Improving contrast of the nonlinear optical microscope

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Focus engineering of coherent anti-Stokes Raman-scattering microscopy enhances biomedical imaging capabilities.

Coherent anti-Stokes Raman-scattering (CARS) microscopy, a nonlinear optical-imaging technique, has attracted the attention of researchers in biomedical imaging. Its popularity is unsurprising given its chemically selective 3D imaging at high resolution with no need for fluorescent labels. CARS microscopy is so fascinating because its optical signal is coherent, which suggests that coherent manipulation techniques developed for ultrafast optical spectroscopy can, in principle, be used to optimize the signals. This makes possible additional controls for improving image contrast that are unavailable in fluorescence microscopy, for instance.

Like techniques developed for ultrafast spectroscopy, many coherent manipulation techniques in CARS are based on shaping the spectral amplitude and phase. This enables higher spectral resolution as well as the ability to selectively probe narrowband vibrational resonances using broadband pulses. The spatial amplitude and phase can be shaped through manipulation of the incident-beam profiles. This approach gives direct control over the phase where the focal molecules are driven into vibrational coherence. Moreover, beam shaping can drive different portions of the focal volume at different phases, offering control over the destructive and constructive interference between CARS-signal waves emitted from distinct focal-spot compartments. In short, this technique provides direct tuning for spatial phase matching of CARS radiation emitted from the focal spot.

Spatial phase shaping (or focus engineering) adds new contrast mechanisms to the CARS-microscopy menu. One example is the generation of differential contrast, which provides background-free images and highlights only the edges between objects. This can be achieved by dressing the incident beam (the Stokes beam) with a sharp transversal phase step, resulting in a beam mode reminiscent of a Hermite Gaussian-01 (HG01) profile (see Figure 1). When this technique is used to drive CARS, it carries the same transversal phase step across the focal volume: see Figures 1(b) and (c).

Under these conditions, the molecules on one side of the focal volume radiate out of phase with their peers on the other

Figure 1. Alternative beam profiles used in focus-engineered anti-Stokes Raman-scattering (FE-CARS) microscopy. (a) Stokes focal amplitude of an Hermite Gaussian-01 (HG01) beam mode, (b) corresponding Stokes focal phase, (c) resulting amplitude for the effective CARS-driving field near the focal volume when using a Stokes beam with a HG01 beam mode, and (d) corresponding spatial phase of the focal driving field.

Figure 2. CARS radiation pattern for (a) regular incident fields and (b) an HG01-engineered focal-driving field. Note that the CARS radiation is phase mismatched along the original propagation axis for the HG01-driving field.

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Figure 3. CARS imaging of mouse connective tissue in the CH-stretching region of CH$_3$ molecules at 2911/cm. (a) Regular CARS image, (b) FE-CARS differential image, and (c) two-photon excited-fluorescence image of the same region, showing elastin fibers.

side. The result is no observed radiation along the propagation axis: see Figure 2(b). Instead, the radiation propagates at angles pointed away from the original propagation axis, effectively changing the phase matching of the CARS radiation. Because the intensity is significantly depleted along the original propagation axis, no signal will be detected from a bulk sample, implying that the nonresonant background radiation is suppressed substantially.

Importantly, whenever a vibrationally resonant object occupies one side of the focus-engineered CARS (FE-CARS) focal volume, the spectral phase shift can outbalance its spatial counterpart, which produces phase-matched radiation from both focal halves on the original propagation axis. This yields an image that highlights lateral interfaces while suppressing the bulk signal.$^7,^8$

This differential-contrast technique is useful in biomedical imaging as it offers a sharper view. For example, where mouse connective tissue is visualized with CH$_3$ vibrational contrast we expect to see CH$_3$-rich structural fibers (see Figure 3). However, the CARS signal is dominated by nonresonant background contributions: see Figure 3(a). However, when the FE-CARS phase shaper is switched on, contrast is improved because only the vibrationally resonant fibers are visible: see Figure 3(b). For comparison we show a two-photon excited-fluorescence image of elastin in Figure 3(c).

Focus-engineering in coherent microscopy is still in its infancy but it holds great promise for providing additional contrast mechanisms in nonlinear imaging. The imaging capabilities offered by FE-CARS are not limited to generating differential contrast. One particularly attractive possibility is the use of these techniques for enhancing the effective resolution of the CARS microscope. Unlike fluorescence microscopy, CARS benefits from the presence of an adjustable phase knob, which provides additional control over the focal volume beyond amplitude shaping alone. Clever use of this phase knob may help reduce the volume from which CARS emission is observed, offering improved contrast resolution.

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