tiff; *.inf; *.info)
- Leica: LCS (*.tif; *.tiff; *.lei; *.raw)
- Leica: Image File Format LIF (*.lif)
- BMP (adjustable file series) (*.bmp)
- TIFF (adjustable file series) (*.tif; *.tiff)

Read only one Time Point

Loads a single time point of a time series.

Button Settings...

In the Settings you specify options for reading certain file formats.

Leica LCS Settings

A LeicaVista data set consists of a number of image stacks (or experiments). A dialog box can be opened to select a specific image stack.

- Select File - Open or click Open in the Main toolbar.
- Select Files of Type: Leica LCS.
- Click on the *.lei file to highlight it and then click on Settings.

Select the required image stack on the left side of the dialog box. The individual images that belong to that stack will display on the right side.

- Click `Ok` to open the image.

A LeicaLIF data set consists of a number of image stacks (or experiments). A dialog box can be opened to select a specific image stack.

- Select File - Open or click Open in the Main toolbar.
- Select Files of Type: Leica LIF.
- Click on the *.lif file to highlight it and then click Settings.

Select the required image stack on the left side of the dialog box. The individual images that belong to that stack will display on the right side.

- Click `Ok` to open the image.

Adjustable Tiff Series Reader Settings

If the data set consists of a series of images, individual images can be sorted according to various dimensions (i.e., slices, channels, time points, dimension sequence). The selected sequence is shown in the File Arrangement panel. The reader can handle tiff series with single and multiple running numbers.

- Select the menu File - Open or click Open in the Main toolbar.
- Select Files of Type: Tiff (adjustable file series) and not Tiff (series) from the drop-down list.
- Open the folder containing the series.
- Click Settings, which is grayed out if the file type selector is on automatic or if the current directory does not contain a series.
- Use Apply Automatic File Filter, Apply, Dimensions, Dimension Sequence, described as follows, to define the series.

Apply Automatic File Filter
This is activated automatically when the dialog is opened. It has the same logics as the classic TIFF series reader of Imaris and will pre-select the first series detected in the directory. Be aware that you may not see all files in the directory.

Button `Apply`
Press this button to use the regular expression to the left and select all files in the current directory that match the criterion, i.e.

- *.tif selects all files with the ending *.tif.
- myfile*.tif selects all files that start with "myfile" and are followed by any letter or digit and by the extension *.tif.
- myfile??.tif selects all files that start with "myfile" and are followed by two letters or digits and by the extension *.tif.

Please note: Depending on the filter, not all files in the directory may be visible. Selecting the required file may take some time because every file is opened but only files with identical xy-dimension are chosen.

Dimensions
- Define the dimensions of the image starting with Slices (Z), Channels (Ch), and Time Points (T).

Please note: The total number of files in the series, as defined by your selection criteria, is displayed below the Time Points input box. Selecting the required file may take some time because every file is opened but only files with identical xy-dimension are chosen.

Dimension Sequence
- Defines how the individual images, which are sorted alphabetically, are to be assigned to Slices (Z), Channels (Ch), and Time Points (T).
- Click `Ok` to return to the Open dialog window.
- Click Resampling Open to open the Resampling dialog box or click Open to open the image.

Button Resampling Open...

The Resampling Open dialog box can be used as preview before loading a data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data. This can be particularly important when reading large data sets over a network.

- Select Menu File - Open or click on the Open button in the Main toolbar.
- Click on a file to highlight it and then click on the button **Resampling File Open ...**.

Original Size
Display of the original file size.

Subsampling Factor

X,Y,Z, Ch, T
You have the option to specify the Subsampling Factor, i.e., the fraction of data points to be retained. The subsampling factor can be specified for the x-, y-, z-directions, the channels (Ch), and the time points (T).

Crop Limits (Min/Max)

X, Y, Z, Ch, T, From, To
The Crop Limits (Min/Max) for the x-, y-, z-directions, the channels (Ch), and the time points (T) can also be specified.

Resampled Size
Display of the resampled file size.

- Click on Open and the data set is cropped and resampled while loading.

Image Preview
The view on the right side displays as image preview a single time point of the data set.
Right-click in the view and move the mouse pointer to the right to increase brightness. Move the pointer upwards to increase contrast.

3.2   Revert to File

Re-opens the actual data set.

3.3   Save as ...

The AutoAligner file format is the same then the Imaris file format (*.ims). Saving in Imaris format (*.ims) is recommended whenever the data set is cropped or the parameters changed. Saving a data set in Imaris file format provides the advantage of a faster loading process and the possibility of using thumbnails. In addition, most parameters are saved with the images.

- In the menu bar select File – Save as... . The Save As window is displayed.
- Select the directory and enter the name for the file to be saved or confirm the suggestion.
- Select the requested file format and click Ok.

The data set is saved.

Available File Formats in AuotAligner

- Bitplane: Imaris 5.5 (*.ims)

- Bitplane: Imaris 3.0 (*.ims)
- Bitplane: Imaris 2.7 (Classic) (*.ims)
- TIFF (series) (*.tif; *.tiff)
- RGBA-TIFF (series) (*.tif; *.tiff)
- Image Cytometry Standard: ICS (*.ics; *.ids)
- Olympus: cell^.R 1.1/standard (*.tif; *.tiff)
- Open Microscopy Environment XML: (*.ome)
- Open Microscopy Environment Tiff: (*.tif; *.tiff)
- BMP (series) (*.bmp)
- Movie file (slice animation) (*.avi; mov.; *.tif; *.tiff)

Advanced Save Options

Button **Format Settings...**
A click on the button **Format Settings...** opens the AutoAligner advanced save options.

Example for the Imaris 5.5. file format:

Enable Compression
Check the box to use a compression.

Add to ImageAccess Database

Check the box to add the file to the ImageAccess database.

3.4 Open Alignment

The alignment can be calculated for a modified version of the data set, e.g. for a filtered version or an edge enhanced version, and later applied to the original data set.

- Open a data set.
- Modify the data set.
- Do an automatic or manual alignment.
- Save the alignment in a text file (Menu File - Save Alignment).
- Now open the original data set again.
- Open the former saved alignment (Menu File - Open Alignment) to perform the same rotations and translations.

3.5 Reset Alignment

Sets all shifts and rotations to zero (Reset for all layers). The old alignment is lost and may be saved before. Reset may be necessary, if a second data set is loaded, which has the same number of layers as that before. Reset Alignment will recover the state before any alignment.

3.6 Save Alignment

In the main bar select File – Save Alignment. The Save As box is displayed. Select the directory and enter the name for the alignment to be saved or confirm the suggestion. We strongly recommend to save the alignment file as *.aln. The settings for Region and Background are saved together with the alignment. Click **Ok** and the alignment is saved.

