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Dispersion-enhanced third-harmonic microscopy

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ABSTRACT

We demonstrate strong enhancements of signal yield and image contrast in third-harmonic microscopy by appropriate choice of driving laser wavelength to modulate the phase-matching conditions of the conversion process by dispersion control. Tuning the laser wavelength in the range of 1010 – 1350 nm at samples containing interfaces with water and glass, we obtained large signal enhancements up to a factor of 19, and improvements in the image contrast by an order of magnitude. The effect is most pronounced at interfaces with media of small and/or not too different nonlinear optical susceptibilities, e.g., as it is the case in typical samples in harmonic microscopy. Beyond the demonstration of this new variant of third-harmonic microscopy, our findings are also of relevance to a proper choice of laser systems for harmonic microscopy setups.

1. Introduction

In the past two decades coherent nonlinear microscopy (CNM) developed into a powerful and broadly applied tool for three-dimensional imaging of transparent samples [1,2]. CNM utilizes frequency conversion processes in strongly focused, ultrashort laser pulses. It even delivers images of otherwise fully transparent samples, which are not accessible by conventional linear microscopy without marking or staining. Examples for CNM processes are second harmonic generation (SHG), third harmonic generation (THG), and coherent anti-stokes Raman scattering (CARS). While SHG and THG microscopy typically apply a single off-resonant laser beam of ultra-fast pulses, CARS requires two laser beams to tune to vibrational Raman resonances for frequency conversion. Thus, SHG and THG microscopy are technically rather simple to implement. Three-dimensional imaging is possible by scanning the laser focus across the sample. We note, that SHG occurs only in non-centrosymmetric media [2], while THG is possible in media of arbitrary symmetry. In the case of tight focussing, the Gouy phase shift in the focus causes destructive interference for third harmonic generation in a bulk sample. Thus, net THG emission occurs only at interfaces [3,4]. This permits high contrast imaging of boundaries in heterogeneous samples.

THG is a parametric effect, i.e. in transparent media the process does not deposit energy in the sample [2]. This otherwise could lead to heating and damage, i.e. an important issue in biological samples. Thus in recent years THG microscopy found a large number of applications, particular in imaging of biological samples [5–7]. However, working under far off-resonant excitation conditions also limits the frequency conversion efficiency. Hence, the THG yield is typically very low, e.g. in the range of 10^{-8} with regard to the intensity of the driving ultra-fast pulses. This also limits the achievable image contrast in any sample.

It would be an obvious idea to increase the THG efficiencies and the image contrast by resonance enhancements. So far, there were only very few experimental attempts to increase the conversion yield and image contrast in THG microscopy using resonance enhancements of the third order nonlinear susceptibility $\chi^{(3)}$ [8]. The investigations were performed in a few specific molecular systems, applying near-infrared lasers with small tuning ranges: Clay et al. investigated possibilities for resonant THG emission in solutions with biologically relevant dyes (e.g. Rhodamin or hemoglobin), driven by ultra-fast laser pulses with center wavelength tunable in the range of 0.75–1.00 μm [8]. They found evidence for two-photon and three-photon resonances of specific solvents and dyes, permitting resonance enhancements by a factor of 2–5. Chang et al. observed resonance enhancements by a factor of 3–5 for THG from hemoglobin, driven by laser pulses at tunable wavelength between 1.2–1.3 μm [9]. Tai et al. demonstrated resonant THG in hemoglobin, applied for in-vivo THM of blood cells at improved image contrast up to 10, by tuning the laser wavelength in the interval 1.2–1.4 μm [10]. As a typical feature of such approaches, resonances in typical liquid or solid samples are usually spectrally very broad, i.e. around 100 nm in the near-infrared regime. The possible resonance enhancements from such broad spectral features are typically small. Thus, the obtained resonance enhancements of $\chi^{(3)}$ (and, hence, the potential gain for the image contrast) are still rather limited. Moreover, resonant excitation can also lead to damage by absorption of the fundamental radiation or THG signal losses by reabsorption.

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Since the first THM experiment of Barad et al. it is well known that the phase mismatch Δk is an important parameter [3]. Débarre et al. show the substantial influence of an index-matching medium on the contrast of a THG image of a human epithelial cell [11]. In the following we present a related approach, using the frequency dependence of the linear index of refraction n to significantly enhance the THG yield in harmonic microscopy. The idea of this "dispersion-enhanced" THG microscopy is to optimize the phase-matching conditions for harmonic generation by appropriately tuning the driving laser wavelength – rather than trying to rely on typically small resonance enhancements of the nonlinear susceptibility. The concept is applicable even quite far off-resonant.

