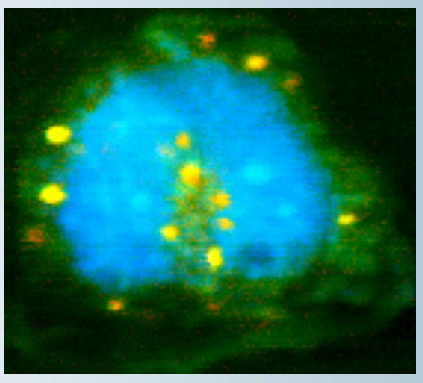
Broadband CARS Microscopy

Objective

The traditional chemical labeling used in optical microscopy can alter cells and materials, thus impeding determination of their true structure, function and response. We are developing broadband coherent anti-Stokes Raman (BCARS) microscopy with the goal of producing a technique that allows label-free and noninvasive functional imaging of materials, cells and tissues. On the materials side, we seek to image statics and dynamics of chemically heterogeneous and structured materials. For biological materials such as cells and tissues, we seek to attain near video-rate acquisition of high-resolution images that contain contrast from chemical processes occurring during cellular processes such as proliferation and differentiation, in order to discriminate normal and abnormal cell behaviors and differentiation states.



PolarOnyx

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Toptica

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Time-Bandwidth

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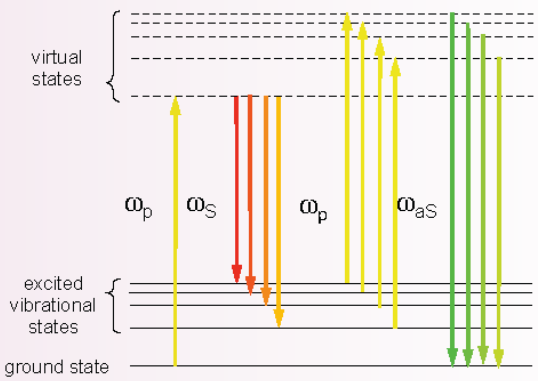
Spectra Physics Laser

Impact and Customers

There is a need for label-free chemical microscopy in medicine, biology and materials science. Most of the current methods use chemical labels that often disturb the distribution and nature of chemical components being investigated. The method we are developing enables noninvasive and rapid collection of Raman spectra for imaging.

- BCARS can be used to track cell signaling processes and can provide functional readouts of cell differentiation, allowing researchers to obtain cell responses to biomaterials in real time and on a cell-by-cell basis.
- BCARS can be used to acquire high-resolution chemical maps of pharmaceutical tablets, including information on morphology of active ingredients, 10 to 100 times faster than spontaneous Raman scattering.
- All researchers who use Raman imaging methods will benefit from our work, including biomedical researchers (and, potentially, clinicians), pharmaceutical industry scientists, geologists and others. Additionally, laser manufacturers have been influenced by our work; PolarOnyx, Toptica, Time-Bandwidth and Spectra Physics Laser have all developed and are marketing laser sources to enable BCARS.

Approach



CARS provides a signal that contains the Raman response of interest for performing label-free chemically sensitive microscopy. In CARS, a vibrational coherence is generated when a pair of photons (pump and Stokes) interact with the sample to excite a vibrationally resonant Raman mode at frequency $\omega_{vib} = \omega_{pump} - \omega_{Stokes}$. A third (probe) photon is inelastically scattered off this coherent excitation, and anti-Stokes light ($\omega_{as} = \omega_{pump} - \omega_{Stokes} + \omega_{probe}$) is emitted from the sample.

The CARS signal has a frequency-independent non-resonant component and a frequency-dependent resonant component. The non-resonant component is entirely in phase with the driving field of the laser and gives us no information about the chemical nature of the sample. The resonant component contains the chemical information and has a frequency-dependent amplitude. It is out of phase with respect to the driving electric field of the laser. The resonant component contains elements with the same bandshape as the spontaneous Raman signal.

We obtain a broadband vibrational spectrum at each laser shot by using broadband Stokes light. The Stokes light contains 3000 cm^{-1} of bandwidth.

Accomplishments

We have had several "firsts" in this project. We were the first to demonstrate broadband CARS microscopy. With this system we obtain 3000 cm^{-1} spectrum at each laser shot, allowing us to rapidly image chemically complex systems, such as the polymer blend shown in the figure below. This image was obtained at 17 ms/pixel, about 50 times faster than can be obtained with spontaneous Raman spectroscopy.

The spectra obtained from CARS includes a resonant and nonresonant component, which made it difficult to extract the Raman spectrum of interest. We have developed a deterministic mathematical approach to extracting the resonant (Raman) signal based on a time-domain Kramers-Kronig (TDKK) transform. The TDKK treatment makes the CARS signal linear in analyte concentration and quantitative. We show, based on imaging of a polymer blend above, that even minor components can be detected and quantitatively accounted for when the CARS signal is transformed in this way.

