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Random-Access, High Speed Confocal Microscope for Functional Imaging

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Technology description

Development of a Novel Confocal Laser Scanning Microscope for Functional Recording of Fast Neuronal Activity

Dendrites of neurons in the central nervous system are the principal sites for excitatory synaptic input. These neuronal dendrites are known to possess active computational properties also. Currently, these properties are mainly studied using well-known patch clamp techniques. However, the small number of sites that can be simultaneously accessed limits this approach. The inventors have developed an imaging system that allows for high-speed, multi-site optical recording from single neurons in living brain tissue by combining acousto-optic (AO) beam deflection with addressable spatial filtering. This combination allows for fast random-access positioning (<20ms) of both the point illumination and point detection necessary for confocal laser scanning microscopy (CLSM). CLSM is a widely used method for studying the structure and function of light scattering tissue such as living nerve cells in the brain.

Conventional CLSM relies on point illumination of the target followed by spatial filtering of the returning fluorescence with a physical pinhole located in an image plane to obstruct scattered light. By raster-scanning a preparation, fluorescence can be collected on a point-by-point basis and a complete image can be reconstructed. Typically, the illumination spot is scanned over the target using galvanometer-driven mirrors. However, due to their inertia, these mirrors scan relatively slowly and can only record several frames per second. Other confocal microscopes rely on scanning multiple illumination spots to decrease the scan time; however, these systems must use an imaging detector, which ultimately limits the acquisition speed to several hundred frames per second. All of these methods are unsuitable for studying fast neuronal activity, which requires capturing several thousand frames per second.

To overcome this speed barrier, the inventors have previously developed a nonconfocal laser-scanning microscope that allows the illumination spot to be scanned inertia-free, using AO deflectors. Because there are no moving parts in these deflectors, the system permits fast random-access laser positioning (<10ms). A critical benefit of random-access beam positioning is that it allows the inventors to only record from user-specified sites-of-interest (SOIs) rather than the entire preparation. By concentrating on SOIs, they can use the available scan time within one frame much more efficiently by ignoring regions of the preparation that do not contain any neuronal structures. This is highly desirable because

it allows them to further increase the effective frame rate without sacrificing the dwell time that is necessary to maintain an adequate signal-to-noise ratio. This nonconfocal system is presently being used in the lab for multi-site recording in neuron cultures with voltage-sensitive and calcium-sensitive fluorescent indicators. To expand this method from neuron cultures to brain slices, it is necessary to use confocal microscopy techniques.
Unfortunately, AO deflection and CLSM are not straightforwardly combined. In a conventional CLSM, the scanning mirrors effectively position both the excitation and fluorescence light. Therefore, fluorescence becomes ï¿½descannedï¿½ and a stationary pinhole can be used after a dichroic mirror separates the emission from the excitation light. With AO deflection, which relies on diffraction, there is a wavelength dependence that prevents efficient positioning of the relatively wide bandwidth fluorescence. To eliminate the need for descanning, the inventors have developed the concept of using an addressable pinhole that is spatially and temporally synchronized with the scanning excitation spot. Furthermore, the particular devices that they use as the addressable pinhole do not have macroscopic moving parts that would slow down the system due to inertia. Two different electronic devices are currently being used as the addressable pinhole: a random-access CMOS detector array and a digital micromirror device (DMD) combined with a single photodetector. The CMOS imager can directly and non-destructively read any of its pixels at high speeds. Its addressability allows the inventors to specify frames composed of several non-contiguous spots, which can then be imaged at very high rates. The DMD is an array of small mirrors (16mm x 16mm), which can be tilted to two different positions thereby directing fluorescence toward or away from a single photodetector. Like the CMOS imager, this device is also capable of studying several sites quasi-simultaneously since the flip time of the mirrors is very fast (~20ms). Spatial filtering is accomplished in both cases by turning one pixel or a small group of pixels on while all neighboring pixels are off. The CMOS imager is advantageous due to the simplicity of the optical pathway since it acts as both the pinhole and detector. The DMD is more useful in low-light situations where a high sensitivity detector is needed to collect fluorescence.
The inventors have been focusing their efforts on the DMD aspect of the apparatus.

Advantages

Currently, they can make fast confocal recordings at a rate that is suitable for recording neuronal activity at several SOIs. The effective frame rate for an arbitrary number of user-selected SOIs is given by: Frame rate = 25,000 Hz/(SOIs); (for SOIs>1). For the limiting case of one SOI, the illumination does not have to be scanned so the fluorescence collection is only dependent of the speed of analog-to-digital conversion of the system. The system is capable of true random-access scanning so the distance between any two consecutive SOIs is of no consequence. This allows the system to adapt between recording from a few SOIs at a fast frame rate, or at many SOIs at slower frame rates.

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