Recent Progress in Analytical SHG Spectroscopy

Sunao YAMADA[†] and I-Yin Sandy LEE

Department of Materials Physics and Chemistry, Kyushu University, Hakozaki, Fukuoka 812-8581, Japan

The progress of laser technology has brought extensive applications of nonlinear optical (NLO) spectroscopy to analytical fields. Especially, optical second harmonic generation (SHG), which is one of the most popular NLO phenomena, has been successfully applied to the study of interfaces (surfaces). Several fine reviews on surface SHG spectroscopies have appeared recently. The present article will concentrate on some new applications of SHG: second harmonic microscopy (SHM) for microstructural characterization, SHG interferometry for the determination of absolute molecular orientation, surface analysis of microstructures, and some molecular interactions at the interfaces.

Keywords Optical second harmonic generation, SHG, interface, SHG interference, second harmonic microscopy, electrostatic interaction

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1 Introduction

The advent of lasers has allowed great progress in the studies of nonlinear optical phenomena. Optical second harmonic generation (SHG), which is one of the second-order nonlinear optical effects, is the conversion of two photons of frequency ω to a single photon of frequency 2ω . In the electric dipole approximation, SHG is electric dipole forbidden in centrosymmetric media, but is allowed in noncentrosymmetric media. The intensity of nonlinear polarization $P(2\omega)$ oscillating at 2ω in the sample materials is responsible for the SHG and is proportional to the square of the intensity of incident electric field $E(\omega)$ as:

$$P(2\omega) = \chi^{(2)} E(\omega)^2, \quad (1)$$

where $\chi^{(2)}$ is called the second-order nonlinear susceptibility. Thus, the second harmonic (SH) signal is larger for higher laser power. In addition, SHG is a coherent process and therefore the SH signal is highly directional and its collection efficiency can be quantitative.

A noteworthy advantage of SHG is the high sensitivity to the interface (surface). At the interface, the chem-

ical species of interest experience different interactions in the direction perpendicular to the interface. Thus, inversion symmetry present in the bulk is broken at the interface region; as a typical case, noncentrosymmetric alignment of molecules is formed at the interface. If the chemical species is present in the bulk as well as the interface, conventional spectroscopic methods (absorption, fluorescence) are generally not able to differentiate optical signals from the interface species with those from the bulk species. As described above, SHG is electric dipole forbidden in centrosymmetric media (that is, bulk), but is allowed in noncentrosymmetric media (that is, interface). Thus, the SHG technique can selectively probe only the interface species even in the presence of a large amount of the bulk species. Accordingly, the SHG technique has been applied for investigations of chemical and physical phenomena at the interfaces (surfaces) by many research groups.¹⁻¹⁵

The SHG technique offers valuable information about events occurring at the interface region. For example, the second-order nonlinear susceptibility $\chi^{(2)}$ of the solid/liquid interface can be expressed as the sum of three possible components:

$$\chi^{(2)} = \chi^{(2)}_{\text{S}} + \chi^{(2)}_{\text{A}} + \chi^{(2)}_{\text{I}} \quad (2)$$

where $\chi^{(2)}_{\text{S}}$, $\chi^{(2)}_{\text{A}}$ and $\chi^{(2)}_{\text{I}}$ are nonlinear susceptibilities of the solid substrate, the adsorbate, and the change in the nonlinear susceptibility of the solid surface induced

[†] To whom correspondence should be addressed.

I-Y. S. L. present address: Department of Chemistry, Toyama University, Gofuku, Toyama 930-0887, Japan.

by surface-adsorbate interactions, respectively. What is measured in most surface SHG experiments is a change in nonlinear susceptibility, $\chi^{(2)} - \chi^{(2)}_s = \chi^{(2)}_A + \chi^{(2)}_I$, induced by adsorption. Thus, the following types of information can in principle be obtained from SHG measurements:

- (i) surface coverage
 - (ii) average molecular orientation
 - (iii) adsorption-desorption processes at the interface (surface)
 - (iv) reactions and interactions at the interface (surface)
- Applications to symmetry measurements of metal surfaces have been summarized elsewhere^{6,8}, and will not be described in this review.

