

# NONLINEAR MICROSCOPY USING BROADBAND LIGHT FROM PHOTONIC CRYSTAL FIBER

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Nonlinear microscopy based on femtosecond lasers including two-photon microscopy, second harmonic generation microscopy, and coherent anti-Stokes Raman scattering (CARS) microscopy, has become an important tool for the investigation of biological phenomena. Most researchers use near-infrared Ti:sapphire lasers, however, spectral width of the laser pulses are limited. By using photonic crystal fibers (PCFs), the generation of broadband supercontinuum spectra that span more than an octave has been demonstrated with low-peak-power pulses from Ti:sapphire oscillators. Several applications of the supercontinuum in the visible and infrared have been demonstrated, such as coherent anti-Stokes Raman scattering [1-3], and confocal microscopy [4-7].

We present the feasibility of supercontinuum as a light source of nonlinear microscopy including CARS and two-photon fluorescence microscopy. Figure 1 shows a schematic diagram of the system used for supercontinuum generation. A mode-locked Ti:sapphire laser produced 65-fs pulses and a repetition rate of 82 MHz. The central wavelength was varied around 800 nm. The laser pulses passed through the Faraday isolator to block reflections from the PCF facet and were coupled into the core of a PCF using a 50× microscope objective (OB1) with a numerical aperture (NA) of 0.55. Before coupling, the laser pulses passed through an SF10 prism sequence to pre-compensate for the dispersion of the OB1 and the Faraday isolator, and the pulse duration at the focal point of the OB1 was thus minimized. We demonstrate that we can obtain desired spectrum of supercontinuum by varying parameters of the PCF and incident laser pulses. By use of wavelength-tunable light from the PCF, we show the tunable CARS microscopy with a simple light source. Moreover, we demonstrate multi-spectral two-photon fluorescence microscopy by use of the PCF. The broadband supercontinuum makes it possible to excite various fluorophores with optimal wavelengths at the same time. We demonstrate that multi-spectral two-photon fluorescence images can be simultaneously acquired from organelles in a cell using the near-infrared region of the supercontinuum [7].

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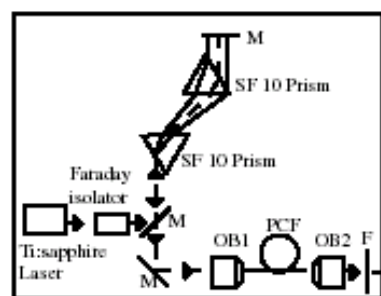


Figure 1: The experimental setup for generation of supercontinuum using a photonic crystal fiber: M, mirrors; OB, objectives; DM, dichroic mirrors; F, filter;