INTENSITY CORRECTION AND NORMALIZATION IN FLUORESCENCE
CONFOCAL MICROSCOPY WITH SECTIONED IMAGING PROPERTY CHARTS
OR SIPCHARTS

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The apparent intensity of fluorescent objects when observing may easily vary by up to 30 %
or more over the lateral confocal image field, while making it nearly impossible to relate the
observed fluorescent intensities (in counts!) of objects when observed under different optical
conditions like different pinholes, objectives with different magnifications or actually
different confocal instruments.

After normalization with SIPchart (see below) data the intensities can be expressed in units
fluorescence of the calibration layer. We have now found that after such calibration with the
SIPchart data the apparent fluorescence can be correlated to a surprising degree: within a few
percent over the lateral image and at present to about 15 % in images obtained with different
pinhole settings or using objectives ranging from 20 to 100 X magnification. This can be
demonstrated both for images of fluorescent beads as well a biological cell cultures. 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.

SIPcharts have been originally developed for the characterization of the imaging conditions in
confocal -or more general sectioning microscopy. They are derived from through-focus data-
sets of thin, uniformly fluorescing reference layers. Parameters like axial resolution, skew of
the axial response, the maximum and integrated fluorescence yield along the optical axis can
be determined at each lateral point in the image, allowing representation of these parameters
as color-coded maps over the imaging field. Assembled in a standard way, a Sectioned
Imaging Property-chart or SIPchart results[1] Thin fluorescent layers were earlier employed
for axial resolution measurements by Schrader et al.[2] and Heintzmann et al. [3]

[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
axial resolution in confocal and two-photon fluorescence microscopy." Journal of Microscopy
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Journal of Microscopy 204(2) 119-137 (2001)