This TDKK transform allows us to take advantage of intrinsic heterodyne amplification of the weak resonant signal and allowed us to be the first group to obtain a full fingerprint and CH-stretch vibrational spectra simultaneously in biological cells. The chemical signatures contained in the combined CH and fingerprint spectral region allow us to discriminate differentiated cells from stem cells, as shown in the figure below.

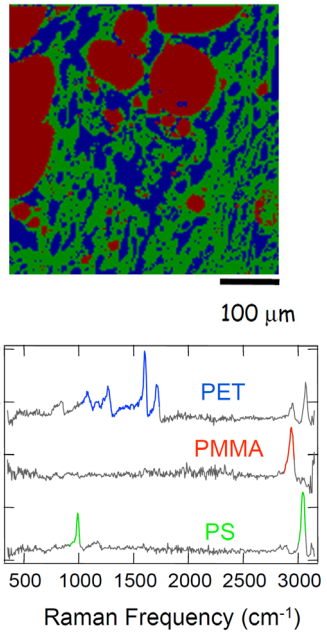


Figure 1. Broadband CARS image of tertiary polymer blend [color coded: polyethylene terephthalate (PET) in blue, polymethyl methacrylate (PMMA) in blue and polystyrene(PS) in red], and examples of spectra obtained at each pixel (bottom).

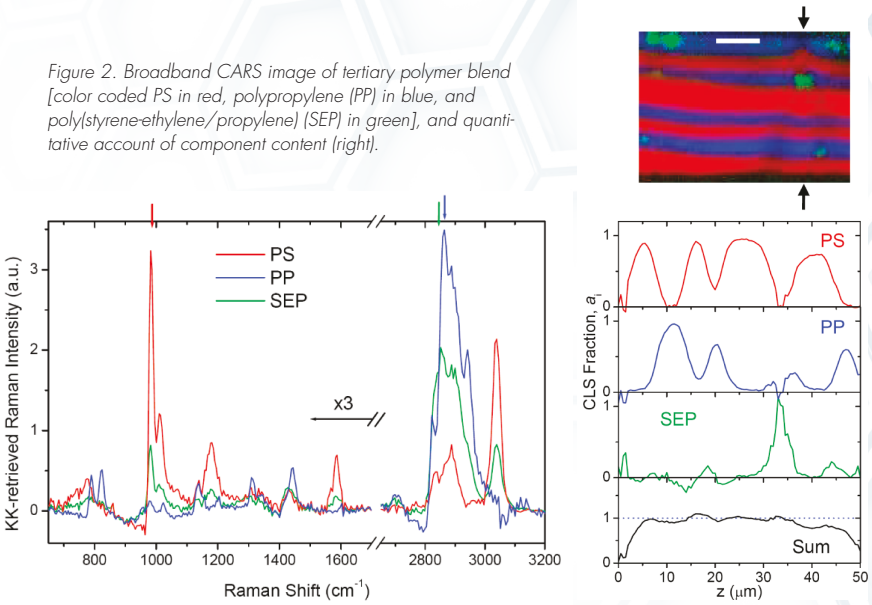


Figure 2. Broadband CARS image of tertiary polymer blend [color coded PS in red, polypropylene (PP) in blue, and poly(styrene-ethylene/propylene) (SEP) in green], and quantitative account of component content (right).

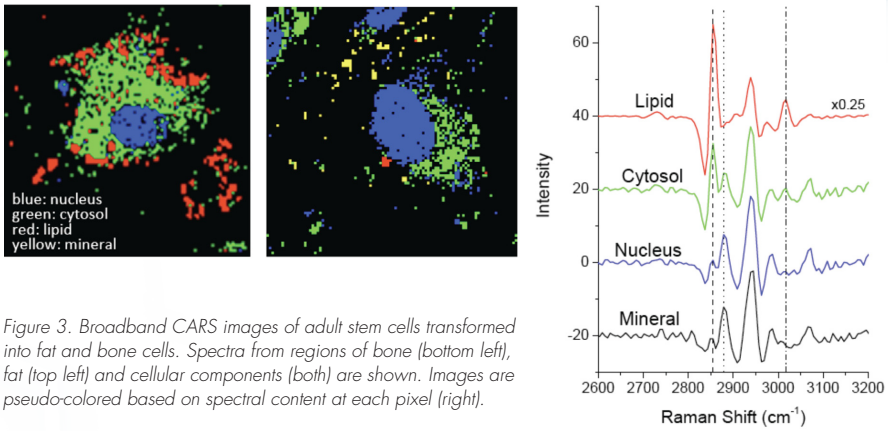


Figure 3. Broadband CARS images of adult stem cells transformed into fat and bone cells. Spectra from regions of bone (bottom left), fat (top left) and cellular components (both) are shown. Images are pseudo-colored based on spectral content at each pixel (right).

Learn More

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Publications

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