Second harmonic generation imaging

with a kHz amplifier [Invited]

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Abstract: Nonlinear optical imaging is a powerful method for observing bulk and interfacial phenomena in time and space. Here, we present a step-by-step description of how to carry out second harmonic generation imaging with a kHz amplifier laser system and demonstrate its applicability for SHG microscopy studies of highly size-resolved colloidal CdSe quantum dots having radii of 1-2 nm deposited on glass slides. It is found that not all quantum dots are SHG active, which suggests that environmental effects and particle distributions are important for SHG activity of quantum dots.

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References and links


1. Introduction

Second harmonic generation (SHG) is a nonlinear optical process that occurs when two incident photons of the same frequency generate a nonlinear polarization in a sample. The oscillating polarization is responsible for producing light at the second harmonic, or half the wavelength, of the incident light. The relationship between the polarization and electric field strength is given by \[ \mathbf{P} = \chi^{(2)}(2\omega) \mathbf{E}(\omega) \mathbf{E}(\omega) \], where \( \mathbf{P} \) is polarization, \( E \) is electric field strength, and \( \chi^{(2)} \), the second order susceptibility, is a tensor that characterizes the extent to which the polarization is coupled to the electric fields. We use the subscripts \( ijk \) to denote any of the Cartesian coordinates, \( \mathbf{x}, \mathbf{y}, \mathbf{z} \). Under the electric dipole approximation [1], which assumes that electric quadrupole and magnetic dipole contributions to the polarization are negligible, SHG is forbidden in centrosymmetric media. This selection rule is due to the fact that changing the sign of the electric field, \( \mathbf{E}(\omega) \), which corresponds to turning a centrosymmetric sample by 180°, must result in the opposite polarization according to \( -\mathbf{P}(2\omega) = \chi^{(2)}(-\mathbf{E}(\omega)) \mathbf{E}(\omega) \mathbf{E}(\omega) \). Hence, \( \chi^{(2)} \) must be zero in a centrosymmetric medium and can be nonzero in a noncentrosymmetric medium. Second harmonic generation is a well-established nonlinear optical coherent method that allows for the study of particles in noncentrosymmetric media.
environments when sample sizes and masses are limited. Such conditions are typical for assemblies of semiconductor quantum dots, whose wurzite structure is noncentrosymmetric and thus SHG allowed. The study of the nonlinear optical properties of such colloidal systems is relevant to energy storage, conservation, or production applications [2–6]. Here, we describe the use of a kHz amplifier laser system for second harmonic generation imaging. We build on the work by Conboy [7], Shen [8], Eisenthal [9], Saykally [10], Baldelli [11], Simpson [12], Floersheimer [13], Loew [14], Scherer [15], Long [16], and Zyss [5], to name a number of SHG, sum-frequency generation (SFG), and $\chi^{(3)}$ imaging pioneers, where $\chi^{(3)}$ indicates that SHG was produced from the interaction of two photons at a sample where a static electric field exists. We test imaging and background-subtraction procedures for our kHz application on sugarcoated glass slides and then demonstrate its applicability for SHG microscopy studies of semiconductor quantum dots having a radius of 1-2 nm spincoated onto glass slides.

Sugar is an ideal starting material for setting up an SHG microscope because it is chiral, and SHG exhibits a very high sensitivity towards chirality [17]. This high sensitivity is manifested by a 100 to 10,000–fold increase of linear dichroism or circular dichroism effects when compared to non-coherent chirality measurements [17–22], and the molecular origins of this effect are discussed in the literature [17]. The SHG signal from sugar is large due to the contribution of a chiral signal in addition to those from noncentrosymmetric environments, such as the surface. In general, the SHG response from chiral species is associated with the $\chi_{xyz}$ tensor element of the nonlinear susceptibility tensor $\chi^{(2)}$, which is unique to all chiral species [23]. $\chi_{xyz}$ specifically represents the SHG signal generated in the x-direction due to incident electric fields oriented in the y- and z-directions, where the xz plane is the plane of incidence. Although chiral sum-frequency generation (SFG) can be observed in bulk liquids [24,25], SHG is forbidden in isotropic chiral media, but allowed at surfaces and noncentrosymmetric environments [26,27]. The intensities scale with the square of the number of oscillators in a given bulk sample [25]. Notably, the SHG response of chiral materials can be strong even when the experiments are carried out off electronic resonance [7,12,18,21]. Important milestones in nonlinear optical imaging of chiral species [7–16,28,29] were reached when Kriech and Conboy imaged functionalized glass slides patterned with R- and S-binaphthol [7], when Shen and associates imaged chiral systems in three dimensions [8], and when Hall and Simpson reported the direct observation of transient Ostwald crystallization ordering from racemic serine solutions [30].

