

# Import of Images to Imaris

A shorter video tutorial you can find at <u>https://imaris.oxinst.com/learning/view/article/importing-data-into-imaris</u>

# 1 General Guidance

It is recommended to try first to import the native file format of the acquisition device. If supported by Imaris this will give you the best experience since you can expect to preserve dimensions, calibration, and channel colours of the native format.

You can find the supporter file formats at 'Imaris main menu -> Help -> Preface -> Getting Started -> Supported File Formats'

Which Import Path to use:

Imaris Arena

If you have only a limited number of datasets to be imported in one go. Imaris Surpass Fiji Plugins

For files were the Imaris reader fail completely

Imaris File Converter

For batch converts of many files.

On machines you have no İmaris license, or you like not to block a license for the conversion.

If Imaris can detect the format but still has problems to import correctly.

# 2 Import using Imaris Arena

If the location of the dataset cannot be already seen in Arena add the folder with the files to be converted to your Arena view using the 'Observe Folder' button



The Arena file browser of the main Imaris program will show you the thumbnails of the detected datasets.

Tip: if you do not see the files or folders immediately press 'CTRL - F5' to refresh the view.

1. With a double click or 'RIGHT-MOUSE -> Convert to Native Imaris File Format' it will be automatically converted and an IMS file will be saved to the same directory. Imaris may ask to enter the Voxels size if it could not extract it.

🚺 Set Voxel Size				?	$\times$
Unknown voxel size.	Please enter the m	issing values.			
Voxel Size [µm]:	0.165	0.165			
			Sav	ve Ca	incel

2. Double click on the Thumbnail to open the IMS file in Imaris Surpass and check for correct dimensions and calibration data at 'Surpass main menu -> Edit -> Image

Properties	-> Geometry'
------------	--------------

-

Channel 2 Channel 2 Parameters Parameters Data Type: 8 bit (unsigned) Size 3 X: Y: Z:	
Parameters Data Type: 8 bit (unsigned) Size 3 X: Y: Z:	
Size 3	
X: Y: Z:	
	T:
Size: 285 218 64	1
Image size: 7,584 MB	
Coordinates [µm]	Time Point 2
X: Y:	Z:
Voxel Size: 0,165 0,165 1,00	Date [yyyy-mm-dd]
Min: 0,00 0,00 0,00	2013-01-06
Max: 46,9 35,9 64,0	Time [hh:mm:ss.ssssss

- If the Voxels sizes [1] or time calibration [2] is not correct you can edit them directly in the dialogue.
- If size in X Y Z or T [3] or the number of channels [4] is not correct you can maybe fix this by using the File Series Reader.
- If the channel colours are not correct you may click on the channel tabs [4] and correct them.

Image Properties		×
Geometry Data Set ▼ Channels Channel 1	Channel 1	Î
Channel 2 Parameters	Name: (name not specified)	
	Description: (description not specified)	
	Emission Wavelength:	
	Excitation Wavelength:	1
	Pinhole Radius:	
	Base Color Mapped Color	
	Red: •  1,000 ^ Green: • 0,000 ^ Blue: • 0,000 ^	
	OK	Cancel

• Alternatively, you can come here by clicking in the name of the channel the 'Display Adjustemt' windows. 'Surpass main menu -> Edit -> Show Display Adjustment'



By default, files with the same base name and size are stored into the same dataset. If the name contains only one running number, it is interpreted as a Z stack or time series. If the name contains multiple running number leaded by one of the below characters, the characters determine which dimensions are used.

\_C### Channels

\_T### Time Points

\_Z### Z Slices X### X Slices

\_X### X Slices

\_Y### Y Slices

### = a running number

If the files are not automatically sorted into the correct dimensions by Arena please read the chapter 'File Series Reader'

#### 2.1 File Series Reader

The Imaris File Series Reader is a tool to handle series of numbered files. It allows you to group the files into datasets with the desired number of sections, channels and time points. The names and sizes of the files are used to arrange them.

- Datasets which files are stored in multiple directories are not supported
- It is available for plain Tiff files **only**, not e.g OME TIFF. Tiff files were Imaris finds metadata about the organisation of the dataset cannot be handled.
- It is recommended to store the files of different datasets into sperate directories to simplify the task.

The File Series Reader can be started from Arena on a thumbnail 'RIGHT-MOUSE -> Configure File Series'

Please note that you can as well use the tool from Imaris File Converter' with a selected input file 'Settings...'

