

Halolmage TBT Toolkit v1.0 – Guide to use

The tool kit has four sets of functions.

- I. Colour Wheel Reference
- II. Convert Halolmage to Fiji RGB
- III. Create Composite Halolmage
- IV. Recommended Halolmage Processing Steps

1. Colour Wheel Reference

Reference colour wheel images for the available for use in the “Create Composite Halolmage” function. Composite Halolmages use a false colour scheme for each channel for two purposes. First, this helps avoid image saturation when composting 15 channel images. Second, the colours can encode spatial information based on the orientation of the light source to the sample to offer insight into sample 3D orientation.

2. Convert Halolmage to Fiji RGB

The Composite Halolmage exported from the HaloVision software environment is a 48-bit colour composite image (16-bit per RGB channel). This function opens and converts the Halolmage into a Fiji RGB image for later use.

3. Create Composite Halolmage

Depending on the sample, users may wish to analyse individual channel images or create a Composite Halolmage outside the HaloVision software environment. Two methods for generating composite images are included in this toolkit as well as some image processing options applied to the channel images prior to build the composite image. The macro imports sequential grayscale TIFF images, assigns colours based on the selected colour wheel, and can generate the following outputs:

- RGB channel stack
- HDR weighted composite
- Direct colour composite

Output	Description
RGB Channel Stack	Creates a coloured stack where each grayscale image is assigned colours based on the selected colour wheel for sequential viewing.
HDR Composite	Recommended for images that do not have a “dark field” background. Generates a high dynamic range (HDR) composite using adaptive pixel weighting. This balances overlapping signals, reduces saturation/clipping, and improves visibility of structures across multiple channels.
Direct Composite	Generates a standard additive composite where all coloured channels are summed directly together.

Workflow

Upload images

Select the folder containing greyscale channel data (.tiff files) exported from the HaloVision software.

Workflow Options

Options	Purpose	Recommended for
Gaussian Blur	Reduces image noise and smooths fine grain before compositing.	Noisy images, grainy acquisitions, low-signal images.

Subtract Background	Removes uneven illumination using rolling-ball background correction. Begin with smaller values for the radius.	Images with non-uniform dark backgrounds, haze, gradual brightness variation, or low-contrast structures that are difficult to distinguish from the background.
Adaptive HDR Sigma	Automatically optimises HDR exposure weighting based on image intensity distribution while ignoring darkfield background pixels.	Recommended for most datasets, especially variable or complex samples.
Create RGB Channel Stack	Generates individually colour-mapped channel slices for sequential viewing.	Channel inspection, quality control, reviewing individual channels.
Create HDR Composite	Creates a balanced HDR composite with improved visibility of overlapping structures and reduced saturation.	Dense multi-channel images, overlapping structures, tissue sections, high dynamic range datasets.
Create Direct Composite	Directly combines all colour-mapped channels into a single additive colour composite.	Images with low channel overlap or when brighter, more vivid colour rendering is preferred.

Output

All generated files are automatically saved to: /Output

Processing Note:

HDR composite generation uses adaptive intensity weighting and therefore requires longer processing time than direct compositing.

4. Recommended Halolmage Processing Steps

The macro applies some often recommended post-processing operations to an existing Halolmage composite image, including:

- CLAHE contrast enhancement
- Unsharp masking

Designed for improving contrast and sharpening already generated composite images.

Workflow

Upload images

Select:

- a HaloVision composite image *or* a previously processed composite TIFF image

Options	Purpose	Recommended for
Enhance Local Contrast (CLAHE)	Improves local contrast and visibility of fine structures.	Low-contrast composites, faint structures, or images with flat intensity distribution.
Apply Unsharp Mask	Enhances edges and fine structural detail by increasing local sharpness.	Soft-edge looking images, blurred structures, or composites requiring improved edge definition.

Output

All generated files are automatically saved to: /output

Generated outputs:

- *_CLAHE.tif
- *_Unsharped.tif