The macroscopic quantity $\chi^{(2)}$ is related to the microscopic molecules noncentrosymmetrically aligned at the interface with the number of N_s and the average molecular hyperpolarizability $\langle\alpha^{(2)}\rangle$ as:

$$\chi^{(2)} = N_s \langle\alpha^{(2)}\rangle. \quad (3)$$

As a simple approximation, the point symmetry of $C_{\infty v}$ is assumed for the molecular hyperpolarizability (that is a single element $\alpha^{(2)}_z$ (z : molecular axis) dominates the SH signal). In this model, the intensity ratio of the transmitted p-polarized SH component induced by p- or s-polarized fundamental light, I_{p-p} or I_{s-p} , respectively, is expressed as:

$$I_{p-p}/I_{s-p} = (3\cos^2\gamma + \chi^{(2)}_{\parallel}/\chi^{(2)}_{\perp}\sin^2\gamma)^2 \quad (4)$$

$$\chi^{(2)}_{\perp}/\chi^{(2)}_{\parallel} = 1/2 \langle\tan^2\theta\rangle \quad (5)$$

where γ is the incident angle of the fundamental light, $\chi^{(2)}_{\parallel}$ and $\chi^{(2)}_{\perp}$ are components of $\chi^{(2)}$ parallel and perpendicular to the molecular axis, and θ is the average tilt angle of $\alpha^{(2)}$. The average molecular orientation can therefore be evaluated from the polarized SHG measurements, and has been carried out for various monolayer assemblies.¹⁻¹³ However, one should keep in mind that the adsorbed molecules are not always aligned in the same direction. The surface SHG reflects only noncentrosymmetric components of the adsorbed molecules.

In this article, new applications concerning (ii) – (iv) will be described.

2 Second Harmonic Microscopy (SHM)

The SHG technique has been applied to probe microscopic regions as second harmonic microscopy (SHM).¹⁶⁻¹⁸ In contrast to linear optical techniques such as ellipsometric, polarization, and Brewster angle microscopy, SHM has an additional advantage of being able to distinguish between a parallel (polar) and an antiparallel (axial, centrosymmetric) order of molecules at the interface (surface) plane. Recently, SHM was applied for the observation of Langmuir monolayers at

the air/water interface.

Three types of optical geometries for SHM experiments are shown in Fig. 1. In the simplest case (a), the area of interest (typically several hundred micrometer region) at the interface is illuminated from below, applying normal incidence of parallel laser beam. In this geometry, the microscope probes the in-plane component of noncentrosymmetry, since the electric fields of the light are polarized parallel to the interface. Using this geometry, SH micrographs of the Langmuir monolayer of SHG-active 2-docosylamino-5-nitropyridine are obtained (Fig. 2). The same position of Langmuir film is observed between parallel polarizers whose orientation is indicated in the figures (horizontal (left) and vertical (right)). Brighter sites show the regions having larger in-plane components of second-order nonlinear susceptibilities. The micrographs clearly show irregular, elongated, dendritic sites with different in-plane orientations. In this geometry, however, results are limited to in-plane noncentrosymmetry.

The normal component of noncentrosymmetry can be determined with oblique (b) or noncollinear (c) geometry. The oblique geometry is disadvantageous in terms of image resolution. The noncollinear geometry where two laser beams cross each other at the interface can improve the resolution, because the SH light is radiated normal to the interface and the coherence of the laser beams is not required. Some other advantages of geometry (c) are: (1) specimens can be observed in the reflection so that even a non-transparent interface can be probed, (2) the laser beam does not impinge on the

Fig. 1 Scheme of the second-harmonic microscopes. The incident laser light (ω) is indicated by solid arrows, and the SH light (2ω) by broken arrows. (a) collinear geometry; (b) oblique geometry; (c) noncollinear geometry.

