

Imaris Stitcher

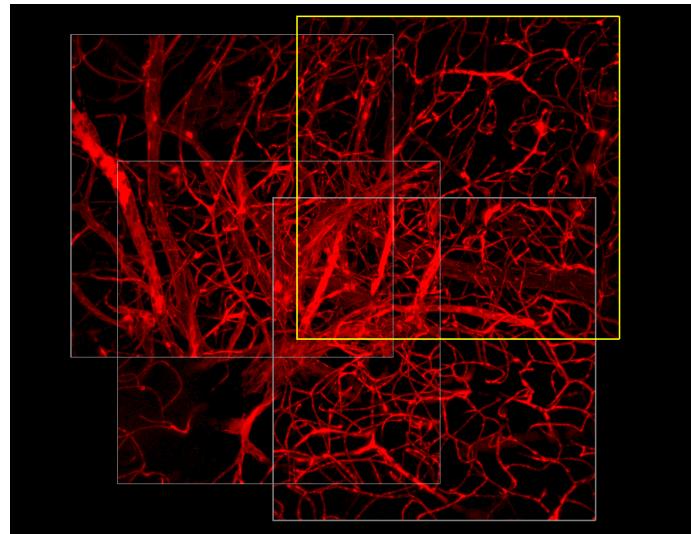
Reference Manual

Section 1 An Introduction to Imaris Stitcher

Microscope stage positioning is not perfect - there may be rotational misalignments with the camera relative to the stage and/or inaccuracies in the distances of the translation of the stage between fields in the x and y axis. Although these misalignments may appear very small being in the order of, for example, a 0.5 degree rotation, it is of particular significance when multiple smaller images are combined into a larger image. Imaris Stitcher lets you take multiple images, correct for such inaccuracies and combine into a single image for further analysis in Imaris.



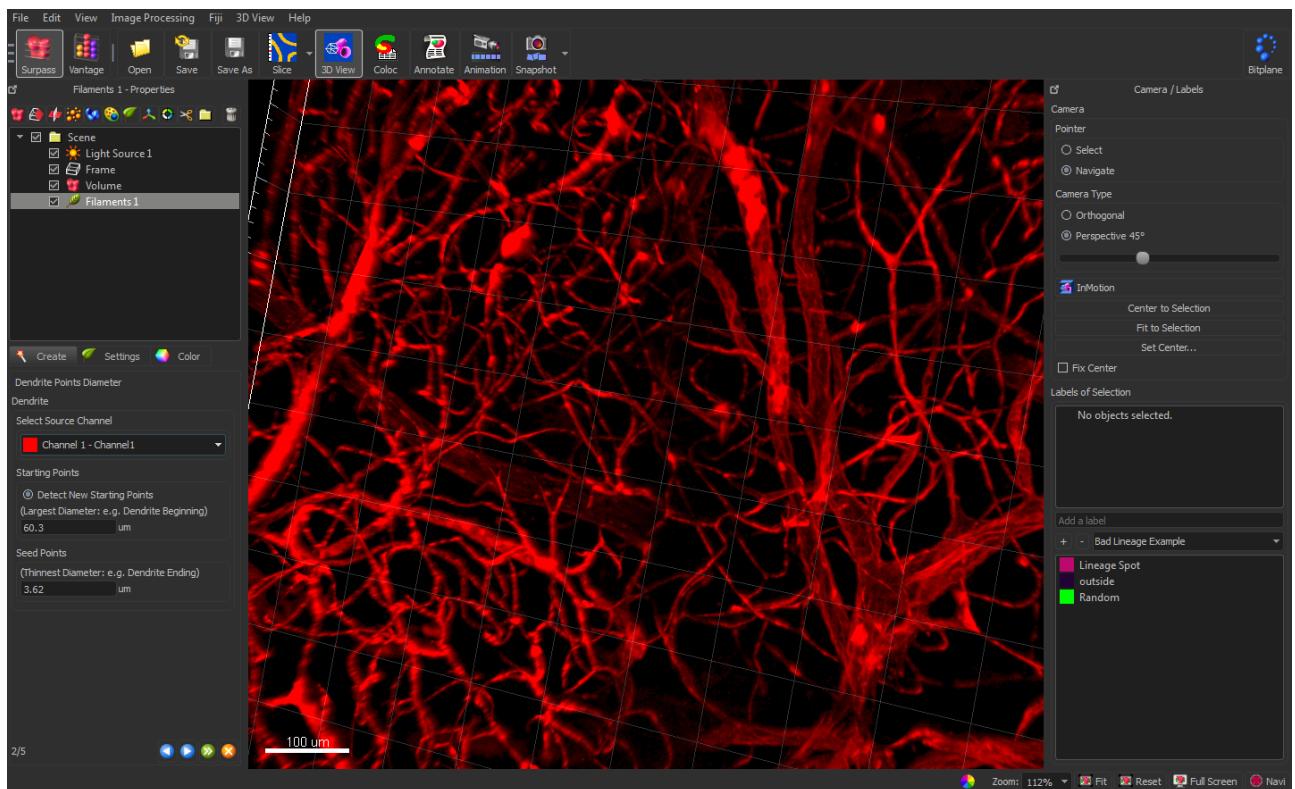
A powerful alignment algorithm works to "stitch" your images together. You can also manually position each image if desired:



Above: Aligning multiple images in Imaris Stitcher

Note: Image files must overlap for the alignment process to work.

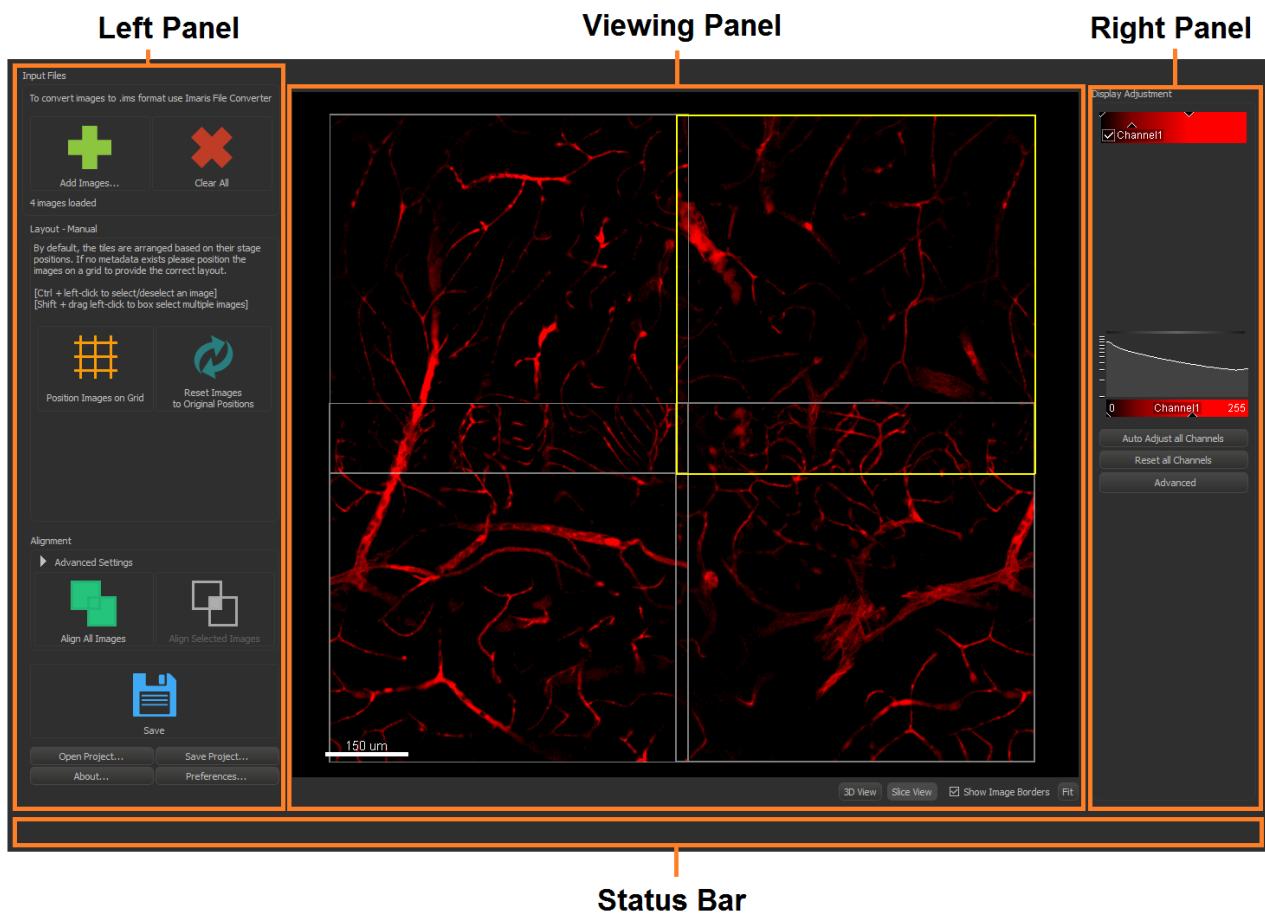
Imaris Stitcher is intuitive and provides all the features required to let you combine the image files in your project for further analysis in Imaris.



Above: Analyze multiple images as a single "stitched" image in Imaris

1.1 An Overview of the User Interface

Imaris Stitcher presents the various functions in a series of panels:



Left Panel

This panel groups the most common functions for your workflow: loading your image files, adjusting their layout, performing alignment and then saving as a single "stitched" image file.

Viewing Panel

This panel enables you to view your images. Images can be viewed in slice (where available) or 3D View. Navigate through the images using the zoom, pan and playback functions.

- If there is more than one image slice (Z-axis), a slider will appear to the left of the images with which you can use to move through the image Z-axis slices. This slice slider is only available for 3D datasets when in slice mode
- If there is more than one time point, a slider will appear under the images with which you can use to move through the time points.

Right Panel

This panel covers the display adjustment options, so you can select and modify how the channels are displayed for the current image files.

Status Bar

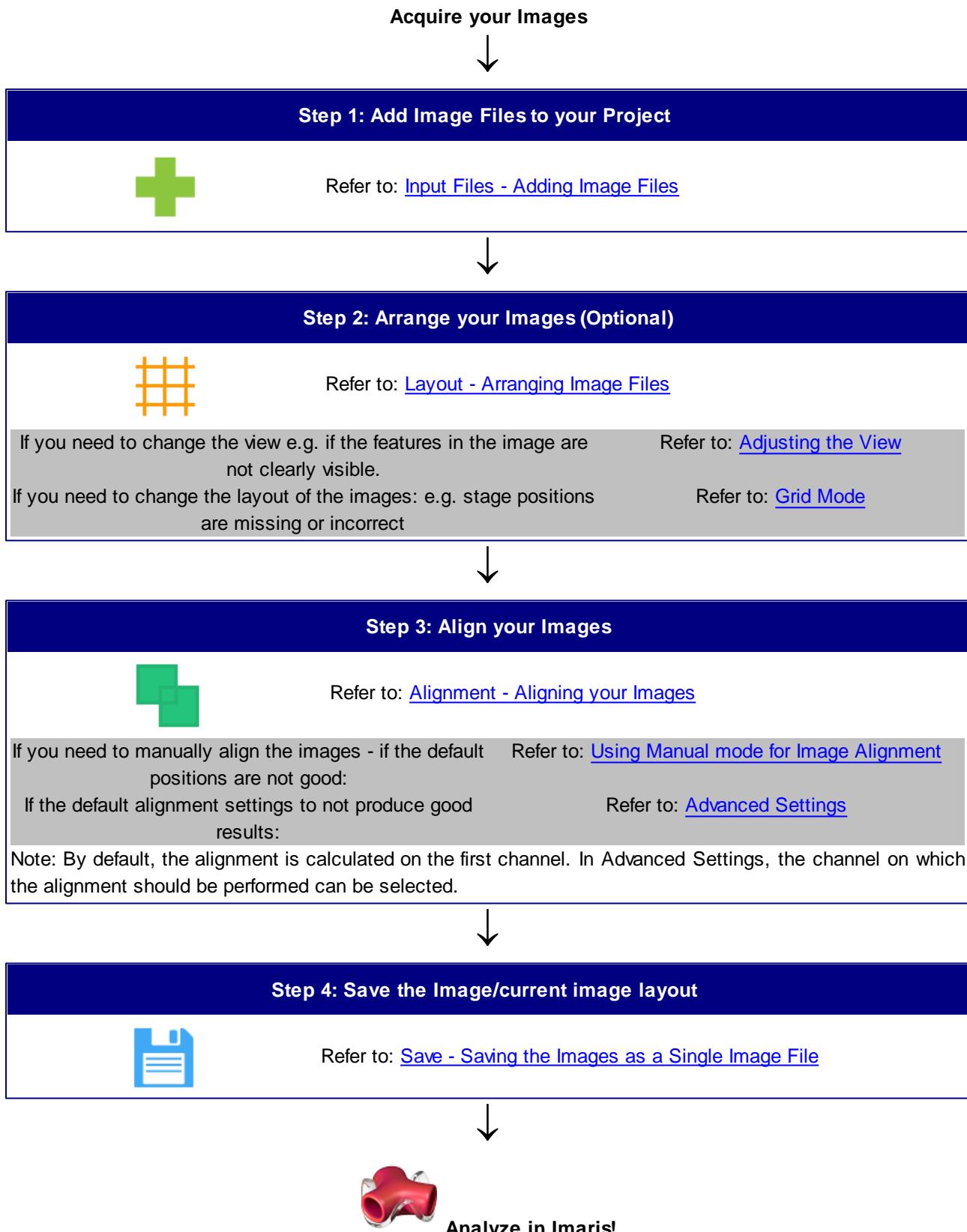
Useful information is presented in this area e.g. progress bar when loading, or combining images.

