

# CONTROLLED LIGHT EXPOSURE MICROSCOPY (CLEM) FOR PROLONGED LIVE-CELL IMAGING

Ron Hoebe<sup>1,2</sup>, Carel van Oven<sup>2</sup>, Winnok de Vos<sup>3</sup>, Peter Zoon<sup>1</sup>, Saskia Lambrechts<sup>1</sup>,  
Patrick van Oostveldt<sup>3</sup>, Dorus Gadella<sup>1</sup>, Ron van Noorden<sup>2</sup>, Erik Manders<sup>1</sup>

E-mail: [e.manders@science.uva.nl](mailto:e.manders@science.uva.nl)

<sup>1</sup>Centre for Advanced Microscopy, Section of Molecular Cytology, Swammerdam Institute for Life Sciences, University of Amsterdam, Kruislaan 316, 1098 SM Amsterdam, The Netherlands.

<sup>2</sup>Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

<sup>3</sup>Department of Molecular Biotechnology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

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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 reduces photobleaching and phototoxicity by a factor of 5 to 10.

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, microscope and imaging parameters and settings of CLEM-electronics. By computer simulation we have investigated the influence of these parameters on the CLEM-factor and image quality [3].

We will present applications of CLEM in cell biology. For example, we will show how we monitor the dynamics of telomeres in human cells for prolonged imaging periods. Application of CLEM in this research leads to biological results that cannot be obtained with non-CLEM (conventional imaging).

Finally, we will discuss quantitative imaging with CLEM, photobleaching and noise properties of CLEM and how CLEM can be applied in wide-field microscopy.

## REFERENCES

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