Fig. 2 Second-harmonic micrographs of a Langmuir monolayer of 2-docosyl-amino-5-nitropyridine at a water surface. The polarizer orientations are indicated. The brighter the color, the higher the noncentrosymmetric alignments.¹⁷

objective, and (3) this setup can be utilized directly as a sum frequency microscope (SFM).

Recently, the SHG technique has been applied for near-field microscopy.¹⁹ The 1064-nm laser light irradiated the surface of silver film. The resultant SH field distribution was probed with an uncoated adiabatically tapered fiber tip, that was scanned over the sample surface. The SH field distribution has been compared with the topographical image. In the near-field region, there is a substantial contribution from evanescent fundamental and SH fields. For s-polarized excitation, the SHG was related mainly to the topology of the surface, while for p-polarized excitation, local enhancement of SH emission has been observed.

3 SHG Interferometry

In SHG, the SH field appears with a certain phase shift relative to the fundamental field. This phase shift can be used for the analysis of molecular orientation at the surface (interface). As a typical case, an interference effect of SH waves between two SHG-active media has been applied to the determination of absolute molecular orientation.²⁰⁻²⁸ Basic experimental arrangements for Langmuir-Blodgett (LB) monolayer assemblies, as an example, are shown in Fig. 3.²¹ Here one assumes that the relative phase shift between SH waves from R- and S-monolayers (denoted as $E_R(2\omega)$ and $E_S(2\omega)$, respectively) is caused only by the difference in the refractive indices (Δn) of air (method A) or glass G (method B) to fundamental and SH lights, and that the phase shift due to the monolayer itself is negligible.

Fig. 3 Experimental arrangements for the measurements of SH interferences: (a) the distance (ΔL) between reference (R) and sample (S) films is changed; (b) the rotation angle (θ) of the quartz glass plate (G) is changed.

In this case, the interference pattern is observed as a function of distance (ΔL) in method A. In method B, both monolayers are fixed at a certain distance and a glass plate G placed midway between the two monolayers is rotated; thus the interference pattern is observed as a function of rotation angle (θ). Figure 4 shows interference patterns of the SH waves from thin polymer films (50–70 nm thickness) in which SHG-active dyes are asymmetrically incorporated in the films, by using method B. The solid line shows the interference

Fig. 4 Interference patterns of SH signals from the RuD-impregnated film (R-film) and the RuD-impregnated film (dotted line) and StI-impregnated film (solid line) as the S-film.

patterns where the R- and the S-films contain the same compound (RuD). When the S-film is changed from the RuD-film to the StI-film, an almost 180° phase shift of the pattern has been observed. This indicates that the overall nonlinear susceptibility of StI with respect to the film surface is oppositely directed to that of RuD. In a similar manner, the interference measurements have been successful for the determinations of absolute orientation or phase shift at the air/water interface²² as well as those of the monolayer assemblies.²³⁻²⁸

4 Applications to Microscopic Structures

As described in the previous section, the relative phase difference between SH waves from R- and S-films is expressed as a function of ΔL , and the interference pattern appears with a period of coherence length l_c . Since the R- and the S-films are symmetrically arranged (C_2 point symmetry), the observed SH signal should be zero when the two films contact each other. However, the SH signal should increase with increasing ΔL up to l_c , according to the following equation:

$$E(2\omega) \propto \alpha^2 E(\omega)^2 \{1 - \exp(i\Delta k \cdot \Delta L)\} \quad (6)$$

where $\Delta k = (4\pi/\lambda)\Delta n$ (λ : wavelength of incident light).²⁹ The l_c value depends on Δn . When the medium between the films is air, as in the case of Fig. 3A, the l_c value is as large as ~ 6.5 cm. When the medium between the films is a quartz glass plate (as a typical case, when the LB films are deposited on both surfaces of a quartz glass plate), on the other hand, the l_c value is as small as ~ 24 μm , because the Δn value of the quartz glass plate becomes much larger than that of air.²⁰