Notes

- Useful information is also displayed on-screen, while clicking an area with the right mouse button will also provide a link to the relevant section of this manual for further information.
- See [Keyboard Shortcuts](#) for a full description of keyboard and mouse shortcuts

1.2 Example Workflow

An example of the Imaris Stitcher workflow is shown in a simplified format below. The following sections of the manual are organized to match this workflow:



1.3 Installation and Licence Information

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

Imaris Stitcher runs on:

- MS Windows 10 x64
- MS Windows 8 x64
- MS Windows 7 x64
- Max OS X 10.11
- Mac OS X 10.10
- Mac OS X 10.9
- Intel based MAC

To find the latest information about minimum and recommended hardware requirements please visit our web site - the Support section.

Installation

To install the software, please proceed as follows:

1. Insert your Imaris CD-Rom in the computer.
2. Follow the instructions on the screen.
3. The installation is completed automatically.

Licensing

To run Imaris Stitcher, the appropriate licence is required. In case of any licence problems, please refer to [License Options](#) and the support information on our website www.imaris.com for detailed instructions.

Starting Imaris Stitcher

Imaris Stitcher can be started by one of the following methods:

- Double-click on the Imaris Stitcher icon
- Drag the icon of an image or a file to the Imaris Stitcher program icon.

The software opens with the main screen.

Supported File Formats

Imaris Stitcher can only read files in the Bitplane: Imaris 5.5 (*.ims) Format. If you have images in a different format, please convert them with Imaris File Converter that is installed along with Imaris Stitcher; this will open automatically if you add non .ims files to Imaris Stitcher. Imaris File Converter can read many formats and produces ims 5 files. Refer to the [Imaris File Converter](#) section for more information.

Notes:

- If another format such as .tiff is added to Imaris Stitcher it will automatically open Imaris File Converter.
- You can then convert the file to .ims format. Refer to Imaris File Converter section for more information.

1.4 About this Manual

Information in this manual is subject to change without notice and does not represent a commitment on the part of Bitplane AG. Bitplane AG is not liable for errors contained in this manual or for incidental or consequential damages in connection with the use of this software.

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For further questions or suggestions please visit our web site at:

www.imaris.com

or contact our support teams:

eusupport@bitplane.com

ussupport@bitplane.com

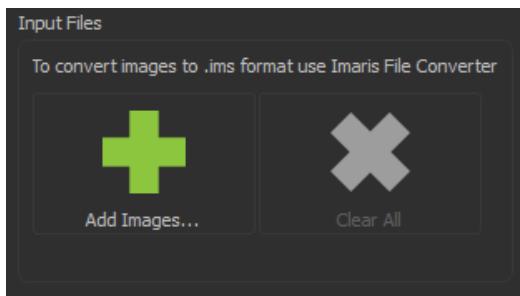
asiasupport@bitplane.com

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Section 2 Input Files - Adding Image Files

The **Input Files** panel provides options for importing image files into your project, and information for the image files in the current project.



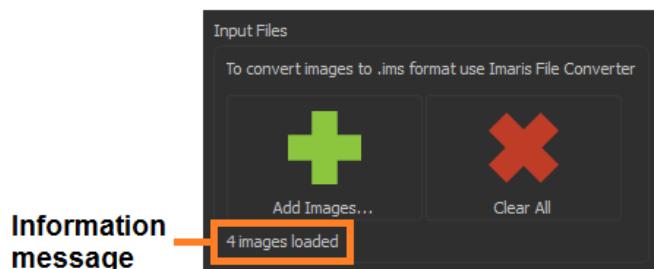
Adding your Images

Images that you want to combine into a composite image can be added in either of the following ways:

1. **Drag and Drop:** Simply drag the image files from a folder location into the Imaris Stitcher application window.
2. **Add Images...** Click the **Add Images...** button and then browse to your required files.

The status bar at the bottom of the screen will indicate the progress. For larger sets of image files this may take some time to load. Images will start to appear as they are loaded.

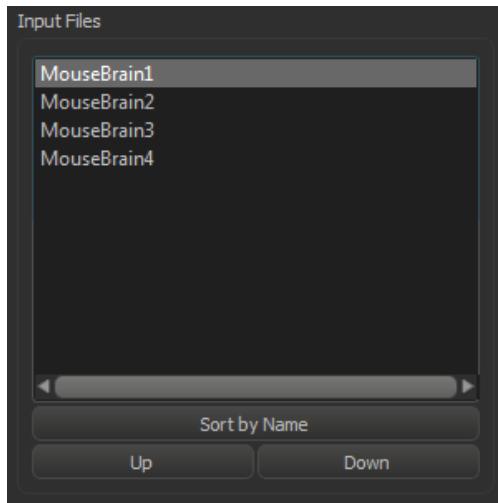
Once the files are successfully loaded a message will be displayed in the **Input Files** information panel and all images will be present in the viewing panel according to the layout settings.



Clear All

Clears all images from the current project (only available when images are loaded).

Once the image files are loaded they can then be adjusted in manual mode, or in grid mode as required. When in grid mode (**Position Images on Grid**) the input files are listed in alphabetical order. In most cases this should match the order in which the images have been captured. However the running sequence of these may need to be modified, depending on your experimental and microscopy set-up. To adjust the sequence please refer to the [Layout](#) section of this manual.



Sort by Name

Click sort by name to revert to the previous file sequence.

Up and Down Buttons

You can use the **Up** and **Down** buttons to move the order of selected image files.

File types

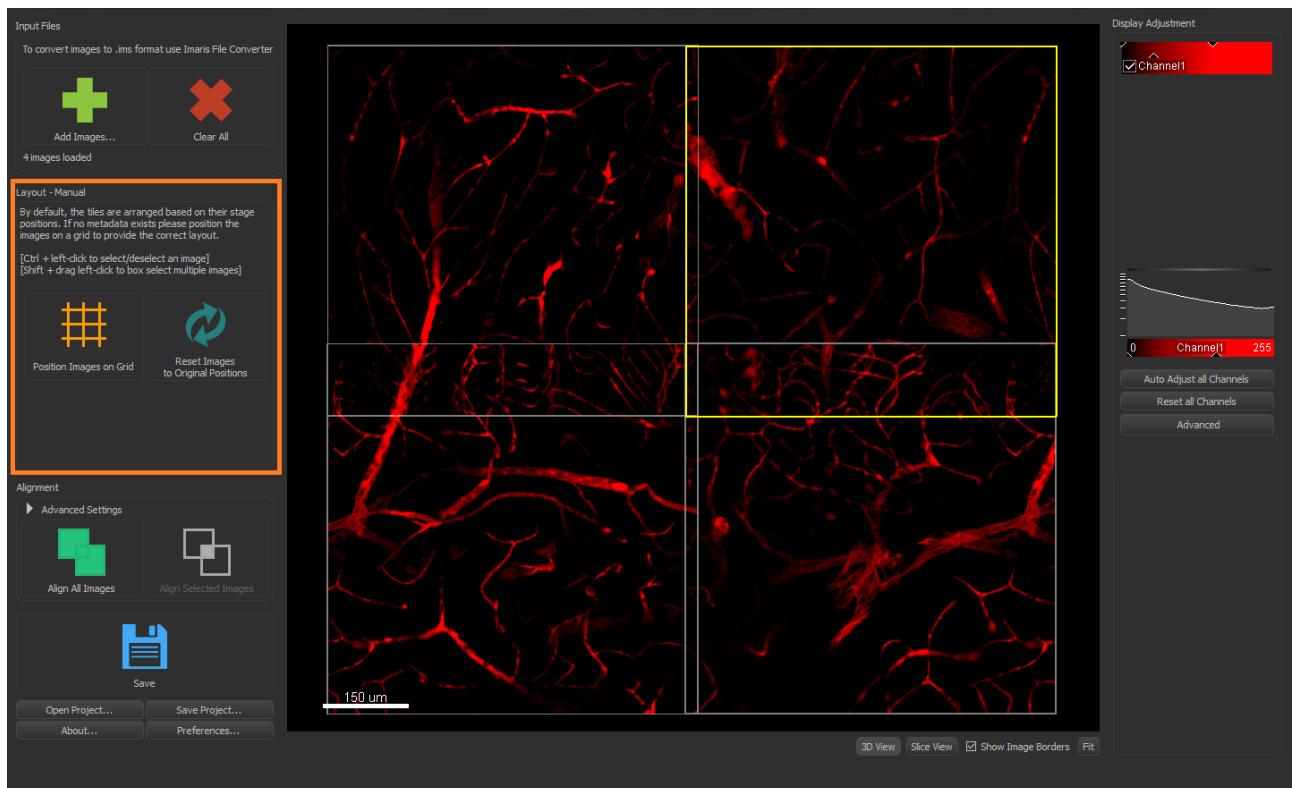
.ims files can be used with Imaris Stitcher. .ims files have additional metadata that includes positional and time information enabling the order of the images to be correctly arranged. Adjustments to sequence and positioning of the image files can be made using the manual or grid layout options. Refer to the [Imaris File Converter](#) section for more information.

Notes:

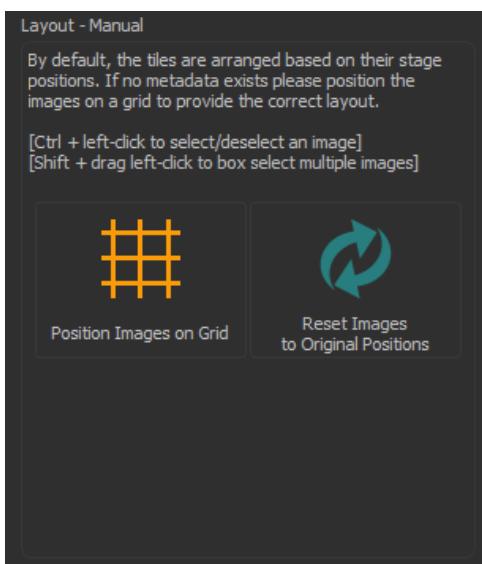
- When in manual mode, image(s) can be deleted by selecting the image(s) in the viewing panel and pressing delete.
- The sequence of the image files depends on the settings defined in the layout panel. Refer to the Layout section for more information.
- If you add additional files they will appear in a default position.
- If you add a file that is already present in the project, this will not overwrite the current file of the same name.
- Use Imaris File Converter to convert other formats such as .tif to .ims.

Section 3 Layout - Arranging Image Files

Once image files have been loaded they can be processed using the tools and settings in the Layout panel (highlighted below).

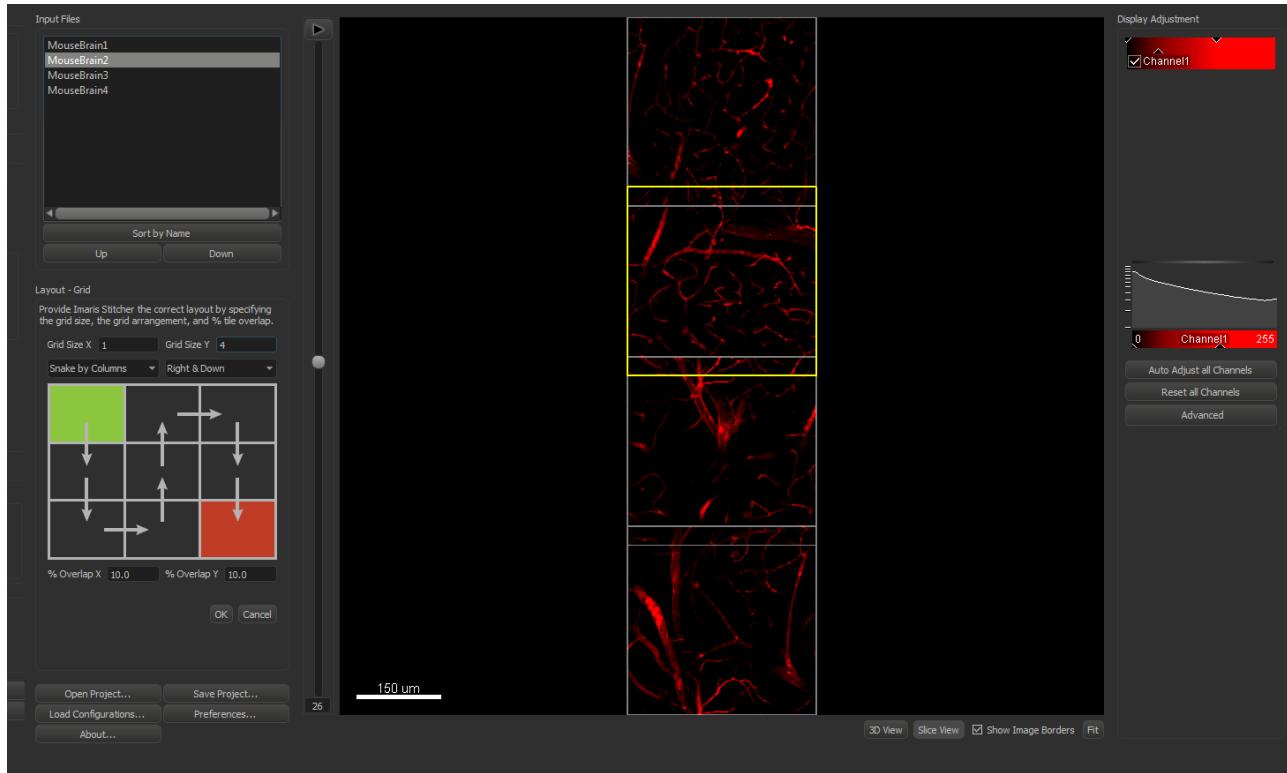


The following options are available:



Position Images on Grid

Images can be arranged according to a grid format according to the defined layout settings. The layout of the image files can be adjusted if they do not have stage positional information, or it is incorrect. In these cases you can use [Grid Mode](#), or adjust manually when out of this mode.



Notes:

- When in manual mode, images can be deleted by selecting the images in the viewing panel and pressing delete.
- Image files must overlap for the alignment process to work.
- Image files cannot be added or deleted in grid mode.
- If image files do not have positional information, they will be stacked on top of each other by default.

Reset Images to Original Positions

Image positions are initially read from the image file metadata, but can be changed by the user or during the alignment process. This button enables the images to be reset to their original positions.

