

Calcium imaging with strongly reduced phototoxicity and photobleaching by Wide-Field Controlled Light Exposure 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. We have used the CLEM concept in wide-field fluorescence microscopy. In wide-field (WF-) CLEM the specimen is illuminated by spatially modulated light source. The illumination and detection images were superimposed by a dedicated alignment procedure to allow proper correction of the CCD image for the spatially modulated illumination.

We have applied the WF-CLEM technology for imaging of Calcium flux in primary hippocampus cultures and HeLa cells expressing a YC3.4 construct (Chameleon). By ratiometric imaging of simultaneously detected CFP and YFP signal, FRET was analyzed as a relative measure for Calcium concentrations. Using the wide-field CLEM technique we were able to reliably measure Ca^{2+} oscillations (induced by histamine) over time spans of greater than 30 minutes, approximately three times longer than in cells imaged without CLEM. Moreover, the rate of indicator photobleaching and phototoxicity-induced cell death was greatly reduced in cells protected by WF- CLEM.

We conclude that the WF-CLEM technique is particularly useful for the study of calcium dynamics in neurons over prolonged periods of time with minimal photobleaching. Furthermore, CLEM technique allows researchers to simultaneously study calcium dynamics soma, axon and dendrites due to strongly increased dynamic range.

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