

WimAutophagy

Specifications for a successful analysis

In order to achieve the best results when using WimAutophagy, these are some guidelines that you should follow while conducting your assay and acquiring images from it. If your images deviate from these guidelines, the accuracy of your WimAutophagy analysis measurements might be negatively affected. Although we work our best to make our solution as adaptable as possible, we do not take responsibility of their accuracy if that is the case.

Please take care of your images, we will take care of the rest.

1. Before acquiring your images

Valid microscopy modalities: WimAutophagy only works with images from epifluorescence and confocal microscopy. Confocal images can be a projection of the z-stack. If you want to analyze images from other modalities, you can ask for a custom solution [here](#).

Moderate cell density: needs a cell density lower than 50% of the image area for proper detection of the background.

Avoid artifacts: any artifacts visible in the image (air bubbles, debris, stains...) may make it harder for the analysis to recognize your autophagosomes and affect negatively your results. Please take care to avoid them when preparing your assay.

2. When acquiring your images

Images in RGB (Red, Green and Blue): images should have each fluorescence dye visible in an independent color channel, either red, green or blue.

Autophagosomes need to be brighter than the cytoplasm: that way both structures can be detected independently.

No visible containers: please avoid images containing parts or whole assay containers (plates, wells...). These structures can be confusing for the detection algorithm and lead to false positives.

Images in focus: images should be properly focused in order to allow autophagosomes to be viewed with the best contrast and distinguish them from the background as clearly as possible.

Homogeneous illumination: homogeneous lighting throughout the image will ensure better results. Please avoid images with areas that are darker or brighter than other.

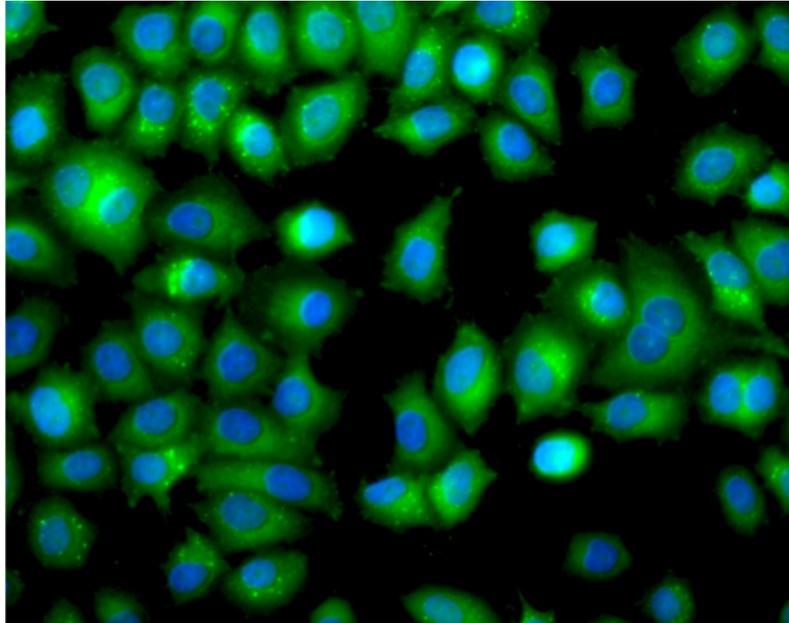


Figure 1: Example of valid image for epifluorescence in stable HeLa GFP-LC3 cells with the autophagosomes in the green layer and nuclei in the blue layer.