3.1 Using Manual Mode for Image Alignment

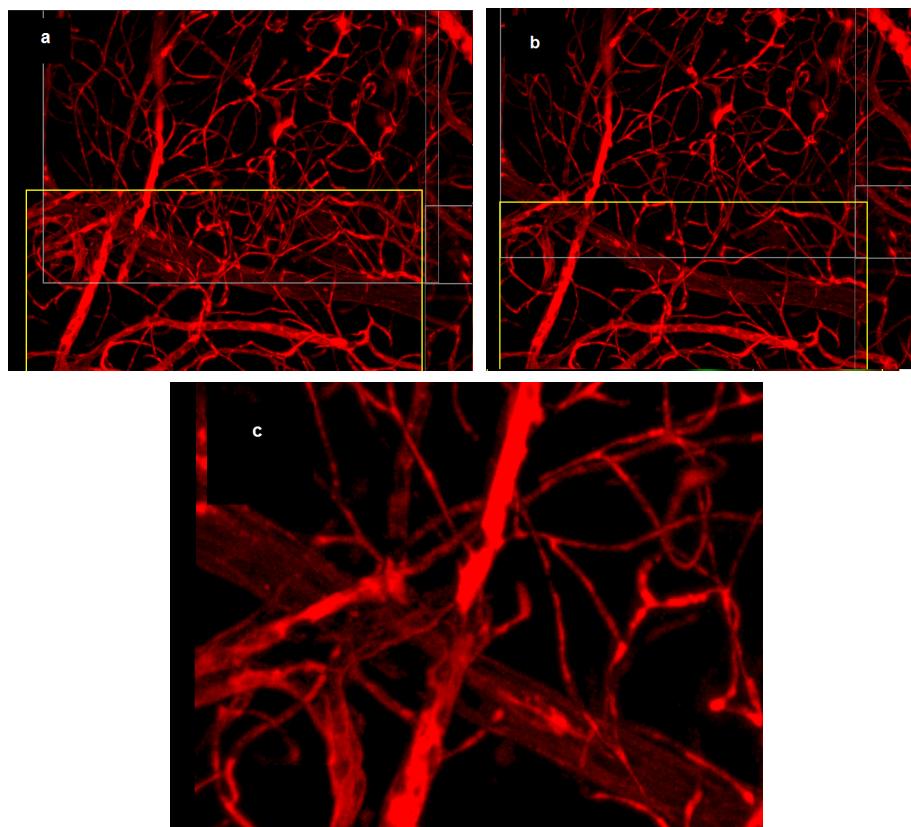
Manual mode can be useful for deleting unneeded images, and manually moving their positions. It can also be used to align images. This can be especially useful if you have a small number of images that were not taken at defined co-ordinates e.g. a number of manual acquisitions of areas of interest taken while scanning through a specimen.

Notes:

- It is always worth trying the in-built Align Images options as the alignment algorithms of Imaris Stitcher should prove very effective with a wide range of images.
- If you are not happy with the result you can still manually adjust the positions afterwards, or Reset Images to Original Positions to start again.
- If you use Align All, manual alignment will be lost.
- Images must overlap for the alignment process to work.

Useful Tips for using Manual Mode for Alignment

- Select a slice view that gives good X and Y information with which to allow for alignment.
- Use the Zoom function (Mouse wheel) to zoom in to an area of overlap for precise alignment.
- Disabling image borders can be useful to confirm precise alignment.
- Once you have the image aligned check in other slices and in 3D view to confirm you are happy with the positioning of each image.

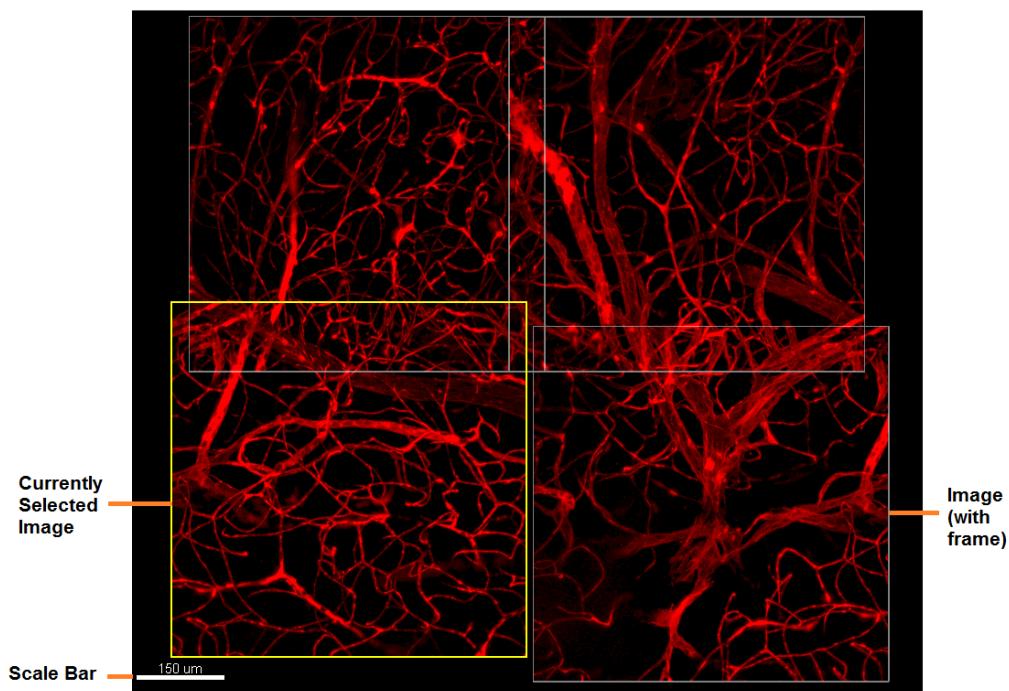


Above: Manually aligning a series of image files.

In the above example, image (a) shows a selected image that is not aligned. In image (b) this has been aligned successfully by manually repositioning the image. In image (c) the images have been checked in other slices and zoomed in, in 3D view with the image borders removed to confirm that alignment is correct.

3.2 Adjusting the View

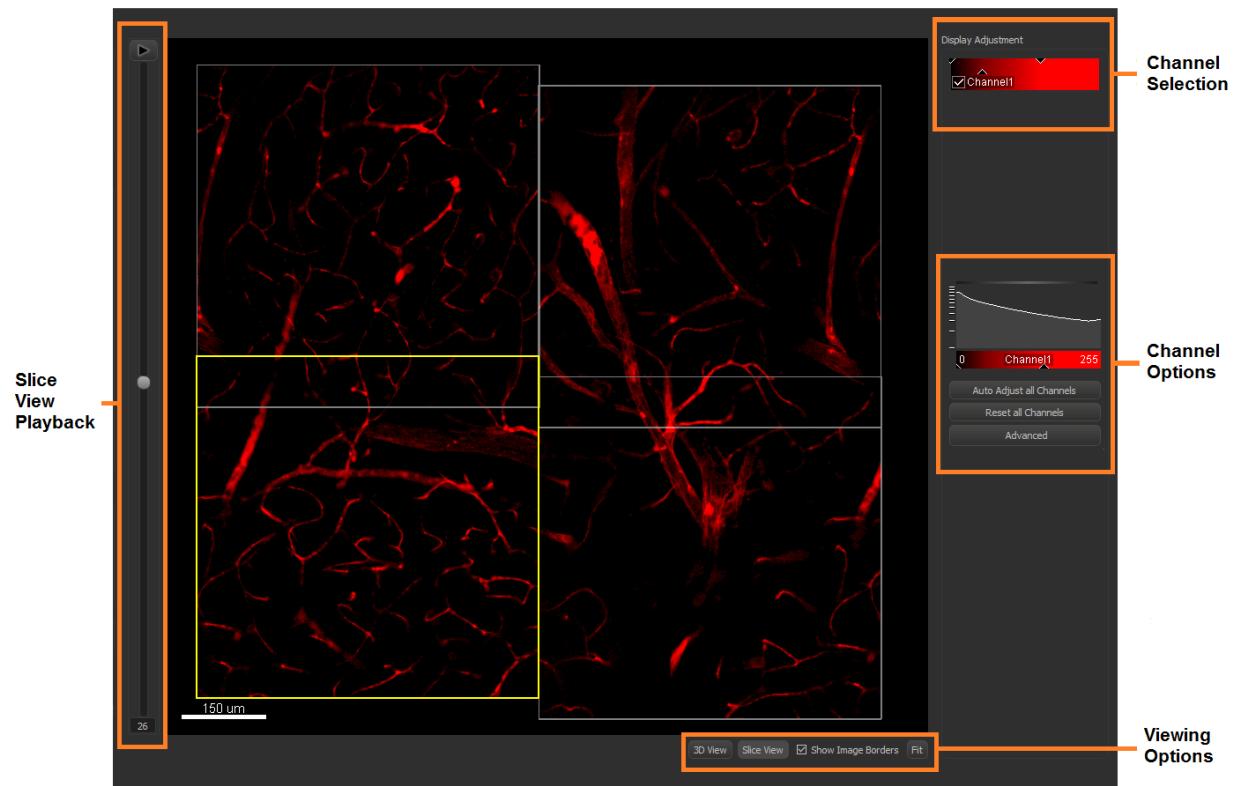
The image files are shown in the viewing area within the viewing panel according to the current options:



Each image file is shown with a frame (which can be disabled) and any selected image file is shown highlighted (in yellow by default). The scale bar will re-adjust to the selected level of image zoom.

Viewing Panel Options

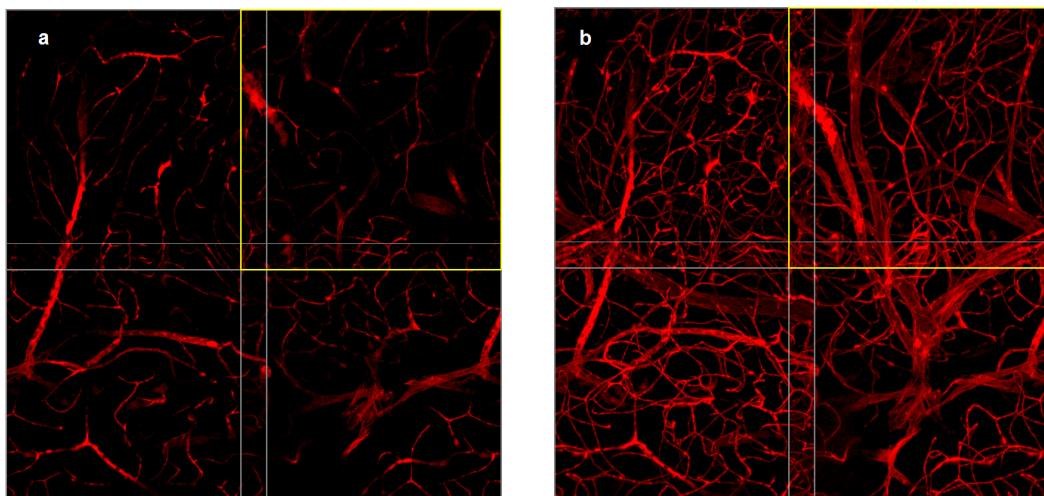
There are a number of options available for changing the view of the images in the viewing panel:



Viewing Options

Toggle between **Slice View** (default) and **3D view** to get the best view of each image:

- If there is more than one slice, a slider will appear in Slice view which you can use to move through each slice, either by clicking the play/pause button or clicking and dragging the slider. The frame/slice ID is displayed at the bottom of the slider bar. (3D view will not appear for selection if there is no depth (Z) points).
- If there is more than one time point, a slider will appear which you can use to move through the time points.
- 3D view will give a view of the composite image through a 3D image stack.



Above: Left (a) - Slice view, Right (b) - 3D View.

Show Image Borders

Toggle the image borders on or off. Image borders help identify the outlines of the images, but may be turned off if desired.

Fit

Click **Fit** to auto-scale the images of the project to the viewing window area.

Selecting Images

- To select between image files – use the left mouse button.
- When you have made an image selection, hold the left mouse button to drag the images, or use the arrow keys.
- A colored outline (default, yellow) helps identify the selected frame. The color can be changed under preferences (see the [Preferences](#) Section).
- To select multiple items - press the control key and left mouse click.
- To select a group of items in a box - press shift and the left mouse button and drag over the required items.

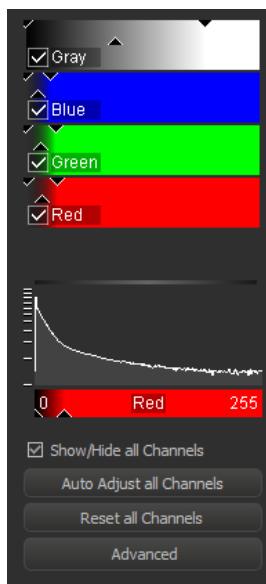
Tip: you can select groups of items and individual items by pressing the control key.

Click the Reset Images to Original Positions button to reset the selected items to the original selection.

3.2.1 Display Adjustments

Channel Selection

It is possible to adjust the visibility of the channels in the same way as the main Imaris application. This may help you get the best visual representation of your image files.



- Clicking the checkbox of a channel will toggle visibility of the available channels from the viewing panel.
- Clicking the channel name brings up a color chart. You can change the color that the channel is displayed in.

Channel Options

The lower and upper thresholds can be adjusted as required by moving them across the x axis of the graph.

Auto Adjust all Channels

Set the values to a calculated optimum value.

Reset all Channels

Reset all channels to their initial threshold values (this will not reset the display color if this was modified).

Advanced

The advanced settings can be toggled on (or off). This brings up **Min.**, **Max.** and **Gamma** values for the selected channel. These can be adjusted as required.

Note: As in Imaris, these adjustments do not change the image data itself, only how it is visualized.

