

INTENSITY MEASUREMENT PRECISION OF FLUORESCENCE DETECTION IN BIOLOGICAL MICROSCOPY

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Standardization and calibration of optical microscopy systems have become an important issue owing to the increasing role of biological imaging in high-content screening (HCS) technology. The proper interpretation of data from HCS imaging experiments requires detailed information about the capabilities of the systems, including their available dynamic range, sensitivity, and noise. Currently available techniques for calibration and standardization of digital microscope commonly used in cell biology laboratories provide estimation of stability and measurement precision (noise) of an imaging system at single level of signal intensity. In addition, only the total noise level, not its characteristics (spectrum), is measured. We propose a novel technique for estimation of temporal variability of signal and noise in microscopic imaging. The method requires registration of a time series of images of any stationary biological specimen. The subsequent analysis involves a multi-step process, which separates monotonic, periodic, and random components of every pixel intensity change in time. By adaptation of photon transfer method we perform simultaneous determination of dark, photonic, and multiplicative components of noise present in biological measurements. Consequently, a respective confidence interval (noise level) is obtained for each level of signal. The technique is validated using test sets of biological images with known signal and noise characteristics. The method is also applied to assess uncertainty of measurement obtained with CCD cameras in a wide-field microscopy and photomultipliers in confocal microscopy. The proposed algorithm may also be used to detect mechanical instability of a microscope and instability of illumination source. In addition, photobleaching kinetics may be characterized at each level of fluorescence intensity.