

**Phototoxicity in live-cell imaging and an effective way to reduce it:
Controlled Light Exposure Microscopy (CLEM).**

Erik Manders², Carel van Oven¹ and Ron Hoebe^{1,2}

¹Department of Cell biology, Academic Medical Centre

²Centre for Advanced Microscopy (CAM), Swammerdam Institute for Life Sciences

University of Amsterdam

Kruislaan 316, 1098 SM Amsterdam, The Netherlands

E-mail : e.manders@science.uva.nl

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Microscopy of living cells has become a method of major importance in cell biology: it tells us where molecules are, at what time, how fast they move, with which molecules they interact and how long they stay attached to these molecules. All these properties we can observe in the molecules natural environment: the living cell. However, it is not easy to follow biological processes in living cells for a long period of time. Especially during mitosis, the cell is extremely sensitive for light [1-3]. Phototoxicity is therefore major limiting factor in live-cell imaging.

We present a new method of microscopy: Controlled Light Exposure Microscopy (CLEM). This new microscopy results in a substantial reduction of phototoxicity (factor of 2-20). The most common strategy to obtain an image of an object is based on a uniform illumination of the object. Light from the object (reflection, transmission or fluorescence) is projected on a detector (CCD, photo-camera or retina) and the detector image gives direct information of the object. This strategy is extremely simple and used in most imaging applications like photography, human vision and microscopy. The basic concept of Controlled Light Exposure Microscopy (CLEM) is to reduce phototoxicity by controlling light exposure in every individual picture element (pixel). After illumination the image is reconstructed by correcting for local light exposure.

In this presentation we will go into the basics of phototoxicity, present methods to reduce it, illustrate the basic concept of CLEM, show the prototype of a CLEM-microscope and discuss the properties of CLEM-images. We show that CLEM, in a specific experiment, reduces phototoxicity by a factor of 7 and we show that CLEM also reduces photobleaching.

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