This situation may be extended to a micrometer-size particle where SHG-active molecules are adsorbed at the surface. If the particle size is much less than the l_c value of the particle material, the SH waves from the two molecules present at the opposite sites will be cancelled. If the particle size is comparable with the l_c value (or λ), the SH waves from the two molecules will be constructive and thus the SH signal will appear. Indeed, Eiseenthal *et al.*^{29,30} found SH signals from the surface (interface) of polymer beads (microemulsions), as shown in Fig. 5. Polystyrene sulfonate (PSS) microspheres of ~ 1.05 μm diameter were suspended in an aqueous solution containing SHG-active Malachite Green (MG) in a sample cell (0.2 cm path length). The pulsed laser light (854 nm, 100 fs) was introduced to the cell and the SH signal was detected in transmission geometry. The SH signal in the presence of PSS beads and MG was two to three orders of magnitude larger than that in the absence of either MG or PSS. The dependence of SH field on the bulk MG concentration showed an approximate fit to the Langmuir isotherm (Fig. 6); the SH signal reaches a constant value at the MG concentration of as low as 10^{-5} M. In this case,

Fig. 5 Schematic representations for adsorption of MG at the PSS particle (a) and the emulsion (b).

Fig. 6 Adsorption isotherm of MG on the PSS particle surface in aqueous solution.³⁰

therefore, depletion of the bulk MG molecule due to adsorption should be taken into consideration. The modified Langmuir model yielded an excellent fit to the experimental results, and the maximum density of adsorbed MG (N_{max}) has been evaluated as $(1.8 \pm 0.2) \times 10^6/\text{particle}$. This method can also be applied to microemulsions. The adsorption behavior of MG at the surface (interface) of an oil-in-water emulsion consisting of sodium dodecyl sulfate (SDS) as a surfactant and 1-dodecanol as a coemulsifier has been investigated in a similar manner (Fig. 5 (b)). The average size of emulsion is ~ 230 nm. As in the case of the PSS particle, the SH signal tended to obey the modified Langmuir model. The N_{max} value has been evaluated as $(1.1 - 1.2) \times 10^5/\text{droplet}$.

Chemical analysis of the supernatant solution after

centrifugation has been usually used to determine the number of adsorbate molecules. Unlike the traditional methods, the SHG technique is a nonperturbing method for the determination of molecular adsorption to the particle surface.

The SH response from the surface of a hanging water droplet (mm size) containing an SHG-active surfactant was reported recently.³¹ The presence of SH response means tilted alignment of the surfactant as to the droplet surface. The SHG response was dependent on the droplet size.

5 Applications to Molecular Interactions

Evaluation of electrostatic and hydrophobic interactions between molecules are important in connection with biological membrane systems, affinity chromatography, and so on. However, *in-situ* evaluation of molecule-membrane interactions at the membrane surface region, for example, is very difficult, and there had been no useful optical methods. The SHG technique has been successful for direct observation of electrostatic interactions by taking advantage of interface specificity, as described in the introduction.

5.1 Complexation at the interface

SHG has been applied for selective observation of ionophore-metal ion complexation at plasticized poly(vinyl chloride)-based membranes.^{32,33} The polymeric liquid membrane (~130 µm thickness) containing a polyether-type ionophore was prepared. Then, one surface of this membrane was put in contact with an aqueous solution containing a cation, and the SH signal was detected by transmission geometry, as schematically

Fig. 7 Schematic diagram of the SHG measurement system for ionophore-incorporated membranes: a, stainless cover; b and e, silicon rubber packing; c, membrane; d, slide glass; f, sample cuvette.³³

shown in Fig. 7. The SH signal was clearly observed when both the ionophore (in membrane) and the cation (in solution) were present. A Langmuir-type saturation profile of the SH signal with increasing cation concentrations suggested the formation of a monolayer of the oriented SHG-active ionophore-cation complex at the membrane surface. The intensity of SH signal depended on the combination the ionophore and the cation.

