Intensity correction and quantification in fluorescence confocal microscopy using Sectioned Imaging Property charts (SIPcharts).

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In fluorescence confocal microscopy intensity measurements are rarely used to do quantitative measurements on specimen simply because it is hard to relate images taken with different microscope conditions. The optical conditions vary too much for each change in microscope settings, making correlation between images difficult. Furthermore, the fluorescence intensity over the lateral confocal image field can easily vary up to 30 percent. It would be greatly beneficial for the confocal user community if it were possible to correct for all these optical variations.
A method is presented here, based on our previous SIPchart work [1], which could account for the differences in intensities measured under different optical settings. It is shown that by making a z-scan of a thin, uniform fluorescent layer, the integrated intensity of this z-stack can be used to correct for the differences in intensities.
The validity of the correction method is shown using fluorescent beads and standard fluorescent labeled cells. It is also argued that when the PMT settings are known and the sampling distance of the z-stack is well within the resolution of the system, the fluorescence intensities of the specimen can be standardized in so called ‘Fluorescence Layer Units’.
We consider this a important development as it indicates that the uniform thin calibration layers and the associated SIPcharts are not only effective for the characterization of confocal instrumentation but also permit objective measurement and correlation of the actual fluorescence observed.

[1] G.J. Brakenhoff; G.W.H Wurpel; K. Jalink; L. Oomen; L. Brocks; J.M. Zwier, "Characterization of sectioning fluorescence microscopy with thin uniform layers: Sectioning Imaging Property or SIPcharts” *Journal of Microscopy*, **219**(3), 122-132 (2005).