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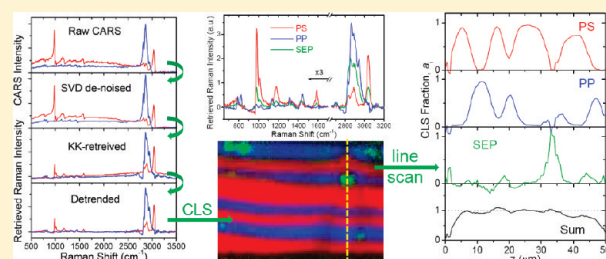
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Quantitative Image Analysis of Broadband CARS Hyperspectral Images of Polymer Blends

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ABSTRACT: We demonstrate that broadband coherent anti-Stokes Raman scattering (CARS) microscopy can be very useful for fast acquisition of quantitative chemical images of multilayer polymer blends. This is challenging because the raw CARS signal results from the coherent interference of resonant Raman and nonresonant background and its intensity is not linearly proportional to the concentration of molecules of interest. Here we have developed a sequence of data-processing steps to retrieve background-free and noise-reduced Raman spectra over the whole frequency range including both the fingerprint and C–H regions. Using a classical least-squares approach, we are able to decompose a Raman hyperspectral image of a tertiary polymer blend into quantitative chemical images of individual components. We use this method to acquire 3-D sectioned quantitative chemical images of a multilayer polymer blend of polystyrene, styrene–ethylene/propylene copolymer, and polypropylene that have overlapping spectral peaks.



Raman microscopy has been widely used to acquire label-free chemical imaging of soft and complex materials, including polymeric and biological samples. However, major weaknesses of conventional spontaneous Raman imaging methods include weak signal intensity and an often strong fluorescence background in the same spectral range as the Raman signal. Many nonlinear Raman methods have been developed to enhance the Raman signal. Successful techniques for microscopy include coherent anti-Stokes Raman scattering (CARS) microscopy^{1,2} and stimulated Raman scattering (SRS) microscopy.³ In both cases, single-frequency (narrow spectral bandwidth) approaches have been widely used to take advantage of high scanning speed. However, single frequency approaches do not facilitate extraction of quantitative composition information from samples with overlapped spectral peaks. On the other hand, in multiplex or broadband hyperspectral imaging, one obtains a Raman spectrum at each spatial pixel, and quantitative analysis of complex samples is possible. While broadband SRS microscopy has not been demonstrated as a useful imaging modality yet due to the limited dynamic range of current multiplex detection systems,⁴ broadband CARS has been fairly well developed and proven as a robust imaging tool. Broadband CARS signal is generated by overlapping a narrowband pulse with a pulse from a femtosecond oscillator or from a continuum source.^{5,6} The CARS signal is generated at higher frequency than any of the input pulses, so there is no interference from fluorescence generated by single-photon processes. Because the CARS process is nonlinear, signal is generated only at the focal point, where the multiphoton process is most enhanced, leading to 3-D sectioning capability.

The CARS signal intensity is dramatically enhanced compared to spontaneous Raman scattering under conditions common to materials imaging. However, a nonresonant background (NRB)

accompanies the resonant CARS signal and very often dominates the weaker resonant signals of interest. The NRB in CARS interferes coherently with the resonant signal, resulting in a dispersive line shape and amplitude, making quantitative analysis difficult. Many sophisticated optical schemes have been devised to minimize the NRB contribution to the measured CARS spectrum.^{7–12} However, in many cases overall CARS signal strength is sacrificed, which can adversely affect the signal-to-noise (S/N) ratio of the detected CARS spectrum. Alternatively, computational methods have been demonstrated to retrieve the resonant signal of interest from the NRB in multiplex or broadband CARS spectra.^{13–17} Unlike other approaches based on iterative fitting,^{13–16} we use a modified Kramers–Kronig (KK) transform method¹⁷ to extract resonant Raman-equivalent spectra from raw CARS spectra and a separately measured NRB spectrum in a straightforward way without iterative fitting. This KK method has been used to retrieve a weak Raman signal from a biological sample rapidly.^{17,18}

Here, we apply this approach to broadband CARS microscopy to solve the problem of quantitative 3-D chemical imaging of tertiary polymer blends. It is critical in this application to know the location of the polymeric components because of the intimate relationship between the processing of the components, the resulting morphological structure, and the material performance. The blending of immiscible polymers by melt mixing is widely used to produce materials with tailored properties. Controlling specific structures during the mixing processes makes

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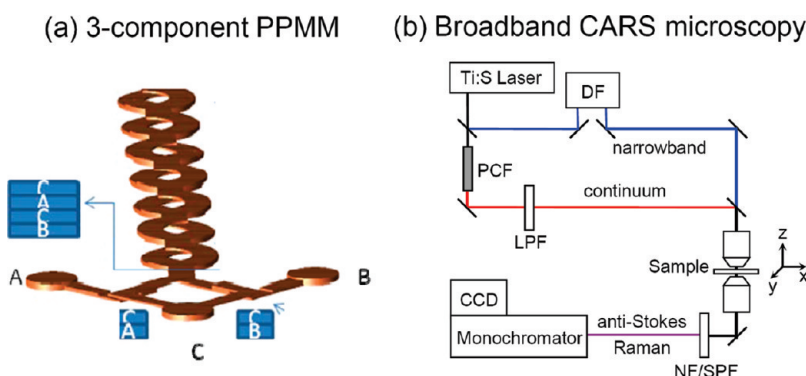