3.7   Snapshot

Saving 2D still images is supported by the Snapshot option. Still images can be stored in TIFF-Format or as database records in ImageAccess in order to manage them more efficiently. The process of creating a snapshot in these formats has been semi-automated in AutoAligner, there is an automatic window size control adjustment implemented.

Image Size

Template
Select Windows, Numerical, Size from Image or an Image Size Template.

There are certain points in the image size to be aware of:
- If you choose as image size a very small or very big sized format an exclamation point appears behind the selected size.
- Use big sizes only if you need the image for a poster, in publications or on covers (print media).

Width
If you select as Template Numerical the width is adjustable. If you select any other Template, the width is displayed.

Height
If you select as Template Numerical the height is adjustable. If you select any other Template, the width is displayed.

Button Lock Aspect Ratio
If you select as Template Numerical you can click on this button to lock the aspect ratio.

Aspect Ratio
Display of the Aspect Ratio.

Image Output

Save to File
Click on the button Save as and select the requested directory. Accept the automatic naming suggestion or enter a new name for the image. Then click on the button Do Snapshot!

Add to ImageAccess Database
Make sure that the Database ImageAccess is started. Select the requested directory and enter a name for the image. Click on the button Do Snapshot! The image is saved on the disk and an entry is added to the database.

Copy to Clipboard
Click on the button Do Snapshot! and the image is copied to the clipboard. Open another application and select the paste function.

File Path and Button Save as...
Here you select the file path for the image output. The image is not saved yet, you just selected the right path and name for the image.

Button Do Snapshot!
Click on the button Do Snapshot! to save the image. Automatically the image number for the next snapshot file is increased in consecutively.

See also:
Menu Edit - Copy Snapshot Image
Menu Edit - Preferences - Display - Off Screen Rendering (for saving Snapshots and Movies)

3.8 Exit

Terminates AutoAligner and returns to the desktop.

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4 Menu Edit

Copy Snapshot Image   Ctrl + C

Image Properties...   Ctrl + I
Show Display Adjustment   Ctrl + D

Preferences...   Ctrl + P

4.1 Copy Snapshot Image

When an image is ready to be stored, make sure that it is fully visible on the screen and not obstructed by any other windows or displays. To save the image, AutoAligner reads from the internal buffer, so other objects on the screen would appear superimposed on the image.

- Select Edit - Copy Snapshot Image and the image is copied to the clipboard.

Open another application and select the paste function. The image is pasted into the new application.

See also:
Menu File - Snapshot

4.2 Image Properties...

Geometry
Data Set
Channels
(Channel 1...n)
Thumbnail
Parameters

When opening a data set, the following parameters should be checked or modified:

- Name and Description (in Data Set).
- Voxel Sizes (in Geometry).
- Channel Colors (in Channel 1...n).

4.2.1 Geometry

The geometrical settings of the actual data set are displayed.

Type

Data Type
Display of the image type.

Size

Size X, Y, Z, T

Display of the image size.

Coordinates

Voxel Size, X, Y, Z
The voxel sizes directly influence the views because they control the height of the image relative to its width. Check the parameters and adjust the **Voxel Size** and/or other settings if necessary.

Min, X, Y, Z
The minimum value of the coordinate axes.

Max, X, Y, Z
The maximum value of the coordinate axes.

Unit, nm, um, mm, m, unknown
Here you can select the unit.

Time Point

First Box
Select the time point.

Date
Enter the collection data.

Time
Enter the collection time.

Button **All Equidistant...**
Only available If the data set is a time series. Enter the date/time for each time point or click **All Equidistant** to open the Set Equidistant Time Points dialog box.

- Enter the **Start Date** and **Start Time** and the **Time Interval**.
- Click **Ok** when finished. AutoAligner will calculate the time for each time point in the series.

4.2.2   Data Set

Name

Data field to type in a data set name.

Description

Data field to type in a data set description.

Numerical Aperture (N.A.)

Reads out the numerical aperture (as defined in the Menu Edit - Image Properties... - Parameters).

Log

Display of processing steps.

4.2.3   Channels

There is no parameter on this card.

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4.2.4　Channel 1...n

In the Index of the Image Properties box click the **Channel** entry (Channel 1, Channel 2 etc.) to select the required channel.

Name

Data field to type in the channel name.

Description

Data field to type in a channel description.

Emission Wavelength

Reads out the emission wavelength.

Excitation Wavelength

Reads out the excitation wavelength.

Pinhole Radius

Reads out the pinhole radius.

Tab Base Color

Red, Green, Blue
Reads out the assigned color. To change the color either adjust the values or move the square in the color circle.

To apply the changes click on `Ok`.

Tab Mapped Color

Selected Color

Button `Edit...`
Click on a square in the grid and click on the button `Edit...`. Select a color and click on `Ok`. The selected color is displayed in the square.

Button `Copy`
Click on a square in the grid and then on the button `Copy` to copy the color.

Button `Paste`
Click on a square in the grid and then on the button `Paste` to paste the color.

Interpolation

Colorspace
RGB
The RGB color model is an additive model in which red, green and blue are combined in various ways to reproduce other colors.

HSV
The HSV (Hue, Saturation, Value) model, defines a color space in terms of three constituent components.

- Hue, the color type.
- Saturation, the vibrancy of the color.
- Value, the brightness of the color.

Button **Interpolate**
Select two squares in the grid and click on the button **Interpolate**. The colors between the two selected squares are interpolated.

Color Table File

Button **Import ...**
AutoAligner comes with a set of pre-defined color tables. Click on **Import...** to open the Import Color Table File window. Select a color table file and click on **Ok**.

Button **Export...**
Click on the button **Export...** to open the Export Color Table File window. Select a destination and click on **Save**.

To apply the changes click on **Ok**.

4.2.5 Thumbnail

Type

None
Select None if no thumbnail should be displayed.

Middle Slice
Thumbnail is the middle slice.

MIP
Thumbnail in the display mode Maximum Intensity Projection. A Maximum Intensity Projection is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.

Blend
Thumbnail in blend projection. Blends all values along the viewing direction and includes their transparency.

Preview

Displays a preview of the thumbnail image.

4.2.6 Parameters

A set of informational parameters is appended to the image file.

Button **Add Group...**
Opens a dialog to add a new group.

Button **Delete Group...**
Deletes a group.

Button **Add Parameter...**
Opens a dialog to add a new parameter to the group.

Button **Delete Parameter...**
Deletes a parameter.

4.3   Show Display Adjustment

The Display Adjustment function lets you choose the channel visibility as well as improve the image display by concentrating on a limited color contrast range of voxels. Usually the color contrast values of the voxels stretch over a wide range (e.g. 0 - 255). The Display Adjustment function lets you set an upper limit for maximum color and a lower limit for minimum color (i.e., black). The range between these two limits is then extrapolated in a linear mode to the full data set range and the new voxel values are calculated.

