SUB-DIFFRACTION RESOLUTION FLUORESCENCE IMAGING OF CELLULAR STRUCTURES WITH STANDARD CONFOCAL MICROSCOPES VIA THREE-PHOTON ABSORPTION OF QUANTUM DOTS

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ABSTRACT

This study improves a recently developed simple technique that allows 3D sub-diffraction imaging based on three-photon absorption of commercially available quantum dots. Quantum dot triexciton imaging (QDTI) utilizes the three-photon absorption ability of CdSe quantum dots (QD655) and the subsequent blue-shifted emission from higher-order excitonic states. This technique has the potential to increase the optical resolution in the axial and lateral direction by approximately 1.7-fold (1). Due to the relatively long lifetime of the QD excited states, this method requires only standard continuous wave lasers and can be easily implemented on any conventional confocal microscope (2).

We applied QDTI in standard confocal fluorescence microscopy to investigate various cellular structures labeled with QD antibodies such as microtubuli (Fig.), lentiviral particles, intermediate filaments in adherent cells and thick multilayered cell clusters. In addition, we adapted a deconvolution algorithm to achieve a further significant improvement in contrast and resolution of the QDTI images.

By careful selection of fluorophores and corresponding excitation wavelengths, we were able to use QDTI in combination with standard green and far-red fluorophores, as well as nuclear stains for multi-color 3D imaging with improved axial and lateral resolution.

In summary, our approach combines imaging of quantum dots by tri-exciton formation at standard confocal microscopes with deconvolution and color multiplexing, resulting in a novel approach for imaging multi-colored samples even of extended axial dimension at a resolution about 2-fold beyond the diffraction limit.