In Confocal Scanning Laser Microscopy (CSLM), an image corresponding to an optical section will contain in some cases blurred, low contrast or over-saturated areas which correspond to the sample regions that are not in the focal plane. For several types of investigations more accurate conclusions can be drawn when based on observations which have as subject images of uniform quality. These types of images will usually allow better morphological observations of the sample details. One method for obtaining such a representation is image fusion. Image fusion will provide an artificial image, which consists of regions coming from different images of the CSLM stack. In our method for multi focus image fusion, each of the images in the stack is divided in a number (n) of square regions, and for each of these regions a focus estimate is calculated. The fused image is built as an image mosaic with n tiles, the tiles of the mosaic representing the square regions with the best focus of all the images in the stack (Fig.1). Depending on the decision criterion based on which it is decided whether a region will be included in the fused image or not, several methods are experimented. These methods are based on both classical focus assessment operators, such as the sum-modified Laplacian, energy of Laplacian, Tenenbaum’s algorithm (Tenengrad), or energy of image gradient [1] and on custom designed quality assessment operators defined for the specifics of CSLM images, which take into consideration aspects such as brightness, edge content, local standard deviation and others.

![Figure 1. Stack of CSLM images (left), correspondence matrix containing the number of the image in the stack whose corresponding square region contributes to the fused image (center), resulted image after fusion (right). Images were split into square regions of 64*64 pixels.](image)