# Biofilm Analysis XTension

Description: This XTension measures the mean thickness, maximum thickness, Roughness Coefficient (Variance) and Standard Deviation of all reconstructed objects in the Surfaces scene. If you have one or many surface objects, it will report a single mean thickness in the Overall Stats Tab, as well as, individual surface object statistics.

The BioVolume is defined as the voxels localized inside of the Imaris isosurface. Thickness of the surface is defined by the number of pixels in vertical column in the BioVolume that lie inside of the surface objects. This localized thickness (for every vertical voxel column) is converted to microns based on the actual Z-voxel scaling set during acquisition, or set in the *Image Properties*. This is done for all columns of voxels in the XY image space that contain BioVolume, through all slices in the dataset. Thus, for each surface, mean and max values can be easily calculated. A sample seen below demonstrates how many pixels (blue) contribute to the local thickness of isosurface.
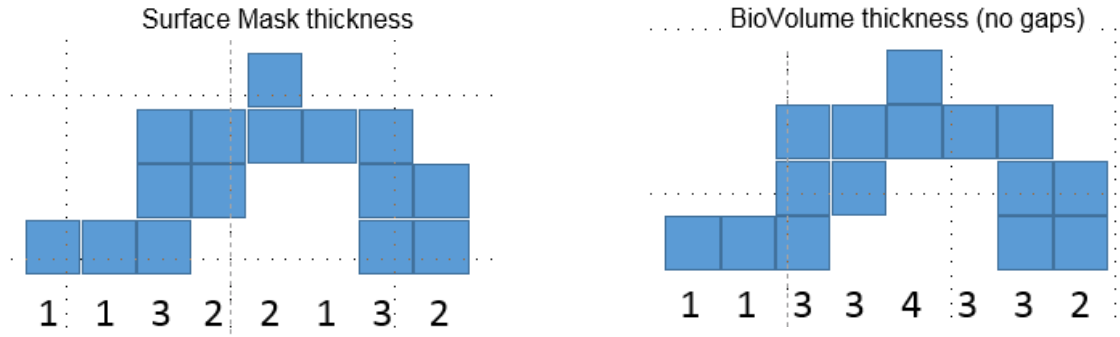
1) **Surface Mask thickness** quantifies only those voxels in vertical column that lie inside the reconstructed 3D volume. If there are gaps in the rendering from poor labeling or poor creation parameters, they will not be included in the thickness measure.

2) **BioVolume thickness** measures from the top of the isosurface to the base (substratum) and includes all gaps present in the reconstructed surface. By default, the substratum is defined as Slice#1, but this can be manually defined, or auto-detected. This Substratum layer will be defined in the name of the surface object in Imaris.

      2a) *AutoDetection* of the substratum start slice defines the first slice as the slice that contains the biofilm surface. There is no minimum threshold in defining start slice (can be defined by single voxel).

 3) **Surface edge to surface edge (subvoxel) thickness** measures the average distance from vertices on the top of the isosurface to vertices on the bottom of the surface. This measure is made from finding the orthogonal distance between vertices. For large surfaces (i.e biofilms), the number of vertices can be reduced to speed up the analysis, as the script processing time is dependent on the number of vertices/surface it samples for the mean thickness. *NOTE: In compiled version, this calculation is slower to process compared to full Matlab version.*

Roughness measures the variability of the localized biofilm thickness.

> Variance: The average of the **squared** differences from the mean.

$$\text{Variance} = \frac{1}{N} \sum_{i=1}^{N} (LT_i - \mu)^2$$

LT = local thickness, μ=mean thickness, N = total number of thickness measures

> ***Roughness Coefficient(surface mask):*** where LT and μ measured from Surface Mask thickness (as defined above)

> ***Roughness Coefficient(no gaps to substratum):*** where LT and μ measured from BioVolume thickness (as defined above)

> ***Population Standard Deviation:*** Square root of Variance (as defined above)

Thickness colormap generates a new channel in the volume, and applies an intensity color-coded LUT to each of the localized vertical voxel columns that are a part of the surface mask (i.e. only those voxels inside the surface). Best viewed either in Slice mode, or with a XY Ortho-Slicer in the Surpass 3D view. This new channel will display either the BioVolume thickness or the surface thickness.

BioVolume is the total volume of the Biofilm for all the surface objects in the dataset

> **BioVolume ImarisSurface:** Internal Imaris surface statistic measure

> **BioVolume (surface mask):** Total number of voxels inside surface mask multiplied by voxel volume

> **BioVolume (surface mask) from substratum:** Total number of voxels inside surface mask (from substratum start slice) multiplied by voxel volume

BioMass is a measure of BioVolume per unit area of the substratum. BioVolume is defined as *BioVolume (surface mask) from substratum. Substratum area* is defined by the mean number of pixels in the surface for the first 5 slices starting at the substratum start slice.

Continuity Ratio is a dimensionless coefficient that measures the continuity of the reconstructed surface. It is measured by taking mean surface mask thickness divided by the mean BioVolume thickness. A value of "1" represents perfect continuity through the Z-stack. Values less than one, define how many gaps (holes) there are in the surface reconstruction.

Notes on Compiled version versus full Matlab version:

Both will do exactly the same functions. However, the if using the "sub voxel" distance measure in the compiled version, the duration to complete the processing can be very very long, even when using the option "AutoReduction for large surface(biofilm)"