QUANTITATIVE ANALYSIS OF THE REDUCTION OF PHOTOBLEACHING AND PHOTOTOXICITY IN CONTROLLED LIGHT EXPOSURE MICROSCOPY (CLEM)

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Photobleaching and phototoxicity are two of the most important limitations of fluorescence live-cell microscopy. Photobleaching and phototoxicity are caused by several photochemical processes after excitation of fluorophores in the sample. The most straightforward way to reduce photobleaching and phototoxicity in fluorescence microscopy is by reducing the dose of light for the excitation of fluorophores. However, reduction of excitation light will lead to loss of image quality (reduced signal to noise ratio (S/N) and/or reduced spatial and temporal resolution).

In Controlled Light Exposure Microscopy (CLEM), a non-uniform illumination of the fluorescent sample allows to adjust the light dose for the excitation of fluorophores for every individual pixel leading to reduced phototoxicity and photobleaching without loosing image quality. We have implemented CLEM in a confocal microscope and have shown that CLEM reduces photobleaching by a factor of 7 in tobacco plant cells expressing GFP-MAP4. In HeLa cells expressing chromatin associated H2B-GFP the production of reactive oxygen species (ROS) is reduced 8-fold causing a 6 times longer scanning time without noticeable cell damage. So CLEM significantly reduces photobleaching and photodamage, by reducing the number of excited fluorophores, without deteriorating image quality.

The reduction of phototoxicity and photobleaching is quantified by a CLEM-factor defined by the ratio of the sum of excited fluorophores in non-CLEM and the sum of excited fluorophores in CLEM. The value of the CLEM-factor depends on the properties of the sample (fluorophore distribution and bleach-rate), microscope and imaging parameters (numerical aperture (NA) of the objective, wavelength, sampling-rate and pinhole size) and settings of CLEM-electronics. By computer simulation we have investigated the influence of these parameters on the CLEM-factor and image quality. We show that the CLEM-factor changes with variations of these parameters but that even in difficult imaging circumstances (e.g. low S/N ratio) the reduction of phototoxicity and photobleaching is significant.