Channel Visibility

Whenever the data set consists of more than one channel, all channels are usually visible concurrently. The channel visibility feature lets you switch the individual channels on or off. Check or un-check the required channel check-box to switch the channel visibility.

Change Channel Color

Click on the channel name to switch directly to the channel properties (Menu Edit - Image Properties - Channel 1...n). Set the requested values and click Ok when finished.

Advanced Settings

Button Advanced
Click on the button to open the Advanced settings.

Select all Channels
Click the requested channel to select it, or check Select all Channels to apply the settings to all channels.

Histogram

The histogram shows a linear voxel representation of the selected channel.

Settings Channel x

Min
Max
Drag the upper or lower handle of the adjustment line to adjust the Min and Max limits or enter direct values in the Min and Max fields. The effect of the change can be seen on the channels (channels appear brighter or darker).

Opacity
The opacity adjustment allows you to change the opacity in real-time. Enter a value or drag the slider bar to adjust the opacity. You can see the effect of the change on the channels (channels appear more or less transparent).

Button Reset
Click this button to set the image back to the original values.

Button Auto
When clicking this button the system detects the real high and low values (e.g. 10 - 150) and sets the Max. and Min. limits automatically to these values.
If you check the parameter **Select all Channels** (see above) all channels have the same Min. and Max. limits.

Button Auto Blend
Click on this button and AutoAligner automatically calculates the optimized Min. and Max. limits. A good portion of the selected image channel becomes transparent.
If you check the parameter **Select all Channels** (see above), all channels are calculated consecutively.

See also:
Menu Edit - Image Properties... - Channel 1...n

4.4   Preferences...

The adjustable parameters in the preferences are application specific and AutoAligner stores this preferences for an individual user.

System
Display
Alignment Display
Alignment
Loading
Update
Advanced

4.4.1   System

Displays the basic system parameters of your computer.

Processor

Number of Processor
Processor Architecture
Processor Speed
Information about the number of Processors, Processor Type and Processor Speed.

Graphics

OpenGL Renderer
OpenGL Version
Pixel Shader
OpenGL Extensions
Information about the OpenGL Renderer, OpenGL Version, Pixel Shader, and OpenGL Extensions.

Operating System

OS
Version (Build)
Service Pack
Information about the operating system, the installed Version and the service pack.

Memory Status

Physical Memory installed
Physical Memory available
Information about the available amount of internal memory.

4.4.2   Display

Select the viewing properties and the basic colors for the background and the selection.

Display

Interpolate
If checked, the images are automatically interpolated for a smoother display.

Texture Cache Limit (MB)
Before displaying any image data, AutoAligner converts the data into a configuration (called textures) that is optimized by the graphics hardware. The value of the Texture Cache Limit determines how many textures can be stored in RAM. Set the value to the memory of your graphics card.

Background Color
Normal background if an image does not completely fill a view.

Button Select...
Click on this button to open the color selection window to change the respective color.

Background Color 2
Background marking the original position if an image is moved.

Button Select...
Click on this button to open the color selection window to change the respective color.

Checkered Background for Blending
When using the blending mode, a checkered background displays.

Tile Size
Allows definition of the tile size for a checkered background.

Linear Color Progress for Blending
The background displays a color gradient in blend progress projections.

Selection Color
Color of selections (e.g. regions rectangle).

Button Select...
Click on this button to open the color selection window to change the respective color.

Show Coordinates Axis
Show Date
Show Scale Bar
Show Time
Show relative Time
Select to display coordinate axis, date, scale bar or time on screen.

Off Screen Rendering (for saving Snapshots and Movies)
Check this box to save only the viewing area. For the Snapshot and Movie AutoAligner hides additional control elements. If you un-check this box the actual screen display is saved, e.g. if the window Display Adjustments is in front of the viewing area this window is saved as well.

Show System Monitors
Check this box to display the system monitors in the Status Bar at the bottom of the screen. The first window displays the "reads per sec", the second the "writes per sec", the third the "write requests in queue" and the last the "percentage read cache hits". These are useful information especially if you work with huge data sets.

See also:
Menu File - Snapshot

4.4.3   Alignment Display

With the Alignment Display parameters you define the basic color parameters of your display and specify the limits of the quality factor display.

The specified limits for Best and Mean quality determine the color coding in the Quality Display Panel of the

main screen (for a detailed description of the Quality Display Panel please refer to chapter Menu Processing - Update Quality).

Best Quality
Layers with a factor between 100 and the limit for Best Quality appear in green.

Mean Quality
Layers with a factor between the limit for Best Quality and the limit for Mean Quality appear in orange.

Bad Quality
Layers with a factor below the limit for Mean Quality appear in red.

Colors

Here you define the color definitions for the colored Overlay Mode in the viewer (for a detailed description of the Overlay Mode please refer to chapter Toolbars and Panels - Overlays Panel).

Overlay Background
The preceding layer is shown in the background with the given color.

Button **Select...**
Click on this button to open the color selection window to change the respective color.

Overlay Foreground
The current (active) layer, which may be manually or automatically aligned, is shown in the foreground with the given color.

Button **Select...**
Click on this button to open the color selection window to change the respective color.

See also:
Quick Start Tutorial - Check Neighboring Slices
Toolbars and Panels - Overlays Panel

4.4.4 Alignment

Alignment

Automatically update quality
Check this box and the automatic execution of the update quality after the alignment calculation is switched On.

Calculation

Number of Processors
Numbers of processors being used for the calculation process.

See also:
Menu Processing - Update Quality (manual execution if the automatic is switched Off)

4.4.5 Loading

How should colors be assigned when loading from other than Imaris data formats?

Take Colors from:

Default Colors
Use the default color selection to display the loaded data set. The parameter set Default Colors (see below) is available.

File Colors (color table or base color if available, otherwise default colors)
Use the original color definition of the loaded data set (usually stored in a lookup table). The parameter set Default Colors (see below) is not available.

Emission Wavelength (from file if available, otherwise default colors)
Use the color according to the emitted wavelength from the file (corresponds to the appearance under the microscope). The parameter set Default Colors (see below) is not available.

Please note that not all file formats support lookup tables and emission wavelength.

Default Colors

The parameter set Default Colors is available, if you select Default Colors in the parameter set Take Colors from (see above).

First Cannel
Second Channel
Third Channel
Other Channels
Display of the defined color.

Button Select...
Click on this button to open the color selection window to change the respective color.

Automatic File Format Detection Sequence

Allows you to specify the order in which the file format readers are called when loading images. Move the most frequently used file format readers to the top of the list to hasten file loading.

