Simultaneous Multi-Region Non-Linear Microscopy Using Spectral Encoding

Technology #2884

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Non-Linear Microscopy can be Too Slow to Capture Multiple, Rapid, Dynamic, and Coupled Events Non-linear microscopy is a powerful tool for in-vivo imaging of structural and functional characteristics of materials including biological tissues by measuring the distribution of fluorescent molecules, or structures exhibiting second or third harmonic generation (SHG/THG). In non-linear microscopy, a laser is focused to excite fluorophores or SHG at specific 3D locations in the tissue sample. By systematically moving the focus point around the sample, 2D or 3D images of fluorescence or SHG/THG contrast can be constructed. Point by point scanning utilized in existing systems is rate-limiting, yet until now has been necessary to obtain optimal depth penetration and sectioning. Rapid scanning of multiple planes is currently implemented by moving the sample or objective in and out of the depth plane at high frequencies, which can disturb the underlying processes being measured, and is still speed-limited by the need to sequentially visit every point in both planes. As a result, existing methods are too slow to capture multiple rapid, dynamic, and coupled events such as intercellular interactions at different 3D locations (e.g. calcium influx of neurons and changes in blood vessel diameter and blood flow). This technology allows for simultaneous imaging of multiple regions of a tissue exactly simultaneously, including imaging multiple depth planes in parallel, regions with differing magnifications, and even imaging of two regions at different frame rates.

Non-Linear Microscopy Technology Achieves Simultaneous, Fast Acquisition Multiple Regions Within Sample

The invention allows non-linear microscopy to achieve simultaneous fast acquisition multiple regions within a sample. To achieve this, multiple incident beams of light, each with a different wavelength are independently focused into and steered within the sample. In the case of fluorescence imaging, these beams excite fluorophores such that specific mixtures of fluorophores yield unique spectral emissions for given excitation beams. A detector system with at least as many spectrally resolved emission channels acquires emerging fluorescence light, and a spectral unmixing approach is used to differentiate emissions originating from each excitation beam, thereby isolating signal from simultaneously illuminated but spatially separated locations. By adjusting the collimation properties of incoming beams, the different regions can be located at different depths within the sample allowing for simultaneous sampling of multiple locations in a 3D volume at high speed. For SHG/THG greater levels of multiplexing can be achieved, since emissions will be narrow-band and at exactly half of the excitation wavelength allowing the possibility of almost continuum sampling of a 3D volume with spectrally resolved simultaneous detection.

Applications:
- Acquire multiple planar images simultaneously at multiple depths
- Improve acquisition speed by scanning simultaneously at multiple locations
- Correlate temporal processes occurring at different depth levels or locations
- Neuroscience applications – monitor change in blood flow at the surface layer while observing changes in intracellular calcium of neurons in deeper layers
– High speed 3D THG imaging of embryogenesis

Advantages:
– Simultaneous acquisition at different depths circumvents the problem of moving the platform or objective
– Significantly improved acquisition speed of acquiring 3D data – two illumination wavelengths will double the effective acquisition speed of a typical system
– Measure concurrent processes at high frame rates and time scales exactly simultaneously.


Licensing Status: Available for Licensing or Sponsored Research Support


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