With 1 W output over 1 mm², a standard Ti:Sapphire amplifier (1 mJ, 120 fsec) produces $8 \times 10^{9}$ W per mm² per pulse, while standard oscillator setups (12 nJ, 120 fsec), such as those employed by Shen and associates and Simpson and associates, yield $1 \times 10^{7}$ W per mm² per pulse. Nanosecond systems, such as the one used by Kriech and Conboy (150 mJ, 7 nsec), yield $2 \times 10^{7}$ W per mm² per pulse. Given that the SHG signal intensities scale with the repetition rate and pulse power, it should be possible to perform SHG imaging with a kHz amplifier, even off electronic resonance. The use of a kHz Ti:Sapphire amplifier allows for SHG imaging with fsec pulses, which is desirable when studying systems with absorptivities at the second harmonic frequency, such as semiconductor quantum dots. The amplifier used in this system provides high peak powers while maintaining low average powers, which can lead to destruction of samples. Because SHG is proportional to peak power, we obtain favorably high signals with minimal sample damage. Finally, imaging with a kHz amplifier provides enough energy per pulse to circumvent the necessity to use laser-scanning setups, allowing for SHG imaging in the far field, which is straightforward, quick, and inexpensive to carry out. Like any fsec laser source, background signal levels and instrument responses are readily evaluated by the straightforward use of a single optical delay line.

2. Experimental

SHG imaging is carried out here using a modified form of Kriech and Conboy’s pioneering crossed-beam design [7], adapted for a Newport/Spectra Physics Hurricane–i Ti:Sapphire amplifier laser system operating at a repetition rate of 1 kHz to produce 120 fs pulses at 800
nm (Fig. 1). The maximum output power of the amplifier is 1 W, which is attenuated to the needs of the system under investigation. We set the polarizations of the two incident beams to be perpendicular (p) and parallel (s) to the sample plane for the left and right beams, respectively, and we set the angle of incidence for each beam to 20°. This geometry is very close to full transmission geometry, for which the incident angles are zero degrees and input and output polarizations are not defined with respect to a sample plane. A variable density filter wheel (Edmund Optics A54-082) located before the last two mirrors before the sample on each of the two arms of the incident beams is used to attenuate and control the power at the sample, typically between 40 and 400 microJ/pulse. At the sample, the overlap of the two defocused incident beams is about 0.25 mm by 1 mm.

With both incident beams arriving at the sample at the same angle and wavelength, the SHG is produced in the forward and backward directions along the bisector of the incident fields. In the present setup, the forward scattering response is detected, and all polarizations are recorded because s- and p-polarization is undefined at zero-degree exigent angle. The samples are imaged through ultra-long working distance epiplan-neofluar 10x and 50x objectives (Zeiss part #4220409902000000 and 4224729900000000, respectively). The 50x objective has a working distance of 9.1 mm. The optical transmission of these objectives is between 80% and 85% at the SHG signal wavelength of 400 nm. The two objectives used here have numerical apertures of 0.2 and 0.55 for 10x and the 50x magnification, respectively, resulting in a maximum resolution of 1.2 µm and 0.4 µm, respectively, at the SHG signal wavelength of 400 nm. We then use a Zeiss tube lens (part #452149-0000) that exhibits 95% transmission at 400 nm, followed by the appropriate optical filter (Thorlabs FB400-10) to isolate the SHG response from the fundamental and scattered light fields, both housed inside a Zeiss binocular phototube (part #4255209030000000) and Axioskope focusing gear box (part

Fig. 1. SHG microscope for a kHz amplifier laser producing 120 fs pulses.
Finally, we image the SHG response onto a thermoelectrically cooled CCD detector (Princeton Instruments PIXIS 1024, 60% quantum efficiency at 400 nm) mounted onto the upper body part of a Zeiss Axioscope (part #4237309030000000) attached to an Axioscope stand column (part #4510179000000000). A Zeiss LED illuminator (part #4239049902000000) allows for optical microscopy of the areas imaged by SHG.

