CONTROLLED LIGHT EXPOSURE MICROSCOPY (CLEM): FLUORESCENCE LIVE CELL IMAGING WITH STRONGLY REDUCED PHOTobleaching AND PHOTodAMAGE.

Ron Hoebe¹,², Carel van Oven² and Erik Manders¹
¹Centre for Advanced Microscopy (CAM), Swammerdam Institute for Life Sciences
²Department of Cell Biology, Academic Medical Centre
University of Amsterdam
Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands
E-mail: R.A.Hoebe@amc.uva.nl

KEY WORDS: Controlled Light Exposure Microscopy, CLEM, Living cells, photodamage, phototoxicity, photobleaching, Confocal Microscopy, Fluorescence Imaging.

Controlled Light Exposure Microscopy (CLEM) is a novel imaging approach that reduces photobleaching and phototoxicity. CLEM reduces the amount of excitation light, without compromising image quality, by spatial control of its intensity thus reducing the production of reactive oxygen species (ROS). We will demonstrate that application of CLEM reduces photobleaching 7-fold in tobacco plant cells expressing GFP-MAP4 associated with microtubules. Production of ROS is reduced 9-fold in HeLa cells expressing chromatin associated H2B-GFP and these cells survive 7 times longer during imaging when CLEM is applied. Therefore, CLEM is essential for live cell imaging.

The basic principle of CLEM is that illumination light is used only where it is needed. Depending on the local fluorescence intensity, the excitation light is locally controlled according to two independent light exposure strategies. The exposure time, and thus the excitation light dose, is reduced in i) fluorophore-dense regions (bright foreground) and ii) background in between objects.

We have implemented CLEM in a standard scanning confocal microscope. Two alterations of the conventional set-up were sufficient to install CLEM. An acoustic-optical modulator (AOM) is placed in the laser beam in order to switch on and off the illumination light. An electronic circuit, which functions as a feedback system, controls the AOM depending on signals from the detector for fluorescence emission light.

Figure 1:
One slice of a 3D “non CLEM” and “CLEM” image of pollen of the spathiphylum phryniifolium with their corresponding illumination.