

SCATTERING OF EXCITING LIGHT BY LIVE CELLS IN SCANNING CONFOCAL MICROSCOPY - ADVERSE EFFECTS IN THE IMAGED AND NEIGHBOURING CELLS, AND RELEVANCE FOR FRAP, FLIP AND FCS STUDIES

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KEY WORDS: Scattered exciting light, live cell imaging, cell damage, phototoxicity, drug efflux, FRAP, FLIP, FCS

BACKGROUND: In FRAP, FLIP and FCS techniques small areas of living cells are illuminated with intense laser beams. However, fluorescently labelled cells may suffer damage as a result of interaction between exciting light and a fluorescent probe. The adverse effects exerted on cells range from an immediate loss of viability or an inability to complete the next mitosis, to various less easily detected disturbances in cell physiology.

GOAL: The purpose of this study was to measure the adverse effects of exciting light in illuminated and unilluminated areas of an imaged cell, and in the vicinity, in unilluminated neighbouring cells.

METHOD: Plasma membrane integrity and the efficiency of drug efflux mechanisms were used as endpoints to quantify the damage exerted on cell physiology. We designed a technique of *in situ* detecting inhibition of drug efflux by measuring the cytoplasmic and extracellular concentrations of acridine orange (AO). At an extracellular AO concentration of 8 $\mu\text{g/ml}$ the colour of cytoplasm (ratio of green/red AO emissions) reflected the intracellular concentration of the dye and thus, the efficiency of pumping out AO. Inhibition of drug efflux by phototoxic reactions resulted in a decrease of a green/red ratio. The following conditions of illuminating cells were used: excitation 457 nm, 150 μW at the specimen, illuminated area 17 x 17 μm , 512x512 pixels, 3.5 s/scan, 10 scans. The illuminated region was placed against a surface of a coverglass without cells, a glass bead resting on a glass surface, a region of a cytoplasm of a cell attached to glass, or the cell nucleus. 20 minutes after illumination the phototoxic effects were investigated in cells located within a radius of 200 μm .

RESULTS: The exciting light incident on cytoplasm, or a cell nucleus, was strongly scattered and caused a loss of integrity of plasma membrane of the imaged cell. In neighbouring cells, that were not exposed to the laser beam directly, inhibition of drug efflux was observed. The intensity of light scattered by the nucleus was comparable with the that of the glass bead and caused inhibition of the drug efflux even in cells located 150 μm away from the incident laser beam. In this test, photodamage was manifested by inhibition of the drug efflux mediated by glycoprotein P, however it is reasonable to expect that other adverse effects on cell physiology occurred as well.

CONCLUSIONS: These data demonstrate that various phototoxic effects of exciting light are not confined to the directly illuminated area, but extend to the whole body of the illuminated cell and far beyond, to neighbouring cells, even though these cells are not exposed directly to a laser beam. Thus, substantial disturbance to cell physiology arising from the scattered light may occur in FRAP, FLIP and FCS studies of live cells.