Button Move Up
Button Move Down
- Highlight a file reader.
- Click Move Up or Move Down to arrange the format readers in the desired sequence.

4.4.6   Update

How often should be searched for updates?

Daily
Weekly
Never
Select either daily, weekly, or never.

4.4.7   Advanced

Displays a window similar to the parameters window. It shows internal settings and parameters in groups and parameter lists.

Please keep in mind: Changing the advanced parameter settings may endanger your system and lead to erroneous shutdowns!

5   Menu View

Please refer to chapter:

Manual	Alt + M	
Automatic	Alt + A	
Fit	Ctrl + B	
100%	Ctrl + A	
Full Screen	F11	
Navi	Ctrl + N	
Main Tool Bar		Toolbars and Panels - Main Tool Bar
Status Bar		Toolbars and Panels - Status Bar
Refresh	Ctrl + F	

5.1   Manual

Moving single layers is only possible in the Manual Alignment mode. By choosing Manual Alignment the viewer mode Overlay is set. This mode allows you to undo and reset movements for single layers. The quality of the manual alignment can be estimated by a Quality Update.

Alignment Panel Manual
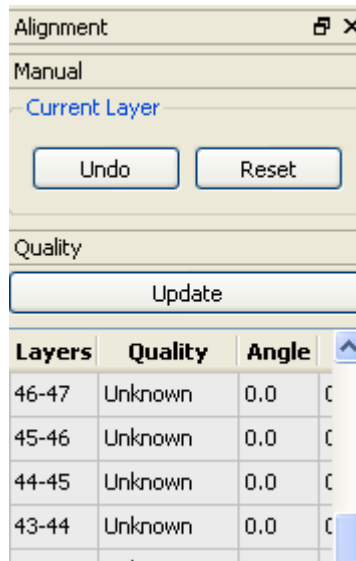
Current Layer

Only the current layer (which is indicated by the number of the slider) can be moved with the mouse.

Please note: If a key or shortcut does not work, it may be necessary to activate the keys and shortcuts first by clicking in the main view.

Buttons Overview

To move the selected layer use the key and mouse combination:

	PC:	Mac:
Translation	Shift + Left-click & drag	Shift + click & drag

Rotation, the angle of the rotation is displayed	Ctrl + Left-click & drag	Ctrl + click & drag

Search for a small detail, which already is in congruence in both layers and should remain as a fix point, and point the mouse arrow to this detail. Then press Ctrl and the left mouse button to indicate this point as rotation center. By horizontally moving the mouse to the right a circle is spanned. By moving the mouse radially, the current layer is rotated by the indicated angle. It is helpful to span a circle with large a radius, because then is it easier to choose the rotation exactly.

Button **Undo**
Undo the last manual alignment (translation or rotation).

Button **Reset**
Resets the alignment of the current layer (rotation angle and shifts are set back to 0). Thus the current layer and the preceding layer become congruent (the boundaries of the images are congruent). If you want to recover the whole unaligned stack (to make all layer boundaries congruent), choose Reset Alignment in menu File.

Quality

Button **Update**
Please refer to chapter Menu Processing - Update Quality.

5.2   Automatic

Basically, whenever possible, you should perform an Automatic Alignment. Manual alignment (Menu View - Manual) is only necessary in exceptional cases or for corrections and fine-tuning of the automatic alignment result. An automatic alignment run may take a while, depending on the size of the data set.

In Automatic Alignment mode layers can not be moved manually. You can chooses parameters and - if required - regions for the alignment. The quality of the automatically aligned layers can be calculated directly by enabling the function (disabled by default) and shown in the Quality field in the left panel of the main screen.

Alignment Panel Automatic
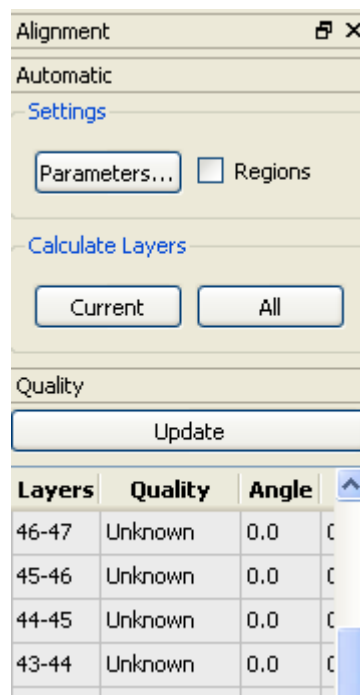
The appropriate choice of some parameters has an important effect on the automatic alignment process. This includes the following settings:

- Alignment on a selected region of the images only
- Parameter for the background intensity
- Parameter for the type of alignment
- Parameter for the accuracy of the movement and the alignment calculation
- Alignment on a resampled version of the images
- Possible restrictions in the movement of the layers

Button **Parameters...**
Please refer to chapter Menu Processing - Auto Alignment Parameters

Check box **Regions**
Please refer to chapter Menu Processing - Select Regions

Calculate Layers

Button **Current**
This function calculates the alignment of the currently visible layer with respect to the previous one. Usually it is used to correct a layer after an automatic alignment has been performed upon the entire data set. Different parameters such as Restrictions, another Background Value or another Region can be chosen for this calculation.

The number of the current layer is shown with the slider in the "Slicer" panel in the main screen. The numbering of the layers always starts with 1, disregarding the given file numbering (e.g., of a TIFF series). Layer number 1 is therefore always the bottom layer in the image stack.

Button **All**
This is the normal processing after having checked and set all previously described parameters. It starts the alignment of the whole stack calculated with the last chosen parameters.

Quality

Button **Update**
Please refer to chapter Menu Processing - Update Quality.

5.3   Fit

Fits the entire scene into the view.

In the Status Bar you find the respective button **Fit**.

See also:
Toolbars - Status Bar

5.4   100%

Select 100% to rotate the image to the original position, center the image in the middle and set the zoom factor to one pixel per voxel.

In the Status Bar you find the respective button **100%**.

See also:

Toolbars - Status Bar

5.5   Full Screen

Displays the viewing area to full size of the monitor. To return to the standard window, click on the button `Full Screen` in the lower right corner.

In the Status Bar you find the respective button `Full Screen`.

See also:
Toolbars - Status Bar

5.6   Navi

The Navigation window displays an overview of the data set in the upper right corner of the viewing area. If you move the data set the overview updates in real-time.

In the Status Bar you find the respective button `Navi`.

Change Position

- In the pointer mode Select you can click and drag the Navigation window to any desired corner.
- Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Re-double-click onto the Navigation window to switch the automatic "snap-to-corner-mode" on again.
- Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

In the Section view the position of the Navigation window is fixed (lower right corner) and can not be moved.

