Novel equipment setup simplifies nonlinear interferometric microscopy

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The interference of two nonlinear optical signals generated in series can improve image quality in coherent anti-Stokes Raman scattering microscopy.

Improving image contrast is a major issue in optical microscopy. According to Abbe’s diffraction theory, the sample acts as a diffraction grating, refracting the incident light such that not all of it reaches the lens. This puts a limit on a spatial resolution.

Coherent anti-Stokes Raman scattering (CARS) microscopy is a nonlinear optical imaging technique in which pump and Stokes laser beams at different frequencies interact with a sample to produce an anti-Stokes (CARS) signal. This signal is strong when the frequency difference of the lasers corresponds to a Raman-active molecular vibration. CARS is significant because of its ability to reveal the invisible in transparent biomedical specimens without staining. The molecularly sensitive CARS process can produce images with strong contrast that chemically distinguish a specimen from its background. Nevertheless, signal strength must be increased for this technique to be sufficiently sensitive to small amounts of target materials.

Another useful technique, optical coherence tomography (OCT), is based on optical interferometry. In OCT, very weak scattering light is coherence gated in a low-coherence interferometer, meaning that only light that has traveled a distance less than the coherence length contributes to the interference. Homodyne amplification makes this signal much more pronounced. Since the CARS signal that results from a parametric process is coherent, like laser radiation, it can also benefit from signal amplification via interferometry. Previous CARS interferometers used conventional setups in which the incident fundamental beams (pump and Stokes) split into two interferometer arms to generate two independent CARS signals: one from the sample; and one from the reference, which acts as a local oscillator field.

The two CARS signals then recombine with some relative phase to produce interference.

As shown in Figure 1, we have set up a different configuration that can be adopted only for parametric nonlinear optical waves. With one set of incident pump and Stokes beams, CARS signals are generated twice successively in two media placed in series along the beam propagation direction. The relative phases of the CARS signals are controlled by a phase-shifting unit (PSU) between the two samples. Our PSU consists of a pair of BK7 glass wedges of the same slope. One of wedges is fixed, and the other can slide on it to change the overall thickness.

The CARS signal generated at the first medium propagates with the fundamental waves through several dispersive materials. These include lenses and the PSU, inside which the fundamental and CARS signal waves travel with different phase velocities. As such, the CARS signal generated at the second medium will be a different phase from that of the first; this phase difference can be adjusted by changing the thickness of the PSU and thus the path lengths. In this kind of interferometer, a bulky optical setup for splitting beams followed by re-

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Figure 2. The interference fringes of two CARS signals are measured as a function of PSU thickness.

We measured the interference fringes of two CARS signals generated at two different points, as shown in Figure 2. The plot of photomultiplier tube output voltages as a function of PSU thickness clearly shows constructive and destructive interference of the two signals. The coherence length is 520 µm, a period of sinusoidal curve.

To produce CARS images of various samples, a scanning galvano mirror system performed raster scans of the laser beams on the samples at the second focal plane. We can obtain 512 × 512 pixel CARS images at a speed of one frame per second. Figures 3(a) and 3(b) show two different CARS images obtained as the laser beams are scanned on 3 µm diameter polystyrene beads distributed over a microscope coverslip. Figure 3(a) was measured without the first glass medium to show a noninterferometric standard CARS image. Figure 3(b) is the interferometric image with the glass slab in place and constructive interference. The beads can be distinguished from the background more clearly because the image contrast has increased. It should be emphasized that the incident laser power has not changed. The image quality improves simply by coherently adding another CARS signal.

Our experimental setup is an easy implementation of interferometric imaging. It is clearly capable of improving image quality in a more convenient and sturdy manner than conventional interferometers. The setup could be adopted for high-performance CARS microscope systems in which molecularly sensitive images can be obtained with much higher contrast. Essentially, different molecules vibrate at different frequencies, and you can measure any change in vibrational state. In CARS, the frequency difference between the pump and Stokes beams is set to a vibrational energy change of the molecule of interest. Doing this increases the CARS signal from that molecule as compared to others, and so should allow us to tune the microscope to focus on any of the molecules in a sample by changing the laser-beam frequencies.

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References