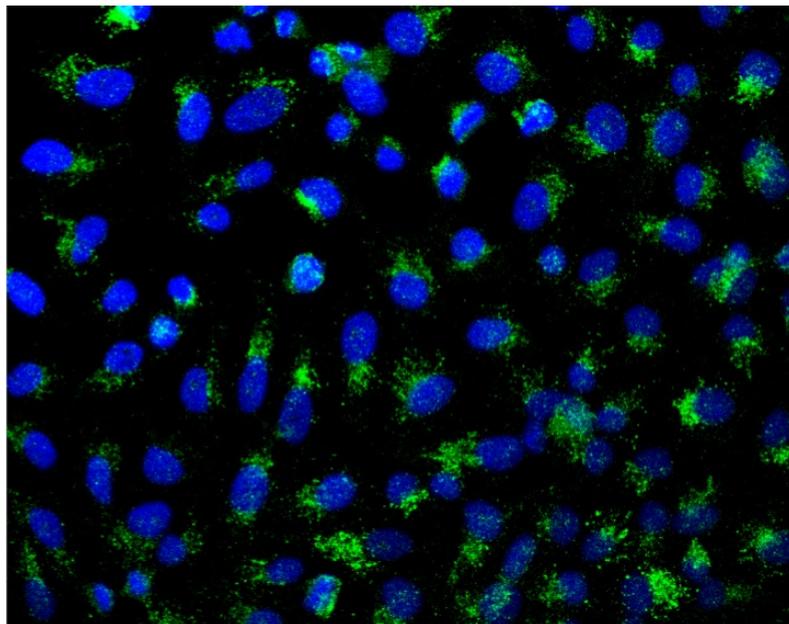
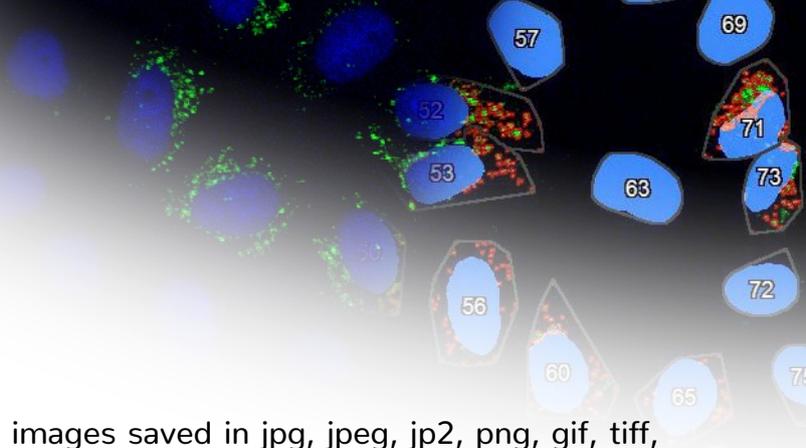


Figure 2: Example of valid image for confocal microscopy using immunofluorescence staining for LC3 with the autophagosomes in the green layer and nuclei in the blue layer.



3. When saving your images

Valid formats: WimAutophagy only accepts images saved in jpg, jpeg, jp2, png, gif, tiff, tif and bmp formats. The image uploader will not let you upload images from other formats. If you want to analyze images from a special image format, you can ask for a custom solution [here](#).

Good image resolution: small images (low resolution) usually show small or poorly defined objects that are difficult to distinguish from the background. The minimum resolution that we accept is 800 pixels by 600 pixels.

No additional information visible on the image: some microscope software add information to the images like a scale bar or a time-stamp, which can make autophagosomes around them more difficult to detect. Please send your images without any extra information on them.

4. When uploading your images to myWim

In order for WimAutophagy to accurately analyze your images and supply you with the best results, you will be asked to enter the following parameters when uploading your images.

Type image acquisition: the microscopy modality of your images, either confocal or epifluorescence.

Layer Autophagosome: the RGB channel in which your autophagosomes are visible, either red, green or blue.

Layer Nuclei: the RGB channel in which your nuclei are visible, either red, green or blue.

(Optional) Scale [$\mu\text{m}/\text{px}$]: the conversion factor between pixels and micrometers for your microscope. If provided, the spatial results will be given in micrometers instead of pixels.

(Optional) Mean Area Autophagosome [px]: the mean area of your autophagosomes, in pixels. If the parameter is not provided, WimAutophagy will calculate it automatically, which is the best option if you are not sure about this parameter.

(Optional) Minimum Area Autophagosome [px]: the minimum area of your autophagosomes, in pixels. Autophagosomes smaller than this parameter will be discarded. If the parameter is not provided, WimAutophagy will calculate it automatically, which is the best option if you are not sure about this parameter.