SPATIALLY-CONTROLLED ILLUMINATION COMBINED WITH RESCAN CONFOCAL MICROSCOPY TO REDUCE PHOTODAMAGE AND ENHANCE IMAGE QUALITY.

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KEY WORDS: Spatially-controlled illumination microscopy (SCIM), rescan confocal microscopy (RCM), live-cell fluorescence microscopy, image quality, photodamage

Fluorescence microscopy is an essential tool for live-cell imaging. However, excitation of fluorophores can induce photodamage. Hence, there is an inherent trade-off in fluorescence microscopy between image quality and photodamage. Recently, we introduced Re-scan Confocal Microscopy (RCM) as a new technology with improved signal-to-noise ratio compared to standard confocal microscopy, while reaching the highest possible confocal lateral resolution [1]. Earlier, we demonstrated that spatial control of illumination (SCIM) leads to reduced photodamage [2]. Here, we show that the combination of SCIM and RCM leads to reduced photodamage while preserving the and/or enhancing image quality. Implementation of spatially-controlled illumination in RCM uses a line scanning-based approach. Using information from previously acquired line images, the spatial illumination profiles for the upcoming lines is calculated by a prediction algorithm, during imaging. As a proof-of–principle, we show images comparing standard RCM to SCIM-RCM.

[1] De Luca, Giulia MR, et al. "Re-scan confocal microscopy: scanning twice for better resolution." Biomedical optics express 4.11 (2013): 2644-2656.

[2] Hoebe, R.A., et al. "Controlled light-exposure microscopy reduces photobleaching and phototoxicity in fluorescence live-cell imaging." Nature biotechnology 25.2 (2007): 249-253.