Six-channel intravital imaging of cancer models by multiphoton excitation at 1300nm and beyond

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Simultaneous imaging of multiple fluorescent probes and morphological features by multiphoton microscopy emerged as a vital tool for the study of live tumors in their micro-environment (1). However, deep inside the tissue this method is hampered by scattering, absorption and chromatic aberration between excitation lines used. The reduced scattering and absorption properties of tissue in the spectral window of 1,300 and 1,700 nm in combination with application of high-energy pulsed laser sources can address these limitations, as was originally demonstrated for in vivo imaging of deep layers in the brain (2, 3). Here, we exploited this method to extend the bandwidth for simultaneously detected parameters and to improve the imaging depth during non-invasive, high-resolution, in vivo imaging of melanoma and fibrosarcoma in mouse models. An optical parametric amplifier was pumped with a 20 W fiber laser to generate nJ laser pulses at 1300 or 1650 nm central wavelengths, repetition rates up to 1 MHz and pulse lengths below 90 fs as measured under the objective. Simultaneous excitation of two, three and four-photon fluorescence, second and third harmonics by one laser line resulted in detailed 6-channel images of the tumor and its micro-environment. In comparison to conventional excitation with an optical parametric oscillator, imaging depth in the tumor could be extended to at least 500 µm without comprising cell viability, due to reduced scattering and absorption, improved signal to background ratio of detected three-photon processes and increased dynamic range of the excitation power. Imaging speed and signal to noise ratio are limiting factors but can be improved by enhanced laser repetition rates, reduced pulse length and tuning of the biological model. Our results demonstrate the potential benefits of these unconventional excitation light sources for multi-parametric intravital imaging of cancer models.

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