

CHARACTERIZATION PROTOCOLS OF OPTICAL MICROSCOPE COMPONENTS TESTED WITH A NOVEL LIGHT SOURCE DEVICE

Edward H. Cho¹, Sylvain Costes², Stephen Lockett¹

¹Image Analysis Laboratory, SAIC-Frederick, National Cancer Institute at Frederick, P.O. Box B, Frederick, MD 21702, ²Life Sciences, Lawrence Berkeley National Laboratory, 1 Cyclotron Rd, Berkeley, CA 94720, USA
E-mail: slockett@ncifcrf.gov

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The optical microscope is an instrument for quantitatively analyzing molecular pathways inside individual living cells, and therefore convenient characterization of its performance is essential. Previous investigations to evaluate the performance of optical microscopy systems have shown the need to be able to characterize specific components of systems to gather useful quantitative data and compare data between microscopy systems. Many quantitative advanced microscopy techniques are coming to the forefront of modern scientific research (e.g. fluorescence recovery after photo-bleaching, fluorescence resonance energy transfer, fluorescence correlation spectroscopy). This makes it imperative for a standard protocol to be in place to characterize one microscope system against another.

We evaluated current protocols and propose an improved protocol, tested using a Zeiss LSM510 and Zeiss LSM410 on an Axiovert 200M and an Axiovert 100 respectively, to measure specific components of an optical system: the sensitivity (i.e. the fraction of emitted photons that are detected), dynamic range of linearity of the detectors (i.e. range of signal intensities over which the instrument response is linear), uniformity over the field of view, and axial resolution of the microscopes. We deem these as the primary factors of quantitative image acquisition from optical microscopes. In order to test several of these components a novel light source device was fabricated. The light source was designed to emit an isotropic source to mimic the fluorescence coming from a real fluorescent sample. One major advantage of the light source for these experiments is that it does not photobleach. As such, the signal to noise ratio could be calculated using this light source as if from a real fluorescent sample without having to correct for photobleaching, a process that can sometimes introduce artifact in the data. A 5 fold increase was observed in signal to noise ratio simply from changing the pixel dwell time from 0.8 μ s to 3.2 μ s. Also, this light source has the potential to yield quantitative data to determine the efficiency of the light path from sample to detector. In that regard, it has the potential to reveal the number of photons per pixel per second (i.e. photon counting). From this, it may soon be possible to determine the number of molecules per unit volume for fluorochromes with a known quantum yield.

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