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References

Accuracy and precision in quantitative fluorescence microscopy

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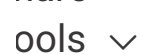

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The light microscope has long been used to document the localization of fluorescent molecules in cell biology research. With advances in digital cameras and the discovery and development of genetically encoded fluorophores, there has been a huge increase in the use of fluorescence microscopy to quantify spatial and temporal measurements of fluorescent molecules in biological specimens. Whether simply comparing the relative intensities of two fluorescent specimens, or using advanced techniques like Förster resonance energy transfer (FRET) or fluorescence recovery after photobleaching (FRAP), quantitation of fluorescence requires a thorough understanding of the limitations of and proper use of the different components of the imaging system. Here, I focus on the parameters of digital image acquisition that affect the accuracy and precision of quantitative fluorescence microscopy measurements.

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accuracy is obvious. Precision is equally important in quantitative fluorescence microscopy because we are often forced to make only one measurement (for ex-ample, one time-point in a live-cell time-lapse experiment). In addition, we are usually measuring biological speci-mens that have some level of natural variability, so variance seen in measure-ments made on different cells will be caused by both biological variability and that which is introduced when mak-ing the measurement. In quantitative fluorescence microscopy, we want to measure the signal coming from the fluorophores used to label the object of interest in our specimen. For example, consider live cells expressing GFP-tubulin in which we wish to mea-sure the amount of tubulin polymer. Fluorescence microscopy has become an essential tool in biology as well as in materials science due to attributes that are not readily available in other optical microscopy techniques. The technique of fluorescence microscopy has become an essential tool in biology and the biomedical sciences, as well as in materials science due to attributes that are not readily available in other contrast modes with traditional optical microscopy. The application of an array of fluorochromes has made it possible to identify cells and sub-microscopic cellular components with a high degree of specificity amid non-fluorescing material. The light microscope has long been used to document the localization of fluorescent molecules in cell biology research. With advances in digital cameras and the discovery and development of genetically encoded fluorophores, there has been a huge increase in the use of fluorescence microscopy to quantify spatial and temporal measurements of fluorescent molecules in biological specimens. Here, I focus on the parameters of digital image acquisition that affect the accuracy and precision of quantitative fluorescence microscopy measurements. The fluorescence microscope is the most used microscope in the medical and biological fields. These types of microscopes use high-powered light waves to provide unique image viewing options. Pictured right: Microscope image of human cancer cell in red fluorescence. When a specimen can be made to fluoresce studying certain structural properties of the specimen is made easier, either emitting this light naturally or achieved through treating with dyes and stains. When the light of a specific wavelength irradiates certain specimens they can emit energy seen as visible light. We have developed a quantitative structured illumination microscopy image processing algorithm (QSIM) as a plugin for the widely used ImageJ software. QSIM can work with the raw images acquired by a traditional structured illumination microscope and can quantitatively measure photon numbers, with noise estimates for both wide-field images and sectioned images. Results and conclusion. We demonstrated the quantitative image processing capability of QSIM by imaging a mouse kidney section in 3D. Waters JC: Accuracy and precision in quantitative fluorescence microscopy. J Cell Biol 2009, 185: 1135–48. 10.1083/jcb.200903097. Article Google Scholar. 10.