3D spots segmentation : a tutorial

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This plugin performs segmentation of spots in 3D. It requires one original image (Spots) and the image of seeds that generally correspond to the local maxima of the original image (Seeds). The plugin 3D Filters can be used to generate local maxima from an image.

The segmentation process uses the seeds from which it successively clusters neighbouring voxels considered as belonging to the object. The main criteria used to decide if a voxel belongs to the object, is an intensity value.

Selection of the seeds that will be considered for object segmentation

Global background : the user can set a threshold intensity value under which seeds are excluded from the segmentation process. This allows the exclusion of seeds from the background, or faint objects.

Computation of the intensity value used as a threshold that defines the extension of the object

This plugin offers three methods to determine the intensity value used as the threshold to stop the voxel clustering. The threshold intensity value can be defined either globally (*Constant*) or locally (*Local mean*, *Gaussian fit*).

Constant : The same threshold will be applied to all the objects. This threshold is defined by the user as Local Background.

Local mean :

Three circles are drawn. The user defines the radius (in pixels) of each circle, given that the first circle should be located within the object, while the two other circles should be located outside of the object. The mean intensities of the object (within the first circle), and of the background (in between the two other circles) are measured and the threshold calculated. By default, the threshold is the mean of the two mean intensity values (weight=0.5). The user can however shift this parameter: for a weight value of 0.75, the threshold will be closer to the background value.

Gaussian fit :

First the radial distribution of the object is computed (see plugin 3D Radial Distribution), as concentric circles centred on the seed define growing regions of interest in which mean intensity values are measured. A gaussian fit of the radial distribution is computed I na given radius around the seed (Radius Max, in pixels)). The standard deviation of the fitted gaussian curve is used to define the threshold. The user enters a factor which is applied to the standard deviation to define the value of the threshold (sd value). As a rule of the thumb, a factor 1.17 will bring

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the threshold to the full width at half maximum while factors 2 and 3 will fill about 90% and 99% of the curve surface, respectively.

Although the gaussian fit assumes the object is spherical, it can be successfully used for determining the local threshold of object of different shapes. The segmentation method (defined below) will respect the original object shape.

Segmentation of the object using the threshold value

When the threshold value has been determined, the plugin offers several methods of voxel clustering that segment the object. The segmentation proceeds by successive examination of voxels, starting from the seed.

Classical : All neighbouring voxels with intensity value higher than the threshold value (Local Background) are clustered to form the object.

Maximum : This mode operates similarly as the previous one, but with one additional constraint : the voxels are clustered in the object if their intensity value is higher than the threshold and if their intensity value is lower than the intensity value of the previously clustered voxels.

Block : The same Maximum procedure is applied, but instead of considering single voxels, block of voxel (all neighbors with value greater than the local threshold) are examined. All voxels must satisfy the constraint to be added to the cluster, else no voxel are added.

Watershed

A watershed can be applied before each of the segmentation method (see plugin Watershed 3D). All objects are simultaneously examined using a 3D watershed procedure to define the area around each seed. The segmentation procedure is then applied within the region defined by the watershed.

The plugin returns an image in which the voxels of each object have the same value (count mask). Using The *Fire* LookUp table, the objects can be visually differentiated. In addition, the plugin opens the 3D ROI Manager with all ROIs corresponding to all segmented objects.

Remarks

In case of apposed objects, the Maximum and Block method may still be unsatisfactory. First, since each object is considered one after each other, the first object considered might have an overestimated size, while the second object will consequently have an underestimated size. Second, artefact structures might appear as the border of the second object might be fused to the first object (typically the first object can encircle the second object). The watershed method resolves those problems, and represents the most advanced method yielding best results. It however requires more computer capacities.