

Quantification for wide field fluorescence microscopy

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Comparison of intensities between fluorescence images taken under different optical conditions is notoriously difficult. Here, we introduce a method for fluorescence quantification based on the use of specially developed calibration layers. The uniform fluorescence and bleaching properties of these layers permit to characterize separately the illumination intensity and the detection efficiency for each spatial point in the image. We find that after correction for these factors image intensities of the same object observed under different microscope conditions can be correlated within a few percent. Inherent in the approach is automatic shading correction, i.e. correction for uneven illumination and detection conditions in a microscopy image. It is also possible to compensate the bleaching in a fluorescent image for the unevenness of the illumination, thus facilitating imaging based on specimen bleaching properties.