

**Fluorescence calibration layers for absolute quantification in 2D and image characterization in 3D fluorescence microscopy.**

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We have shown -this conference- that the intensities in fluorescence images can be calibrated against uniform fluorescent reference layers. Such calibrated images are shading corrected by the calibration procedure itself and also permit correlating fluorescent images obtained under different imaging conditions. These calibrations were relative to the used reference layers. In the present contribution we report the first steps towards absolute quantification by the calibration of the local illumination or excitation intensities over the image in terms of photons/sec per surface area.

Also the extension of the calibration approach to 3D - confocal, multi-photon- will be discussed. It is well known that the applicable axial PSF and apparent fluorescence intensities are dependent on pinhole conditions and vary in confocal microscopy over the image field. As a first result we found that the thin - approx. 100nm- calibration layers are convenient for evaluating these parameters in practice for a particular confocal imaging situation.