When started you need chose how the type of the naming of the files

File Names with Delimiter File Names without Delimiter

 If the file name has multiple running numbers for different dimensions chose 'File Names with Delimiter' e.g. retina\_ChO\_layer000.tif retina\_ChO\_layer001.tif retina\_ChO\_layer002.tif retina\_Ch0\_layer003.tif retina\_Ch1\_layer004.tif retina\_Ch1\_layer005.tif retina\_Ch1\_layer006.tif retina\_Ch1\_layer007.tif retina\_Ch1\_layer008.tif retina\_Ch0\_layer009.tif retina\_Ch0\_layer010.tif

retina\_ is the base name. Only files with this base name will be converted. Ch0 contains the running number for the channels layer001 contains the running number for the layers (slices).

- If the files with the identical base name have one running number chose the Tab 'File Names without Delimiter' e.g.:

retina\_001.tif retina\_002.tif retina\_003.tif retina\_004.tif retina\_005.tif retina\_007.tif retina\_008.tif retina\_009.tif retina\_009.tif retina\_010.tif

retina\_ is the base name. Only files with this base name will be converted. 001 is a single running number for all files of the dataset

#### Notes:

- It is recommended to store the files of different datasets into sperate directories to simplify the task.
- Datasets which files are stored in multiple directories are not supported

#### 2.1.1 File Names with Delimiter

To be used if filenames contain multiple running numbers, e.g. retina\_Ch0\_layer000.tif

The TIFF reader uses some patterns \_C?number \_T?number \_S?number. That \_[letter] codes are used to assign the different dimensions and sort the files automatically into channels, time points, slices etc.

\_C?number -C \_CH \_W Channel\_T?numberTime\_Z?number -Z \_SSlice\_X?number -YColumn\_Y?number -YRow

The ? can be any character or absent (\_Cnumber) It does not distinguish between upper and lower case. The letter codes you can change at 'Preferences – File Conversion'

If the automatic assignment does not work, you can use the 'Series Reading Sequence' tool to further tune it for specific files.

III Series Re	eading Sequence - C:\tmp\delimiter\،روtina_Ch0_layer000.tif	×
Reference F C:\tmp\del	ile limiter\retina_Ch0_layer000.tif	
File Names	s with Delimiter File Names without Delimiter	
retina_Ch ( 2	C Jayer Z Jayer Z Itif Filename	
File Arrange	ement Amount of items	
Z 1 - C 1	retina_Ch0_layer	
Z 2	retina_Ch0_layer	
Z 3	retina_Ch0_layer	
Z 4	retina_Ch0_layer Preview table	
Z 5	retina_Ch0_layer	
Z 1 - C 2	Second channel starts	
Z 2	retina_Ch1_layer	
Z 3	retina_Ch1_layer	
Z 4	retina_Ch1_layer	
Z 5	retina_Ch1_layer	
Voxel Size		
X: 0,165	μm Y: 0,165 μm Ζ: 0,00	μm
		OK Cancel

Imaris will show the filename and at the place of the running number a menu to select how the number should be interpreted:

**BLANK** Ignore

- X Slices
- Y Slices
- Z Slices

\_

- C Channels
- T Timepoints

F (Split) Split the files into the next dataset if the number changes.

Below the number of items are shown which will be assigned to the chosen dimensions. In the screenshot this are 2 channel and 5 Z layers.

The table 'File Arrangement' shows a preview of the resulting first dataset which will be created.

Here are a few examples:

File Names	with Delimiter File Nam	nes without Delimi	ter
retina_Ch <mark>1</mark> 2	∎ <mark></mark> layerZ	<mark>,</mark> tif	Summary 10 of 10 Files used
File Arrange	ment		
		T 2	
Z 1 - C 1	retina_Ch0_layer000.tif	retina_Ch1_layer0	00.tif
Z 2	retina_Ch0_layer001.tif	retina_Ch1_layer0	01.tif
Z 3	retina_Ch0_layer002.tif	retina_Ch1_layer0	02.tif
Z 4	retina_Ch0_layer003.tif	retina_Ch1_layer0	03.tif
Z 5	retina_Ch0_layer004.tif	retina_Ch1_layer0	04.tif

First number T, second number Z -> 1 datasets with 1 channel, 2 time points and 5 Z layers will be created.

