Performance Benchmarking a Widefield Fluorescence Microscope Using Fluorescent Glass

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***Abstract*— Widefield fluorescence microscopy is a highly used tool for visually assessing biological samples and for quantifying cell responses. Despite its widespread use in high content analysis and other imaging applications, few published methods exist for evaluating and benchmarking the analytical performance of a microscope. Easy-to use benchmarking methods would facilitate the use of fluorescence imaging as a quantitative analytical tool in research applications, and would aid in instrument validation for commercial product development applications. We describe and evaluate a method to characterize a fluorescence imaging system's performance by benchmarking the detection threshold, saturation and linear dynamic range to a physical artifact. The benchmarking procedure is demonstrated using two different materials as the physical artifact, uranyl-ion-doped glass and Schott 475 GG filter glass. Both are suitable candidate reference materials that are homogeneously fluorescent and highly photostable, and the Schott 475 GG filter glass is currently commercially available. In addition to benchmarking analytical performance, we also demonstrate that the reference materials provide for accurate day to day intensity calibration.**

A script written in MicroManager, an open-source microscopy control software, has been developed to automate the procedure and return the benchmarked parameters. The MicroManager script is publicly available (http://www.nist.gov/mml/bbd/cell_systems/fluorescence-microscope-benchmarking.cfm). Ideally, this work will result in a common operating procedure that will help to assure that quantitative microscopy results are meaningful, traceable, and comparable from day to day and between laboratories.

***Index Terms*— microscope benchmarking**