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Extended Abstract

Light Exposure in Microscopy—How can Cell Survival be Increased?

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Light microscopy is an indispensable tool to study cell function and metabolism. However, an excess of light may induce phototoxic damages or even cell death, so that scientists should consider a maximum light dose for maintaining cell viability. This dose will determine the kind of experiment which can be performed including resolution, 3D imaging and exposure times[1]. All light doses are related to solar irradiation of about 1kW/m² corresponding to 100 mW/cm² or 1 nW/µm².

Using a colony formation assay as well as a neutral red absorption test, we found a pronounced dependence of cell viability on the wavelength of irradiation as well as on the application of fluorescent markers or proteins. While non-incubated cells remained viable up to light doses between 25 J/cm² (375 nm) and 200 J/cm² (633 nm), viability was often limited to about 10 J/cm² when applying green fluorescent dyes or proteins (GFP). An example for excess of this dose limit is given for 3T3 fibroblasts incubated with the lysosomal marker acridin orange, when after application of 55 J/cm² lysosomes are damaged causing a release of this marker to the cytoplasm and the cell nucleus with pronounced green fluorescence.

Limitation of light dose has a large impact on 3D microscopy. Confocal laser scanning microscopy or structured illumination microscopy with axial resolution require illumination of the whole sample for measurement of each single plane, so that the whole available light dose may be consumed upon recording of about 20 planes needed for reconstruction of one 3D image. Novel methods of super-resolution microscopy based on single molecule detection or on stimulated emission depletion microscopy (STED) even do not permit to record a single image under viable conditions. Therefore, *Light-Sheet based Fluorescence Microscopy (LSFM)* is regarded to be the only 3D method permitting a larger number of exposures, because illumination and detection are always restricted to the same layer

of the sample. Also, *Total Internal Reflection Fluorescence Microscopy (TIRFM)* permits a larger number of exposures, since illumination is limited to the plasma membranes and adjacent parts of the cells, so that phototoxic damages (taken as a whole) may be lower. Due to their low phototoxic potential, methods and applications of 3D microscopy described in this paper are concentrated on LSFM and TIRFM.

For TIRFM a special microscope condenser is described which permits variable-angle illumination and, therefore, variation of the penetration depth of the evanescent electromagnetic field. In addition to selective measurements of the plasma membrane, cell-substrate topology can thus be assessed in the nanometer range. This permits to distinguish between cancer cells and less malignant cells due their differences in surface topology, which appears rather plane for cancer (glioblastoma) cells and more folded after application of tumor suppressor genes. The condenser unit includes an additional path for transillumination and phase contrast microscopy for adjustment of the samples and further morphological measurements[2].

For LSFM experiments a miniaturized module has been developed and adapted to various commercial microscopes[3]. In addition to 3D microscopy this device can be easily combined with spectral imaging, fluorescence lifetime imaging (FLIM) and Förster resonance energy transfer (FRET) measurements. Preliminary applications[4,5] include

- 3D imaging of necrotic cells in a tumour cell spheroid after inhibition of the mitochondrial respiratory chain;
- 3D measurements of apoptosis using a FRET based membrane associated sensor;
- Uptake and cellular interaction of a cytostatic drug;
- Nanosecond ratio imaging of a genetically encoded redox sensor.

For all experiments as well as related applications light doses were below the limit of phototoxic cell damages.

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