Fi	le Names	with Delimiter File	Names without Deli	imiter		
r	etina Ch	z v lav	er T 👻	<sub>tif</sub> Summary		
	- 2		5	10 of 10 Files us	ed.	
Fil	e Arrange	ment				
		T 1	T 2	T 3	T 4	T 5
Z	1 - C 1	retina_Ch0_layer	retina_Ch0_layer	retina_Ch0_layer	retina_Ch0_layer	retina_Ch0_layer
Z		retina_Ch1_layer	retina_Ch1_layer	retina_Ch1_layer	retina_Ch1_layer	retina_Ch1_layer

First number Z, second number T -> 1 datasets with 1 channel, 2 Z layers and 5 timepoints will be created.

File Names	with Delimiter File Nam	es without Delimit	ter
retina_Ch	(Split) ▼_layerZ	▼.tif	Summary 10 of 10 Files used
2			
File Arrange	ment		
Z 1 - C 1	retina_Ch0_layer000.tif		
Z 2	retina_Ch0_layer001.tif		
Z 3	retina_Ch0_layer002.tif		
Z 4	retina_Ch0_layer003.tif		
Z 5	retina_Ch0_layer004.tif		

-

First number 'F- (Split), second number Z -> 2 datasets with 1 channel and 5 Z layers will be created. Please note that the table show always only the layout of the first IMS dataset.

File Names	with Delimiter File Nam	nes without Delimiter			
retina_Ch	C	,tif Sumn 10 of	nary 710 Files used.		
File Arrange	ment				
	T 1	Т 2	Т 3	T 4	Т 5
Z 1 - C 1	retina_Ch0_layer000.tif	retina_Ch0_layer001.tif	retina_Ch0_layer002.tif	retina_Ch0_layer003.tif	retina_Ch0_layer004.tif
Z 1 - C 2	retina_Ch1_layer000.tif	retina_Ch1_layer001.tif	retina_Ch1_layer002.tif	retina_Ch1_layer003.tif	retina_Ch1_layer004.tif

First number C, second number T -> 1 dataset with 2 channels, 5 time points and 1 Z layer will be created.

#### 2.1.2 File Names without Delimiter

To be used if filenames contain a single running numbers, e.g. retina\_**000**.tif

III Series Re	eading Sequence - C:\tmp\running humber\retina_001.tif	×
Reference F	File	
C.\timp\iun		
File Names	s with Delimiter File Names without Delimiter	
Z	Summary	
<del>•</del> 10	$\bullet$ $\bullet$ 1 $\bullet$ 1 $\bullet$ 1 $\bullet$ 10 Files used.	
File Arrange	ement	
Z 1 - C 1	retina_001.tif Preview table	
Z 2	retina_002.tif	
Z 3	retina_003.tif	
Z 4	retina_004.tif	
Z 5	retina_005.tif	
Z 6	retina_006.tif	
Z 7	retina_007.tif	
Z 8	retina_008.tif	
Z 9	retina_009.tif	
Z 10	retina_010.tif	
Voxel Size		
X: 0,165	μm Y: 0,165 μm Z: 0,00 μm	
	OK Ca	ncel

[1] Oder to loop inside the dimensions. The available settings are:

- BLANK Ignore
- X Slices
- Y Slices

- Z Slices
- C Channels
- T Timepoints

F (Split) Not needed. May crash Imaris when set!

At [1] you can set the dimensions used for the dataset and in which order the files are assigned to them.

At [2] you can set the number of items which will be assigned to the dimension above. In the above screenshot this is 1 dataset with 1 channel and 10 Z layers.

If you use multiple of the entries Imaris loops through the dimensions and assigns specified number of files to them in each round. The most right entry is for the inner loop and the most left for the outer. First the files with consecutive numbers will be added to the inner loop before the next dimension to the left starts to get filled.

Here are some examples:



Inner loop will be used for 2 channels. So files 01 and 02 go into the two channels of Z layer 1, time point 1. Middle loop is for Z layers so the files 03 and 04 go into Z layer 2, into the two channels. Now all dimensions of the inner two loops are filled and the outer loop starts to fill the second time point in the same order as the first one.

[1] Imaris found 10 files with the same base name but we now use only 8. That means something is wrong with our configration of the dimensions, some files were lost after acquisition or the base name was not correctly detected.



The inner loop will be used for 2 Z layers. So file 01 and 02 goes into the two layers of the channel 1. Since Z can hold only two items the files 03 and 04 go into the the second channel. Since there are 5 channels to be filled this is repeted for the remaining channels. The third loop is not used in this example since no dimension is assigned to the most right entry.