Figure 1. (a) Schematic diagram of the three-component planar polymer micromixer (PPMM). For a PS/PP blend, A = C = PS and B = PP; for a PS/SEP/PP blend, A = PS, B = PP, and C = SEP. (b) Schematic diagram of broadband coherent anti-Stokes Raman scattering (CARS) microscope: PCF, photonic crystal fiber; DF, dispersionless filter; LPF, long-pass filter; NF, notch filter; and SPF, short-pass filter. In this paper, the *y*-axis is parallel to the flow direction; the *z*-axis is the normal direction of the layers; and the *x*-axis is perpendicular to the *y*- and *z*-axes.

new physical, chemical, and bioengineering properties available. For example, a multilayer structure can reduce gas transport,¹⁹ a cocontinuous structure of a polymer scaffold can be used for tissue engineering,²⁰ and a domain/matrix structure can be used to control mechanical properties such as brittleness.²¹ In these applications, two incompatible polymers that have synergistic properties are often mixed together in the presence of a compatibilizer, which resides at the interface of the incompatible polymers.

Typical methods for imaging polymer blend structures at high spatial resolution include scanning electron microscopy (SEM) and tunneling electron microscopy (TEM),²² but these methods generally do not provide chemical phase information, so auxiliary methods such as solution extraction²³ are frequently used. Solution extraction is not amenable for use with multilayer blends due to the potential for destruction of the original morphology during solution extraction. Therefore, sample cross sections of multilayer blends are typically prepared for SEM analysis by cryofracture,²⁴ where chemical contrast is derived from differences in texture of broken surfaces. For TEM analysis, additional heavy metal staining is necessary for chemical image contrast.²² These electron microscopy techniques require complicated and invasive sample preparation procedures and it is very challenging to obtain reliable spatiochemical information with only SEM/TEM analysis, especially for tertiary polymer blends.

The need for a chemically sensitive microscopic imaging technique is furthered by the development of the planar polymer micromixer (PPMM). Compared to traditional melt mixing, the PPMM, which is a split and recombine mixer,²⁵ features tighter control of flow conditions and works in situations of limited sample quantity. Due to these features, the PPMM is well-suited for studies designed to elucidate the relationships between structure, processing, and properties of multicomponent polymer blends.^{24,25} A variety of targeted structures was demonstrated on binary blends, such as multilayer, cocontinuous, domain/matrix, and multiple coaxial structures. Good contrast between a polystyrene and polypropylene was achieved using cryofracture SEM. In the current work, we seek to utilize the PPMM in tertiary blends to understand the role of compatibilizers in the mixing process.

We demonstrate here that broadband CARS microscopy can meet the requirement of 3-D imaging of all three polymers without the additional sample preparation steps required for electron microscopy imaging. We perform quantitative image

analysis of Raman spectra of both the fingerprint and C–H regions using a classical least-squares approach after the CARS spectra have been denoised using singular value decomposition and transformed into background-free Raman spectra using a modified Kramers–Kronig phase retrieval and baseline detrending. This paper focuses on the methods to apply CARS microscopy to a polymer blend whose components have overlapping spectral features, and a companion paper focuses on the fundamentals of the three-component mixing and structuring in the PPMM.^{24,25}

EXPERIMENTAL SECTION

Sample Preparation. The PPMM is designed to passively mix relatively small quantities of two (or three) components by forcing the molten polymers through a series of splitting and recombining units. In previous work on a binary blend, a multilayer structure was formed over the first several units, but then interfacial tension forced the morphology into that of a domain/matrix or cocontinuous structure.^{24,25} We are now interested in creating a three-component blend to understand how compatibilizers behave in model processing operations.^{24,25} Briefly, as shown in Figure 1a, the three-component PPMM consists of the entrance region and a series of the splitting and recombining (SAR) units. At the entrance region, the components A and C form a vertically displaced parallel flow in one “T” junction and the components B and C form another parallel flow in the other T junction. The two parallel flows of C/A and C/B join at another T junction to form a parallel flow of four layers with a C/A/C/B sequence. The thickness of the flow channel inlet for the component C is 50 μm , while the thickness of the other channels for the components A and B are 75 μm . This parallel flow enters the series of SAR units, where the layered flow is split into two perpendicular channels and recombined into one channel by placing one split stream on top of the other. As the layered flow passes *n* SAR units, the number of layers in the flow increases in principle by 2^n . The width of the mixing channel is 750 μm and the depth varies from 50 μm (split and reorientation region) to 125 μm (recombination region), which is determined by the combination of the top and bottom shims (50 μm in thickness) and the middle shim (25 μm in thickness). Polystyrene (PS, Styron 666D, DOW Chemical), polypropylene (PP, Exceed 4062, Exxon), and poly(styrene–ethylene/propylene) diblock copolymer (SEP, Kraton G 1701, Shell Chemical) were

used as received. The hot polymer blend was slowly cooled down in the air before it was detached from the mixer for further analysis.

