**Detailed code description:**

This code calculates the total biovolume of a biofilm as well as the biovolume above a specific z-plane, and the ratio between the two.

The specific z-plane is determined by the parameters *maxZ* and *imagesliceID*. The parameter *maxZ* is always set by the user (default: 2 µm), while *imagesliceID* can either be set manually or calculated automatically by identifying the plane with the highest biovolume density. The final z-plane above which biovolume will be calculated for the saved values is *maxZ*+*imagesliceID*.

Code lines 5-12: Choosing calculation parameters

In line 5, the user can set *maxZ*, an offset for the height above which biovolume will be measured. *maxZ* should be given in µm. The default value is 2 µm.

In line 10, the user can manually set the z-position corresponding to the substrate (bottom of biofilm) in µm. Alternatively, the variable *imagesliceID* can be set to zero for automatic detection.

Line 12 converts the µm value of *imagesliceID* into the corresponding z-stack position.

Code lines 16-24: Determining the brightest slice

In line 16, the abstract "objects" variable is converted into an image *w*. In this image, every pixel with a value of 1 or greater is considered biomass.

Lines 19-24: If no manual value for the substrate level was given, for each plane of the image *w*, the total number of non-zero pixels are counted. The plane with the highest number of non-zero pixels is used to replace *imagesliceID*. The height of this plane in µm is saved.

Code lines 27-36: Biovolume calculations

In line 27, the height value *maxZ*+ *imagesliceID*, which is given in µm, is converted into the corresponding slice in the z-stack.

In line 30, the number of pixels in the image *w* with value greater than zero is counted. This is the total biovolume.

In line 31, the number of pixels with value greater than zero is counted for all slices above maxZ+1 µm. This is the biovolume above maxZ+1 µm.

Lines 34-36 saves the following data: The total biovolume, the biovolume above maxZ+1 µm and the ratio between the two.