#### 2.1.3 Voxel Size and Channel colours

At the bottom of the window you find the voxel size.

Voxel Size			
X: 0,165	µm Y: 0,165	μm Ζ: 0,00	μm

Set the Voxel size to have the correct calibration of the dataset. You may need to open the original dataset in the acquisition software to find the correct values. The calibration is very important to set **before** you start any analysis of the dataset. For a 2D dataset use 1. 0 will be not accepted by Imaris.

The units, here micrometer, are a global setting in Imaris main menu 'Preferences -> Display -> Unit of Length'.

Note: The time calibration you must set after the conversion in Surpass main menu 'Edit -> Image Properties... -> Geometry -> Time Point'.

Typically, all channels will be displayed in red. Please open the dataset in 'Surpass main menu -> Edit -> Image Properties -> Geometry -> Channel #'.

Image Properties		×
Geometry Data Set	Channel 1	
<ul> <li>Channels</li> <li>Channel 1</li> </ul>		Î
Channel 2 Parameters	Name: (name not specified)	
	Description: (description not specified)	
	Emission Wavelength:	
	Excitation Wavelength:	
	Pinhole Radius:	
	Base Color Mapped Color	
	Red: •  1,000 • Green: • 0,000 • Blue: • 0,000 •	
	OK	Cancel

Alternatively, you can come here by clicking in the name of the channel the 'Display Adjustemt' windows. 'Surpass main menu -> Edit -> Show Display Adjustment'



# 3 Fiji Plugin From Imaris Surpass

Mainly to be used for formats which are not at all supported by Imaris or Imaris File Converter. Switch from Arena to an empty Surpass from main Imaris bar of Icons



# 3.1 Open files with the standard Fiji 'File – Open' tool

To be used first.

<u>'Surpass Main Menu -> Fiji -> File -> Open'</u>



When you select a file, it will be opened by Fiji and directly send to Imaris.

3.2 Open files with the Fiji 'Plugins – Bio-Formats Importer' tool This gives you more options for the import.

'Surpass Main Menu -> Fiji -> Plugins -> LOCI -> Bio-Formats Importer'

retina_with_objects.ims - Honey	Com	ponent Analysis.png - In	naris	x64			•		
File Edit View Image Processing	Fiji	3D View Help							
Arena Surpass Vantage Open	U U	Image From Fiji Image To Fiji		<b>sto</b> View	Slice	Section	n Image Proc	Coloc	Animation
ත් No Object Sele	Ð	Labkit Segmentation							
智母神深い 🏵 🔨 🙏 🔍 🖿		File							
		Process	Þ						
		Plugins	Þ	l	LOCI	Þ	😈 Bio-Form	ats Expo	orter
		Options		ſ	Process		🗊 Bio-Form	ats Imp	orter
		Help		I	Registra	tion 🔸			
				9	Skeletor	ı ≯			

When you select a file, the Bio-Formats Import Options can be used, the datasets will be opened by Fiji and directly send to Imaris.

Stack viewing	Metadata viewing	Information —
View stack with: Hyperstack	🗖 Display metadata	View stack with - The type of image
Stack order: XYCZT 💌	Display OME-XML metadata	viewer to use when displaying the dataset.
	🗖 Display ROIs	Possible choices are:
	ROIs Import Mode: ROI manager 💌	<ul> <li>Metadata only - Display no</li> </ul>
Dataset organization	Memory management	pixels, only metadata. • Standard ImageJ - This option
□ Group files with similar names	Specify range for each series	is deprecated (i.e. intended for use by old macros only). Please
□ Swap dimensions □ Open all series	Crop on import	<ul> <li>Hyperstack - Display the pixels in ImageJ's built-in 5D viewer.</li> </ul>
Concatenate series when compatible	Split into separate windows	Data Browser - Display the pixels in the multidimensional Data Browser viewer. The Data
Stitch tiles	🗖 Split channels	Browser has some additional
Color options	Split focal planes	features on top of the normal
Color mode: Default 💌	Split timepoints	• Image5D - Display the pixels in
▼ Autoscale		Joachim Walter's Image5D

## 3.3 Open files with Fiji using any Plugin and or pre-processing

Use in case you need any other special plugin of Fiji to read the file.

- 1) Open Fiji using 'Surpass Main Menu -> Fiji -> Image from Fiji'
- 2) Use any desired function of Fiji to open and pre-process the file.
- 3) Transfer the dataset using 'Surpass Main Menu -> Fiji -> Image from Fiji' again.

