DOI: 10.1017/S1431927605509346
Microsc Microanal 11(Suppl 2), 2005

Confocal Microscopy System Performance: Spectroscopy and Foundations for Quantitation

Robert M. Zucker [1]

[1] Reproductive Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Key Words: Confocal microscope, Quality assurance, Spectroscopy, Calibration

The confocal laser-scanning microscope (CLSM) has enormous potential in many biological fields. The reliability of the CLSM to obtain specific measurements and quantify fluorescence data is dependent on using a correctly aligned machine that contains a stable laser power. For many applications it is useful to know the CLSM system's performance prior to acquiring images so the necessary resolution, sensitivity and precision can be obtained in the experiment. Applications of deconvolution, FRET and quantification necessitate that the confocal is correctly configured and operating at the highest performance levels to achieve the desired results.

One of the most common methods that many laboratories use to measure system performance involves the use of a histological slide to create a "pretty picture". Although this test evaluates many parameters that can influence a CLSM image in a crude manner (i.e. laser power, field illumination and lateral resolution), the interpretation of this histological image is very subjective making the range of what constitutes an acceptable image and performance extremely variable. In fact, many confocal microscopes can obtain "pretty pictures" even when they are operating at sub-optimal levels. It is impossible to compare the performance of similar machines if only an image is used as the reference standard. If a scientist wants to determine whether the CLSM is indeed working at appropriate performance levels, it is essential that these types of CLSM QA performance tests be applied on their system. This tutorial will illustrate a number of different tests that can be applied to a CLSM to determine its functionality and performance.

These tests methods have been devised on the Leica TCS-SP1 confocal microscope systems to ensure that it is operating correctly. The CLSM tests measure the following: field illumination, lens functionality and lens clarity, spectral registration, total laser power, laser stability, dichroic reflectance, spectral registration of the beams, axial resolution, scanning stability, overall machine stability, galvanometer function colocalization, and system noise (1-3). It is anticipated that by applying these QA tests, different performance standards for the confocal microscopes will be determined, thus eliminating some of the current subjectivity that exists in evaluating CLSM performance. Hopefully, these tests will serve as guidelines for other investigators to assess both the performance of their machines and the quality of their data. These confocal tests have also been applied successfully to Zeiss 510 confocal system. It is anticipated that with minor modifications they may be adaptable to the other CLSM manufactures' machines.

Modifications and improvements in these QA tests that were previously described in our publications (1-3) will be described in this tutorial.

Recently, we have developed a test to measure spectral performance of a CLSM (3). 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. The derived spectra include features of contrast, wavelength ratios and spectral resolution. From these results we feel that using an absolute reference light source (such as the MIDL lamp) will provide a simple, sensitive and inexpensive method for any researcher to test and validate the performance of their instrument. This test will help investigators to assess both the performance of their instruments as well as the accuracy of their spectral data (3). It also serves as a stable universal reference spectrum to compare instrumental performance with colleagues in different laboratories. The spectral characterization test is well suited to all wavelength dispersive CLSM systems including the Leica SP, Zeiss 510 Meta, Olympus FV1000 and the Nikon C1 spectral confocal microscopes.

References
1. Zucker, RM. Price OT. Cytometry 44:273-294 2001
2. Zucker, RM, Price OT. Cytometry 44:295-308 2001
3 Lerner JL Zucker, R.M: Cytometry 62A:8-34 2004
This abstract of a proposed presentation does not necessarily reflect EPA policy

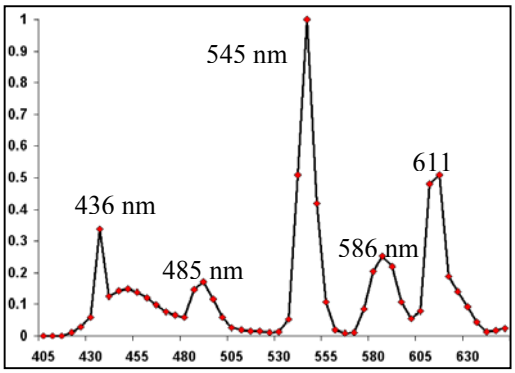

Figure 1

Figure 1 MIDL spectra illustrating reference peaks on a Leica SP1 confocal microscope. The position and shape of the peaks serve as reference points for a properly functioning CLSM machine.

https://doi.org/10.1017/S1431927605509346 Published online by Cambridge University Press