For SEM imaging, the molded strip was detached manually from the shims and embedded into epoxy for a supporting medium. Then, the sample was frozen by liquid nitrogen and broken at target locations. The cross sections prepared by the cryofracture method were imaged by a scanning electron microscope (SEM; Hitachi, S-4700).

Broadband CARS Imaging. The experimental setup of the broadband CARS has been described previously.^{18,26} Briefly, as shown in Figure 1b, the output (70 fs, centered at 830 nm, 80 MHz) of a Ti:S laser oscillator (MaiTai-DeepSee, Spectra-Physics) was split into two parts. One part was introduced into a photonic crystal fiber (Crystal Fiber, Femtowhite) to generate a continuum pulse. The continuum pulse spectrum was cut with an 850 nm long-pass filter. The bandwidth of the remaining oscillator output (the narrowband pulse) was reduced by adjusting slit width in a 4-f dispersionless filter to 10 cm⁻¹ full width half-maximum (fwhm) with the center wavelength at 830 nm. The two beams were introduced collinearly and with parallel polarization into a 60× 1.35 NA oil immersion objective lens (Olympus) or a 40× 0.95 NA objective lens (Olympus) and focused on the sample. The CARS signal generated from the sample was collected in the forward direction and passed through a set of an 830 nm notch filter and an 810 nm short-pass filter and was analyzed using a charge-coupled device (CCD; DU920-BR-DD, Andor) attached to a monochromator (SP-2300, Acton) with a grating with a groove density of 300 groove/mm. The spectral resolution was 12 cm⁻¹, determined by the spectral bandwidth of the narrowband pulse. The spatial resolution was laterally 500 nm and axially 1 μm at the glass coverslip surface and became larger as the focus moves into a polymer sample due to spherical aberration. The average laser power at the sample was kept below 10 mW for each pulse, to avoid photodamage. The CCD exposure time is typically 20 or 50 ms per pixel.

Multivariate Image Analysis. The broadband CARS image data were acquired in the (x, z, ω) matrix at each y section, where the z -axis is normal to layer interfaces, the y -axis is parallel to the flow direction, the x -axis is perpendicular to the y - and z -axes, and ω indicates the Raman shift frequency dimension. Singular value decomposition (SVD) was performed using a user-built code based on LabView (National Instruments). The Kramers–Kronig phase retrieval¹⁷ and baseline detrending processes were also performed using the same user-built LabView code. The retrieved Raman spectral image of a polymer blend was analyzed with a classical least-squares (CLS) method by a commercial package for multivariate image analysis (Solo+MIA, Eigenvector). The Raman spectra of pure components were used as basis functions for the CLS analysis.

RESULTS AND DISCUSSION

We first consider the case of the binary blend composed of PS and PP, as there is good contrast between the two components in both the cryofractured surfaces (for SEM images) and in Raman spectra. The SEM images in Figure 2a,b show layer structures with a high spatial resolution, sufficient to provide a detailed texture of the cross section surfaces. In particular, the SEM images of the cryo-fractured cross section show clear difference in texture among the layers. Limited chemical identification of the individual layers was achieved by comparing with the textures of

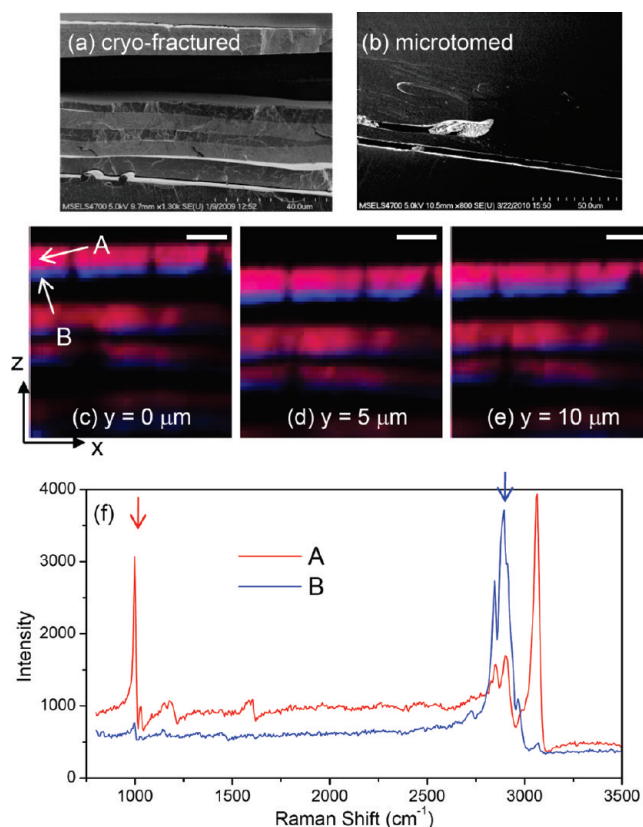


Figure 2. SEM images of (a) cryofractured and (b) microtomed cross sections at the third SAR segments of a PS/PP blend prepared by PPMM. (c–e) CARS hyperspectral image of an optical cross section at the third segment of the PS/PP blend. The raw CARS data was used to construct the false color image, where the red and blue color indicates the intensity at 990 and 2880 cm⁻¹, respectively. (f) The CARS spectra measured at the locations A and B, as indicated in the first image. The scale bar in the CARS images indicates 20 μm. The 40× 0.95 NA objective lens was used. The exposure time was 50 ms per pixel.

