

Non-invasive multiphoton imaging as very useful diagnostic technology for cardiovascular research

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In the studies we describe here, a novel application of high-resolution near infrared (NIR) laser scanning microscopy has proved successful in the non-invasive cross-sectional diagnostic analysis of living native and tissue-engineered cardiovascular structures.

Low-energy (< 2 nJ) femtosecond laser pulses in the spectral range of 760 nm to 840 nm, provided by compact, turnkey Ti:sapphire lasers, have been used to analyze the structural features of extracellular matrix (ECM) components, principally collagen and elastin, of human (healthy and pathological), porcine (fresh and cryopreserved), ovine and tissue-engineered heart valves and blood vessels in their natural state, with no need for embedding or staining. The non-linear induced autofluorescence originates from naturally endogenous fluorophores and protein structures such as reduced nicotinamide adenine dinucleotide phosphate (NADPH), flavins, collagen and elastin. Second harmonic generation (SHG) was used to detect collagenous structures.

The quality of the resulting three-dimensional (3-D) images allowed exact differentiation between collagenous and elastic fibers. In addition the samples could be recovered and further experiments performed, including the implantation of the tissue-engineered constructs into an animal. The analysis of heart valve tissues of patients with cardiomyopathy revealed a dramatic loss of SHG, indicating a structural deformation of the collagenous fibers, which was virtually impossible to obtain by routine histological or immunohistological staining. Cryopreserved porcine heart valves showed serious alterations and destruction of the ECM structures compared to the matrix properties of fresh tissues, explicitly verifiable only by NIR laser scanning microscopy.

These experimental results indicate that NIR femtosecond laser scanning microscopy can be employed as a novel non-contact optical technology for 3-D resolved extracellular matrix component imaging and tissue state diagnosis. NIR microscopy provides significant advantages over conventional fluorescence microscopic techniques and should therefore become a favored method in biotechnology, tissue engineering and medicine.