Improving sensitivity and resolution in confocal microscopy.

Giulia De Luca, Ronald Breedijk, Eelco Hoogendoorn, Marten Postma, Erik Manders

Van Leeuwenhoek Centre for Advanced Microscopy, University of Amsterdam

Fluorescence microscopy of living cells has become an instrumentation of major importance in life science. The major limiting factor in live-cell imaging is phototoxicity. The most effective way to reduce phototoxicity is reducing the dose of excitation light. However, reduction of the dose of illumination implies reduction the number of detected photons and consequently reduction of image quality. By spatially controlling the intensity of the excitation light we can strongly reduce the total illumination light dose without compromising image quality. A substantial (5-10 fold) reduction of phototoxicity is achieved by spatially controlled excitation (Hoebe et.al, Nature Biotechnology, 2007).

Currently, we are further developing this technology for wide-field microscopy and super-resolution microscopy. By combination of several techniques such as controlled excitation and Structured Illumination Microscopy (SIM) we will increase fluorescence detection sensitivity in confocal microscopy by a factor of 50 to 100 moreover we will improve the resolution of the confocal microscope by a factor of 2.