# 4 Imaris File Converter

Imaris File Converter is a stand-alone application for converting many datasets in one go. It is installed with Imaris but on the customer portal there is a as well a version to be installed alone. You do not need a license so you can install it e.g. on your file server.

# 4.1 Graphical User Interface

Imaris File Converter			-	- 🗆 X
Input Drag & Drop Files, or click Button below to add Files. 1 Add Files	Output Same Folder      Specific Fold	as Input File er: D:\Documents\ 2	Browse	Download ImarisConvert- Bioformats Plugin
Input		Output	Voxel Size	Selected Input File Settings
C:\tmp\running-number\nummern_0	1.TIF C:\tm	np\running-number\nummern_01_1.ii	ms ?, ?, ? 3	Set Voxel Size
				Remove Selected
				Remove All
Correct voxel s				Start All
Input		Output		
Preferences About				

Add files to be converted either by Drag&Drop or using the 'Add Files' button.

- 1. Add files to be converted either by Drag&Drop or using the [1] 'Add Files' button.
- 2. Chose were you like to store the output. If you chose [2]'Specific Folder' all files will be stored there no matter from which directory level they origin.
- 3. If there are question marks in the column [3] 'Voxel Size' Select the lines which should get the same calibration and click [4]'Set Voxel Size'. For a 2D dataset use 1. 0 will be not accepted by Imaris.
- 4. Press [5] 'Start All' to start the conversion.

## 4.2 If you do not get the desired result:

#### 4.2.1 Using BioFormats Readers

ImarisFileConverter9.9 can use the open source program <u>ImarisConvertBioformats</u> to convert images from other formats to the IMS format.

At ,Preferences -> Bio-Formats -> Configuration' you can select per file format which converter you would like to use.

O Choose File Converter per File Format:						
File Format	ImarisConvert	ImarisConvertBioformats				
Abberior Instruments: OBF/MSR (OBF, MSR)	۲	0				
Aperio (SVS, TIF): single-file pyramidal tiled TIFF, with non-standard metadata and		0				
Zeiss: AxioVision / ZVI (ZVI)						
Big Data Viewer (XML, H5)		0				
Biorad: MRC (PIC)	۲					
BMP (BMP)	0					

For those formats, for which you choose ImarisConvertBioformats, file format detection is a twostep process. When a file is added to ImarisFileConverter first it tries to detect the format. If it is a format for which ImarisConvertBioformats is selected as the converter, then the file will be processed by the plugin. This means If ImarisFileConverter can not detect the format at all this method will not work.

The two-step process can take noticeably longer for some formats (compared to file format detection by ImarisConvert alone) as the speed with which ImarisConvertBioformats queries the format and the voxel size varies a lot for different file formats.

#### Setup:

The program ImarisConvertBioformats itself uses the open source <u>Bio-Formats</u> file readers to read images and it uses the open source <u>ImarisWriter</u> to write IMS files. You may fist to download and then detect the plugin at Preferences -> Bio-Formats -> Installation'.

Installation	
Ownload ImarisConvertBioformats	
2 Detect Installed ImarisConvertBioformats	
Application:	Browse

Hint it may be that you see the above, but the plugin is already installed but just not connected so you may press (2) first. Only if this detection fails press (1) and install the Plugin ImarisConvertBioformats.exe followed by pressing (2) again.

#### 4.2.2 If there are problems arranging Tiff files

It is recommended to try first to import the native file format of the acquisition device. If supported by Imaris this will give you the best experience since you can expect to preserve dimensions, calibration, and channel colours of the native format.

You can find the supporter file formats at 'Imaris main menu -> Help -> Preface - Getting Started -> Supported File Formats'

If there is no other choice and you need to import series of tiff files the converter tries to deduct the correct dimensions from the file names. By default, files with the same base name and size are stored into the same dataset.

If the name contains only one running number, it is interpreted as a Z stack or time series. If the name contains multiple running number leaded by one of the below characters, the characters determine which dimensions are used.

Basename\_C?number Channel Basename \_T?number Time Basename \_Z?number Slice Basename \_X?number Column Basename \_Y?number Row

The ? can be any character or absent (\_Cnumber) It does not distinguish between upper and lower case. There are more latter codes you can change at 'Preferences – File Conversion'.

A preview of which files will be used to build a IMS file you can hover over the Input entry.

