

# Determination of the Spectral Characteristics of Confocal Spectral Imaging Systems and Comparability of Data

Ute Resch-Genger<sup>1</sup>, Katrin Hoffmann<sup>1</sup>, Roland Nitschke<sup>2</sup> and Axel Engel<sup>3</sup>

<sup>1</sup>Federal Institute for Materials Research and Testing, Richard-Willstätter-Str. 11, D-12489 Berlin, <sup>2</sup>Albert-Ludwigs-Universität, Life Imaging Center, Institute of Biology I, Developmental Biology, Hauptstr.1, D-79104 Freiburg, Germany, <sup>3</sup>Schott AG, Mainz, Germany.

E-mail: [ute.resch@bam.de](mailto:ute.resch@bam.de)

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Confocal and wide-field fluorescence microscopy have been developing into some of the most frequently used methods in medicine, forensics, and bioanalysis. Instrument-specific effects, that limit the direct comparison of fluorescence data obtained on different fluorescence microscopes, in different laboratories, and at different times, are linked to the spectral irradiance at the sample position, the light collection properties, the aberration correction of the microscope, and the spectral responsivity of the emission detection system, respectively [1-4]. Aside from the choice of suited standards, these effects also play a role for analyte/fluorophore quantification from measurements of fluorescence intensities.

The desired comparability of microscopic data accordingly implies determination and correction for the time-dependent variability in sample illumination as well as for the time-dependent spectral responsivity of the detection system. To achieve this and to check on the complex instrumentation linked to confocal and high-end wide-field imaging microscopy, there is an urgent need for easy-to-use standards for the characterization and validation of these instruments. This includes standards and ideally standardized procedures for control of instrument specification, day-to-day and long-term performance as well as for the comparability—and in some cases the combination of microscopic data—across instruments and laboratories [5]. The demand for standardization of instrument characterization and measurement procedures is further enhanced by the increasing use of fluorescence microscopy in areas like medical diagnostics.

Here, as a first step into this direction, we discuss a simple approach to the determination of the spectral characteristics of confocal imaging systems relying on easy-to-operate liquid fluorescence standards and fluorophore-doped glasses [6], that can be made traceable eventually. The potential and limits of these materials to compare the instrument performance and instrument characteristics have been evaluated on several commercially available confocal microscopes.

## References

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