

## Quantitative image calibration for confocal fluorescence microscopy using thin reference layers and SIPchart based calibration procedures

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The fluorescence intensity image of an axially integrated through-focus series of a thin standardized uniform fluorescent layer can be used for image intensity correction and calibration in sectioning microscopy. This intensity image is in fact available from the earlier introduced Sectioned Imaging Property or SIP-charts [1]. It is shown that the integrated intensity of a z-stack from a biological sample, imaged under identical conditions as the layer, can be calibrated in terms of fluorescence layer units or FLU's of the calibration layer.

The imaging after such calibration becomes, in a first approximation, independent of microscope system and imaging conditions. This is demonstrated on axially integrated images of standard fluorescent beads and standard BPAE Fluorocells. It is also shown that with the present approach the actual underlying three dimensional (3D) fluorescence data set itself can be corrected for variations in point spread function (PSF) imaging efficiency over the imaging data cube. To realize such calibration between imaging conditions/systems requires basically only the 2D fluorescer molecule density of the reference layer and the section distance with which the layer data are collected.

Presented calibration approach is shown to be effective for a range of biological sectioned imaging situations, providing practical means to quantitatively correlate fluorescence intensities observed under different imaging conditions. These range from shading affected images to images obtained with different magnifications and even different instruments. With the specimen fluorescence intensities expressed in units of the fluorescer density of the calibration layer, the method opens up the possibility for a molecular type of quantitative microscopy. See for further details.[2].

[1] Brakenhoff, G. J., Worpel, G. W. H., Jalink, K., Oomen, L., Brocks, L. & Zwier, J. M. Characterization of sectioning fluorescence microscopy with thin uniform fluorescent layers: SIPcharts, *J. Microsc.* 219, 122-132,(2005).

[2] J. M. Zwier, L. Oomen, L. Brocks K. Jalink, G..J. Brakenhoff. Quantitative image correction and calibration for confocal fluorescence microscopy using thin reference layers and SIPchart based calibration procedures. *J. Microsc.* accepted for publication Jan. 2008.