Abstract:

We demonstrate a novel line scanning multiphoton microscope with a single element detector, potentially allowing fast imaging deep into scattering tissue. Second harmonic imaging of ex vivo rat tendon is presented.

Summary of Research:

Point scanning multiphoton microscopy (MPM) is widely used for optical sectioning deep into scattering tissue since nonlinear optical processes are confined to the focal volume of the microscope [1]. The imaging acquisition speed of point scanning MPM, however, is typically slow and fundamentally limited by the maximum fluorescence generation rate, i.e., fluorescence saturation. Current technologies for fast imaging are based on parallel excitation of multiple pixels in space such as line-scanning microscopy (LSM) [2] and multifoci multiphoton microscopy (MMM) [3,4]. While both methods are satisfactory in a neat sample or a thin slice of tissue, the signal emitted from different resolution volumes will be completely mixed due to scattering, a well-known image smearing problem when strong scattering is present. Thus, none of the existing fast imaging techniques is compatible with optical sectioning deep in scattering tissues.

A new approach is required to provide point-resolved imaging in a LSM or MMM without imaging the signal photon. Here, we demonstrate line scanning MPM without imaging the excited nonlinear signal. This is accomplished by modulating each point in our sample at a different frequency, collecting all the nonlinear signal emitted by the sample onto a single element detector (PMT), and demodulating the signal to reconstruct the image.

The experimental set-up is described in Figure 1. A mode-locked Ti:sapphire laser is used as the excitation source ($\lambda = 780$ nm, approximately 100 fs pulse width, and 80 MHz repetition rate). We first create a focused line illumination using a cylindrical lens (CL). This line illumination then impinges onto a one-dimensional spatial light modulator (SLM), generating a linear array of point sources, with different point sources modulated by different frequencies. The excited nonlinear signal is epi-collected through the objective and reflected off a dichroic mirror onto a large area photomultiplier tube (PMT) detector.
(Hamamatsu R7600U-200). The detected signal is then processed as a spectrogram to reconstruct the image: the y-axis is proportional to RF modulation frequency, x-axis is the time during the line scan, and the intensity of the pixels is the amount of power in the RF spectrum at a given time during the line scan.

High modulation rates are required (~ 1 MHz) to resolve distinct points along the line. Since commercially available linear SLMs cannot modulate at such speeds, we created a dispersion-free, polarization independent free-space optical chopper (Figure 1a, dashed box) that can modulate an array of point sources at MHz rates by scanning a focused laser beam over a small (~ 10 µm period) mirror grating on a photolithography mask. Each horizontal line on the photolithography mask had a different spatial frequency. The reflected light is then descanned by the same scan mirror, and is imaged onto the sample by the line scanning microscope.

The concept of the modulation microscope is demonstrated by imaging a 1951 USAF Resolution Test Chart in transmission mode. The transmitted light signal is collected by a biased silicon photodiode with a 3.6 mm × 3.6 mm active area.

The processed image is shown in Figure 2. The modulation frequencies are between 350 kHz and 650 kHz and the scan is over 0.5 s. The frame is approximately 230 × 300 pixels. The feasibility of multiphoton LSM with a single element detector is clearly demonstrated by imaging the intrinsic second harmonic generation (SHG) from tendons extracted from the tail of a rat ex vivo (Figure 3). Excited nonlinear signal is epi-collected through the objective and detected by a PMT. This technique can also be extended by parallel acquisition of data for fluorescence lifetime imaging microscopy, significantly increasing frame rates for FLIM imaging of long lifetime dyes.

We demonstrated a novel line scanning MPM with a single element detector. By avoiding the need for imaging the signal photons, our technique opens the possibility for fast imaging deep into scattering tissue.

References: