

Single-Beam CARS Imaging for Reacting Flow Diagnostics

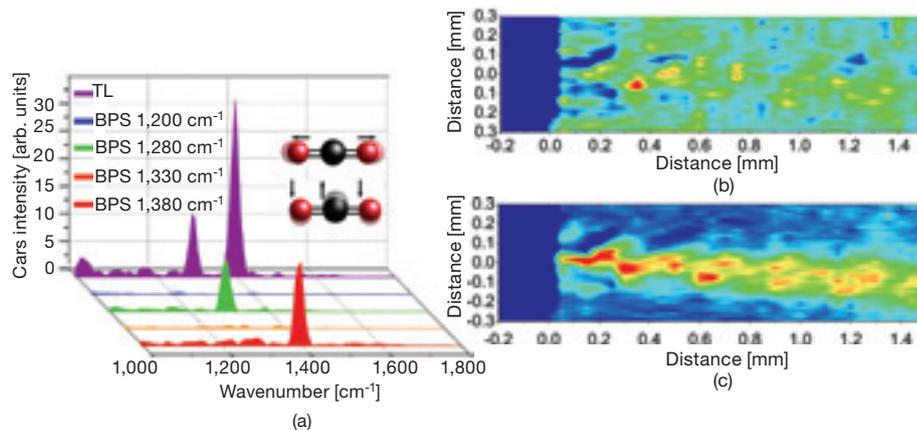
Paul J. Wrzesinski, Dmitry Pestov, Vadim V. Lozovoy, Suresh Roy, James R. Gord and Marcos Dantus

Coherent anti-Stokes Raman Scattering (CARS) is a long-standing diagnostic technique of choice in the combustion community for measuring temperature and major species concentration in gas-phase-reacting flows. Additionally, the coherent excitation of Raman-active molecular vibrations offers intrinsic chemical specificity and high spatial resolution.

The application of traditional nanosecond and picosecond CARS is hindered by its cumbersome experimental implementation, requiring multiple laser beams that are spatially and temporally overlapped in a complex beam geometry. However, advances in laser and pulse-shaping technologies have made it possible to measure the CARS signal using a single laser beam.¹ The use of supercontinuum sources allows for acquisition of an entire Raman spectrum without wavelength scanning or changes to experimental parameters.

In single-beam CARS, researchers use spectrally broad, ultrashort pulses that contain the pump, Stokes, and probe components. In our work, sub-10 fs amplified pulses are capable of impulsively exciting Raman transitions with wavenumbers up to 2,500 cm^{-1} range.^{2,3} A narrow region on the high-frequency side of the spectrum is selected as the probe. Its polarization is rotated by 90° and a π -phase step is applied to minimize the nonresonant contribution due to the instantaneous electronic response. Additional phase-modulation techniques can be used to selectively excite a single vibrational mode. More importantly, mode-selective phase modulation allows for the elimination of multichannel detection. Single-channel acquisition is desirable for imaging applications that require high-speed beam scanning.

One proven method for mode-selective excitation is the use of pseudo-



Selective single-beam CARS: (a) CARS spectra of pure CO₂ gas in a cell for a set of phase functions targeting Raman transitions at various wave numbers. Excitation selectivity is accomplished using BPS. (b) Image of a CO₂ jet taken without mode-selective excitation. The CARS signal is integrated over 300–2,500 cm^{-1} range. (c) Image of the same CO₂ jet with the selective excitation of the symmetric stretch (1,280 cm^{-1} band).

random binary sequences.⁴ We have applied binary phase shaping (BPS) for mode-selective CARS in liquids and, more recently, in gas-phase samples.^{2,5} We have demonstrated independent excitation of CO₂ Fermi dyads and nearly complete suppression of the nonresonant four-wave mixing, as illustrated in (a).

To highlight the advantages of selective Raman excitation for species imaging, we have focused the beam of shaped pulses into a jet of CO₂ flowing from a rectangular nozzle mounted on a motorized 2-D translation stage. The spectrally integrated CARS signal is collected at each XY position of the stage. Two different pulse shaping methods are used: (i) impulsive excitation for all Raman-active vibrational modes using transform limited pulses and (ii) selective excitation of the symmetric stretch of CO₂ at 1280 cm^{-1} using BPS.

The image obtained using impulsive excitation (b) exhibits poor contrast due to the resonant contribution from N₂

and O₂ molecules, abundant in ambient air, and the nonresonant response that dominates the low-frequency part of the integrated CARS spectrum. The second phase shaping approach (c) has the advantage of suppressing excitation at Raman frequencies other than the target frequency as well as nearly complete elimination of the nonresonant background. It provides high imaging contrast for the jet of CO₂ gas relative to ambient air. Furthermore, static turbulent modulation of the CO₂ flow is clearly visible. Δ

Paul J. Wrzesinski (paul.wrzesinski@gmail.com), Dmitry Pestov, Vadim V. Lozovoy and Marcos Dantus are with the department of chemistry, Michigan State University, East Lansing, Mich., U.S.A. Suresh Roy is with Spectral Energies, and James R. Gord is with the Air Force Research Laboratory Propulsion Directorate.

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Snapshot Image-Mapping Spectrometer for Hyperspectral Fluorescence Microscopy

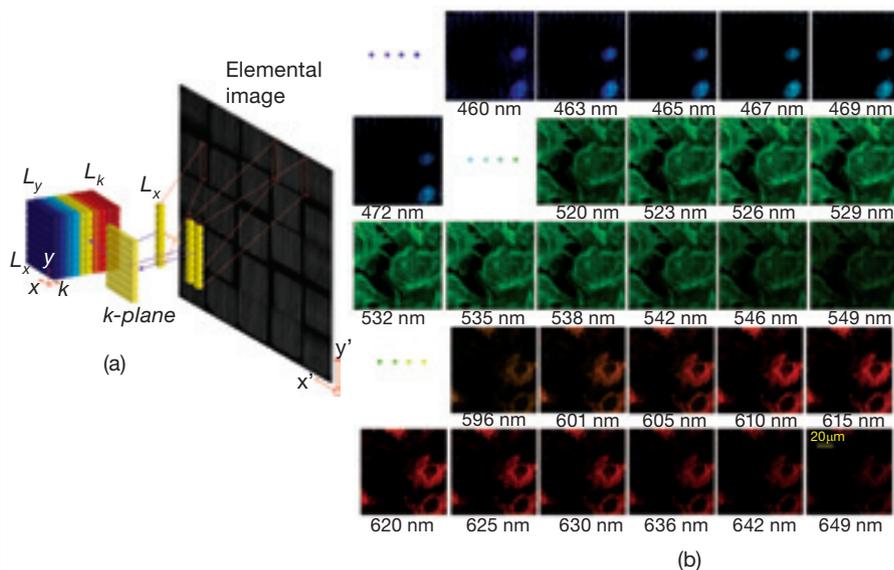
Liang Gao, Robert T. Kester, Nathan Hagen
and Tomasz S. Tkaczyk

We recently developed a snapshot hyperspectral imaging device called the image-mapping spectrometer (IMS) for dynamic cellular imaging applications.^{1,2} The IMS replaces the camera in a digital imaging system, allowing one to add high-speed snapshot spectrum acquisition capability to a variety of macroscopic imaging modalities—e.g., microscopy, endoscopy, etc.—to maximize collection speed.

The motivation to develop a snapshot hyperspectral imaging technique comes from the high temporal resolution requirement in time-resolved multiplexed fluorescence imaging.³ Conventional instruments acquire hyperspectral datacubes (x, y, λ) through scanning, either in the spatial domain (as in confocal laser scanning microscopes) or in the spectral domain (using acousto-optic or liquid-crystal tunable filters). Because scanning instruments cannot collect light from all elements of the dataset in parallel, there is a loss of light throughput by a factor of N when measuring N spectral channels.

To some extent, one could compensate for this by increasing the intensity of illumination, such as with the high-power lasers used in confocal microscopes, but this produces photobleaching and photodamage to the sample. Once all of the fluorophores have been boosted to their excited state—a situation commonly reached by existing confocal systems—even this method falters. For demanding applications that require imaging of fast dynamic scenes, scanning hyperspectral fluorescence microscopes thus provide poor performance.

The IMS is a parallel acquisition instrument that captures a hyperspectral datacube without scanning. It also allows full light throughput across the whole spectral collection range due to its snapshot operating format. Its operation is based on redirecting image zones through



Assembling the full datacube by IMS and its cellular fluorescence imaging results. (a) Data produced by an image of a monochromatic spatially uniform field; 60 of them are used to produce the full calibration dataset. (b) The acquired spectral channel images of bovine pulmonary artery endothelial cells. Cellular nuclei labeled with DAPI are visible in the blue spectral channels; filamentous actin labeled with Alexa Fluor 488 phalloidin are seen in the green; and mitochondria labeled with MitoTracker Red CMXRos are visible in red.

the use of a custom-fabricated optical element known as an image mapper.⁴ The image mapper is a complex custom optical component comprised of high quality, thin mirror facets with unique 2-D tilts.

These mirror facets reorganize the original image onto a single large-format CCD sensor to create optically “dark” regions between adjacent image lines. The full spectrum from each image line is subsequently dispersed into the void regions on the CCD camera. The entire datacube (x, y, λ) is acquired instantaneously in a single integration event. This mapping method establishes a one-to-one correspondence between each voxel in the datacube and pixel on the CCD camera requiring only a simple and fast remapping algorithm for data reconstruction.

The current IMS acquires a datacube of size $285 \times 285 \times 60$ (x, y, λ) , and a spectral range sampled by 60 spectral channels from 450 to 650 nm. For

demonstration, (b) of the figure shows 27 of the 60 total spectral channel images of triple-labeled bovine pulmonary artery endothelial cells, which were captured by the IMS in a single snapshot. For future applications, we intend on using high-speed sCMOS detector arrays⁵ inside the IMS, allowing for time-resolved fast imaging of action potentials and full-field Raman spectroscopy—two demanding applications that lie at the limit of what current instruments can measure. Δ

Liang Gao (lgao@rice.edu), Robert T. Kester, Nathan Hagen, Tomasz S. Tkaczyk are with the department of bioengineering, Rice University, Houston, Texas, U.S.A. Gao and Tkaczyk are also with the Rice Quantum Institute at Rice University. T. Tkaczyk is also affiliated with the department of electrical and computer engineering of Rice University.

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