UV-ACTIVATED CONVERSION OF DNA DYES, HOECHST AND DAPI, INTO BLUE-EXCITED, GREEN EMITTING FORMS

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INTRODUCTION
Commonly known DNA-binding fluorescent dyes, such as DAPI, Hoechst 33342 and Hoechst 33258 or Vybrant DyeCycle™ Violet are excited by UV and emit in the blue region of the light spectrum, thus leaving the reminder of the spectrum for detection of other cellular targets by probes emitting in green, yellow or red. However, an exposure of a biological specimen stained with DAPI, Hoechst 33342, Hoechst 33258 or Vybrant DyeCycle™ Violet to ultraviolet light (metal halide lamp) or the 405 nm laser light during a typical fluorescence or confocal microscope observation leads to photoconversion of these dyes to the forms which can be excited by blue light and emit green fluorescence (emis. max. approx. 520 nm), with detectable signal intensities in yellow to orange region of the spectrum.

RESULTS
Photoconversion process of Hoechst or DAPI is independent of the presence of DNA and can also take place in solution. For Hoechst a conversion into a blue-excited, green-emitting product can also be detected following an exposure to H2O2 in the dark. Photoconversion of Hoechst 33342 has been observed in fixed as well as live cells. The rate of photoconversion is independent of the initial concentration of the dye or pH. The photoproducts of Hoechst 33258 and DAPI are stable in time. The amount of the photoconverted product is directly proportional to the dose of UV light delivered to the sample. Mass spectrometry analysis of Hoechst 33258 in solution following exposure to UV demonstrated the presence of a protonated form of the dye [1].

CONCLUSIONS
Photoconversion of UV-excited dyes bound to DNA may be exploited in super-resolution microscopy. In standard fluorescence microscopy, however, photoconversion of these dyes in biological samples may pose problems for image analysis in multicolor fluorescence microscopy. Even relatively small doses of UV-light result in creating a false positive - a green fluorescence signal derived from the DNA-bound dye, which is expected to fluoresce only in the blue region. Following photoconversion, the unexpected emission arising from Hoechst or DAPI may be mistakenly interpreted as a green fluorescent signal expected from another fluorescent probe used in the same experiment.