

Problem-Adaptable Multifunctional Calibration Tools for the Traceable Characterization of Fluorescence Measuring Systems

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Photoluminescence techniques are amongst the most widely used tools in the material and life sciences, with new and exciting applications continuously emerging. Advantages include their comparative ease of use, unique sensitivity, non-invasive character, and potential for multiplexing, remote sensing, and miniaturization. Generally recognized drawbacks, however, are signals, that contain unwanted wavelength- and polarization contributions from instrument-dependent effects, which are also time-dependent due to aging of instrument-components, and difficulties to measure absolute fluorescence intensities, thus rendering the use of intensity standards mandatory for quantification [1-4].

Recent developments in fluorescence-based assays in clinical, pharmaceutical, biotechnological and other areas, in conjunction with the increasing need for instrument performance validation and global trends to harmonize measurements have boosted the demand for robust, easy-to-use, readily-available, reliable, and well documented fluorescence standards. This includes e.g. fluorescence standards for the consideration of instrument-specific spectral and intensity distortions of measured signals and instrument performance validation as well as fluorescence intensity standards for the quantification from measured intensities and for signal referencing, thereby accounting for excitation light-induced intensity fluctuations [1,4-6].

This encouraged us to develop liquid and solid standards for different fluorescence parameters and fluorescence techniques for use under routine measurement conditions in different formats or measurement geometries focusing on the determination and control of the spectral responsivity of detection systems, wavelength accuracy, homogeneity of illumination, and intensity referencing for e.g. spectrofluorometers, fluorescence sensors and confocal laser scanning fluorescence microscopes [1-4,7]. Here, we will present design concepts and examples for mono- and multifunctional fluorescence standards that provide traceability to radiometric units and can be used for in different formats / measurement geometries and thus for different fluorescence techniques. The eventual goal is a toolbox of method-adaptable standards for the standardization of fluorescence measurements.

References: [1] P.C. DeRose et al. in: *Standardization and Quality Assurance in Fluorescence Measurements I: Techniques*, U. Resch-Genger (ed.), *Springer Series on Fluorescence*, O. S. Wolfbeis (ed.), vol. 5, **2008**, pp. 33. [2] K. Hoffmann, R. Nitschke, U. Resch-Genger in: *Standardization and Quality Assurance in Fluorescence Measurements II: Bioanalytical and Biomedical Applications*, U. Resch-Genger (ed.), *Springer Series on Fluorescence*, O. S. Wolfbeis, vol. 6, 2008, pp. 89. [3] U. Resch-Genger et al., *J. Fluoresc.* **2005**, 15, 337. [4] U. Resch-Genger, K. Hoffmann, D. Pfeifer in: *Reviews in Fluorescence*, Geddes, C. D. (ed), Springer Science Business Media, Inc., New York, **2009**, vol. 4, pp 1. [5] L. Wang et al., *Biophotonics Int.* **2005**, 42. [6] P. C. DeRose, U. Resch-Genger, *Anal. Chem.*, in press. [7] D. Pfeifer et al. *J. Fluoresc.* **2006**, 16, 581.