

## **Characterization of sectioning fluorescence microscopy with thin uniform fluorescent layers: SIPcharts.**

G. J. Brakenhoff<sup>##</sup> & G.W.H. Wurpel<sup>#</sup>, K. Jalink<sup>+</sup>, L. Oomen<sup>+</sup>, L. Brocks<sup>+</sup>, J. M. Zwier<sup>#</sup>

<sup>#</sup>*Swammerdam Institute for Life Sciences, Section of Molecular Cytology and Center of Advanced Microscopy, University of Amsterdam, Kruislaan 316, 1098 SM Amsterdam, The Netherlands,* <sup>+</sup>*Division of Cell Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam, The Netherlands*

**Email:** [brakenhoff@science.uva.nl](mailto:brakenhoff@science.uva.nl)

In confocal microscopy image formation depends both on user controlled factors as well as the optical properties of the actual instrument used. In particular, off-axis aberrations -like spherical or chromatic- may cause confocal imaging conditions to vary laterally over the image.

It is shown that thin, uniformly fluorescing reference layers can be used for characterization of the imaging conditions in confocal -or more general sectioning microscopy. Through-focus data-sets of such layers, obtained by standard microscope routines, are the basis for the approach. A set of parameters derived from these data sets is developed for defining a number of relevant sectioned imaging properties. These include the axial resolution, the asymmetry or skew of the axial response, the maximum and integrated fluorescence yield along the optical axis and the axial height of the sectioned plane imaged. They can be determined at each lateral point, allowing representation of these parameters as color-coded maps over the imaging field. The main characteristics of a particular imaging situation can be summarized in a Sectioned Imaging Property-chart or SIPchart. We propose such charts for the general documentation of imaging conditions in sectioning microscopy.

It is shown that with these charts the dependence of above parameters can be followed with good sensitivity as a function of imaging conditions like pinhole size, alignment, objectives used and excitation/detection wavelength settings. Particularly the latter is relevant for Fluorescence Resonance Energy Transfer (FRET) studies where co-localization of the imaging at various spectral settings is important. Applications include image calibration for confocal microscopy, quality control, documentation and maintenance of confocal imaging conditions or for instance optimization of de-convolution procedures.

The uniform layers have been previously used for calibration of the image formation in regular wide-field fluorescence microscopy. (Zwier et al. 2004) J. M. Zwier, G. J. van Rooij, J. W. Hofstraat and G.J. Brakenhoff, *J. of Micr.*, 216, 15-24, 2004.