

WIDE FIELD CONTROLLED LIGHT EXPOSURE FLUORESCENCE MICROSCOPY (WF-CLEM)

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Controlled Light Exposure Microscopy is a novel and simple technology that strongly reduces phototoxicity and photobleaching in live-cell imaging without compromising image quality [1,2]. This technology is based on a non-uniform illumination of the fluorescent sample that allows tuning the light dose for every individual pixel. Results show that CLEM applied in confocal microscopy reduces photobleaching and phototoxicity by a factor of 5 to 10.

After successful implementation of CLEM in a confocal microscope, we have used the same CLEM concept in wide-field fluorescence microscopy. In wide-field (WF-) CLEM the specimen is illuminated by spatially modulated light source. After detection with a CCD camera, the images are corrected for the illumination pattern in the excitation light path. WF-CLEM reduced photobleaching approximately by a factor of 3. To measure phototoxicity we have developed an assay and dedicated software to evaluate the results. Phototoxicity was reduced to a negligible level by WF-CLEM.

After a short introduce the concept of CLEM, we will show our first WF-CLEM results, discuss quantitative imaging with CLEM [3] and noise properties of CLEM [4].

REFERENCES

- [1] Manders, E.M.M. (2004) Method and apparatus for formation of an image of an object. *Patent WO2004102249*.
- [2] Hoebe, R.H., C H Van Oven, T.W.J. Gadella, P.B. Dhonukshe, C.J.F. Van Noorden and E.M.M. Manders (2007) Controlled light-exposure microscopy reduces photobleaching and phototoxicity in fluorescence live-cell imaging, *Nature Biotechnology*, **25**, 249 – 253
- [3] Hoebe, R.A., H.T.M. van der Voort, J. Stap, C.J.F. van Noorden, E.M.M. Manders (2008) Quantitative determination of the reduction of phototoxicity and photobleaching by controlled light exposure microscopy (CLEM). *J. Microscopy*, **231(1)** 9-20
- [4] Hoebe and E.M.M. Manders (2008) Werkwijze voor het vormen van een afbeelding van een object. *Patent NL-47665-VB*