5.2 Coadsorption at the interface

Paul and Corn³⁴ studied electrostatic coadsorption of biopolymer and surfactant at the water/1,2-dichloroethane interface. Schematic representation for coadsorption is shown in Fig. 8. A SHG-active, cationic surfactant (D^+-CH_3 ; $R=CH_3$) is dissolved in the 1,2-dichloroethane phase. Figure 9 shows time course of SH signal changes, upon injection of anionic polypeptide pGlu₂₄₀ into the water phase. The signal did not

Fig. 8 Schematic diagram of the biopolymer (pGlu₂₄₀)-surfactant coadsorption process at the water (H₂O)/1,2-dichloroethane (DCE) interface.

Fig. 9 Time course experiments showing the signal evolution as pGlu₂₄₀ is injected into samples containing D^+-CH_3 (filled circles) or $D^+-(CH_2)_3-SO_3^-$ (filled diamonds) adsorbed at the water/1,2-dichloroethane interface.³⁴

increase immediately after the injection, because the transport of pGlu₂₄₀ to the interface depended on both convection and diffusion. Once pGlu₂₄₀ reaches the interface, electrostatic coadsorption of pGlu₂₄₀ and D⁺-CH₃ occurs. Thus, the SH signal due to noncentrosymmetric alignment of D⁺-CH₃ at the interface appeared and increased with time. When a neutral surfactant (D⁺-(CH₂)₃-SO₃⁻; R=(CH₂)₃-SO₃⁻) was dissolved in the 1,2-dichloroethane phase, on the other hand, the SH signal did not change even after injection of pGlu₂₄₀; in this case, electrostatic coadsorption does not occur at the interface.

5.3 Charge-transfer interactions

SHG has been verified to serve as a probe for charge-transfer (CT) interaction at the interface. A phenothiazine (electron donor)-viologen (electron acceptor) (Fig. 10 (a)) pair shows a strong CT interaction.³⁵ First, the surfaces of a quartz glass plate were modified with an amphiphilic viologen ECV, with a submonolayer level. Then, this modified-plate was immersed into a hexane solution containing phenothiazine (PTH), resulting in a clear *in-situ* SH response. No appreciable SHG signals were observed either with the ECV-modified plate alone, or with the unmodified (bare) plate in the presence of PTH. The SH response due to the CT interaction has also been verified by using a phenothiazine-viologen linked compound, PTHC8V. The SH response from the PTHC8V-modified plate was larger in a solvent with lower dielectric constant; the CT interactions in PTHC8V are schematically shown in Fig. 10 (b). The *in-situ* SHG technique thus has been powerful as a probe for intra- and inter-molecular CT interactions at the interface. Also, the method may be applicable to evaluate the polarity at the interface region.

(a) Compounds

(b) Schematic structure of PTHC8V

Fig. 10 (a) Structures of compounds for CT experiments. (b) Possible structures for intramolecular CT interactions at the modified-glass/solvent interface.

6 Epilogue

In spite of the restricted area of topics in this article, it is clear that the SHG technique is extremely useful in studying chemical and physical phenomena occurring at the interfaces (surfaces). The noteworthy advantage of this technique is that only asymmetric perturbations (*e.g.* molecular ordering, reactions, and transportation at the interfaces (surfaces)) can in principle be measured directly even in the presence of unwanted bulk species. At the same time, the application to microscopy is quite fascinating, because the SHG technique offers detailed information about the degree of molecular ordering. Furthermore, the SHG microscopy will be applicable to the direct observation of phenomena occurring at the membrane surface of a biological cell. Of course, applications to the studies of equilibria, dynamic processes, and interactions at molecular levels will continue to grow.

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