See also:
Toolbars - Status Bar

5.7   Refresh

Refresh the screen.

6   Menu Pre-Processing

Find Background
Invert
Normalize

The Pre-Processing menu contains the image processing functions for the alignment preparation.

6.1   Find Background

In order to perform the automatic alignment process, all images are embedded in a larger field. This field, which is called the background, should have a gray value that is similar to a mean gray value along the borders of all images to be aligned. The appropriate choice of the background value is crucial for a good automatic alignment. AutoAligner is able to perform an automatic detection of the mean intensity in an area along the image borders. This function is called "Find Background".

Automatic Background Determination

There are three ways to activate the function:

A. In the menu bar select Pre-Processing – Find Background.
B. In the menu Processing - Auto alignment parameters. In the section Basic select **Other** and click on the button `Find Background`.
C. In the Automatic Alignment Mode on the Alignment panel in the section Settings click on the button `Parameters...` to open the Auto alignment parameters (see B.). In the section Basic select **Other** and click on the button `Find Background`.

The mean gray value is determined automatically along the border of all images in the data set. If the values of the single layers do not differ significantly, the resulting value is filled automatically in the respective field of the Parameters window.

Please note: If the values differ too much, you must define the background value manually. Please refer to Menu Processing - Auto Alignment Parameters for further details.

See also:
Menu Processing - Auto Alignment Parameters (for manual background adjustment)

6.2   Invert

It is of the utmost importance that the structures, the automatic alignment has to be performed upon, show the highest gray values, i.e., are the brightest and therefore easiest to detect on the image. If these structures appear to be darker than the other areas the image can be inverted for the alignment process. If required, the layers can be turned back to normal after the alignment has been completed and applied, but before saving the data set.

Check the Image Gray Values

The actual gray values in the single layers are shown in the status bar on the bottom left. Move the mouse pointer over the regions of interest on the image and observe the constantly changing display to determine, whether the images have to be inverted.

"24 at (13.44 36.80 1.00)" means „gray value 24 at the image coordinates (x y z)".

Invert Image

There are two ways to invert the image.

A. Select in the Menu Pre-Processing the menu entry Invert.
B. Click on the icon Invert in the main toolbar.

6.3   Normalize

Incidentally single layers can be acquired with different exposure times or have different thickness. In this case some of the images differ visibly in the average gray values. This can be seen and checked when animating the data set by clicking on the button `Play` in the Slice Panel (Toolbars and Panels - Slice Panel). The images are shown one after one.

In case such images are detected, normalization should be performed, to automatically calculate and apply an average contrast and brightness (linear normalization) to all layers.

Normalization is not necessary for a proper alignment but it improves the visualization and helps to improve possible manual corrections of the alignment.

Normalize Image

In the Menu Pre-Processing select Normalize to calculate and apply a mean value for both brightness and contrast on all images. The function is executed automatically and applied to the image stack.

7 Menu Processing

Select Regions
Auto Alignment Parameters
Calculate Current Layer
Calculate All Layers

Update Quality
Find Quality of All Layers

Straighten Alignment

Apply
Undo

The Processing menu contains all functions needed during the alignment processing stage including the parameter definition.

After finishing the preparation of the image layers in the pre-processing stage, the next step is the alignment.

Alignment does not take place on the original data set but on a copy of the image stack stored in the memory. The original data remains unchanged until the result of the alignment is applied to it. Basically, whenever possible an automatic alignment should be performed. Manual alignment is only necessary in exceptional cases or for corrections and fine-tuning of the automatic alignment result. An automatic alignment run may take a while, depending on the size of the data set.

The alignment result (together with the settings for Region and Background) is saved in a text file. If necessary a correction of single layers by manual alignment can follow.

Further optimization may be achieved by a second, restricted automatic alignment run, which performs the best fit within user-defined limited movements.

If the alignment is satisfactory, it has to be applied to the original data set: the layers are rotated, shifted and embedded into a larger image stack, which can now be saved. This produces a new aligned data set, which then can be post-processed.

Before applying the alignment the information about rotation and shifting is only administrated in an internal data format, which is shown in the Quality field in the left panel of the main screen. It may be saved at any time with Save Alignment (even before Apply Alignment). The settings for Region and Background are saved together with the alignment.

7.1 Select Regions

The automatic alignment can be calculated on:

- The entire data set. This is time consuming and does not necessarily lead to a better result.
- A user-defined rectangular part of the image layers, called a region. This region should cover the main structures needed for a proper alignment.

What is a Region?

A region is defined in its size and shape on one of the layers and is then positioned on all other layers. The dimensions of the region remain the same on all images but the position may differ on the layers.

The alignment is then calculated on this region only and the result applied to the whole stack. The quality is calculated not only in the regions but also for the entire image size in the original stack.

This option can be used on one hand for shortening the calculation time significantly. On the other hand it must be used for aligning distorted images, which still contain undistorted parts, e.g., sectioned tissue, where parts of the sample have been ruptured during mechanical sectioning, but other parts are still intact and can be used for the alignment.

Activate Regions
There are two ways to activate the Regions option in the AutoAligner.

A. In the menu Processing click on Select Region.
B. In the Automatic Alignment panel of the main screen click the checkbox Regions (in the Settings).

A rectangular shape is displayed on the image in the viewer.

Define Size and Position of the Region

Size
- Point with the mouse on the region's contour and the cursor changes into a cross sign.
- Press the left mouse button and move the mouse to change the shape of the region.

Position
- Point with the mouse into the center of the region and the cursor changes into a cross with arrows at the ends.
- Press the left mouse button and move the mouse to shift the region on the image.

Shifting all Regions
If you want to shift all regions on all layers at the same time, press the left Alt-key and the left mouse button and move the mouse to shift the regions.

For rotational alignment it is recommended to choose the region nearly quadratic in order to use all information in the chosen region.

7.2   Auto Alignment Parameters

The Alignment Parameters box is displayed with the two tabs Basic and Advanced.

- The Basic parameters are pre-defined and should be checked or adapted before starting the alignment.
- The Advanced parameters are used in special cases only, however, the Accuracy parameter should be checked as well before any alignment is started.

Basic Parameters

Background Intensity

In order to perform the automatic alignment process, all images are embedded in a larger field. This field, which is called the background, should have a gray value that is similar to a mean gray value along the borders of all images to be aligned. The appropriate choice of the background value is crucial for a good automatic alignment.

Mean value
Check this box and the mean value of all dark and bright areas in the background and foreground is set as background intensity.

Zero
Check this box if the image shows a homogeneous black background (gray value=0).

Other

If you select Other you can either enter the value manually in the data field or click on the button `Find background` for the automatic background determination (see below).