3.3 Grid Mode

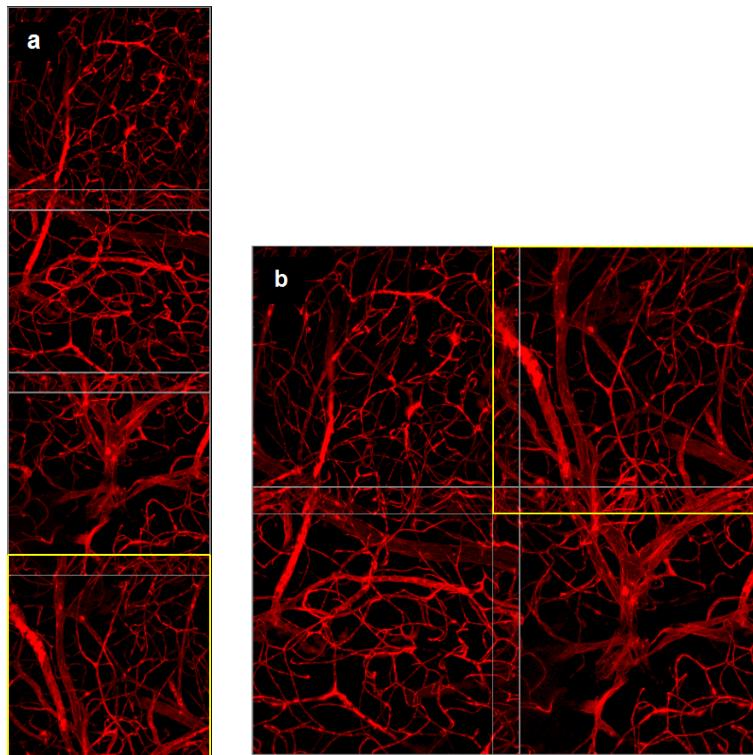
If the images do not contain stage positions, or if they are incorrect, these can be corrected using **Position Images on Grid**. This lets you define the layout of the images in a grid format, and set the overlap between images. Grid mode is enabled by clicking the **Position Images on Grid** button.



To change the grid size from the default, change the x and y size fields as required.

- **Grid Size X:** The number of images in a column
- **Grid Size Y:** The number of images in a row

In the following images we can see that the default order (below left) of Grid Size X of 1 (columns) by Grid Size Y 4 (rows) does not match the images. This can be adjusted, in the example (below right) to Grid Size X of 2 (columns) by Grid Size Y 2 (rows).



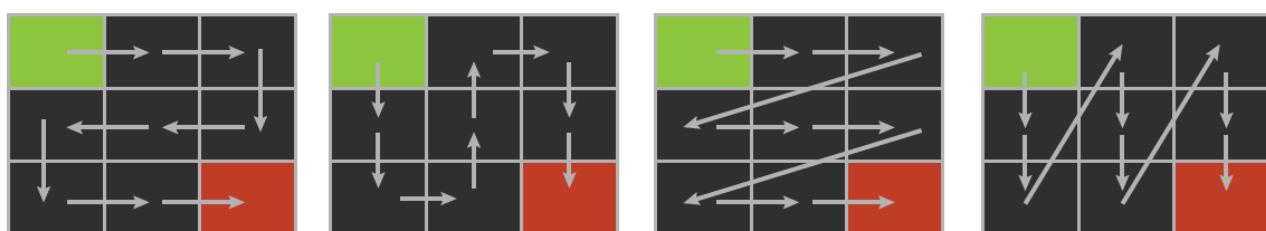
Above: Left (a) - Images in 1 X (column) by 4 Y (rows), Right (b)- Images in 2 X (columns) by 2 Y (rows).

Notes:

- If the current layout does not match the images required for the grid size, an information message will be displayed.
- To move the position of individual image files use manual mode.

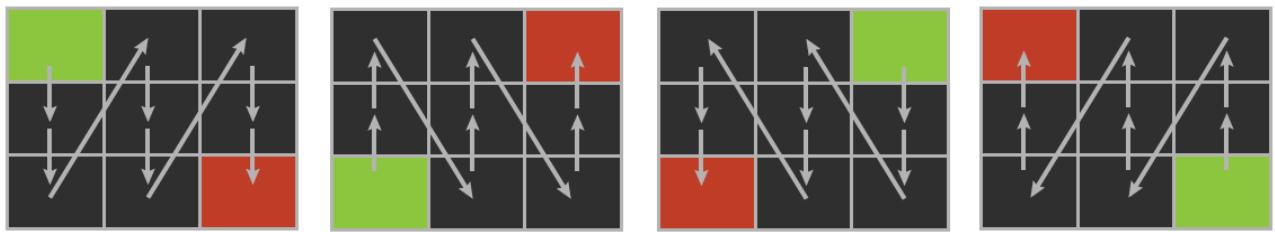
Set the order of the files by row or by column from the following options:

- Snake by Row
- Snake by Column
- Row by Row
- Column by Column



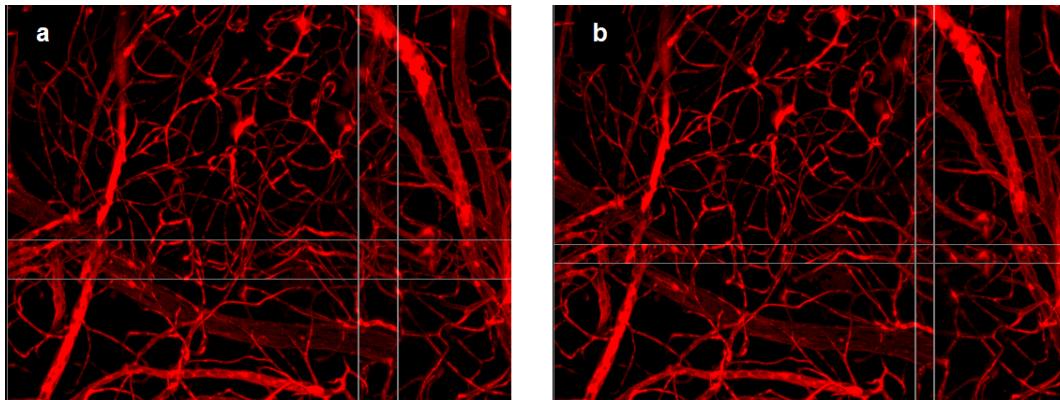
The direction can also be set from the following options:

- Right & Down
- Right & Up
- Left & Down
- Left & Up



The percentage overlap can be adjusted for both the x and y dimensions set a smaller or larger overlap between the images. This should match your acquisition setup.

- **% Overlap X**
- **% Overlap Y**



Above: The % overlap can be adjusted. In this example the image on the right (b) has the overlap % reduced from 10% to 5%

Click **OK** to confirm the adjustments

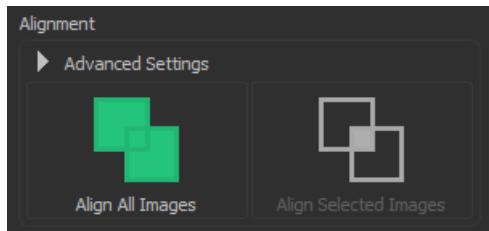
Click **Cancel** to cancel the changes

Notes

- Click **sort by name** to revert to the original file sequence.

Section 4 Alignment - Aligning your Images

Once the images have been positioned in the correct layout and sequence, image alignment can then be performed using the Imaris Stitcher Alignment Algorithm (or manually, see [Manual Mode](#)).



Advanced

View the advanced settings (see [Advanced Settings](#)).

Align All Images

Perform image alignment on all images in the current project. The alignment algorithm is a powerful tool for aligning your images precisely, however you can manually position images if desired.

Align Selected Images

Align only the selected images.

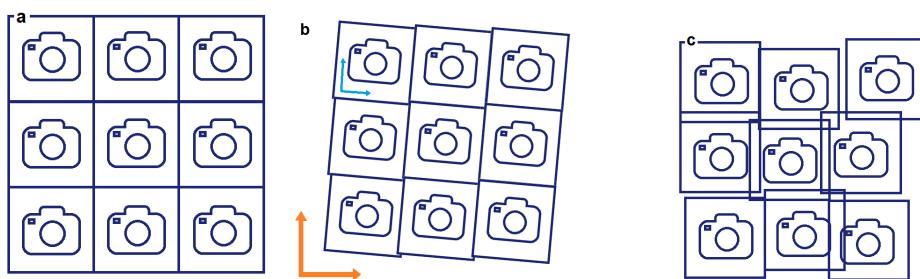
Notes:

- If you had manually aligned images, clicking the align buttons runs alignment according to the current image positions and alignment parameters.
- Imaris Stitcher will not be able to align images if there is no overlap.
- For large files image alignment may take some time to process. Progress may be viewed in the status bar in the bottom of the screen with the process shown at the left and the progress bar at the right.



About the Image Alignment Method used in Imaris Stitcher

The Imaris Stitcher alignment process corrects for both rotational misalignments of the camera relative to the microscope stage axes and stage x-y positional variances. These are represented in the following illustrations:



Above: (a) Ideal image positions. In practice, errors can include (b) Rotational errors of camera relative to the microscope stage axes, (c) Positional errors accumulated during stage movement through x and y axes. Imaris Stitcher corrects errors in (b) and (c) to provide image shown in (a).

The alignment process is outlined below:

1. The overlapping images may be preprocessed with a Gaussian Filter or a Derivative of Gaussian Filter.
2. For every pair of overlapping images a best pairwise translation is computed based on the image content (using phase correlation).¹
3. The camera rotation is estimated based on all the pairwise translations and stage coordinates.²
4. All pairwise translations are corrected for camera rotation, producing “residual pairwise translations”.
5. A global optimizer takes all “residual pairwise translations” and computes the best global repositioning of all images. This is done as described by Preibisch et al., (2009).
6. Untrustworthy positions that exceed the “typical” translation are rejected.
7. The results from the trustworthy global positions are applied to the camera-rotation-corrected positions.

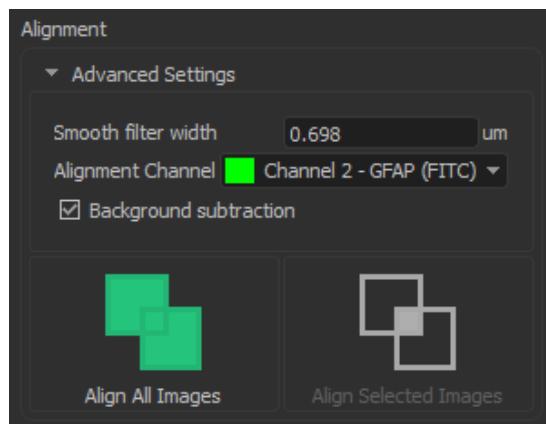
1 This pairwise translation is the vector that when added to the existing offset between the two lower left hand corners of the images in order to produce a positioning with maximal cross correlation.

2 More precisely: the coordinates used as input for alignment which could come from “set position to grid”

Stephan Preibisch, Stephan Saalfeld and Pavel Tomancak (2009) **Globally Optimal Stitching of Tiled 3D Microscopic Image Acquisitions** *Bioinformatics 2009*, **25** (11), 1463-1465.

4.1 Advanced Settings

Click the **Advanced** drop-down button to view the Advanced settings for the image alignment process. Here you can change the default values for smoothing and which channel should be used for alignment.



The following options can be adjusted:

- Smooth filter width
- Alignment Channel
- Background subtraction

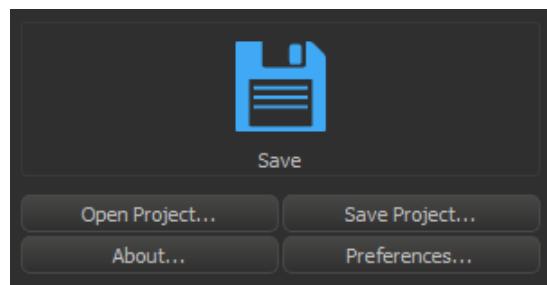
Smooth filter width is the only adjustable numerical parameter of the alignment method. Increasing or decreasing this value will correspondingly increase or decrease the smoothing effect.

The **Alignment Channel** option lets you select which channel is to be used for performing alignment.

Background Subtraction can be disabled/enabled from the checkbox. This applies a Gaussian filter to define the background at each voxel and then performs a Baseline Subtraction of this variable background.

Section 5 **Save - Saving the Images as a Single Image File**

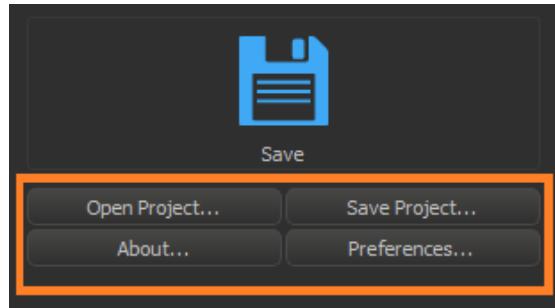
Once alignment has been performed you can then save the image. Save will save the collection of aligned image files as a single .ims file. (This is based on a weighted average depending on the distance from the border of the image). This may then be analysed using Imaris. If the file saving option "Automatically open saved file in Imaris" has been enabled in [Preferences](#) the saved file will open automatically in Imaris. You can also save the current layout of loaded images before alignment has been done using the save option. For example, a set of image files can be saved for alignment at a later time.



Note: for large files saving may take some time to process. Progress may be viewed in the status bar in the bottom of the screen.

Section 6 Preferences and Project Options

The preference and project options are located below the Save option:



Project Options

The project options let you save your work as a project so that you may return to continue work on the project at a later time.

Open Project

Open an existing project.

