Enrico Gratton, Peter So, Todd French, Weiming Yu, Laboratory for Fluorescence Dynamics, University of Illinois at Urbana Champaign, Illinois, USA: "A Two-Photon Lifetime Resolved Fluorescence Microscope."

A two-photon scanning fluorescence microscope with submicron section capability has been built using a Coherent Mira Titanium-Sapphire laser. The microscope is built around a Zeiss M35 inverted microscope. The scanning system is from Cambridge Technologies. The z-axis control is home built using a stepper motor with electronic feed-back control to give an accuracy of 100 nm in the z direction. For lifetime imaging, the frequency domain technique was adopted. The Ti:Sa laser is used to synchronize the electronics composed of two frequency synthesizers and a 12-bit digital acquisition card. At every pixel the phase and modulation of the fluorescence emission is calculated with respect to the intensity modulated excitation. In intensity mode, the frame speed is limited by the scanner speed, and generally it is of the order of one second for a 256×256 pixel frame. In lifetime acquisition mode, the frame rate is about a factor of four slower. Frame integration is possible. The histogram of phase values on a reference flat image has a dispersion of about 1 degree. At a modulation frequency of 80 MHz, this phase dispersion corresponds to subnanosecond lifetime resolution. Several biological systems have been investigated and the results are presented.