If the image shows a highly structured pattern of dark and bright elements the Find background function is not recommended, because the border region also contains many bright pixels and will result in an overall mean value of all structures which is not representative for the darker background value.

To manually find out the background intensity do the following:

- Zoom in on the image to a smaller region, preferably the region of interest for the alignment.
- Evaluate manually the mean value within the darker parts of the small region by moving the mouse pointer over these fields on the image. Observe the changing display in the status bar (button left corner) to determine the mean value.
- In the section Basic select Other and enter the chosen value as Background Intensity in the data field.

Automatic Background Determination

Button `Find background`
This button is only available if Other is selected (see above).

AutoAligner is able to perform an automatic detection of the mean intensity in an area along the image borders. Click on the button and the mean gray value is determined automatically along the border of all images in the data set. If the values of the single layers do not differ significantly, the resulting value is filled automatically in the respective data field.

Type of Alignment

Alignment is usually performed by both rotational and translational movements.

Translational
If the experimental setup allows shifts but no rotations of the acquired layers, you can choose translational alignment (only).

Translational and rotational
Rotational and translational alignment movements are performed..

Channel (multi-channel images only)

If the image is composed of three channels, it can be a stack of colored images (RGB) or a data set with three independent channels. The various channels may be viewed separately in the Display Adjustments (Menu Edit - Show Display Adjustment). The quality is also calculated on the chosen channel or on a gray version of the RGB-image, respectively. The alignment will be applied to all channels of the data set.

Select channel number
In case of three or any other number (>1) of independent channels the number of the channel used for the calculation has to be selected.

RGB image
Choose this option if you have a colored image. This converts the colored image into a gray value image for calculation of the alignment, but then applies the alignment again to the original color data set.

Click on `Ok` to save the changes.

Advanced Parameters

Resampling Factor

For a faster calculation the alignment can be calculated in a smaller, resampled version of the loaded data

set (e.g., Resampling Factor = 2). Resampling affects the calculation only. The rest of the program, especially also the Update Quality, still works with the original dimensions and factor of the data set. If you press Apply Alignment, it will be applied to the loaded original data set.

Accuracy

Rotation [degree]
The accuracy of the translational alignment is 1 pixel by default. You can choose the accuracy of the rotational alignment. The chosen number may be rounded internally and will be shown in the Parameters window after having pressed Ok. However, you have to be aware that a small value (like 0.2 degree) results in a longer calculation time.

Restrictions

Please note: The restricted alignment needs the same calculation time as the free alignment. You do not save time by restricting.

The first automatic alignment of a data set should be performed without any restrictions. Restrictions are recommended only for correcting single layers. For example, if the result of the automatic alignment of one layer is bad, it can be corrected manually, followed by a finer automatic search for the best alignment with set restrictions. Restriction values are no absolute values - they are defined relative to the actual alignment. It is recommended to use only rotational restrictions or, when only shifts are possible in the experiment, translational restrictions.

Limited x-shift +/- [um]
Enter the value for the x-shift.

Limited y-shift +/- [um]
Enter the value for the y-shift.

Limited rot. angle [degree]
Enter the limited rotation angle.

Example

Perform a manual alignment as best as possible. Then choose a restricted rotational range, e.g., 5°, and calculate the alignment by clicking Calculate Current. In most cases a further limitation of the shifts is not necessary.
If so, reduce the rotational range to 0° and choose appropriate translational restrictions, depending on the pixel size of your data set. **Type of Alignment** must be **Translational and Rotational** (see above).

Click on Ok to save the changes.

See also:
Menu Edit - Show Display Adjustment

7.3   Calculate Current Layer

This function calculates the alignment of the currently visible layer with respect to the previous one. Usually it is used to correct a layer after an automatic alignment has been performed upon the entire data set. Different parameters such as Restrictions, another Background Value or another Region can be chosen for this calculation.

The number of the current layer is shown with the slider in the "Slicer" panel in the main screen. The numbering of the layers always starts with 1, disregarding the given file numbering (e.g., of a TIFF series). Layer number 1 is therefore always the bottom layer in the image stack.

See also:
Menu View - Automatic

7.4   Calculate All Layers

This is the normal processing after having checked and set all previously described parameters. It starts the alignment of the whole stack calculated with the last chosen parameters.

See also:
Menu View - Automatic

7.5   Update Quality

Quality

Update
The quality is always calculated from the entire loaded image stack, even if regions are selected or a resampling factor has been chosen. It is therefore possible that the Update Quality process takes longer than the actual calculation of the alignment.

The automatic execution of the update quality can be switched On or Off.

- Select Edit – Preferences... – Alignment.
- Set or remove the checkmark for Automatically update quality and click **Ok**.

Only images that have been moved are processed in the update quality function. Images that have not been moved display "unknown" in the Quality panel of the main screen. If the automatic execution is switched off, moved images display "need update" in the Quality panel of the main screen. Otherwise the actual quality index value is indicated. A green indication means a high quality value. An orange indication means an average quality. Indication in red means the value is rather poor. The limits for these values can be set in the Edit – Preferences... – Alignment Display.

Find Quality of All Layers
If you need to calculate the quality not only of the moved layers but of all layers in the image stack, the Find Quality of All Layers function can be used. This function calculates the quality index of the "need update" and of the "unknown" layers in the stack and produces therefore a complete quality overview of the entire image stack.

The function is called by Processing – Find Quality of All Layers.

Find Quality of a Multi-Channel Data Set
If a multi-channel data set is loaded, the quality is calculated either:

- of a selected channel or
- of a gray-valued version of the RGB-image

The same parameters are used as chosen for the automatic alignment. If the layers have not yet been aligned automatically, please select carefully the parameters for Background intensity and Channel in Menu Processing - Auto Alignment Parameters - Basic.

Quality Index
The quality of the current alignment is expressed by a quality index (normalized cross-correlation coefficient). Identical layers with no shift and rotation show a quality index of 100. Properly aligned similar layers should achieve figures between 96 to 98. The chosen background value ("link") has a strong influence on the quality index. If you have not changed the background value, it will be the same as in the alignment calculation.

Once the alignment is calculated and shows a satisfactory result, it can be applied to the image stack ("link to Apply").

See also:
Menu View - Manual
Menu View - Automatic

7.6   Find Quality of All Layers

The quality of all layers is calculated.

7.7   Straighten Alignment

You can use this function to correct an image layer shift.

Translation
Select this function if the image stack is horizontally shifted (like the Leaning Tower of Pisa).

Rotation
Select this function if the image stack winds like a spiral stair.

7.8   Apply

Once the alignment is calculated and shows a satisfactory result, it can be applied to the image stack. This causes the single layers to be shifted and rotated and embedded into a larger image stack. Therefore no original data will be lost, but the resulting data set can be much larger than the original one.

