Two-dimensional molecular imaging by coherent Raman spectroscopy with quadrature phase modulation

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Abstract: We developed efficient phase modulation in phase sensitive method for Coherent Anti-Stokes Raman Scattering (CARS) spectroscopy. This method enables us to extract broad vibrational spectra from intense background noise by only 4 spectra with different modulation phases. We demonstrated two-dimensional density maps of small molecules, which difficult to label, by referring their intrinsic vibrational spectra. As a demonstration, we clearly obtained sevoflurane droplets embedded in water. Intense background noise generated from water is selectively suppressed with time-resolved technique.
OCIS codes: (120.3180) Interferometry; (120.5060) Phase modulation; (300.6230) Spectroscopy, coherent anti-Stokes Raman Scattering; (300.6310) Spectroscopy, heterodyne; (320.5540) Pulse shaping; (320.7150) Ultrafast spectroscopy.

Observation of molecular density distribution identifying molecular species is quite important for many scenes. To find target molecules in observation area, one generally use fluorescent or any other index molecules as labels for molecules, however it limits subject molecules and fields. In fact, suitable labeling molecules are not necessarily prepared. Moreover, even if one can use label molecules, the molecules should not affect physical and chemical property of target molecules. Small molecules which have molecular weight of less than 900 Dalton are well known as typical unobservable molecules because label molecules are much heavier than small molecules and may spoil their mobility, chemical reactivity, and molecular functions. Inhalation anesthetics, for example, are typical small molecules therefore their dynamics in human body is difficult to study so far. [1]

To obtain two-dimensional molecular image without labelling, observation of specific signals for each target molecules is required. Here, we used intrinsic vibrational spectra of molecules to identify molecular species. We have developed sensitive Raman spectroscopy in which a broad Raman spectrum were constructed from a few tens of spectra with randomly modulated input spectral phase [2]. In this study, we improved the spectroscopy to obtain broad Raman spectra only from 4 spectral measurements with quadrature modulation phases. We demonstrated the method for two-dimensional molecular imaging of small molecules.

2. Method

We developed Phase-Sensitive Coherent Anti-Stokes Raman Scattering (PS-CARS) spectroscopy which measures broad vibrational Raman spectra with high sensitivity and high frequency resolution [2]. In this method, single broadband pulse is divided into narrowband and broadband component. Broadband component causes molecular vibration, however generates nonresonant background due to third order electro susceptibility of sample. A delayed, narrowband pulse probes molecular vibration generating anti-Stokes Raman Scattering (CARS) signals. Nonresonant background and resonant CARS signals are interfered spectrally each other in a spectrometer. As a result, weak CARS signals are enhanced by intense background as a local oscillator of heterodyne detection.

In this study, we modulate spectral phase of the narrowband components to extract CARS signals from intense background. We measured 4 spectra ($I_0, I_{\pi/2}, I_{\pi}, I_{3\pi/2}$) with four different phase such as $0, \pi/2, \pi, 3\pi/2$. From the four spectra, resonant CARS signals I_{CARS} is

$$I_{CARS} = \sqrt{(I_0 - I_{\pi})^2 + (I_{\pi/2} - I_{3\pi/2})^2}\,. \qquad (1)$$

Here, absolute value of the relative phase between the narrowband and the broadband component does not play important role.

3. Experimental and results

We use femtosecond laser oscillator (Vitara-T-HP, Coherent Inc.) as a broadband light source. The center wavelength and bandwidth were 800 nm and 125 nm respectively. The pulse energy at sample is less than 1 nJ with repetition rate of 80 MHz. The output pulse is sent into Michelson interferometer in which an optical bandpass filter is used instead of a half mirror typically used. The bandwidth of the filter is 4 nm at 780 nm. The 780 nm component (narrowband component) and other (broadband component) are divided and combined in the interferometer. For the

