

# THE ROLE OF OXYGEN AND PHOTON FLUX IN PHOTOBLEACHING DURING CONFOCAL MICROSCOPY OF FLUORESCENT PROBES BOUND TO CHROMATIN

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Photobleaching limits the use of fluorescence and confocal microscopy in biological studies. Loss of fluorescence decreases the signal-to-noise ratio and so image resolution; it also prevents the acquisition of meaningful data late during repeated scanning (e.g., when collecting three-dimensional images). The aim of this work was to investigate the role of oxygen in the photobleaching of fluorophores bound to DNA in fixed cells, and to explore whether anoxia could minimize such bleaching.

Anoxia significantly reduced bleaching rates and changed the order of reaction of both propidium iodide (an intercalator) and chromomycin A3 (a minor-groove binder) bound to DNA; it afforded the greatest protection at low photon fluxes. However, it had no effect on the bleaching of the green fluorescent protein (GFP) covalently attached to a histone and so bound to DNA, probably because the protein shielded the chromophore from oxygen. Bleaching of all three fluorophores depended on photon flux. The optimal signal to noise ratio could be achieved only at a certain value of excitation light flux and image acquisition time (or the number of frames collected). On one hand, for high-order reactions (e.g., involving eGFP and intercalated PI) it was advantageous to use a long image acquisition time (or accumulate a large number of frames) while keeping the exciting flux low. On the other hand, for (rare) reactions with order close to unity, the opposite strategy produced the highest signal-to-noise ratio. For most fluorophores (which are characterized by high order bleaching reactions), the dependence on photon flux of signal loss suggests that delivering the dose required for image acquisition will yield a better signal stability, a higher signal-to-noise ratio, and so a better image if it is divided into the smallest possible portions, with averaging of the resulting images. Practical ways of minimizing bleaching were examined, and examples of three-dimensional images of DNA marked by propidium and GFP (collected under standard and optimized conditions) will be presented.

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