

Technology Offer

Multi-Channel Time-Multiplexed Multiphoton Microscope

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Abstract

The invention relates to an advanced multiphoton microscope and microscopy method designed for high-resolution, multi-depth imaging in biological samples. By distributing excitation light pulses across multiple optical channels and focal spots, the system enables temporally separated irradiation and detection of signals from different spatial regions. This approach overcomes limitations of conventional multiphoton microscopy, such as restricted imaging depth and temporal resolution. The technology integrates optical fibers, beam splitters, and scanning mechanisms to achieve flexible spatial and temporal control of excitation. Particularly suited for neuroscience and live imaging, the invention allows simultaneous or rapid sequential observation of multiple layers within a sample. Its miniaturizable design further enables use in compact or head-mounted microscopes for in vivo applications.

Background

Multiphoton microscopy has become a critical tool for imaging biological tissues, especially for monitoring neuronal activity in vivo. It provides non-invasive optical sectioning and enables imaging at greater depths compared to conventional microscopy. However, existing systems face challenges related to limited imaging depth, constrained temporal resolution, and trade-offs when imaging multiple planes. Techniques such as sequential scanning reduce temporal resolution, while simultaneous multi-plane imaging often requires bulky setups that are difficult to miniaturize. Additionally, optical losses and complexity increase when splitting laser pulses or introducing delay lines. Current systems struggle to efficiently image multiple depths without sacrificing speed or compactness. Therefore, there is a strong need for a microscopy solution that enables multi-plane imaging with high temporal resolution while remaining suitable for miniaturized and flexible implementations.

Technology

The proposed multiphoton microscope is based on a multi-channel excitation architecture that enables controlled delivery of light pulses to multiple focal spots within a sample. The system uses a light source, typically a pulsed laser, whose output is directed into an excitation beam path and distributed across several excitation channels via a dedicated distribution unit. Each channel includes an optical fiber terminating at an exit end, where light is focused into a distinct spatial position within the sample. The spatial arrangement of these fiber ends determines the relative positioning of the focal spots.

A central aspect of the invention is the temporal sequencing of excitation. Instead of illuminating all simultaneously, the system irradiates focal spots sequentially by directing pulses into different channels in a time-controlled manner. This ensures that fluorescence or emission signals generated at each focal spot are temporally separated and can be distinguished during detection. High-speed detectors capture the emitted signals, which are then de-multiplexed based on their arrival times.

The system allows different implementation strategies, including energy splitting of pulses into multiple channels or directing entire pulses into individual channels using fast optical switches. Additional flexibility is achieved through optional delay elements, wavelength multiplexing, and scanning components that enable spatial variation of the excitation

region. The overall design supports compact integration, including miniaturized microscope heads suitable for portable or in vivo applications.

Advantages

- Enables multi-plane imaging with high temporal resolution via sequential excitation and signal de-multiplexing.
- Reduces system size by eliminating long optical delay lines required in conventional approaches.
- Provides flexible excitation control through energy or time-based pulse distribution across channels.
- Improves signal separation and minimizes cross-talk using nanosecond-scale temporal discrimination.
- Compatible with miniaturized and head-mounted microscope designs for in vivo imaging applications.

Potential applications

- In vivo neuronal activity imaging in freely moving animals using compact head-mounted microscopes.
- High-resolution multi-depth imaging in biological and biomedical research laboratories.
- Functional imaging of layered tissues such as brain cortex or organoid structures.
- Advanced fluorescence microscopy with multi-wavelength excitation for multiplexed labeling studies.
- Portable diagnostic imaging systems requiring compact, high-performance optical sectioning capabilities.

Patent Information

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