Imaris File Converter					-		×
Input Drag & Drop Files, or click Button below to add Files. Add Files	Output O Same Folder as Specific Folder:	Input File D:\Documents\		Browse		ownload narisConv ioformats lugin	/ert-
Input		Output		Voxel Size	Sele	ected Inpu Settings.	nt File
C:\tmp\delimiter\retina_Ch0_layer000.	tif	D:\Documents\retina_Ch0_layer000.ims		0.165, 0.165, ?			ize
Con Input	File is ready. Detected file forma Using C\Program I Series of 10 Files: "retina_Ch0 layer00 "retina_Ch0_layer00 "retina_Ch0_layer00 "retina_Ch1_layer00 "retina_Ch1_layer00 "retina_Ch1_layer00 "retina_Ch1_layer00	t is TIFF (adjustable file series), Files\Bitplane\Imaris 10.0.0 alpha1\ImarisConvert.4 20.tif" 21.tif" 22.tif" 24.tif" 01.tif" 01.tif" 21.tif" 23.tif" 21.tif" 24.tif" 24.tif"	exe for file	e conversion.		move Sele Remove <i>J</i> Start All	ected All
Preferences About							

In case the automatic detection of the series still fails use [1] Settings button to open the File Series Reader. Please read the 'File Series Reader' chapter.

#### 4.3 File Series Reader

The Imaris File Series Reader is a tool to handle series of numbered files. It allows you to group the files into datasets with the desired number of sections, channels and time points. The names and sizes of the files are used to arrange them.

- Datasets which files are stored in multiple directories are not supported
- It is available for plain Tiff files **only**, not e.g OME TIFF. Tiff files were Imaris finds metadata about the organisation of the dataset cannot be handled.
- It is recommended to store the files of different datasets into sperate directories to simplify the task.

The File Series Reader can be started with a selected input file [1] 'Settings...' Please note that you can as well use the tool from Arena on a thumbnail 'RIGHT-MOUSE -> Configure File Series'

When started you need chose how the type of the naming of the files

- If the file name has multiple running numbers for different dimensions chose 'File Names with Delimiter' e.g.

retina\_ChO\_layer000.tif retina\_ChO\_layer001.tif retina\_ChO\_layer002.tif retina\_ChO\_layer003.tif retina\_Ch1\_layer004.tif retina\_Ch1\_layer005.tif retina\_Ch1\_layer006.tif retina\_Ch1\_layer007.tif retina\_Ch1\_layer008.tif retina\_Ch0\_layer009.tif

\_

retina\_Ch0\_layer010.tif

retina\_ is the base name Ch0 contains the running number for the channels layer001 contains the running number for the layers (slices).

- If the files with the identical base name have one running number chose the Tab 'File Names without Delimiter' e.g.:
  - retina\_001.tif retina\_002.tif retina\_003.tif retina\_004.tif retina\_005.tif retina\_006.tif retina\_007.tif retina\_008.tif retina\_009.tif retina\_010.tif

retina\_ is the base name 001 is a single running number for all files of the dataset

Notes:

- It is recommended to store the files of different datasets into sperate directories to simplify the task.
- Datasets which files are stored in multiple directories are not supported

#### 4.3.1 File Names with Delimiter

To be used if filenames contain multiple running numbers. E.g. retina\_ChO\_layerOOO.tif

The TIFF reader uses some default patterns \_C?number \_T?number \_S?number. That \_[letter] codes are used to assign the different dimensions and sort the files automatically into channels, time points, slices etc.

\_C?number -C \_CH \_W Channel

	TITLE
_Z?number -Z _S	Slice
_X?number -Y	Column
_Y?number -Y	Row

The ? can be any character or absent It does not distinguish between upper and lower case.

If the automatic assignment does not work, you can use the 'Series Reading Sequence' tool to further tune it for specific files.

🚺 Series Re	eading Sequence - C:\tmp\delimiter\rctina_Ch0_la	ver000.tif ×
Reference F C:\tmp\del	ile limiter∖retina_Ch0_layer000.tif	
File Names	with Delimiter File Names without Delimiter	
retina_Ch	C · _layer Z · .tif Filen 2 5 10 of	ame 10 Files used.
File Arrange	Ement Amount of items	
Z 1 - C 1	retina_Ch0_layer	
Z 2	retina_Ch0_layer	
Z 3	retina_Ch0_layer	
Z 4	retina_Ch0_layer	ible
Z 5	retina_Ch0_layer	
Z 1 - C 2	Second channel	starts
Z 2	retina_Ch1_layer	
Z 3	retina_Ch1_layer	
Z 4	retina_Ch1_layer	
Z 5	retina_Ch1_layer	ion
Voxel Size		
X: 0,165	μm Y: 0,165 μm Ζ: 0,00	
		OK Cancel

Imaris will show the filename and at the place of the running number a menu to select how the number should be interpreted:

**BLANK** Ignore

- X Slices
- Y Slices
- Z Slices

\_

- C Channels
- T Timepoints

F (Split) Split the files into the next dataset if the number changes.