identifiable regions of the same or other cross sections. SEM images allow for precise measurement of physical dimensions and observation of structural morphology. However, the cryofracturing technique requires very careful sample handling to achieve a cross section at a desired location in a small micromixer sample, but more importantly, it is impossible to obtain multiple cross sections separated by well-defined distances, which is crucial for studying morphology evolution along the flow direction in forced assembly mixers. Alternatively, a microtome method can provide multiple cross sections separated by well-controlled distances. However, image contrast is significantly reduced in the SEM image of the microtomed surface, as shown in Figure 2b, where it is almost impossible to recognize even the layer boundaries. The loss of fine texture used for image contrast makes the microtome method not applicable for conventional SEM imaging of layered polymer blends. In addition, the embedded epoxy for support during cryofracturing or microtoming can potentially alter the morphology of a multilayer polymer blend.

We acquired CARS image data of a PS/PP blend produced in the PPMM at three optical cross sections separated by 5 μm without any additional sample preparation steps. The CARS spectra look very different between the two regions and are easily identifiable as PS and PP, as shown in Figure 2f. PS is

characterized by the phenyl ring breathing mode at 1000 cm^{-1} and the C–H stretching mode on the phenyl ring at 3100 cm^{-1} , while PP is characterized by the strong CH_2 and CH_3 modes at $2800\text{--}2900\text{ cm}^{-1}$. The false color images are constructed at the frequencies of 990 cm^{-1} (red) and 2880 cm^{-1} (blue), which represent the frequencies of the most intense CARS peaks of PS and PP, respectively. The images show clear layer structure of PS and PP, with some delamination and cracks within layers. However, as mentioned above, the NRB contribution, which appears as a slowly varying baseline in the CARS spectra of Figure 2f, prevents quantitative chemical analysis of the CARS images. Due to the peak shift and the nonlinear dependence on concentration, caused by the NRB interference, single frequency-based image analysis cannot provide quantitative information.

Quantitative Imaging of Tertiary Blends. The above method is not only qualitative but it is also not suitable for tertiary blends where there is spectral overlap between components. Here, we describe four sequential data processing steps that overcome both limitations: (1) singular value decomposition (SVD), (2) modified Kramers–Kronig (KK) phase retrieval, (3) baseline detrending (BD), and (4) classical least-squares (CLS) analysis. All the data processes require multiplexed spectra and are not applicable to single-frequency-based CARS microscopy.

Singular Value Decomposition (SVD). The SVD method has been used as an extremely powerful means to reduce noise from hyperspectral images.²⁷ SVD factors a $(m \times n)$ -matrix \mathbf{D} into a product of three matrix factors, $\mathbf{D} = \mathbf{U}\mathbf{S}\mathbf{V}^T$. For CARS hyperspectral image data, the original data matrix \mathbf{D} is arranged as $(\omega \times xy)$, where ω is the number of frequency elements and x and y indicate the numbers of image pixels on the x and y axes, respectively. The matrix \mathbf{U} contains the so-called basis spectra, \mathbf{V} is a matrix containing the amplitude vectors as an image, and \mathbf{S} is a diagonal matrix containing the singular values. Some of the spectral and spatial vectors in \mathbf{U} and \mathbf{V} are dominated by random noise. Removing only those noise-dominating components will reduce noise without losing information of interest. Mathematically, one can transform \mathbf{S} into a reduced matrix \mathbf{S}' by replacing singular values corresponding to random noise with zeros. The reconstructed \mathbf{D}' from $\mathbf{D}' = \mathbf{U}\mathbf{S}'\mathbf{V}^T$ will contain noise-reduced spectral information of interest. The challenge in using SVD is to objectively decide on which singular values are statistically and physically significant. Several numerically based criteria have been used to determine which singular values to keep for SVD reconstruction. For example, the criteria used in ref 28 are (i) distinctive change in declining slope of singular values, (ii) the first-order autocorrelation function for columns of the \mathbf{U} and \mathbf{V} matrices, and (iii) the randomness of residual plots for the difference between the original \mathbf{D} and a reconstructed \mathbf{D}' calculated with trial singular values. These criteria were demonstrated to be useful for spectral noise reduction of UV spectra of DNA melting profiles.²⁸ The validity of those criteria for our broadband CARS analysis is tested by using an example of CARS hyperspectral data of a PS/SEP/PP polymer blend with 569 frequency elements and 101×141 image pixels. There is no distinctive change in the slope for the singular values shown in Figure 3b, so criterion i does not provide an unambiguous metric. Applying criterion ii to the first-order autocorrelation functions plotted in Figure 3c with a threshold of 0.8 leads to use of either the first two or the first seven singular values, depending on whether columns of the \mathbf{U} or \mathbf{V} matrices are used for the autocorrelation. We reconstructed new \mathbf{D}' using two and seven

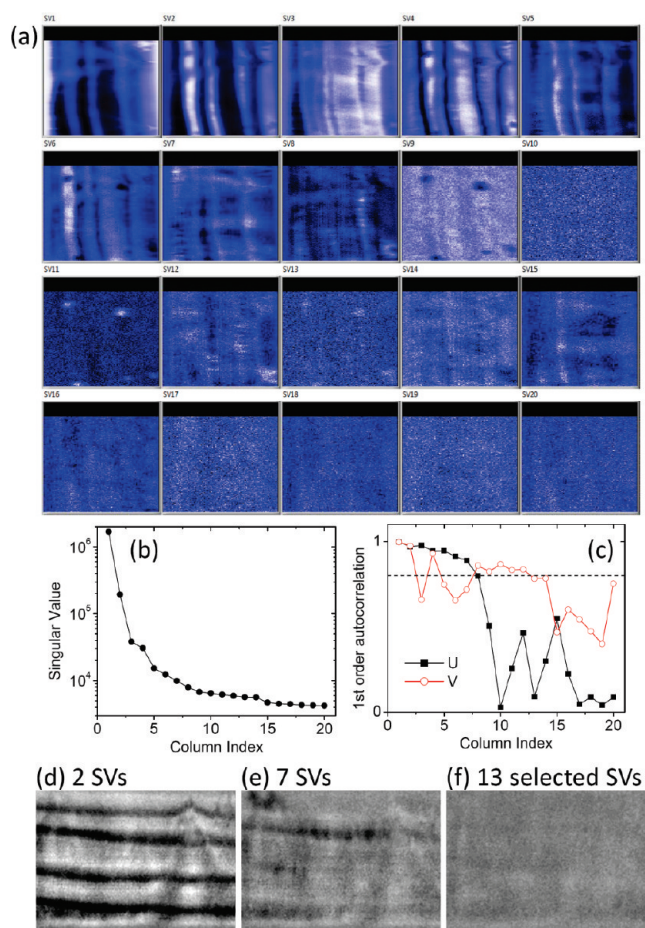


Figure 3. Singular value decomposition (SVD) of CARS hyperspectral data of a PS/SEP/PP blend. (a) The images of the first 20 columns of the generated \mathbf{V} matrix. (b) Plot of singular values in the matrix \mathbf{S} . (c) The normalized first-order autocorrelation function of the columns of the \mathbf{U} and \mathbf{V} matrices. (d–f) Total spectral intensity images of the residual matrix $(\mathbf{D} - \mathbf{D}')$ generated with various number of singular values determined by different criteria (described in the text). For the residual image (f), 13 singular values (indices of 1–9, 11, 12, 14, and 15) are used as nonzero to reconstruct \mathbf{S}' and \mathbf{D}' .

singular values and generated the residual matrices $(\mathbf{D} - \mathbf{D}')$ as suggested by the criterion iii. The images of residual matrixes $(\mathbf{D} - \mathbf{D}')$ with singular values of two and seven, shown in parts d and e of Figure 3, respectively, exhibit noticeable structural features, indicating that the numerical criteria can cause loss of meaningful physical information through SVD filtering. The excessive truncation for \mathbf{S}' with the numerical criterion ii must be related with the characteristics of the hyperspectral image data: for the \mathbf{U} matrix, sharp spectral peaks will cause the first-order autocorrelation function to be underestimated, and similarly for the \mathbf{V} matrix, small features compared with pixel-to-pixel distance will be regarded as noise in the first-order autocorrelation consideration.

Therefore, we use a rather qualitative criterion based on the randomness of images of the individual columns of \mathbf{V} and confirm whether to determine the significance of their corresponding singular values. Figure 3a shows the images of the matrix \mathbf{V} columns corresponding to the first 20 singular values of the CARS image of a PS/SEP/PP polymer blend. Among them, 13 images are selected to be meaningful based on recognition of meaningful features by the user. It is clear that the reconstructed

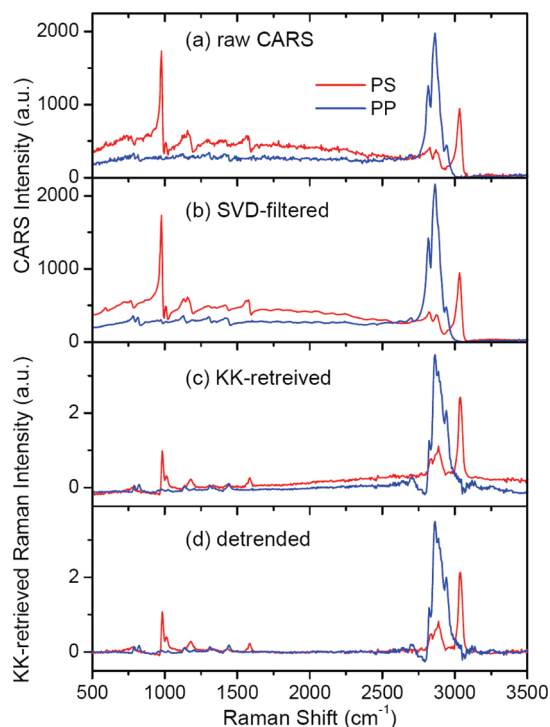


Figure 4. Examples of data processing of CARS spectra taken from PS and PP homopolymers. (a) The raw CARS spectra were taken by single measurements for 20 ms exposure time. (b) The SVD filtering was performed over 101×101 pixels for a CARS hyperspectral image of a homopolymer layer on a glass coverslip. The singular values were 4 and 5 for PS and PP, respectively, following the criteria explained in the text. (c) The KK-retrieval was performed for the SVD-filtered CARS spectra with the SVD-filtered NRB spectra measured from the glass coverslip. (d) The KK-retrieved spectrum was subtracted by a baseline determined by fitting it with a fourth-order polynomial over three separate frequency regions where Raman resonance is absent ($500\text{--}600$, $1600\text{--}2600$, and $3250\text{--}3500\text{ cm}^{-1}$).

image of the residual matrix ($\mathbf{D} - \mathbf{D}'$) of Figure 3f contains mostly random noise, compared with parts a and b of Figure 3.

Figure 4 shows spectra at various stages of data processing for PS and PP CARS spectra measured from $50\text{ }\mu\text{m}$ thick homopolymers located on a glass coverslip. Figure 4a shows raw CARS spectra of single spatial pixels taken from CARS images with 101×101 pixels and 622 frequency elements. As shown in Figure 4b, application of the SVD filtering process with the criterion based on the \mathbf{V} column images allows significant reduction of high-frequency spectral noise with minimal loss of meaningful spectral information.

Kramers–Kronig (KK) Phase Retrieval and Baseline Detrending (BD). The total CARS signal intensity is proportional to the squared modulus of the complex third-order susceptibility, $|\chi(\omega)|^2$, where $\chi(\omega)$ contains a resonant (χ_R) and nonresonant (χ_{NR}) part:

$$|\chi(\omega)|^2 = |\chi_{NR}(\omega)|^2 + 2\chi_{NR}\text{Re}[\chi_R(\omega)] + |\chi_R(\omega)|^2$$

A modified Kramers–Kronig relation is used to retrieve the resonant Raman spectrum from the total CARS spectrum $|\chi(\omega)|^2$ and the nonresonant spectrum $|\chi_{NR}(\omega)|^2$, as described in detail previously.¹⁷ A separately measured CARS spectrum of a glass coverslip is used as a nonresonant spectrum in the KK process. Figure 4c shows the KK-retrieved Raman spectra

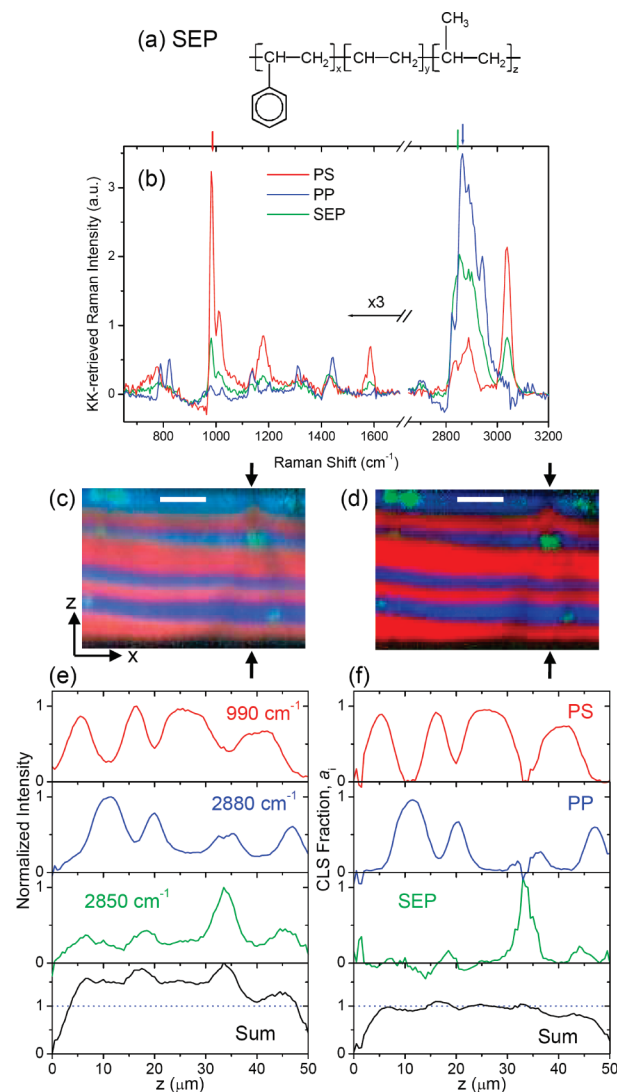


Figure 5. (a) Chemical structure of SEP. (b) Raman spectra generated with SVD, KK, and BD from CARS data of PS, SEP, and PP homopolymers. CARS hyperspectral data from the third segment of a PS/SEP/PP blend produced by PPMM were processed with SVD, KK, and BD in sequence. (c) The false color image constructed by single-frequency intensities at 990 cm^{-1} (red), 2890 cm^{-1} (blue), and 2850 cm^{-1} (green). (d) The false color image constructed by CLS fractions (a_i): PS (red), PP (blue), and SEP (green). The scale bar indicates $20\text{ }\mu\text{m}$. The exposure time was 50 ms per pixel.

from the SVD-filtered CARS spectra of PS and PP and the SVD-filtered NRB spectra from the glass coverslip in the same measurements. While a total CARS signal is not proportional to sample concentration, a Raman signal in a KK-retrieved spectrum is linearly proportional to the concentration of molecules of interest, making quantitative data analysis possible.

The KK-retrieved Raman signal contains a slowly varying baseline. This is particularly evident in the region around 2000 cm^{-1} , where the signal is nonzero, although there are no resonant peaks. The KK algorithm assumes that the reference NRB is identical to the NRB generated in the sample, and the presence of the nonzero baseline simply indicates that NRB associated with the sample is not the same as that measured in the glass. Differences in NRB from the slide glass and the sample and

can arise either from changes in the continuum pulse spectrum that occurred in the time interval between the separate measurements or from intrinsically different NRB response functions between the sample and glass. Currently, we have no way to measure the CARS and NRB spectra simultaneously at the same sample location. A simple and practical way to remove the baseline is to subtract a baseline determined by polynomial fitting over three Raman quiescent regions in the KK retrieved spectrum. Figure 4d shows the KK-retrieved Raman spectra of PS and PP after baseline detrending (BD).

We now turn to analysis of the retrieved Raman spectra. If each chemical component was associated with a unique Raman peak, it would be straightforward to discriminate the chemical components of essentially any blend by simply quantifying the amplitude of each identifying peak at each spatial pixel. Such a simple analysis is not possible for our PS/SEP/PP blend, however. As shown in Figure 5a, SEP, used as compatibilizer, is copolymer including both styrene (the monomer unit of PS) and propylene (the monomer unit of PP) units. All Raman peaks from SEP are also shared by either PS or PP, as shown in Figure 5b. We attempt a simple spectral analysis, the results of which are shown in the false color image of Figure 5c. This image was constructed using contrast from peaks at frequencies of 1000 cm^{-1} (red), 2880 cm^{-1} (blue), and 2850 cm^{-1} (green) for PS, PP, and SEP, respectively. Line scans at the indicated location in Figure 5c are normalized with the maximum intensities and plotted to represent the fraction of each component. The sum of individual line scans is larger than 1.5 at most of the region, indicating that the image constructed Raman intensity at single frequencies is not properly calibrated. As expected from the spectral overlap shown in Figure 5b, the single-frequency image analysis can provide qualitative information on which chemical components are dominant but cannot provide quantitative information about how much of the chemical components is present.

Classical Least Square (CLS) Analysis. We applied a multivariate approach to analyze the hyperspectral data quantitatively. Among many different multivariate regression methods, classical least-squares (CLS) is the most often used to determine the concentrations (a_i) of multiple components (i) from a mixture spectrum, $\Phi(\omega) = \sum a_i \phi_i(\omega) + E(\omega)$,²⁹ where $E(\omega)$ is the error, when the individual spectra, $\phi_i(\omega)$, of all of the pure components are known. We use separately measured pure Raman spectra of PS, PP, and SEP homopolymers, shown in Figure 5b, to perform CLS analysis using the pure spectra to determine concentrations (a_i) or fractions of the three components at each image pixel. Figure 5d shows a false color image of the a_i for the three components determined with CLS analysis. As the line scan of Figure 5f shows, the concentrations of all three components range from 0 to 1 and the sum is close to unity, indicating that the fractions a_i are complementary. This result supports the idea that the CLS multivariate analysis can provide quantitative information on the 3-D chemical image by broadband CARS hyperspectral data.

It should be noted that several optical artifacts can cause errors in a quantitative CLS. These artifacts include spherical aberration and sample scattering. Spherical aberration caused by refractive mismatch of the sample and a glass coverslip increases as the focus moves farther from the surface of the glass coverslip, and the reduced effective light intensity at the focus will lower the nonlinear CARS signal intensity. Similarly, the CARS signal will be reduced as the focus moves deeper from the sample entrance surface in a turbid sample. The z -dependent deviation can be seen in Figure 5f; the sum of the spectral components becomes