Save Project

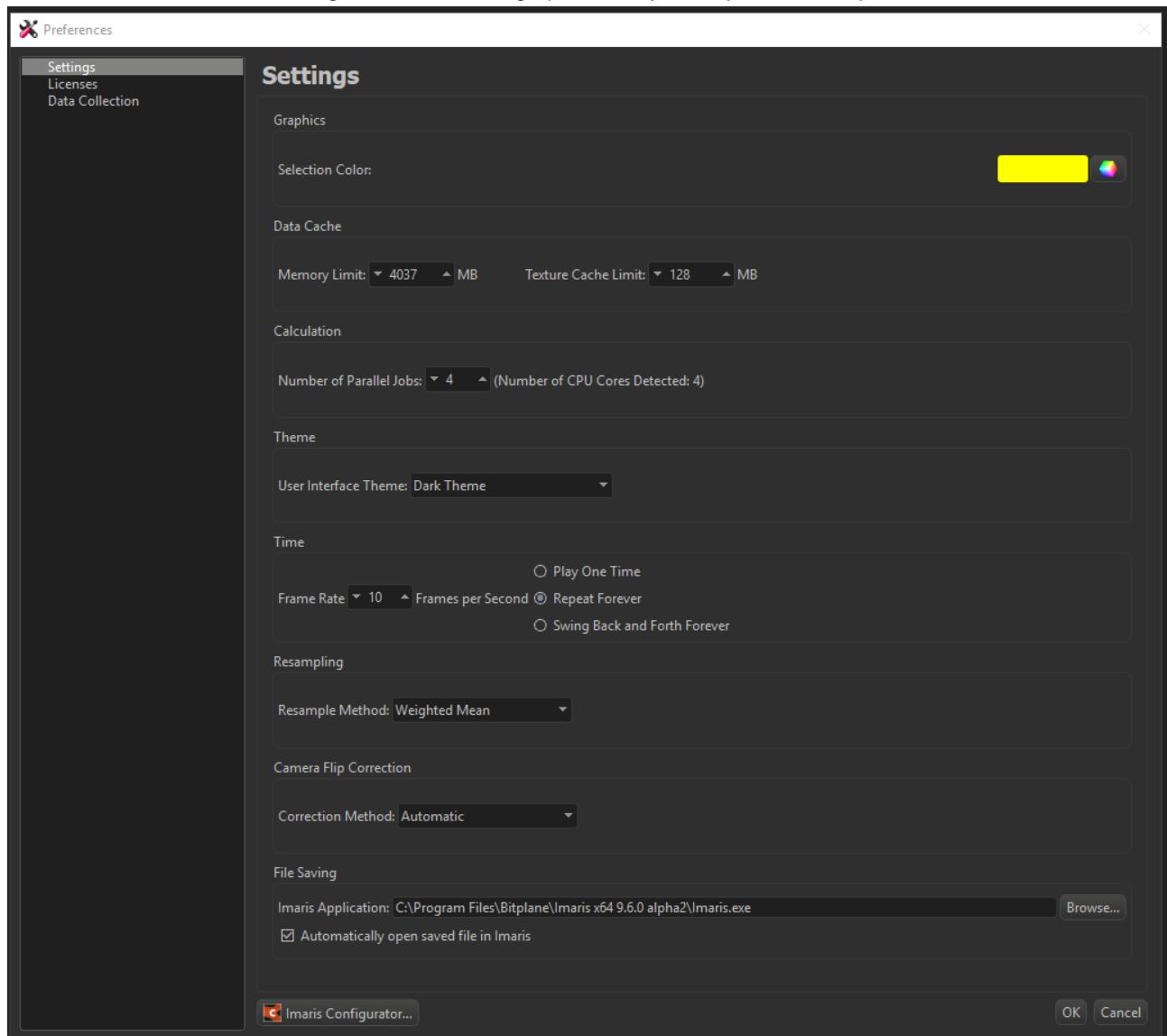
Use this option to save the settings and layout of the images as a project.

About...

Provides version information about the current version of Imaris Stitcher installed.

6.1 Preferences - Settings

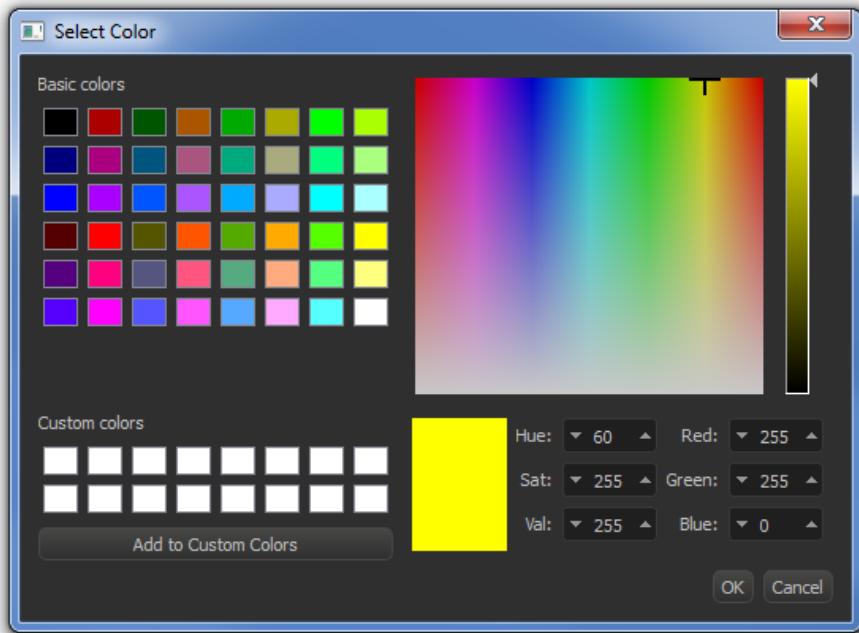
Within the Preferences - Settings tab the following options may be adjusted as required:



The following settings can be adjusted under Preferences:

Graphics

Change the color used to identify the selected image (Selection Color) from the default from either the basic colors or a custom option from the color chart.



Data Cache

Adjust the memory allocated for the Memory Limit and the Texture Cache Limit:

- **Memory limit:** The value of Data cache limit controls the amount of data blocks Imaris Stitcher will keep in memory at any time.
- **Texture Cache Limit:** Before displaying any image data, Imaris Stitcher 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. The Texture Cache Limit is automatically determined from the available memory of the graphics board when Imaris Stitcher is used for the first time. You can also manually set the value to the memory of your graphics card.

Calculation

Adjust the Number of Parallel Jobs from the default value. This is based on the Number of CPU Cores detected. This may be reduced to increase PC responsiveness on performing other tasks outside of Imaris Stitcher - at the expense of increasing the computation time required for Imaris Stitcher processes.

Theme

Change the User Interface Theme between Dark Theme (default) and Native Theme from OS.

Time

Adjust the Frame rate (FPS) and loop options for image sets that have time data. The following options are available and are activated when the Play button is played.

- Play One time All time points of the data set are shown one time. The play back stops when the last time point is reached.
- Repeat Forever Once the play back has reached the last time point, it starts at the first time point again (continuous loop).
- Swing Back and Forth Forever When the last time point is reached, the time sequence is shown in reverse until the first time point is reached.

Resampling

Adjust the resampling method between Weighted mean and Sample closest. Weighted mean is the default

value.

- Weighted mean In the overlapping regions the voxels of the stitched image are computed as a weighted average of the respective input image voxels. The weight is a linear function of the distance to the border of each input image.
- Sample closest In the overlapping regions the voxels of the stitched image are sampled from the input image whose border is farthest from the respective voxel position.

Camera Flip Correction

Adjust the correction method between Automatic, Always flip and Never flip. Automatic is the default value.

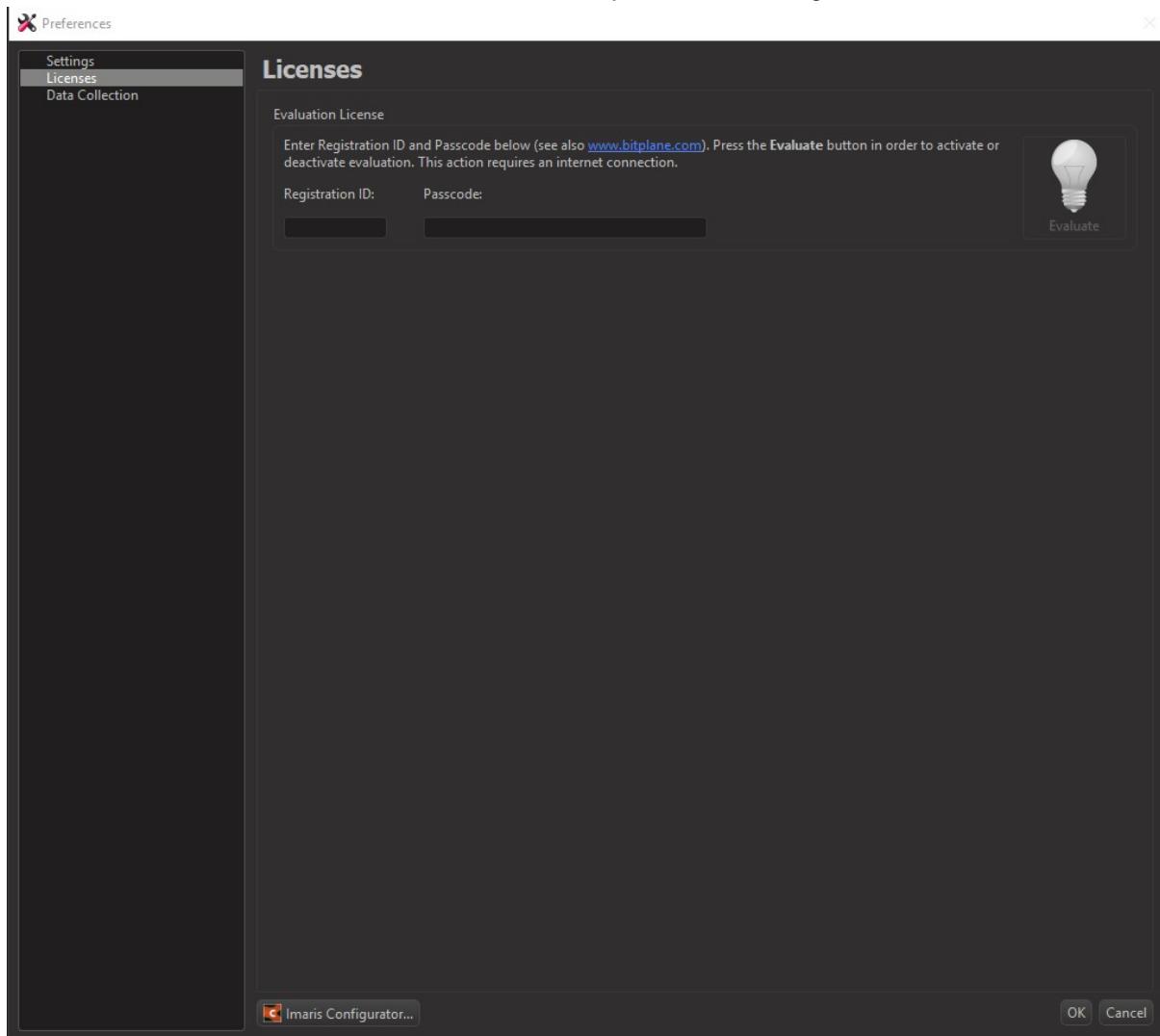
- Automatic Flipping is automatically estimated from image content.
- Always flip Image coordinates are always flipped after reading from file.
- Never flip Image coordinates from the input files are used as initial tile coordinates.

File Saving

The **Automatically open saved file in Imaris** option enables you to set whether new image files created in Imaris Stitcher are automatically opened in the latest version of Imaris installed on your PC. This is set to on by default - it can be disabled by unchecking the "Automatically open saved file in Imaris" checkbox. If the checkbox is checked/enabled, Imaris Stitcher will search for the latest version of Imaris that is installed. You can override this search and specify a specific version of Imaris which will be used to open the file saved from Imaris Stitcher.

6.2 Preferences - Licenses

Within the Preferences - Licenses tab, the user can adjust license settings.



Evaluation Licenses are temporary licenses allowing customers to explore all fascinating possibilities Imaris Stitcher offers before actually purchasing it. The license is only limited in time (10 days), but not in features. Additionally an Evaluation License is issued per user.

To get an Evaluation License you first have to register at:

<https://imaris.oxinst.com/downloads>

An automatic mail response should provide you with your personal RegistrationID and its corresponding Passcode. These credentials can then be entered during the application's start up.

After entering your RegistrationID and Passcode you can click on the Light Bulb button  to activate the Evaluation License.

This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the light bulb will turn on .

You can also disable your Evaluation License by clicking again on Light Bulb button and the light bulb will turn off. This indicates that you aren't using any Evaluation Licenses.

Any change of licenses will require a new start of Imaris.

RegistrationID

Please enter your RegistrationID you received by mail previously.

Passcode

Please enter your Passcode you received by mail previously.