Below the number of items are shown which will be assigned to the chosen dimensions. In the screenshot this are 2 channel and 5 Z layers.

The table 'File Arrangement' shows a preview of the resulting first dataset which will be created.

Here are a few examples:

File Names with Delimiter File Names without Delimiter					
retina_Ch <mark>1</mark> 2	∎ <mark></mark> layerZ	<mark>,</mark> tif	Summary 10 of 10 Files used		
File Arrange	ment				
		T 2			
Z 1 - C 1	retina_Ch0_layer000.tif	retina_Ch1_layer0	00.tif		
Z 2	retina_Ch0_layer001.tif	retina_Ch1_layer0	01.tif		
Z 3	retina_Ch0_layer002.tif	retina_Ch1_layer0	02.tif		
Z 4	retina_Ch0_layer003.tif	retina_Ch1_layer0	03.tif		
Z 5	retina_Ch0_layer004.tif	retina_Ch1_layer0	04.tif		

First number T, second number Z -> 1 datasets with 1 channel, 2 time points and 5 Z layers will be created.

Fi	File Names with Delimiter File Names without Delimiter							
r	retina Ch Z V laver T V tif Summary							
	2 5 10 of 10 Files used.							
Fil	e Arrange	ment						
		T 1	T 2	T 3	T 4	T 5		
Z	1 - C 1	retina_Ch0_layer	retina_Ch0_layer	retina_Ch0_layer	retina_Ch0_layer	retina_Ch0_layer		
Z		retina_Ch1_layer	retina_Ch1_layer	retina_Ch1_layer	retina_Ch1_layer	retina_Ch1_layer		

First number Z, second number T -> 1 datasets with 1 channel, 2 Z layers and 5 timepoints will be created.

File Names	with Delimiter File Nam	es without Delimi	ter
retina_Ch	Split) ▼_layer Z	.tif	Summary
2			To of to thes used.
File Arrange	ment		
Z 1 - C 1	retina_Ch0_layer000.tif		
Z 2	retina_Ch0_layer001.tif		
Z 3	retina_Ch0_layer002.tif		
Z 4	retina_Ch0_layer003.tif		
Z 5	retina_Ch0_layer004.tif		

-

First number 'F- (Split), second number Z -> 2 datasets with 1 channel and 5 Z layers will be created. Please note that the table show always only the layout of the first IMS dataset.

File Names with Delimiter File Names without Delimiter								
retina_Ch	retina_ChC Jayer , tif Summary 2 5 10 of 10 Files used.							
File Arrange	File Arrangement							
	T 1	Т 2	Т 3	T 4	Т 5			
Z 1 - C 1	retina_Ch0_layer000.tif	retina_Ch0_layer001.tif	retina_Ch0_layer002.tif	retina_Ch0_layer003.tif	retina_Ch0_layer004.tif			
Z 1 - C 2	retina_Ch1_layer000.tif	retina_Ch1_layer001.tif	retina_Ch1_layer002.tif	retina_Ch1_layer003.tif	retina_Ch1_layer004.tif			

First number C, second number T -> 1 dataset with 2 channels, 5 time points and 1 Z layer will be created.

#### 4.3.2 File Names without Delimiter

To be used if filenames contain a single running number. E.g. retina\_**000**.tif

Series Re	Series Reading Sequence - C:\tmp\running wumber\retina_001.tif					
Reference File C:\tmp\running-number\retina_001.tif						
File Names	File Names with Delimiter File Names without Delimiter					
Z • 10	v v v S⊌mmary v 1 v 1 v 1 1 10 Files used.					
File Arrangement						
Z 1 - C 1	retina_001.tif Preview table					
Z 2	retina_002.tif					
Z 3	retina_003.tif					
Z 4	retina_004.tif					
Z 5	retina_005.tif					
Z 6	retina_006.tif					
Z 7	retina_007.tif					
Z 8	retina_008.tif					
Z 9	retina_009.tif					
Z 10	retina_010.tif					
Voxel Size						
X: 0,165	μm Y: 0,165 μm Ζ: 0,00 μm					
	OK Ca	ncel				

[1] Oder to loop inside the dimensions. The available settings are:

- BLANK Ignore
- X Slices
- Y Slices

\_

- Z Slices
- C Channels
- T Timepoints

F (Split) Not needed. May crash Imaris when set!

