

NOISE REDUCTION IN CONTROLLED LIGHT EXPOSURE MICROSCOPY (CLEM)

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Photodamage (i.e. photobleaching and phototoxicity) are major limitations of fluorescence live-cell microscopy. A straightforward way to limit photodamage is reduction of the light dose for the excitation of fluorophores, but image quality is reduced when the amount of excited fluorophores is restricted. Confocal fluorescence microscopy is based on uniform illumination of the field of view. The principle of CLEM is to illuminate and thus excite fluorophores only as much as needed for imaging and is based on two strategies. The first strategy of CLEM is to use significantly less excitation light by limiting the time of illumination in a pixel via a feed-back system when background is imaged. This strategy not only reduces excitation of the background that is in focus but also and most importantly fluorophores that are out-of-focus. The second strategy of CLEM is to stop illumination when local fluorophore levels in a pixel are high (bright foreground) and a similar acceptable S/N as in the weak foreground is obtained. The resulting image has a lower S/N in the background (without signal) and a S/N in the bright foreground that is similar as the S/N of the weak foreground.

The decisions in the CLEM strategies are based on signal levels at the background decision time for strategy 1 and the bright foreground threshold for strategy 2. S/N of these intermediate signals can be considerably lower than the S/N of the expected signal at the end of the pixel exposure time. A CLEM strategy decision whether a pixel belongs to the background, weak foreground or bright foreground can be incorrect due to noise. In the presentation it will be shown that at a S/N value of 7 or higher the effect of noise on CLEM decisions is low enough to prevent loss of image quality. However, this noise at S/N values of 7 or less due to wrong CLEM decisions can be reduced. First, the previous scanned pixels can be taken into account. Second, a special image processing filter can be used that removes nearly all the extra noise. These two noise reduction steps improve CLEM imaging overall and enables CLEM even for high noise imaging.

References

Controlled light-exposure microscopy reduces photobleaching and phototoxicity in fluorescence live-cell imaging

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Quantitative determination of the reduction of phototoxicity and photobleaching by controlled light exposure microscopy (CLEM)

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