3. Results

Figure 2A shows the SHG image from a 1 mm thick z-cut quartz window, collected over 500 ms and oriented for maximum SHG intensity. The vertically aligned area where SHG occurs is due to the crossed-beam geometry employed in the microscope. Figure 2B shows that the SHG occurs at 400 nm, and that signal sources at wavelengths other than the fundamental probe light are negligible. When we translate the z-cut quartz window in the z-direction, i.e. into and out of the cross-sectional volume of the two incident beams, while maintaining focus on the top surface of the z-cut quartz window, we find that the SHG intensity goes through a maximum but does not decay to zero over the distance range studied with our translational stage (Fig. 2C, empty circles). This finding indicates that the length of the cross-sectional beam overlap in the z-direction exceeds the beam waist due to the steep incident angle. When we repeat this experiment using a plain glass microscope slide coated with sugar, we find that the SHG response is markedly more localized on the edge of the glass microscope slide (Fig. 2C, filled circles). This situation is summarized in Fig. 2D.
Figure 3A shows the optical image of a glass microscope slide onto which Domino confectioners' sugar, which is combination of cane sugar and cornstarch, was sprinkled. We removed a horizontal section of sugar by dragging a spatula across the slide, as seen in the figure. This process revealed the glass underneath, which appears light in the image. Superimposed onto the optical image is the corresponding SHG image, where the areas producing high SHG intensity are color coded such that red corresponds to the highest SHG intensity. Clearly, strong SHG responses are obtained from areas that show sugar present in the optical image, and negligible SHG is obtained from plain glass during the 3-minute image acquisition time. We note here that SHG responses up to 50 counts over background per pixel are obtained in the overlap area of the two fundamental probe light fields when plain microscope slides are imaged over 40 minutes. The data in Fig. 3B shows the corresponding power study, which is consistent with a second order optical process and indicates that optical breakdown does not occur in these studies.
Having tested SHG microscopy with a kHz amplifier using a bulk material (z-cut quartz) and a chiral oligomer on an achiral substrate (sugar on glass), we moved on to studying highly size-resolved colloidal CdSe quantum dots (QD) ranging from 1 to 2 nm in radius spin coated onto glass microscope slides from a $1 \times 10^{-6}$ M solution in hexane. While chiral structures on inorganic materials such as silver and gold have been imaged using SHG [31–33], as well as core/shell CdTe/CdS semiconductor quantum dots having radii of 10-15 nm [5], CdSe quantum dots having radii of 1-2 nm have not been studied until now. The QDs used in this work were prepared according to previously published procedures [34,35]. Briefly, we synthesized CdSe QDs from cadmium stearate, trioctylphosphine oxide, and hexadecylamine (HDA) into which trioctylphosphine selenide was injected at 300 K, following a procedure modified from the one published by Qu et al [36]. After arresting the growth of the resulting QDs by adding room temperature hexane to the reaction mixture, we allowed the samples to rest in hexane overnight. After three precipitation and centrifugation cycles [34] using hexane as the solvent and methanol as the non-solvent, which removes excess ligand, we determined the sizes and concentrations of the QDs in representative samples using TEM and the spectroscopic method of Yu et al [37]. Figure 4A shows the UV-vis spectrum and corresponding TEM image of CdSe QDs having radii of 1.2 and 1.3 nm, respectively. The presence and structure of the ligands, which include n-octylphosphonate, P'P'-(di-n-octyl)-pyrophosphonate, and, in one case outlined below, HDA, was verified using nuclear magnetic
resonance (NMR), x-ray photoelectron spectroscopy (XPS), and the vibrational analogue of SHG, sum-frequency generation [38] (SFG) spectroscopy. This analysis indicates the presence of the alkyl chains of the ligands that are associated with the Cd ions at the surface of the QDs [39]. The presence and physical properties of the ligand are important because they contribute to QD-QD interactions, as described by Murphy and associates [40]. Such interactions lead, presumably, to the SHG hotspots discussed below.

**Fig. 4.** (A) UV-vis spectrum of 1.22 nm radius CdSe quantum dots in hexane. Inset: TEM of CdSe quantum dots that have an average radius of approximately 1.3 nm and a bandedge absorption of 522 nm. (B) Vibrational SFG of 2.0 nm radius CdSe quantum dots spin coated from hexane onto a microscope glass slide.

