Confocal Microscopy System Performance: Foundations for Calibration, Quantitation and Spectroscopy

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The confocal laser-scanning microscope (CLSM) has enormous potential in many biological fields. The goal of a CLSM is to acquire and quantify fluorescence and in some instruments acquire spectral characterization of emitted signals. The accuracy of these measurements demands that the system be in alignment with stable laser power and spectral registration, however the most common method to check the performance of a CLSM system is to characterize a histological slide to create a “pretty picture”. We have developed a series of tests to replace this subjective approach which include objective measurements of field illumination, spectral registration, lens function and clarity, total laser power, laser stability, dichroic reflectance, axial resolution, scanning stability, overall machine stability, and system noise (1-2). Improvements in these tests will be described. In addition recently we have developed a test to measure spectral performance which will serve as guidelines for investigators to assess both the performance of their instruments as well as the quality of their data (3).

The spectral characterization test is well suited to all wavelength dispersive CLSM systems including the Leica SP, Zeiss 510 Meta, Olympus FV1000 and the new Nikon spectral confocal microscopes. We used an inexpensive, eye-safe, battery operated, multi-ion discharge lamp (MIDL) (LightForm, Inc., Hillsborough NJ) containing mercury ions and inorganic fluorophores as an absolute reference light source because it emits stable, reproducible, spectral features between 400 and 650 nm. The lamp was simply positioned on the microscope stage above, or below, the objective lens. An acquired MIDL spectrum enables the user to determine contrast, wavelength ratios and spectral resolution.

In addition to the other tests we feel that using an absolute reference light source, such as the MIDL lamp, provides a very simple, sensitive and inexpensive method for any researcher to test and validate the performance of a spectral confocal instrument. It also serves as a stable universal reference spectrum to compare instrumental performance with colleagues in different laboratories.

References

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