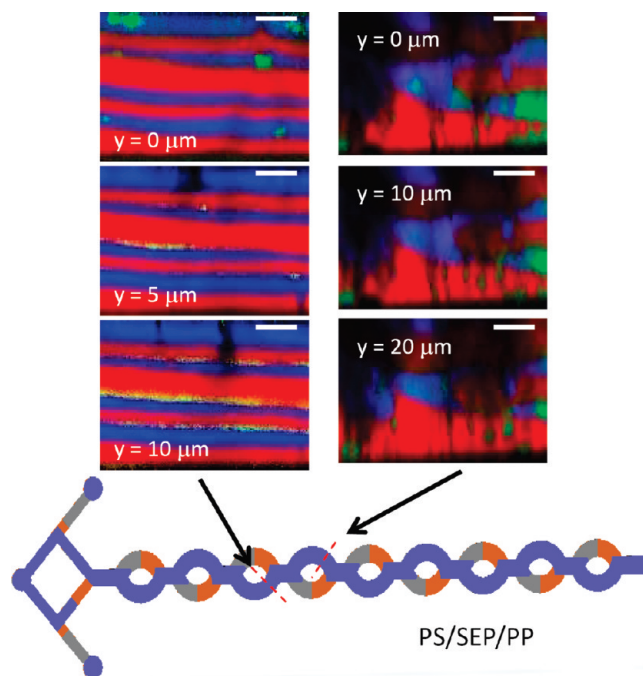


Figure 6. 3-D sectioned CARS images at the third and fourth segments of the PS/SEP/PP blend. All the hyperspectral data were processed with SVD, KK, and BD, and the resulting CLS fractions were used for image construction. The false colors represent PS (red), PP (blue), and SEP (green), respectively. The scale bar indicates $20\text{ }\mu\text{m}$. The exposure time was 50 ms per pixel.

smaller as the z value increases, that is, as the focus moves farther from the entrance. This is simply due to diminished signal level at increased depth. In principle, this reduced signal level could be corrected. These artifacts notwithstanding, multivariate image analysis of broadband CARS images provides a much better quantitation than can be achieved with single-frequency-based image acquisition or analysis.

Figure 6 shows quantitative chemical images of the third and fourth SAR segments at separated optical cross sections of the PS/SEP/PP blend. The 3-D sectioned images of the third segment look very different from the images of the fourth segment. The breakdown of the laminar phase occurs earlier in the presence of SEP compared to the equivalent PS/PP binary blend, which is a clear indication that SEP is acting as a compatibilizer.³⁰ The results indicate that the viscosity ratio of the compatibilizer relative to the other polymers controls the multilayer stability (earlier breakdown of the layered morphology) during flow while its reduced interfacial tension controls the final domain size.

It can be noted that the volume of SEP in the images of Figures 5 and 6 is much smaller than that of PS and PP. This follows from the fact that the flow rate must be proportional to the cross section of the inlet channel and be inversely proportional to the viscosity of the fluid when the same inlet pressure is applied for all components. The height of the SEP channel ($50\text{ }\mu\text{m}$) is lower than those of the PS and PP channels ($75\text{ }\mu\text{m}$). In addition, separate viscosity measurements³⁰ of the same polymer stocks show that the viscosity of SEP ($60\text{ }000\text{ Pa s}$) is about 3 times larger than that of PS ($15\text{ }000\text{ Pa s}$) or PP ($20\text{ }000\text{ Pa s}$) at the mixing temperature ($207\text{ }^\circ\text{C}$). Therefore, both the smaller cross section and higher viscosity of the SEP are consistent with the small volume fraction of the SEP component. We expect

accumulation of SEP at interfaces between PP and PS, based on the observed compatibilizer effect. We observe it by CARS in some interfacial regions, but not all. The fact that we do not see it at all interfaces suggests that in some regions the amounts are below the detection limits of CARS.

CONCLUSIONS

We have demonstrated that broadband CARS can be used to acquire quantitative chemical images of tertiary polymer blends. While lacking the spatial resolution of electron microscopy, this nonlinear optical imaging method based on Raman scattering provides 3-D sectioned chemical information without additional sample preparation steps, such as staining or fracturing, which are required for conventional electron microscopy imaging. The CARS hyperspectral data of multilayer polymer blends were transformed into background-free Raman spectral data with singular value decomposition, Kramers–Kronig phase retrieval, and baseline detrending, and then a multivariate analysis method (classical least-squares) was used to decompose the whole Raman spectra into the sum of each chemical component and to construct a quantitative chemical image of each component. We have discussed the location of the compatibilizer, SEP, in a PS/SEP/PP blend and are in position to study the compatibilizer effect on the evolution of morphology in polymer mixing. These optical methods are complementary to traditional electron microscopy and should be applicable to any system of spectrally distinct polymers.

Imaging speeds such as demonstrated here represent advancements in full spectral imaging; however, broadband CARS affords further opportunities for significantly faster signal acquisition. Imaging speed is limited by sample damage threshold, signal generation efficiency at a given laser power, and data transfer rate of the detector. Advances in laser technology—specifically, better-optimized laser pulses (wavelength and pulsewidth)—will allow more efficient CARS signal generation. A faster multielement detector (CCD or CMOS) will also improve the CARS imaging speed.

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Notes

Certain equipment, instruments, or materials are identified in this paper in order to adequately specify the experimental details. Such identification does not imply recommendation by the National Institute of Standards and Technology nor does it imply the materials are necessarily the best available for the purpose.

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