After Apply Alignment further (manual or automatic) alignment is no longer possible, because the original data was interpolated by applying the alignment. Only Post-Processing operations can be performed now on this data set.

We recommend saving the alignment first before pressing Apply Alignment. Since the Apply will take some time, it is recommended to use it only if the aligned data set is saved afterwards.

Apply Alignment virtually overlays all layers and then generates an enlarged image stack in a size which covers entirely all moved layers. The area outside the original layers is then filled with the defined background gray value.

7.9   Undo

Undo Apply Alignment recovers the state of the data set just before clicking Apply Alignment. The possibly modified data set (normalized) is recovered with the last alignment. You can now perform again all Pre-Processing and Processing steps.

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8   Menu Post-Processing

Interpolate Current Layer
Crop Largest Common Region

Show 3D

Post-Processing actions are performed on the aligned and saved data set. This can be an interpolation of missing or bad layers or the generation of a masked, more compact data set.

8.1   Interpolate Current Layer

You should use this option only, if a "bad" layer has to be replaced. This can be a heavily distorted layer or a layer showing scanning artifacts.

Interpolate a Layer

- If layer 5 is "bad", select it as current layer in the view.
- Select in the Menu Post-Processingthe the menu entry Interpolate Current Layer.

Layer 5 will be replaced by an artificial layer, which is interpolated from layer 4 and layer 6.

8.2   Crop Largest Common Region

For 3D visualization a compact image stack is usually preferred. Crop Largest Common Region leaves the gray values in the common region, which is defined in all aligned layers. The rest is set to zero and cut to a smaller image stack, which then allows a nice 3D-volume rendering.

- Select in the Menu Post-Processing – Crop Largest Common Region.

The cropping is performed automatically and the result is displayed in the main screen. Crop detects the common region of all overlaid layers and draws the smallest possible rectangle around this shape. All information outside this rectangle is cut away. Inside the rectangle the area that is not common to all layers is set to 0 and appears therefore in black.
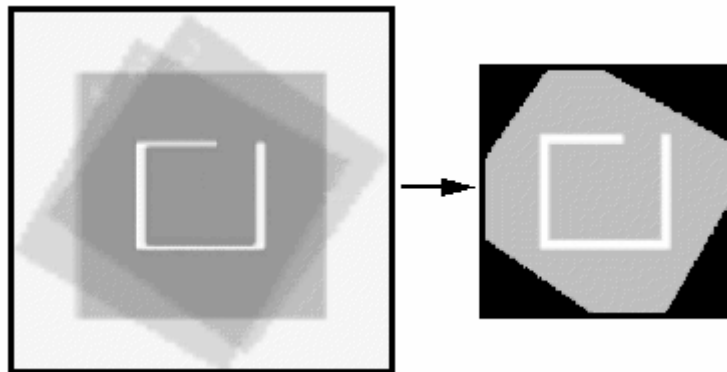

Fig.: Cropping function and result

8.3   Show 3D

This menu entry is only accessible if Imaris is installed on your computer. To do some further analysis you can load the aligned data set in Imaris. Select the menu entry Show 3D and Imaris starts automatically.

For further information about Imaris please visit our web page: www.bitplane.com.

9   Menu Help

Quick Start Tutorials   Ctrl + F1
Keyboard and Mouse   F1
Reference Manual

License...   F2
Check for Updates now...   F3

Bitplane
About AutoAligner ...

9.1   Quick Start Tutorials

The online version of the AutoAligner Manual displays in your browser. The chapter Quick Start Tutorial displays. In this Quick Start Tutorial you will learn the AutoAligner basic working steps.

9.2   Keyboard and Mouse

Camera

	PC:	Mac:
Translate	Right-click	Ctrl + click
Zoom	Middle-click	Ctrl + shift + click

Region

	PC:	Mac:
Resize	Leftt-click on corner & drag	Click on corner & drag
Move	Leftt-click in region & drag	Click in region & drag
Move in all layers	Alt + left-click	Alt + click

Slice Alignment

	PC:	Mac:
Translate	Shift + leftt-click & drag or Shift + cursor keys	Shift + click & drag or Shift + cursor keys
Rotate, the angle of the rotation displays	Ctrl + leftt-click & drag or Ctrl + cursor keys	Command + click & drag or Command + cursor keys

Display Values (Status Bar)

Move mouse over image	Display pixel gray value at defined image point

Example: 128.5 at (20.3 35.7 4.0)

128.5	Gray value
20.3	X-coordinate
35.7	Y-coordinate
4.0	Z-coordinate

9.3 Reference Manual

The online version of the AutoAligner Manual displays in your browser.

Overview

Fig.: AutoAligner Manual, contents screenshot

The AutoAligner Manual provides you with the basic information about AutoAligner: The viewing area, toolbar (s), menu(s), the mouse and keyboard shortcuts and all adjustable parameters in the program. On the left hand side, click on a chapter in the Table of Contents to display a list with the available subchapters.

Context Sensitive Help

For PC User:
With a right-click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a left-click on the massage the AutoAligner Manual opens in the actual browser and displays the corresponding page.

For Mac User:
With a Ctrl + click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a click on the massage the AutoAligner Manual opens in the actual browser and displays the corresponding page.

Search

To find a specific parameter in the online version of the AutoAligner Manual use the "Search function" on the left hand side.

Fig.: AutoAligner Manual, search screenshot

9.4 License...

You receive your license by email and also printed together with your CD. The licence is a text string you have to insert in a file of the AutoAligner software. To perform the installation, please proceed as follows:

- Insert your AutoAligner CD-ROM in the computer.
- Follow the instructions on the screen.

Manual Installation

- Display the contents of the CD-ROM.
- Select the Windows directory.
- Start the AutoAligner executable file (.exe).
- Follow the instructions on the screen.

The installation is completed automatically.

9.5 Check for Updates now ...

If your computer is connected to the Internet, AutoAlicner can directly check, if a software update is available.

9.6 Bitplane

If your computer is connected to the Internet, AutoAligner can directly open the Bitplane AG homepage (www.bitplane.com).

9.7 About AutoAligner

The AutoAligner start screen displays.

AutoAligner
Version number and release date.

© Bitplane 2007

10   Toolbars and Panels

In the standard AutoAligner view there are five different toolbars and panels available. The Main Toolbar, the Alignment Panel, the Slice Panel, the Overlays Panel and the Status Bar.

