

Saving Formats in Huygens

- HDF5
- ICS / ICS2
- OME
- (Big) TIFF 8-bit & 16-bit & 3*8-bit RGB - Single, multi-layer or File series (Leica Tiff style numbering scheme)
- Biorad PIC
- Imaris classic
- Delta Vision IMSubs (r3d) files
- AVI
- Huygens Professional only: plain text (csv, txt)
- Huygens Core only: JPEG (for web applications)

Depending on the file format chosen to save the image the file data type will be 32 bits, 16 bits or 8 bits. The image, as read by Huygens, has either 32 bits, 16 bits or 8 bits as data type. This presents a number of combinations from the data types of the open image to data types of the saved image. This situation is summarized in the following table, where the cells show the data type of the resulting file given a source image and a destination file format:

	hdf5	ics	ics2	tiff 8	tiff 16	tiff-rgb	imaris	biorad 8	biorad 16	ome-xml	delta r3d
8 bit Image	8 bit	8 bit	8 bit	8 bit	16 bit	8 bit	8 bit	8 bit	-	8 bit	8 bit
16 bit Image	16 bit	16 bit	16 bit	8 bit	16 bit	8 bit	8 bit	8 bit	16 bit	16 bit	16 bit
32 bit Image	32 bit	32 bit	32 bit	8 bit	16 bit	8 bit	8 bit	8 bit	16 bit	32 bit	32 bit

When the chosen file extension implies fewer bits than those of the image, for example saving a 32 bit image to a 16 bit file, the software will prompt for a conversion method choice: Contrast stretch, Linked stretch or clipping.

Please note that writing to TIFF files may alter the data, see Tiff scaling. JPEG, being a lossy format, i.e. not all the original information is saved, is only intended for visualization purposes and is therefore not listed in the above table.

Data scaling when saving in TIFF format

The TIFF export File Formats available in the Huygens Software may require that the data is rescaled to make the intensities fit the destination format's Dynamic Range. If not rescaled, a data clipping or a loss of dynamic range (*intensity resolution*) may occur. If you are interested in quantifying your data, you may want to pay attention to the scaling of images and how to treat them properly. See also Signal Quantification.

The basic TIFF format can store 8-bit data only.

The 16-bit TIFF variant can hold unsigned (positive) 16-bit data.

- Huygens Essential converts 16-bit Tiff files always to 16-bit signed integer, dividing it by 2 if necessary.
- Huygens Professional always asks the user what to do with 16-bit Tiff files; convert them to 8-bit, 16-bit, or 32-bit float?
- The Batch Processor, present in both, converts all 16-bit Tiff images, irrespective their range, to 32-bit float.

This means that restoration results with dynamic range higher than 8-bit will need a downscaling when saved in RGB TIFF to avoid clipping, scaling the maximum to a value of 255.

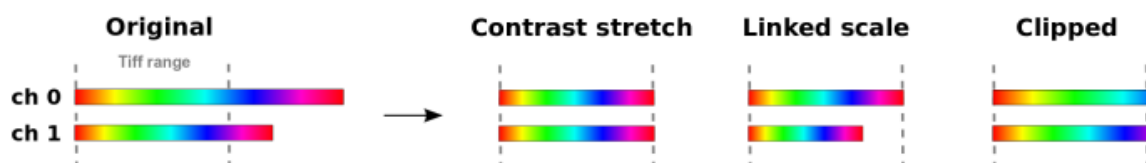
Similarly, 32 bit Float Type images may need stretching when saved in 16 bit TIFF format, whose maximum scales to 65535.

In both cases, only the positive intensities present in the image are saved.

If you want to export to TIFF files for further analysis you may be interested in having more control on how the data is scaled. It is of course possible with the advanced tools present in Huygens Professional and Huygens Scripting. Since version 3.1 of Huygens Essential some options are available when exporting to TIFF to give priority to analysis, instead of the previous priority to visualization and contrast stretching.