high spectral resolution, we add another bandpass filter in the narrowband path to narrow bandwidth to 1 nm. Narrowband component can have arbitrary delay for time-resolved techniques. Optical path for the Narrowband component have an electro-optic modulator (EOM) to set the relative phase of these two components. The broadband and narrowband components are set to be collinear and sent into a sample cell which is placed on a two-dimensional piezo-stage. Transmitting pulse is introduced into spectrometer with a coolant CCD camera with typical exposure of 17 msec. We used a grating pair and a 4f pulse shaper for compensation of dispersion of all optical components and a microscope. As a test target, we used sevoflurane molecules (200 Daltons) which are common small bioactive compounds for inhalation anesthesia.

Figure 1 shows typical spectra of sevoflurane. Left top panel is overlaid of four spectra obtained with different modulation phases. Right bottom panel is a typical CARS spectrum constructed from the four spectra according to equation (1). As shown in fig. 1, only resonant CARS signals are clearly extracted from intense background.

As a demonstration of two-dimensional imaging, we used sevoflurane droplets embedded in water. A sample image taken by CMOS camera is shown in fig. 2 (a). In order to construct a two-dimensional Raman spectral image, we measured 4-different-phase spectrum at point by point in observation area. By using time-resolved technique, intense background noise generated from water was suppressed. Figure 2(b) shows a constructed Raman spectral image. We used summation of integrated intensities of two Raman peaks of sevoflurane (as shown fig. 1) for pixel intensities of the image. Sevoflurane droplets are clearly measured suppressing intense noise generated from surrounding water. The imaging speed is estimated to be 80 s/frame with exposure of 17 ms for each spectral acquisition.

We also added taurine at water part of the sample and measured their spatial distribution. We obtain Raman signals form taurin not only in water area but also in sevoflurane droplets. This shows that taurine molecules penetrated in sevoflurane droplets.

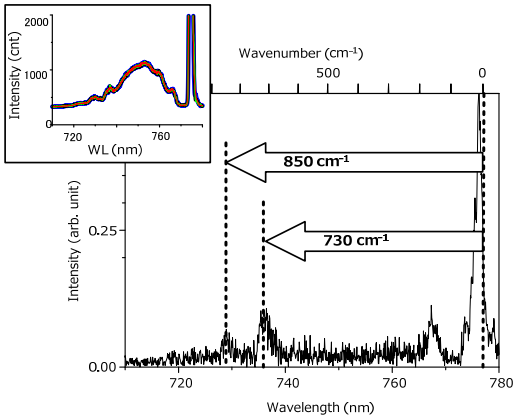

Fig. 1 Typical CARS spectrum of sevoflurane molecules obtained by quadrature phase-modulation method

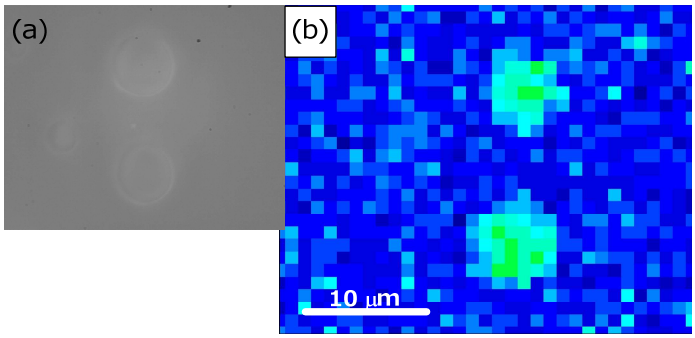

Fig. 2 Two-dimensional CARS image of sevoflurane droplets in water. (a) CMOS image of the observation area and (b) Raman spectral image.

4. Conclusion

We improved phase sensitive method for CARS spectroscopy. The improvement enables us to construct CARS spectra only from 4 raw spectra. By using the method, we successfully obtain two-dimensional image of small anesthetic molecules without label molecules. Intense noise generated from water is clearly suppressed by a time-resolved technique.

References

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