At [1] you can set the dimensions used for the dataset and in which order the files are assigned to them.

At [2] you can set the number of items which will be assigned to the dimension above. In the screenshot this is 1 dataset with 1 channel and 10 Z layers. If you use multiple of the entries Imaris loops through the dimensions and assigns specified number of files to them in each round. The most right entry is for the inner loop and the most left for the outer. First the files with consecutive numbers will be added to the inner loop before the next dimension to the left starts to get filled.

Here are some examples:



Inner loop will be used for 2 channels. So files 01 and 02 go into the two channels of Z layer 1, time point 1. Middle loop is for Z layers so the files 03 and 04 go into Z layer 2, into the two channels. Now all dimensions of the inner two loops are filled and the outer loop starts to fill the second time point in the same order as the first one.

[1] Imaris found 10 files with the same base name but we now use only 8. That means something is wrong with our configration of the dimensions, some files were lost after acquisition or the base name was not correctly detected.



The inner loop will be used for 2 Z layers. So file 01 and 02 goes into the two layers of the channel 1. Since Z can hold only two items the files 03 and 04 go into the the second channel. Since there are 5 channels to be filled this is repeted for the remaining channels. The third loop is not used in this example since no dimension is assigned to the most right entry.

4.3.3 Voxel Size and Channel colours At the bottom of the window you find the voxel size.

Voxel Size		<i>'</i>	
X: 0,165	µm Y: 0,165	µm Z: 0,00	μm

Set the Voxel size to have the correct calibration of the dataset. You may need to open the original dataset in the acquisition software to find the correct values. The calibration is very important to set **before** you start any analysis of the dataset. For a 2D dataset use 1. 0 will be not accepted by Imaris.

The units, here micrometer, are a global setting in Imaris main menu 'Preferences -> Display -> Unit of Length'.

Note: The time calibration you must set after the conversion in Surpass main menu 'Edit -> Image Properties... -> Geometry -> Time Point'.

Typically, all channels will be displayed in red. Please open the dataset in 'Surpass main menu -> Edit -> Image Properties -> Geometry -> Channel #'.

Image Properties		×
Geometry Data Set	Channel 1	
Channel 1 Channel 2 Parameters	Name:       (name not specified)         Description:       (description not specified)         Emission Wavelength:       (description)	
	Excitation Wavelength: Pinhole Radius: Base Color Mapped Color	
	Red: • 1,000 • Green: • 0,000 • Blue: • 0,000 •	
		OK Cancel

Alternatively, you can come here by clicking in the name of the channel the 'Display Adjustemt' windows. 'Surpass main menu -> Edit -> Show Display Adjustment'



### 4.4 Command Line Version of Imaris File Converter

there is a command line version for the FileConverter which can be used e.g. in your own scripts.

It is in your Imaris and ImarisFileConverter installation folder. E,g, c:/program files/Bitplane/Imaris 9.9.1

You get a first help by running this command from the command line: ImarisConvert.exe --help

If you need some inspiration, you can have a look at the log files that the FileConverter creates. Enable the logging at: 'Imaris File Converter -> Preferences -> Settings -> Logger'. There is a link (show log) in the Preferences to open the Log directly from there. Now convert a file and have a look into the log. The file will be placed on Windows at: "C:/Users/USERNAME/AppData/Local/Temp/ImarisFileConverter.log" There is a link (show log) in the Preferences to open the Log directly from there.

A typical command line could look like this:

```
C:\Program Files\Bitplane\Imaris 9.9.1\ImarisConvert.exe" -lp -i
"D:\Source\WB-Live_F12_w1_s1_z1_t1.tif" -ii 0 -ps 8000 -nt 24 -of Imaris5 -
o "D:\MyOutput.ims" -vs "0.000 0.000 0.000" -il "D:\Test.txt" --log
D:\temp\Log.LOG
```

Please Note: This is really only a raw backend of the GUI so it e.g. does not warn when it overwrites files.