In case the current range of the image data does not fit in the range of the selected Tiff type, the following choices to reduce the range are offered:

- **Contrast stretch** (positive data range for each channel is adapted to the Tiff range, the different per-channel factors are not reported)
- **Linked scale** (as stretch, but now the relation between channels is retained: the same factor is applied to all channels that stretches the one with maximum intensity to the Tiff range. The factor is reported by Huygens.)
- **Clip** (positive data outside the Tiff range is clipped to that range)



If you have a single channel image, the first two modes are equivalent, but only in the second one the scaling factor is reported.

Again, remember that in all three cases the negative values are always clipped.

See below for examples and details.

Keeping the information

What determines the necessity of the scaling is not only the value of the maximum, but mostly the difference in Dynamic Range between the image and the file format to export to. Imagine for example that you have a Float Type image with a maximum intensity of 220, and some other pixels have intensities like 219.0, 219.2 and 219.6. The maximum of 220 is smaller than 255, thus it could be stored directly in an 8-bit TIFF, but then the other mentioned values would have to be rounded to either 219 or 220, losing the *intensity resolution*. This happens even if you save the intensities directly in a 16-bit TIFF.

By applying a contrast stretching and exporting to 16-bit TIFF you can preserve the contrast, at least partially. If you scale the maximum up to 65535, the other mentioned values could be 65237, 65297 and 65416 respectively, and you can still distinguish them.

If the image data type matches the selected TIFF format type, no stretching is necessary.

Visualization purposes

One must be careful with Multi Channel images when a stretching is applied. The default behaviour is to stretch each channel independently to obtain maximum contrast. One may think that this is changing the information too much, but in many cases the users are interested in the TIFF format for visualization purposes. Moreover, further analysis might not be affected by this scaling, as happens with many of the most extended colocalization coefficients, that are insensitive to these scaling factors different for each channel.

This is not the case, of course, of ratiometric Images, where the ratio between channels is directly affected by independent scaling. If you are saving float images in low dynamic range formats like TIFF, you should do it carefully to take care of this adaptative rescaling.

Analysis purposes

Since version 3.1 of the Huygens Software an option is available to couple the channels when saving to TIFF, to preserve intensity ratios in case a scaling is necessary. In Huygens Professional this can be already done manually, properly scaling the whole dataset and converting the image to Integer Type before saving it to 16-bit TIFF.

Moreover, saving to TIFF can be done without any scaling, writing the actual values directly (and probably losing dynamic range). The user must be careful because clipping will happen if the image intensity range exceeds the destination file's.

An example

Having the original (raw) data in 8 bits doesn't mean that the deconvolution result really fits the 8-bit range. Actually, it is quite unlikely. The deconvolution collects blurred light and puts it back to the original sources, preserving the total intensity while increasing the intensity of some pixels. This increased value can go

beyond the 8-bit dynamic range if the original data filled it well. The deconvolution result will require more bits than 8 to keep the intensities.

When deconvolving a particular 8-bit test image, the highest intensity increased from 246 to 370 after a few iterations. 370 needs at least 9 bits to be stored, thus the Huygens Software will return the result in 16-bit Integer Type. You can't save this directly into an 8-bit file format anymore.

The intensities will be preserved when the data is saved in the recommended ICS file Format. But when you export the dataset to a TIFF format, some scaling will occur depending on the destination Dynamic Range. When saved in 8-bit TIFF, it is downscaled by a factor < 1 . Otherwise the data would be clipped.

When saved in 16-bit TIFF stretching is in principle not necessary. Still, you have to notice the difference between Huygens 16-bit (Integer Type) mode, and Tiff format 16 bit. Huygens' is signed (allowing positive and negative values), and Tiff's is unsigned (only positive values allowed). When exporting to Tiff negative values, if existing, are lost (clipped to zero). What the program does now with the remaining range depends on your saving options: you can multiply it by 2 or leave it as it is.

Another example

Saving a 32-bit (Float Type) image from Huygens to an unsigned 16-bit Leica Tiff file may require a downscaling to make the data fit in there. Otherwise one could obtain a Clipped Image. In Huygens Float Type, intensities can have any real value (positive or negative) with absolute value between approximately 10^{-38} to 10^{38} , with 7 digits precision. But in 16-bit unsigned integer format, the possible values range from 0 to 65535 only. Even in the case the maximum in the 32-bit image falls inside the 16-bit range, keeping the contrast as much as possible requires a scaling, as explained above.