Imaris Configurator button

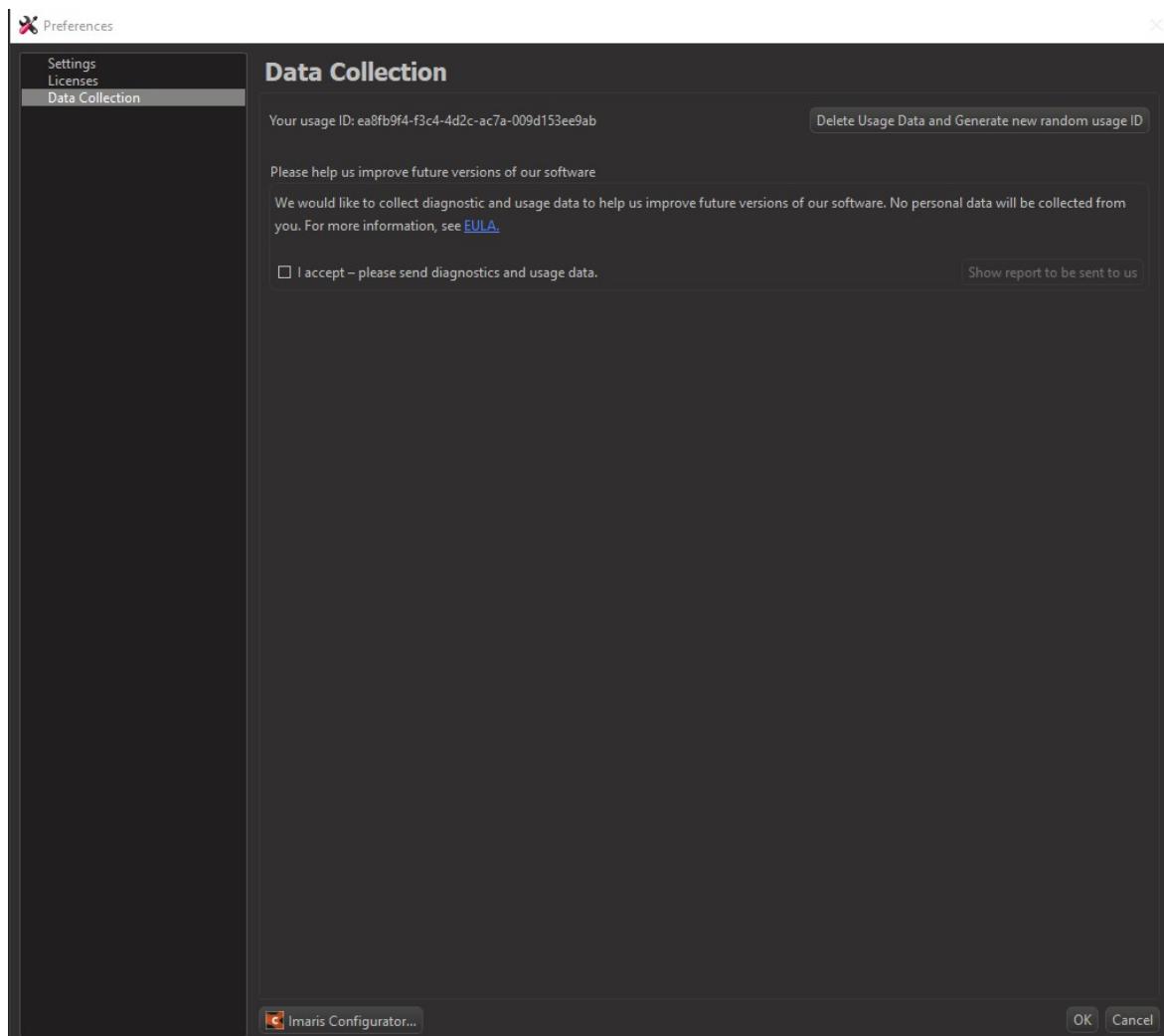
This starts the Imaris Configurator. Please see [Imaris Configurator for Stitcher](#).

6.3 Preferences - Data Collection

Within the Preferences - Data Collection tab the user can adjust data usage preferences.

Usage Data

The first time that Imaris Stitcher is started, you are requested to enable or disable capture of usage data. When enabled, Imaris Stitcher will send usage data to Bitplane, which will help the further development of the Imaris Stitcher software. To update usage data preferences please use this tab.



6.4 Imaris Configurator for Stitcher

Enter the Imaris Configurator for Stitcher to view and modify the account details and select a license file.

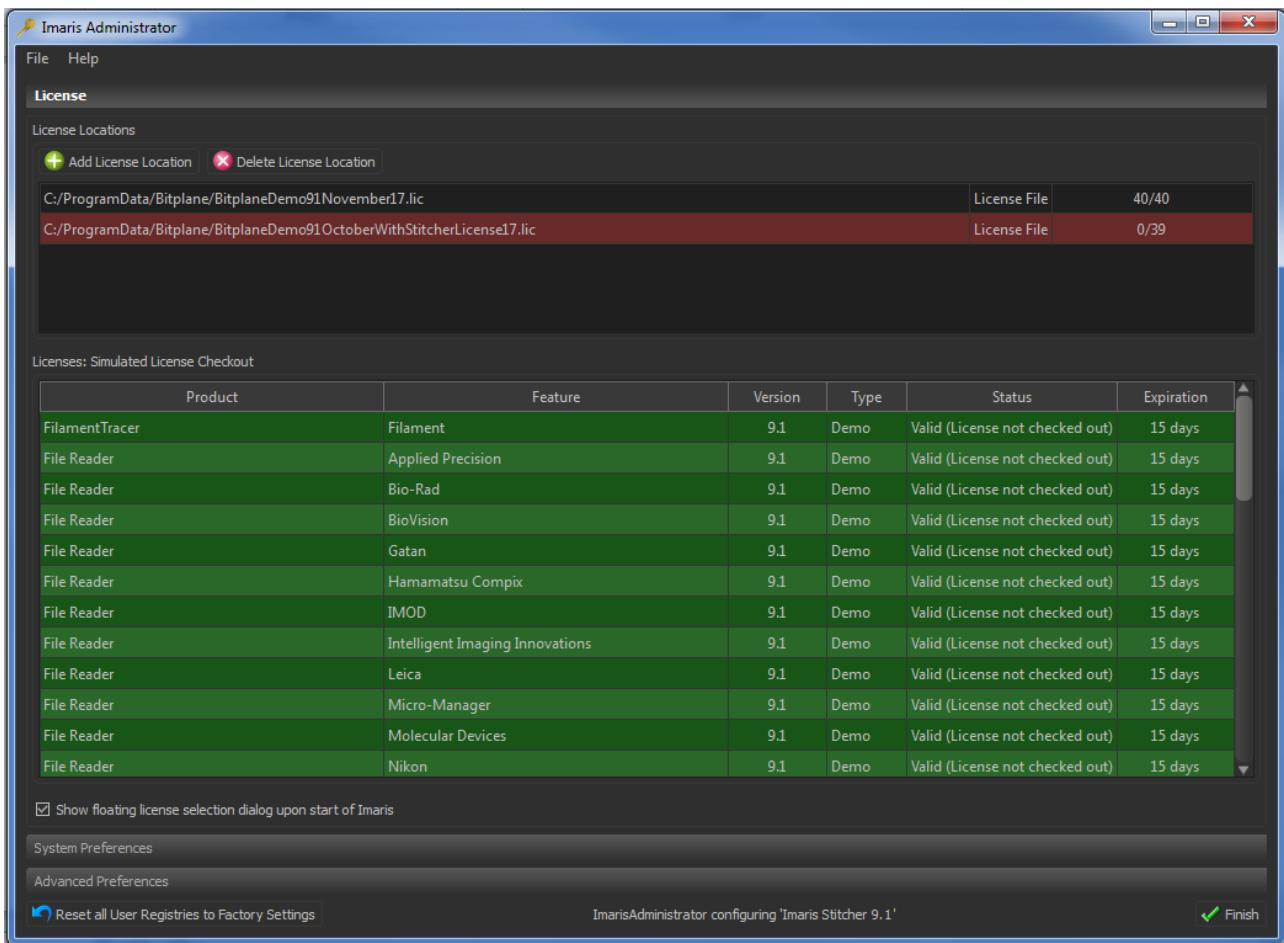


Notes

- You'll need administrative privileges in order to start the Imaris Configurator for Stitcher.
- You can use the "Reset" options to reset either a selected option, or all options to the default factory values.

6.4.1 License Options

Imaris Configurator for Stitcher provides a simple interface to add or delete any licenses. When a licence is added, the licence file gets copied to a system folder where it is secure from (accidental) manipulation by standard users. Although it is possible for administrators to modify licence files directly in this system folder we strongly advise you not to do so. It is recommended to go through the Imaris Configurator for Stitcher application instead.



Add Licence Location

Select the licence file for use of Imaris Stitcher.

Delete Licence Location

Delete an obsolete or unrequired licence file.

The table displays all existing Imaris licenses on the system and additional information for each feature:

Version

The latest Imaris Stitcher version this license can be used with.

Type

Which type of licensing scheme is used (an overview of the different licences is given later in this section).

Status

Displays the status of a license.

Expiration

Shows the number of days until this license expires.

License colors

In order to gain a quick overview, the validity of a license is also indicated by different colors:

- Green: Licence is valid.

- Yellow: Licence is valid, but all licences are currently in use.
- Red: Licence has expired.
- No color: Licence is not available (e.g. this additional licence feature hasn't been purchased).

Reset all User Registries to factory Settings button

Reset all user registry information back to the default factory settings.

Finish button

This button is only enabled if you have added at least one licence location. It is an indicator when all necessary steps to set up a licence are done. If all features in the above Licence table are highlighted in green (or only some of them) you successfully managed to add a licence. If you click the button, it just closes Imaris Configurator for Stitcher and you can proceed using Imaris Stitcher.

Licence Types

Node-locked Licence

Node-locked licences grant permission to run the licensed software on one computer (node) only. Node-locked licences are the easiest way to license the software. You can use the Imaris Configurator for Stitcher to directly download a node-locked licence from the Bitplane server. If your computer is not connected to the Internet it is also possible to download this licence from another computer and then copy it to the target machine. Please refer to the offline licence method for further instructions.

Floating Licence

Floating licences grant permission to run the licensed software on several computers. One floating licence can activate only one instance of the program at any given time. Floating licences require the installation of a separate licence server that runs on a Windows computer. The server installation requires more technical know how.

The Imaris Configurator for Stitcher application easily let you enable a floating license.

Demo Licence

A Demo Licence is a time-limited (usually one month) license. It is not an official licensing scheme and therefore can't be purchased. It's main purpose is for internal Testing and to grant customers with licensing issues an easy and quick workaround so that their productivity can continue until the problem is solved. Simply open the Imaris Configurator for Stitcher application and open the Demo Licence you received from your local support representative.

Evaluation Licence

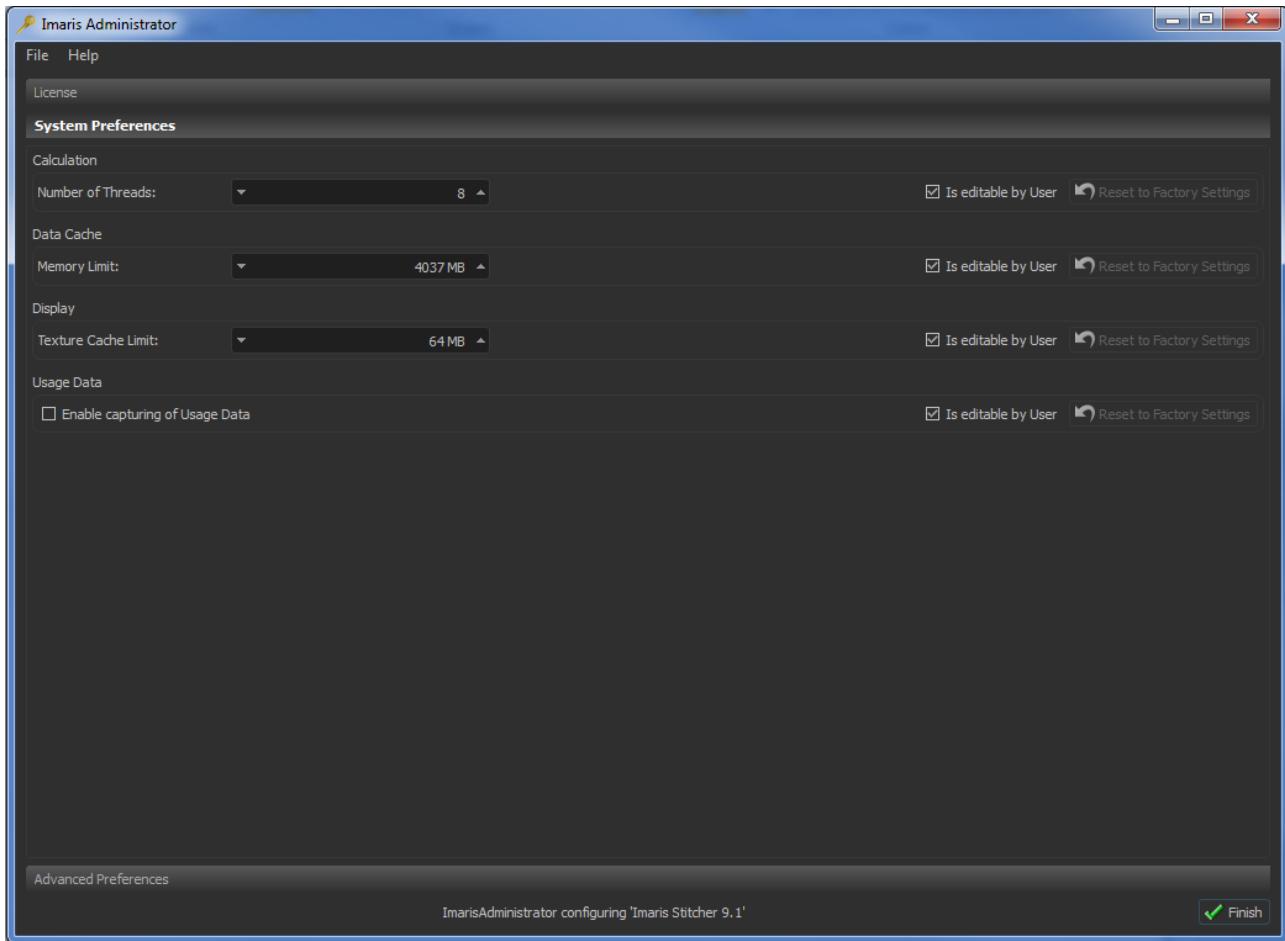
Evaluation Licences are temporary licences allowing customers to explore all the features Imaris Stitcher offers before actually purchasing it. The licence is only limited in time (10 days), but not in features. Additionally an Evaluation Licence is issued per user.

Notes

- You'll need administrative privileges in order to start the Imaris Configurator for Stitcher.
- You can use the "Reset" options to reset either a selected option, or all options to the default factory values.

6.4.2 System Preferences

Make adjustments to the system preferences. Care must be taken when making changes as changes to parameters may affect performance of Imaris. It is therefore recommended to use the default values for most situations.



Calculation

The number of processing threads can be adjusted from the default value depending on the system resource requirements of the computer running Imaris.

Data Cache

Adjust the size of the **Data Cache Memory Limit** to be used.

Display

Set the maximum memory limit allocated to the texture cache used for display. Adjusting the Texture Cache Limit can be used to improve image quality or performance or according to the size of the images and the computer resources available.

