TWO-PHOTON IMAGING AND NANOPROCESSING OF STEM CELLS WITH 12 FEMTOSECOND LASER PULSES

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Human and animal stem cells (rat and human adult pancreatic stem cells, salivary gland stem cells, human dental pulp stem cells) have been investigated by femtosecond laser 5D two-photon microscopy. Autofluorescence and second harmonic generation have been imaged with submicron spatial resolution, 270 ps temporal resolution, and 10 nm spectral resolution. In particular, NADH and flavoprotein fluorescence was detected in stem cell layers and stem cell spheroids. Major emission peaks at 460 nm and 530 nm with typical mean fluorescence lifetimes of 1.8 ns and 2.0 ns, respectively, were measured using spectral imaging and time-correlated single photon counting. Differentiated stem cells produced the extracellular matrix protein collagen which was detected by SHG signals at 435 nm. Nanoprocessing was performed with 12 femtosecond laser pulses and low picojoule pulse energies to realize targeted transfection and optical cleaning of human adut stem cell populations.
Multiphoton sub-20fs microscopes may become novel non-invasive tools for marker-free optical stem cell characterization, for on-line monitoring of differentiation within a three-dimensional microenvironment, and for optical manipulation.