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Ultra-Deep Imaging with Cellular Resolution: Enhanced Two-Photon Fluorescence Microscopy with the Use of a Wide Area Photodetector

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We have previously shown that the use of a wide photocathode area PMT as a detector in a two-photon fluorescence microscope allowed us to image in turbid samples up to the depth of about 2.5 mm with cellular resolution. This detection scheme enables a very efficient collection of fluorescence photons directly from the wide (1" diameter) area of the sample, which considerably increases the detection system sensitivity in comparison to a traditional two-photon microscope, where fluorescence is collected by the same objective lens used for excitation. Because the imaging depth depends on the ability of the system to sense weak fluorescent signals, this new detection method significantly enhances the imaging depth. We have recently built a new experimental system that works in the upright configuration, which is best suited for experiments on live animals. The system employs a high power Ti:Sa Mai Tai laser with a group velocity dispersion compensator (DeepSee) for two-photon fluorescence excitation that allows us to extend the imaging depth to 3mm in samples simulating brain tissue optical properties. Imaging experiments in vivo and in vitro have also been conducted on live animals (mice) and tissues (skin, colon, small intestine).

With the aid of the new high speed response PMT that we are currently incorporating in the system, we will be able to perform fluorescence lifetime imaging microscopy (FLIM) on whole animals and tissue samples at a few mm depth. This double feature will particularly aid in vivo neuron imaging.

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