Usage Data

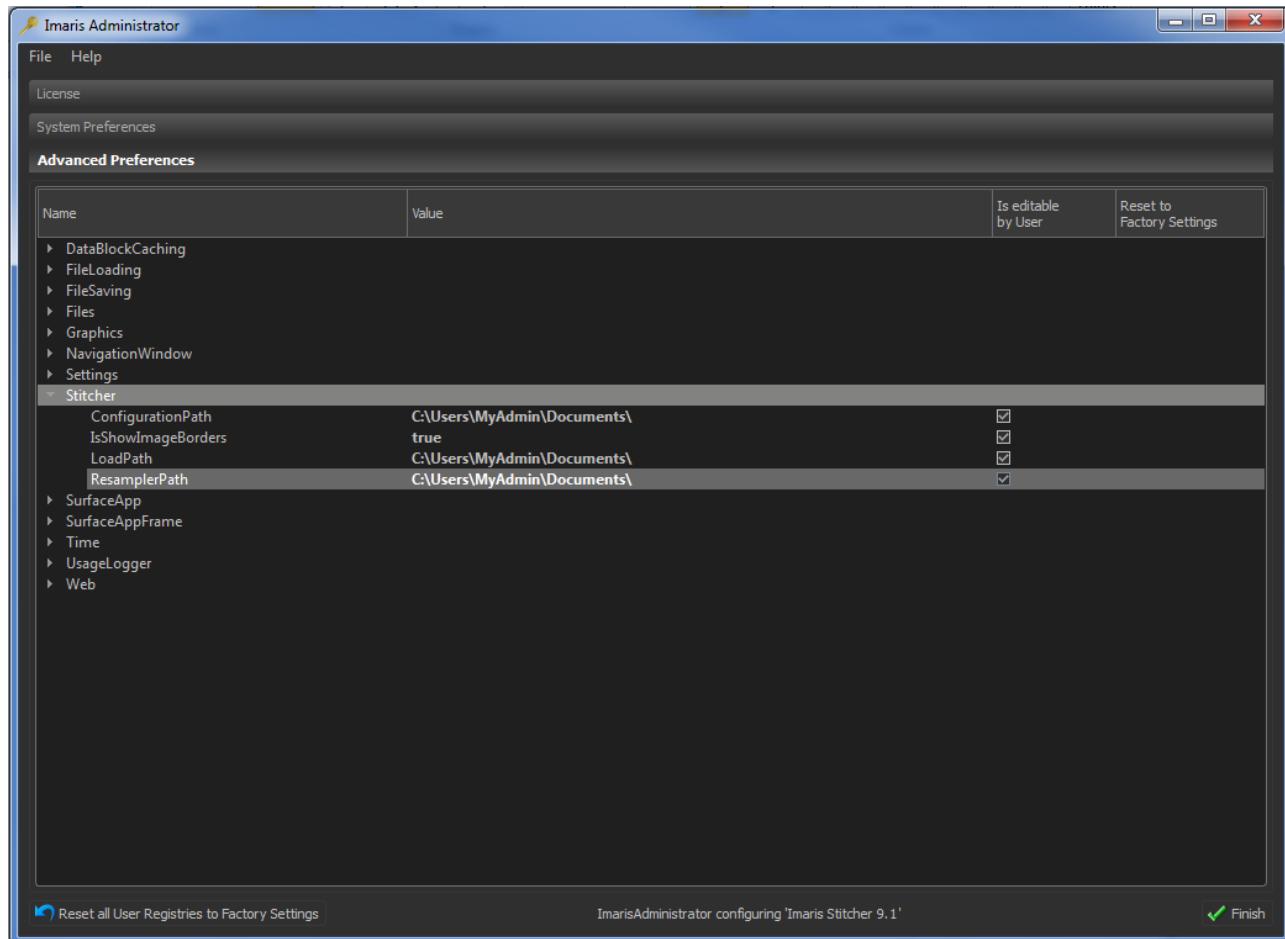
Enable or disable the capturing of usage data.

Notes

- You'll need administrative privileges in order to start the Imaris Configurator.
- You can use the "Reset" options to reset either a selected option, or all options to the default factory values.

6.4.3 Advanced Preferences

Further changes can be made using the Advanced Preferences menu:



In Advanced Preferences it is possible to edit parameters in Imaris Stitcher and set whether they may be editable by the user under the Advanced option of preferences. Care must be taken when making changes as changes to parameters may affect performance of Imaris Stitcher. It is therefore recommended to use the default values for most situations. To return values to the original default values select **Reset to Factory Defaults**. Click the **Finish** button when you are finished making changes. You will need to restart Imaris Stitcher for any changes to take effect.

Notes

- You'll need administrative privileges in order to start the Imaris Configurator for Stitcher.
- You can use the "Reset" options to reset either a selected option, or all options to the default factory values.

Section 7 Keyboard Shortcuts

This section outlines the keyboard and mouse shortcuts that can be used to quickly access or perform useful functions in Imaris Stitcher.

Keyboard Shortcuts

The following keyboard functions are available:

Function	Description	PC	Mac
3D View	Switch to 3D View	Ctrl + 1	Cmd + 1
Slice View	Switch to Slice View (if available)	Ctrl + 2	Cmd + 2
Fit	Fit images to fit viewing window	Ctrl + A	Cmd + A
Add Images	Add an extra image	Ctrl + O	Cmd + O
Save	Save current image layout and settings or aligned image	Ctrl + S	Cmd + S
Preferences	View Imaris Stitcher preferences	Ctrl + P	Cmd + P

Mouse Functions

The mouse provides shortcuts to the Imaris Stitcher Manual and a range of useful functions to work with images in the viewing window:

Function	Action
To access the Help Manual	Click an area within Imaris Stitcher with the right mouse button, then left click to open the relevant section of the Imaris Stitcher manual
Select image	Select with left mouse button.
Select additional images	Hold Ctrl + select or deselect each additional image.
Select multiple images	Hold Shift + left mouse button
Select group of images in a box	Hold Shift + left mouse button, then drag over images
Move image(s)	Select image(s) then left click and drag to the required position
Zoom in to image	Mouse wheel up
Zoom out of image	Mouse wheel down
Pan Image	Hold right mouse button and drag in required direction

Note: Selected image(s) will be highlighted by a box (default color is yellow).

Section 8 Troubleshooting

This section provides some useful troubleshooting scenarios with possible causes and actions to check.

Issue	Cause	Action
Multiple images have loaded but the positions do not appear to match the expected sequence/layout from acquisition.	<ul style="list-style-type: none"> Inaccurate stage position information. 	<ul style="list-style-type: none"> Check stage position/acquisition settings for microscope. Use Grid mode to correct the layout (see section Grid Mode).
Images have loaded but only one is visible.	<ul style="list-style-type: none"> Inaccurate or no stage positional information – images are stacked in a default position. 	<ul style="list-style-type: none"> Check stage position/acquisition settings for microscope. Move images into correct positions manually (see section Layout - Arranging Image Files) or use grid mode (see section Grid Mode).
Images are not visible.	<ul style="list-style-type: none"> Images are not within the viewing area. Channels are not set to be visible. 	<ul style="list-style-type: none"> Click the Fit button to adjust the view to fit images on screen. Check channels are set to be visible (Adjusting the View).
Image alignment does not happen.	<ul style="list-style-type: none"> Images do not overlap - selected, images must overlap for alignment to be possible. Image data is not suitable for effective alignment to occur. 	<ul style="list-style-type: none"> Ensure images overlap (see section Grid Mode). Select a different channel with which to perform alignment (see section Alignment - Aligning your Images).
Image alignment is not accurate.	<ul style="list-style-type: none"> Image data is not suitable for effective alignment to occur. Images may be too far out of position to allow alignment. 	<ul style="list-style-type: none"> Reposition images manually in required position (see section Layout - Arranging Image Files) Select a different channel with which to perform alignment (see section Alignment - Aligning your Images).
Alignment does not occur for all images.	<ul style="list-style-type: none"> Some images may be in the incorrect location/sequence. Some images may not have overlap. 	<ul style="list-style-type: none"> Ensure images are in the correct position and sequence relevant to the other images (see section Grid Mode).

If you are unable to resolve an issue, please contact your nearest Bitplane customer support team:

eusupport@bitplane.com

ussupport@bitplane.com

asiastoprt@bitplane.com

Section 9 Imaris File Converter

Imaris Stitcher can only read files in the Bitplane: Imaris 5.5 (*.ims) Format. If your images are in a different format you can convert them with Imaris File Converter to .ims format. Imaris File Converter is installed along with Imaris Stitcher and also as part of Imaris. Imaris File Converter can read many image file formats and produce ims files that can be subsequently used by Imaris Stitcher and Imaris.

Input

To add files, Drag & Drop Files, or click the **Add Files...** Button. Imaris File Converter will also launch if non .ims format files are added to Imaris Stitcher.

The following file types are supported:

- Bitplane: Imaris 3.0 (*.ims)
- Bitplane: Imaris 2.7 (*.ims)
- Andor: iQ ImageDisk (*.kinetic)
- Andor: Multi-TIFF- (Series} (*.tif;*.tiff)
- Big Data Viewer (*.xml;*.h5)
- Applied Precision: DeltaVision (*.r3d;*.dv)
- Biorad: MRC (series) (*.pic)
- BioVision: IPLab IPL (series) (*.ipl)
- BioVision: IPLab IPM (*.ipm)
- Gatan: Digital Micrograph (series) (*.dm3)
- Hamamatsu Compix: Simple PCI (*.cxd)
- Intelligent Imaging Innovations: Slide Book (*.sld)
- IMOD: MRC (*.mrc;*.st;*.rec)
- Leica: Image File Format LIF (*.lif)
- Leica: TCS-NT (*.tif;*.tiff)
- Leica: Series (*.tif;*.tiff;*.inf;*.info)
- Leica: Vista (*.tif;*.tiff;*.lei;*.raw)
- Micro-Manager: (*.tif;*.tiff;*.txt)
- Molecular Devices: STK (series) (*.stk)
- Molecular Devices: MetaMorph (*.nd)
- Nikon: Image Cytometry Standard ICS (*.ics;*.ids)
- Nikon: ND2 (*.nd2)
- Olympus: cellR (*.tif;*.tiff)
- Olympus: FluoView OIB (*.oib)
- Olympus: FluoView OIF (*.oif)
- Olympus: FluoView OIR (*.oir)
- Olympus: FluoView TIFF (*.tif;*.tiff)
- Olympus: VSI (*.vsi;*.tif)
- Prairie Technologies: Prairie View (*.xml;*.cfg;*.tif;*.tiff)
- Open Microscopy Environment: TIFF (*.tif;*.tiff)
- TIFF ImageJ (adjustable file series) (*.tif;*.tiff)
- Open Microscopy Environment: XML (*.ome)
- LCS
- PerkinElmer: Improvision Openlab LIFF (series) (*.tiff)
- PerkinElmer: Improvision Openlab RAW (*.raw)
- PerkinElmer: UltraView (*.tim;*.zpo)
- TILL Photonics: TILLvision (*.rbinf)
- Zeiss: AxioVision I ZVI (*.zvi)
- Zeiss: LSM710. LSM510 (*.lsm)
- Zeiss: LSM410. LSM310 (*.tif;*.tiff)
- BMP (adjustable file series) (*.bmp)
- TIFF (adjustable file series) (*.tif;*.tiff)
- Zeiss: CZI (series) (*.czi)
- Bitplane: Imaris Scene File (*.imx)

Button Add Files ...

Click the **Add Files ...** button to display the Select Files for Conversion window. Then navigate to and select the required file(s).

Output

Same Folder as Input File

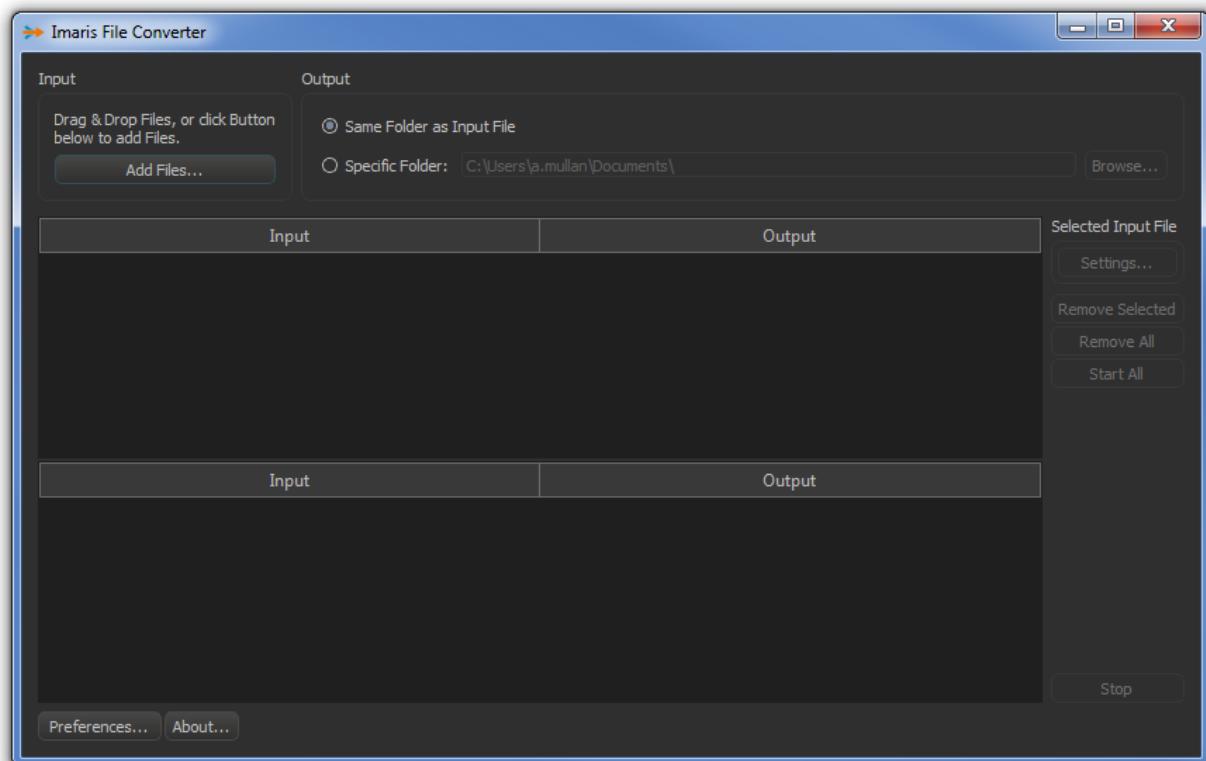
You find the converted image(s) in the same folder as the input files.