Fig: AutoAligner Overview.
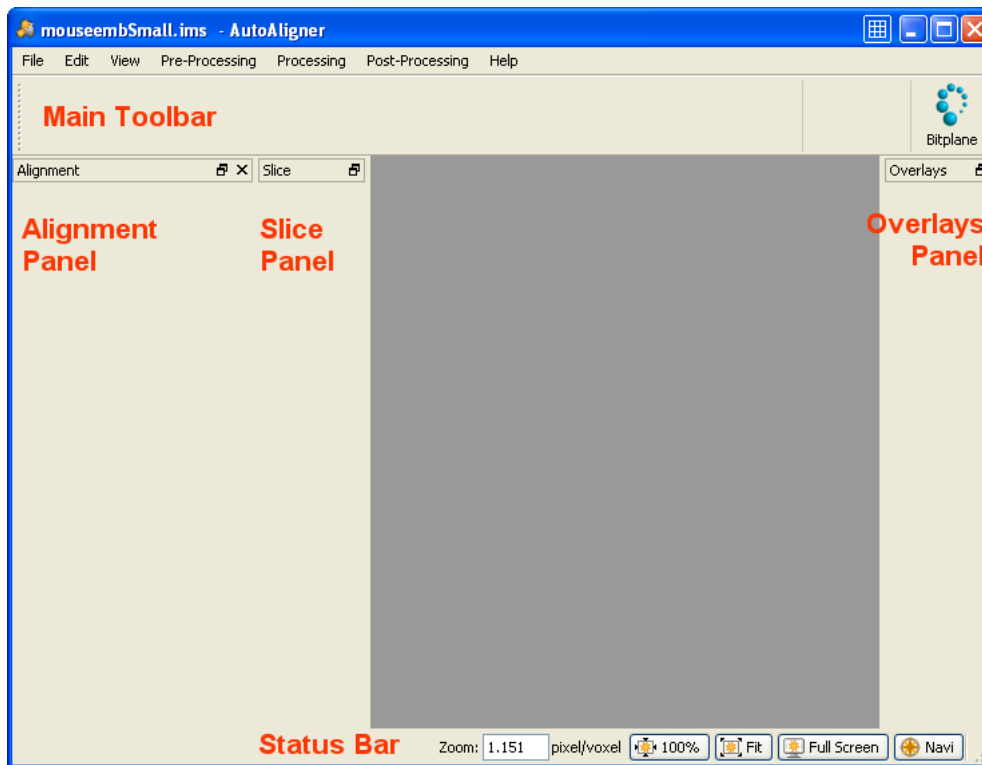
10.1   Main Toolbar

Please refer to chapter:

Open	Menu File - Open
Open Alignment	Menu File - Save as
Save as	Menu File - Snapshot
Save Alignment	Menu File - Save Alignment
Snapshot	Menu File - Snapshot

Manual	Menu View - Manual
Automatic	Menu View - Automatic
Invert	Menu Pre-Processing - Invert
Apply	Menu Processing - Apply
Show 3D	Menu Post-Processing - Show 3D
Bitplane AG	Menu Help - Bitplane AG

10.2   Alignment Panel

Alignment Panel Manual

Please refer to chapter Menu View - Manual.

Alignment Panel Automatic

Please refer to chapter Menu View - Automatic.

10.3   Slice Panel

The slice concept in AutoAligner is very intuitive to understand. Whenever you load a data set with several slices automatically the slice window is displayed at the left hand side of the viewing area.

Fig. Slice panel, slice 6 displays in the viewing area.

Play Button

On top of the Slice window you find the button **Play** (arrow to the right). Click on the button **Play/Pause** to see a continuous display through all the slices in the stack. To pause re-click on the button.

Slice Slider

To inspect any slice in the data set use the slider. Each bar represents one slice of the data set. In the example the slider is at slice 6.

Slice Number Field

Additionally, the slice number of the actual image is displayed at the bottom of the time slider in the slice number field. To change slices enter the desired slice number in the number field at the bottom of the slider.

Slice Panel Overview

▶	Play/Pause through All Slices
Click Slider + Drag Slider	Go to Previous/Next Slice
Click Slider (Select) + Right Arrow on Keyboard	Go to Next Slice
Click Slider (Select) + Left Arrow on Keyboard	Go to Previous Slice
Click Slider (Select) + Home-key on Keyboard	Go to First Slice
Click Slider (Select) + PageDown-key on Keyboard	Go to First Slice
Click Slider (Select) + End-key on Keyboard	Go to Last Slice
Click Slider (Select) + PageUp-key on Keyboard	Go to Last Slice
Enter number in Slice Number Field	Go to Slice with Respective Number

10.4   Overlays Panel

To check the neighboring slices AutoAligner provides several viewing aids. The parameters are grouped in the Overlays Panel.

Overlays Panel

Enable
Check this box and the current layer is transparent and you see it overlaid on the preceding layer. In this function you can choose between two modes (see below).

Mode

Normal
Only available if the parameter Enable (see above) is checked. Check this box and the current layer and the preceding layer are shown in their color.

Colored
Only available if the parameter Enable (see above) is checked. Check this box and the current and the preceding layer are shown in different colors. You can select the colors in the "Preferences" dialog (Menu Edit - Preferences... - Alignment Display) .

Note: Another way to easily compare layers is flipping between two layers by means of the Slice panel. Overlays must be disabled. Then click into the slider of the Slice panel to activate it. You can now flip between the layers with the up and down arrow keys of your keyboard.

See also:
Quick Start Tutorials - Check Neighboring Slices
Menu Edit - Preferences ... - Alignment Display (to change the color definition of the Overlays Mode)

10.5   Status Bar

At the bottom of the screen you find the Status Bar.

Zoom ... pixel/voxel
Enter the zoom factor.

Button `100%`
Click on this button to rotate the image to the original position, center the image in the middle and set the zoom factor to one pixel per voxel.

Button `Fit`
Click on this button to pan the position to best fit in the window and adjust the zoom factor.

Button `Full Screen`
Click on this button to maximize the viewing area to full size of the monitor. To return to the standard window, click on the button Full Screen in the lower left corner.

Button `Navi`
Click on this button to toggle the display of the Navigation window of the data set in the upper right corner of the viewing area. If you move the data set, the overview updates in real-time. In the pointer mode Select you can click and drag the Navigation window to another position in the viewing area. The Navigation window is placed to the nearest corner.

Change Position of the Navigation Window

- In the pointer mode Select you can click and drag the Navigation window to any desired corner.
- Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Re-double-click onto the Navigation window to switch the automatic "snap-to-corner-mode" on again.
- Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

Mouse Clicks to Zoom

- Click with the middle mouse button on the image, hold down the middle mouse button, and drag the mouse up or down.
- Hold the Shift-key and click with the right mouse button on the image, and drag the mouse up or down.

See also:
Menu View - Fit
Menu View - 100%
Menu View - Full Screen
Menu View - Navi