PHOTOBLEACHING AND SENSITIVITY OF LIVE CELL IMAGING SYSTEMS

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KEY WORDS: Confocal, laser scanning microscopy, multi-dimensional imaging, photobleaching, sensitivity, signal-to-noise-ratio

Today’s bio-medical applications are mainly driven by the need to understand function and interaction of structures and molecules in living biological model systems. Such applications address questions about the development of cells and organs, the investigation of ion signalling processes within cells and across cell membranes, as well as vesicle and protein transport within cells at different time scales.

Depending on the specific application a high-speed live cell imaging needs to fulfill several requirements: First, it needs to image samples with a temporal resolution that matches the dynamic processes that are investigated. Second, it needs to provide subcellular optical resolution and optical sectioning. Third, the sensitivity needs to be as high as possible in order to not bleach the faintly stained samples. Currently available confocal microscope systems require to make different tradeoffs regarding these parameters. In order to match the right confocal scanning and illumination schemes to a specific application, there is a need to characterize all three parameters carefully. To measure optical performance several methods have been established [1,2] including signal-to-noise ratio and background rejection evaluation [3]. However, procedures for measuring sensitivity and photobleaching are still under discussion [4].

The talk will focus on methods to measure and compare photobleaching and sensitivity parameters using different illumination and scanning concepts. Results from line scanning and point scanning confocal microscopes are presented and conclusions for applications are drawn. We show that in many cases the photobleaching depends on the illumination photon dose only and is therefore independent of the pixel dwell time that is dramatically different for line and point scanning systems. It means that the photobleaching is fundamentally limited by the sensitivity of the detection and therefore by the photon noise in optimum-designed systems.