Executive Summary

Enhancing Imaging Resolution and Depth

With Adaptive Optics Focal Modulation Two-Photon Microscopy

Optical microscopy has been a critical tool for scientific discoveries ever since it was invented. In 2014, three scientists won the Nobel Prize for their superior work in the development of optical microscopy for bio-imaging. Biological tissues quite often appear opaque and are subject to strong scattering and absorption. Imaging the detailed structure of these tissues is similar to visualizing a building number through fog. A novel method was developed in this research to remove the “fog” and “unwanted light from street lamps a couple of doors away”.

When a spatial time-variant phase modulation was applied to the illumination light, the desired signal from tissue structures oscillates with the input signal, but the background scattering (“fog”) does not. A fast algorithm was developed to separate the oscillatory signal from the background. Adaptive optics removed the system aberration so that illumination light can be brought to a tight focus. Only photons from the very small focal region can be detected, which substantially increases image sharpness. Special phase patterns were applied to the illumination light so that both out-of-focus and near-focus fluorescent backgrounds (“street lamps”) are suppressed.

The method of Adaptive Optics Focal Modulation Two-Photon Microscopy was developed and demonstrated experimentally by imaging fluorescent microbeads. The measurement results showed that the signal-to-noise ratio was improved by 7 dB and the image resolution was doubled at a depth of 500 micrometers. This novel method significantly increased the imaging resolution, contrast, and depth for thick tissues.