Figure 5 shows the false color optical (A) and SHG (B) images of CdSe quantum dots (1.2 ± 0.1 nm radius) spin coated from hexane onto a glass microscope slide. The presence of agglomerated particles is clearly visible in the optical images, which were obtained using a 10x objective. The corresponding SHG image shows that some of the quantum dot agglomerates are SHG active. We routinely find only about 10% of the optically visible particles to exhibit nonlinear activity. Our results agree well with earlier highly size-resolved Hyper-Raleigh scattering studies of CdSe quantum dots having radii of 1-5 nm that were obtained in transmission geometries [6]. Figures 5A and 5B demonstrate the clear advantage of spatial information obtained from imaging vs. bulk measurements in the case of studying nonlinear optical responses from semiconductor quantum dots: the measurements discussed here clearly show that only a small subset of particles is SHG active, and that there appear to be hotspots of SHG that will be the subject of future investigations by our group.

When reflecting upon the fact that only a small population of the sample produces nonlinear optical signals, the question arises as to whether there are specific geometries and sizes of colloidal agglomerates that promote SHG action in CdSe quantum dots. We therefore zoomed in on a particular agglomerate and investigated it further with larger magnification. Figures 5C and 5D show an optical and SHG image of quantum dots (1.2 ± 0.1 nm radius, purified of excess ligands) which agglomerated into a structure having dimension of about 5 μm x 20 μm that was imaged using 50x magnification. The SHG image, which was obtained using ten minutes data acquisition time, shows an SHG hotspot near the top right corner of the structure, clearly indicating that SHG activity is not uniformly distributed among the optically visible quantum dot agglomerate. With the given SHG resolution of 400 nm, we find that the SHG hotspot is localized in an area of about 2 μm x 2 μm. This finding indicates that not all quantum dots are SHG active and suggests that environmental effects are important. These environmental effects are likely to involve contributions from the organic ligands, whose
structure and order are readily measured by SFG (Fig. 4B). We emphasize that this information would not have been available without SHG microscopy.

We note that knowledge about the presence of the ligands associated with the colloidal quantum dots in these experiments is important for two reasons: 1) control experiments (not shown) indicate that pure hexadecylamine (HDA) spincoated onto glass microscope slides is present in a polymorph that results in weak SHG activity that can be observed if the signal acquisition time is increased to two hours. The other ligands and chemicals used for the CdSe synthesis, after deposition onto glass slides, do not show this effect. 2) The quantum dot samples used in the experiments that resulted in the SHG images shown in Fig. 5D contained no HDA as verified by XPS and NMR. While individual agglomerates showed high SHG activity over ten minute signal integration time, we found, on average, fewer SHG active agglomerates when HDA was absent than when it was present as a minor component in the samples. At this point, the role of HDA in this system is not known. Future experiments are directed towards quantifying its role, as it can be a common component of CdSe quantum dot samples.

![Fig. 5. Optical (A) and SHG (B) images of 1.2 ± 0.1 nm radius CdSe quantum dots spin coated from hexane onto a glass microscope slide, obtained with a 10x objective. Optical (C) and SHG (D) image of quantum dots (1.2 ± 0.1 nm radius), purified of HDA ligands, obtained with a 50x objective.](image-url)
4. Conclusions

In conclusion, we have presented a step-by-step description of how to carry out second harmonic generation imaging with a kHz amplifier laser system and demonstrated its applicability for SHG microscopy studies of semiconductor quantum dots on glass slides. We emphasize that the use of nanosecond or picosecond pulses would have most likely led to optical breakdown on these immobilized systems. The use of a kHz amplifier laser system that delivers several tens of microJ of energy per 120 femtosecond pulse allowed us to defocus the beam to avoid optical breakdown while still maintaining reasonable signal acquisition times. The approach presented is not limited to the techniques described here, but is also applicable to materials that allow for SHG or higher-order resonance enhancement. Our findings indicate that not all quantum dots are SHG active and suggest that environmental effects, particle distributions, and ligands are important for SHG activity of CdSe semiconductor quantum dots. Such information would not have been obtainable with ensemble measurements. Future work will focus on measuring the SHG response as a function of quantum dot size, which may also be used to quantify the degree of sample homogeneity in a straight-forward fashion. In addition, this work opens up the possibility for correlating the intensity and occurrence frequency of the SHG hotspots with ligand order and disorder, which is QD size dependent. We are currently expanding these experiments towards the study of chiral materials other than confectioners' sugar, including synthetic and natural chiral aerosol particles relevant for atmospheric chemistry and oligonucleotide-functionalized surfaces that are relevant for biodiagnostics. Future work focuses on isolating the chiral tensor element, $\chi_{xyz}$, by using an appropriately placed polarizer.

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