HIGH-SPEED 2-PHOTON MICROSCOPY WITH ISOTROPIC RESOLUTION

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A new technique for high-speed and very gentle 2-photon laser-scanning microscopy with isotropic optical resolution is presented. It is based on an adaptive temporal and spatial beam splitter that converts the excitation beam into eight individual beams that are recombined and either focused to a single spot or flexibly distributed in space. If a PMT is used for detection the foci pattern acts like a single enlarged spot. At the cost of a slightly reduced lateral resolution the fluorescence yield and therefore the imaging speed can significantly be increased and photodamage is reduced while the signal-to-noise ratio is maintained. As the lateral resolution in laser-scanning microscopy is anyway higher than the axial this approach can be used to create isotropic resolution. For photo treatment applications this greatly enhances the efficiency as the pattern of foci can be adapted to the structure of interest: Complete cells or cell compartments can be treated simultaneously. Results for pure temporal and spatio-temporal multiplexing and comparison to conventional single-beam multi-photon microscopy will be presented in different imaging and line-scanning modalities.