Specific Folder

Here you can select another folder for the converted image(s). Either type in the respective path or use the button **Browse**.

Button **Browse**

Click on this button to browse for the specific folder.



Input

Here you can see the selected input file path(s).

Output

Here you can see the selected output file path(s).

Settings

Provides additional settings for the selected file type e.g. to specify voxel size and file names with or without delimiters (see [Settings](#) section).

Remove Selected and Remove All buttons

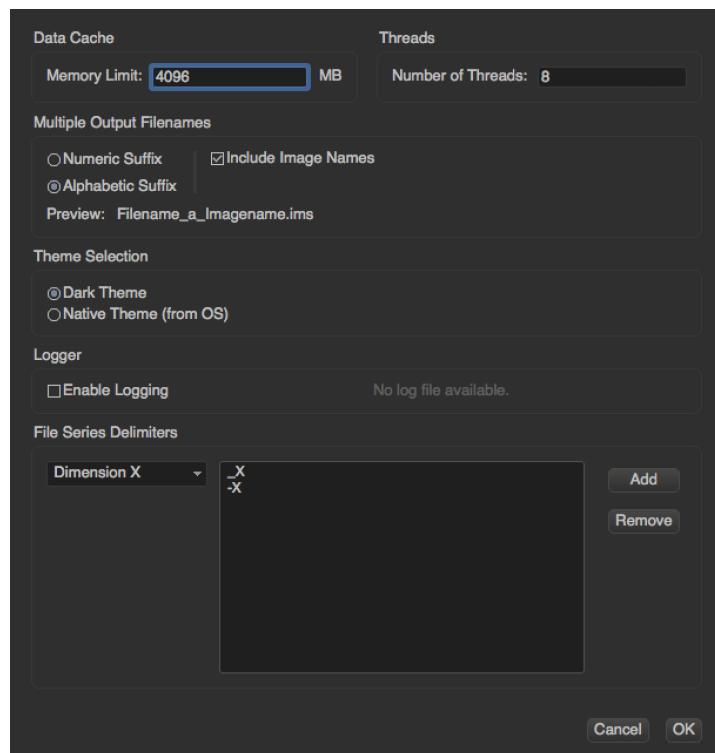
Click on these buttons to clear either a selected row or all rows in the table respectively.

Button **Start All**

Click on this button to start the conversion.

When the conversion starts, files are moved to the bottom table. This displays the progress of the conversion for both read progress and write progress. It is possible to add new files and configure settings while conversion is running.

Preferences



Data Cache

Adjust the memory allocated for the Memory Limit. The value of Data cache limit controls the amount of data blocks Imaris File Converter will keep in memory at any time.

Threads

The number of processing threads can be adjusted from the default value depending on the system resource requirements of the computer.

Multiple Output Filenames

Select the default filename format . This includes either numeric or alphabetic formats and option to include the image name as part of the filename. The currently selected option is shown in a preview.

Theme Selection

Change the User Interface Theme between Dark Theme (default) and Native Theme from OS.

Logger

Select whether to enable or disable usage data logging.

File Series Delimiter

Refer to the [Settings](#) section.

About...

View the current software version information (Click anywhere to exit).

9.1 Settings

In the Settings you can specify options for reading certain file formats. Examples are detailed below:

Leica LCS Settings

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

1. Click on the *.lei file to highlight it and click **Settings**. A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).
2. By checking the box **Thumbnail Preview** you can see an image preview. The individual images that belong to that stack will display on the left side.
3. Select the required image stack on the left side of the dialog box.
4. Click **OK** to open the image.

Leica LIF Settings

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

1. Click on the *.lif file to highlight it and click **Settings**. A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).
2. By checking the box **Thumbnail Preview** you can see an image preview. The individual images that belong to that stack will display on the left side.
3. Select the required image stack on the left side of the dialog box.
4. Click **OK** to open the image.

Series Reading Sequence

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 reader can handle Tiff series with single and multiple running numbers.

1. The **Settings** button only becomes enabled when a TIFF file is selected.
2. In the Series Reading Sequence window you can define the options to combine multiple files into one multi-dimensional image.

Reference File

Display the full path of the selected file.

Depending on the structure of the file system and the file name extension there are two options you can use:

1. File Names with Delimiter
2. File Names without Delimiter

File names with Delimiter

You can use this option when your files are numbered in sequential order and contain fields of information. The dialog allows you to specify the order of the dimension within the image series and defines how they should be arranged in the final sequence. These are Slices: Z, channels: C, time points: T and to split into multiple datasets/images: F (Split).

You may apply as many or as few dimensions as you wish, depending on your image file requirements.

Assign the appropriate dimension by clicking on the arrow and selecting a dimension from the drop-down menu.

The selected dimensions are applied to the series of images.

Summary

Displays the total number of files selected within the file cluster, as defined by your selection criteria, to be used to create multi-dimensional image.

The summary number is updated dynamically.

File names without Delimiter

You can use this option when the files are not numbered and contain no separator or delimiter.

The dialog allows you to specify the order of the dimension within the image series and defines how they should be arranged in the final sequence. If the file is being loaded, the number of dimensions must be specified by the user so that the algorithm will know when to finish reading the first dimension and continue with the second.

You may apply as many or as few dimensions as you wish, depending on your image file requirements.

Assign the appropriate dimension by clicking on the arrow and select a dimension from the drop-down menu.

Summary

Displays the total number of files selected within the file cluster, as defined by your selection criteria, to be used to create multi-dimensional image.

The summary number is updated dynamically.

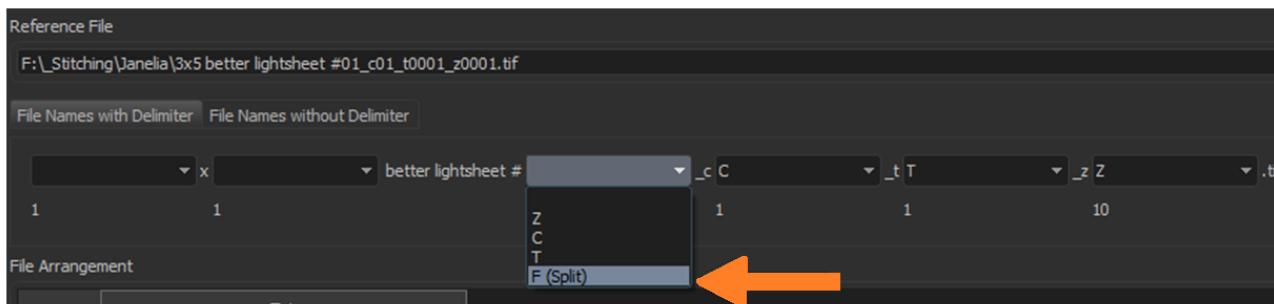
File Arrangement

In the File Arrangement window, the selected sequence and dimension order is shown in tabular form. This display can be used to validate the sequence parameters.

Validate this information before you apply and open the TIFF series.

F(Split)

The split option F (Split) lets you split a collection of images files into multiple DataSets / Images.



This Settings Dialog is present both in Imaris and in Imaris File Converter, this option is available in the two applications:

- In Imaris File Converter, it allows to convert file series into multiple output IMS Files (with the benefit of

configuring the layout and applying the X, Y, Z size)

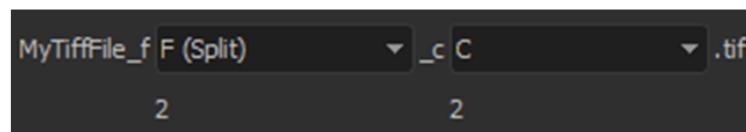
- In Imaris, it allows, to read a single file (or a subset of files) from file series, or load multiple images from subsets of the series for batch run.

The procedure for how to split a number of files into two ims images, with two channels each is as follows:

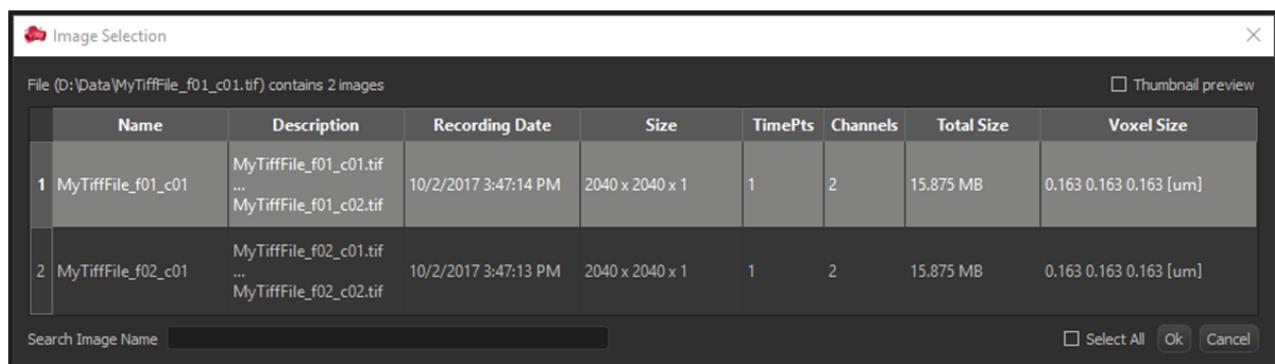
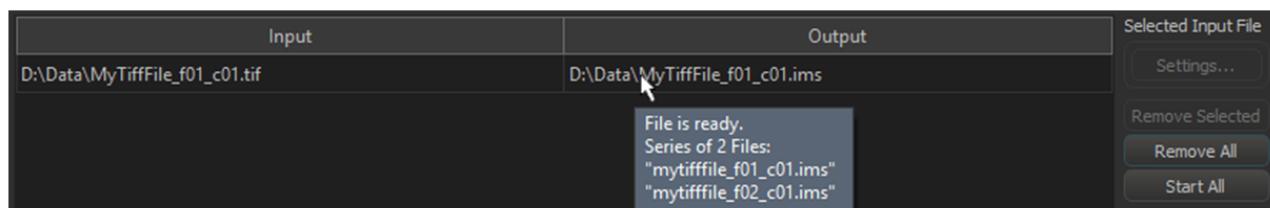
In this example there are 4 TIFF files:

- MyTiffFile_f01_c01.tif
- MyTiffFile_f01_c02.tif
- MyTiffFile_f02_c01.tif
- MyTiffFile_f02_c02.tif

1. Add one of one of the TIFF files to Imaris File Converter.
2. Click Settings the settings button.
3. Select "F (Split)" for the File Split delimiter. In this case it is "_f".
4. Select "C" for the channel delimiter. In this case it is "_c".



5. Click OK.
6. By letting the mouse pointer hover over the output, you can see that two IMS Files will be generated (see below).



- The Name and Description categories show which file corresponds to which DataSet.
- The behavior is then the same when loading a multi-image file.

When adding image files as part of a file series, Imaris and Imaris File Converter will try to automatically detect the layout of image files in the file series. If the detected layout is not as desired, it is possible from to change it from the Series Settings option. It would be cumbersome if you were working with a number of similar image data sets with an incorrect structure to do this each time a new image was opened.

The File Series Delimiters configuration lets you configure the delimiters that are used for the automatic detection. This lets you adjust the process so it matches the image files. (The File Series Delimiters

configuration is also accessible in Imaris from Preferences/Loading and in the Imaris File Converter application under Preferences).

On the left-hand side, you can select the dimension that you want to configure from the drop-down list. The corresponding delimiters will appear in the central area.

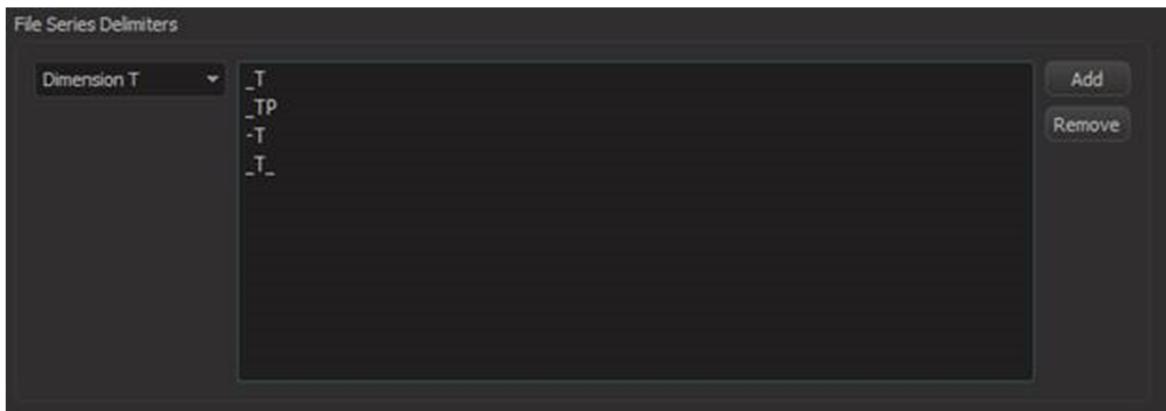
For the example above, with "Dimension T" the following images would be correctly detected as time series, because the _T delimiter is in the Dimension T delimiters' list:

- MyFile_t1.tif
- MyFile_t2.tif
- MyFile_t3.tif
- MyFile_t4.tif

But the following images would not:

- MyFile_t_1.tif
- MyFile_t_2.tif
- MyFile_t_3.tif
- MyFile_t_4.tif

To have these files detected automatically as a time series, the _T_ delimiter must be added:



Dimension F (Split) is by default not detected automatically (it does not contain any default delimiter). Therefore to have File Series Split detected automatically, some delimiters must be added (e.g. _F, _F_, _FLD).

Notes

- The delimiters are case insensitive.

OXFORD
INSTRUMENTS

IMARIS

imarist.com

Image credit: Embryo capsella is
acquired by Dr Bruno Combettes.

