

2-Photon Laser Scanning Microscopy on Native Human Chondrocytes for Tissue Engineering

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Abstract: We used 2-photon laser scanning microscopy (multifocal NIR 2-photon excitation microscope, TRIM-Scope, La Vision Biotec) to characterize native and unlabelled cartilage tissue and biomedical collagen scaffolds via laser-induced autofluorescence and second harmonic generation detection (SHG).

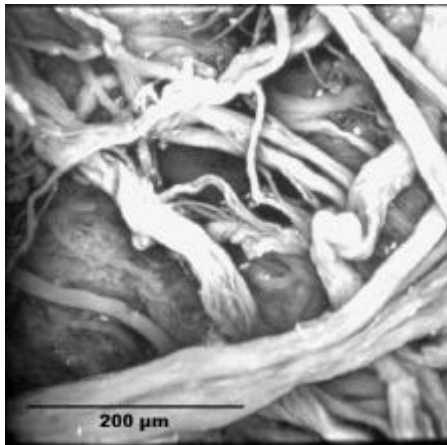
By spectral sensitive colocalization detection the fibrillogenic nature of these strongly scattering tissue materials was resolved and discrimination between extracellular matrix components and chondrocytes was possible.

Our setup guarantees careful treatment of the sample, as it is kept in culture medium during the measurement and as the use of stains is obsolete. In addition, the laser power for the excitation of autofluorescence could be limited to prevent from photodamage of the human chondrocytes.

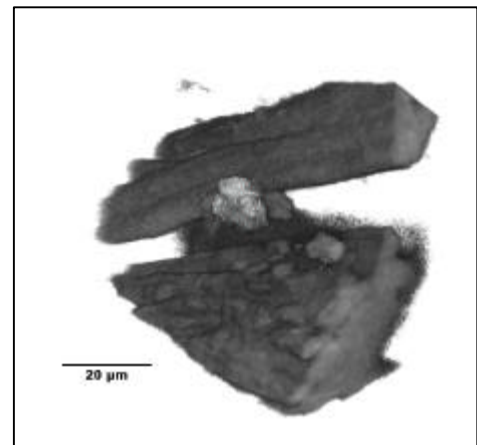
We found SHG on collagenes even at low levels ($< 5\text{mW}$ @ 100 ps pulses and 820 nm).

Therefore it was possible to spectrally identify chondrocytes of the collagen membrane, although their autofluorescence after 2-photon excitation with wavelengths between 780 nm and 860 nm is essentially identical.

Our results indicate that these experimental findings could lead to new microscopical method for on-line monitoring and screening of cell-matrix tissue activities.



(left) Autofluorescence
Image of Collagen
Scaffold (@800 nm)



(right) Chondrocyte on
collagen fibril, digital
reconstruction -
autofluorescence and
SHG signal (@ 820 nm)