

# Huygens - Background

## Background estimation for deconvolution

In the deconvolution wizard in [Huygens Essential](#) and [Huygens Professional](#), estimating the [BackGround](#) is part of the deconvolution procedure. After the estimation you can indicate a percentage of correction to be applied to the automatic value. For example, set this to zero to accept the estimated value as is, or set it to -10 to reduce the estimated value in 10%. If you manually modify the background absolute value, the change will be also reflected in the percentage correction. This relative background value is especially useful for timeseries, which intensity could decay due to bleaching.

In [Huygens Professional](#), there are multiple ways you can estimate the [BackGround](#):

### Automatic estimation

The mean background can be estimated automatically in [Huygens Professional](#) with the `Estimate background` tool in the Analysis menu of the Operations window. Use a search area of 0.7 micron radius. If your image does not contain large open areas, decrease the radius.

This tool reports the found value to the Task report window along with the coordinates of the area in which the value was found, so you can confirm this value visually in e.g. the [Twin Slicer](#).

The reported value is a conservative estimate of the average background. For instance, when there is appreciable bleaching there is high probability that the tool will find the last recorded, darkest layer. You can exclude such layers from the search by [cropping](#) the image.

The Estimate background tool tries to evade off focus light of the object in conventional images. Still, it is a good idea to inspect the location where the tool reported the lowest average value.

This automatic estimation will be executed also if you run a deconvolution command like `cmle` or `qmle` with any [Background Mode](#) other than `manual`, but you can correct the estimated values by a percentage as defined by the [Background Per Channel](#) parameters.

### Selecting a region of interest

You can have more control if you extract the interesting portion of the image to a new image by using the [Intelligent Cropper](#), and then ask for the statistics of that independent chunk (Edit > Statistics): you will obtain among other things the average value. Still, the cropper only allows you to crop or extract regions of cuboid shape.

But the ROI selection in the [Object Analyzer](#) can be used for more complex shapes. When you have a ROI defined anywhere in the image and click the "Analyze all" button, also information about the ROI is printed on the table, among others (by default, but you can change this) the sum intensity and the number of voxels. By dividing these two figures you can easily get an average value for the intensity inside the ROI.

Because defining a ROI in the background where not objects are normally found may be tricky, you may want to activate in the MIP pipe the same channel you are analyzing, so that you have a visual reference on where to define the ROI.

### Background Mode

Background Mode is a [Restoration Parameter](#) in the [Huygens Software](#). It establishes how the [BackGround](#) of an image is estimated in a deconvolution procedure.

The following choices are possible here:

- **Lowest value (default):** The image is searched for a 3D region with the lowest average value. The axial size of the region is around 0.3 micron; the lateral size is controlled by the radius parameter which is default set to 0.5 micron.
- **In/near object :** The neighborhood around the voxel with the highest value is searched for a planar region with the lowest average value.
- **Widefield (WF) :** First the image is searched for a 3D region with the lowest values to ensure that the region with the least amount of blur contributions is found. Subsequently the background is determined by searching this region for the planar region with radius *r* that has the lowest value.

The size of the region where the background is looked for can be controlled by the radius parameter in the [estimate background tool](#), but for the deconvolution commands a default value is used.

If background mode is `lowest`, `object` or `wf` a background value will be estimated for each channel and frame in the image. In the [Batch Script](#), with the mode set to `auto` the most appropriate method for the image is selected.

The estimated values can be modified by a percentage as defined by [Background Per Channel](#).

If the background mode is `manual` then the value in [Background Per Channel](#) is taken as an absolute value, and will be removed.