Let us consider an interface between two media "A" and "B" in a heterogeneous sample, to be imaged by THG microscopy. We assume the Rayleigh length b/2 to be shorter than the sample size and a beam satisfying the paraxial approximation. In this case the generated third harmonic power at the (AB) interface is given by the well-known relation from nonlinear optics [8]:

$$P_{AB}(3\omega) \propto \frac{3\omega}{2\epsilon_0}\left|\frac{\chi_A^{(3)} b_A J(b_A \bullet \Delta k_A)}{n_A c} - \frac{\chi_B^{(3)} b_B J(b_B \bullet \Delta k_B)}{n_B c}\right|^2 \quad (1)$$

$$J(b \bullet \Delta k) = \int_0^\infty \frac{e^{ib \bullet \Delta k\, z}}{(1 + 2i\, z)^2} dz \quad (2)$$

with the nonlinear susceptibility $\chi^{(3)}$ or THG, the confocal parameters b, the phase mismatches $\Delta k = 3\omega \cdot (n\omega - n3\omega)/c$ between fundamental radiation and third harmonic, and the phase-matching integral $J(b \cdot \Delta k)$. As Eqs. (1,2) show, changes in the linear index of refraction modulate mainly the phase-matching integral in the THG process. The indices of refraction enter the integrals $J(b \cdot \Delta k)$ in a highly nonlinear fashion. Hence, they have a larger impact on $J(b \cdot \Delta k)$ compared to the linear dependence of P_{AB} upon the indices of refraction in the denominators (see equation (1)). Hence, modulation of the index of refraction by tuning the laser frequency in dispersive media should enable a strong variation of the THG yield via $J(b \cdot \Delta k)$.

Moreover, as Eq. (1) shows and as it is well-known in harmonic microscopy, the THG signal is large, when the difference between the nonlinear susceptibilities $\chi_A^{(3)}$ and $\chi_B^{(3)}$ is also large, i.e. when the media show very different nonlinear response. However, many media in microscopic samples still exhibit rather similar nonlinear susceptibilities. As simple examples, the third-order susceptibilities of water and fused silica are almost equal, and the third-order susceptibilities of water and ethanol differ by a factor of two only [12]. Thus, signals can be quite weak in THG microscopy. As an interesting feature, the possible THG enhancement via modulation of the indices of refraction is largest when THG signals are weak, i.e. when the contributions from the two media A,B in Eq. (1) are similar. In this case, small changes in the absolute signal yield cause large relative changes, i.e. large signal enhancements (compare also experimental data in Fig. 3, discussed below). However, the enhancement is possible in any kind of medium with arbitrary susceptibilities. The benefits from THG enhancements via dispersion modulations will be broadly applicable. Small variations of the refractive index (e.g., by tuning the driving laser wavelength) will have a big impact upon the possible THG enhancement.

We note, that the above approximations are only valid for moderate numerical apertures (NA) of the focussing lens. For large numerical aperture the phase mismatch caused by the Gouy phase $\Delta k_G \approx \pi/2b$ in the focal region becomes larger than the phase mismatch $\Delta k = 3\omega \cdot (n_\omega - n_{3\omega})/c$ due to dispersion [13]. In this case, dispersion will play a minor role only. To estimate the maximal NA, which still permits the observation of pronounced dispersion effects, we must compare the confocal parameter b (i.e. the interaction length) and the coherence length $l_C = \pi/\Delta k$. Dispersion plays a role, when b is larger than l_C. At our experimental conditions, we have $l_C \approx 5$ µm. Hence, dispersion effects are relevant for moderate focussing with numerical apertures NA ≤0.4.

In the following, we demonstrate the above concept by monitoring the THG yield from hybrid samples with interfaces between different media. As we will see, appropriate choice of the laser wavelength permits substantial modulation of the THG emission, yielding strong suppression of perturbing or large enhancements of desired THG signals. In particular, we observe THG enhancements up to a factor of 19, and significantly improve the image contrast in a microscopy sample by an order of magnitude. As an important feature, the optimal laser wavelength depends upon the media at the interface. This is of relevance for any THG microscopy setup.

2. Experimental implementation

The experiments require a laser system, providing ultra-fast laser pulses with tunable center frequency, driving THG in demonstration samples in a nonlinear optical microscope setup (see Fig. 1). We apply a picosecond optical parametric oscillator (OPO FAN, APE), synchronously pumped by a titanium sapphire oscillator (MIRA 900 P, Coherent). The laser system provides pulses with tunable center wavelength in the range 1010 – 1350 nm, at an average output power of 360 mW, pulse duration 1.5 ps and 76 MHz repetition rate. The laser beam is focused by an aspheric objective lens (O, A240TM-C, Thorlabs) with a numerical aperture of 0.5 (partly illuminated: 0.2) and a focal length of 8 mm into the sample (S). The focus diameter is roughly 2 µm and the Rayleigh length is 10 µm (measured in air). The objective lens is mounted on a translation stage, which permits scanning of the laser focus parallel to the optical axis (z-axis). A galvanic scanner (GS, Nutfield, OFH-5 SQ-7) enables steering the laser focus in the transversal axis (x and y-axes) across the sample. The third harmonic generated in the focus is collimated by a condenser lens, separated from the fundamental radiation by dichroic mirrors (DM) and interference filters (IF) (FB340-10 or FB430-10, Thorlabs), and detected by a photo-multiplier tube (PMT) (R4220, Hamamatsu). In experiments which require larger tunability of the laser wavelength, we

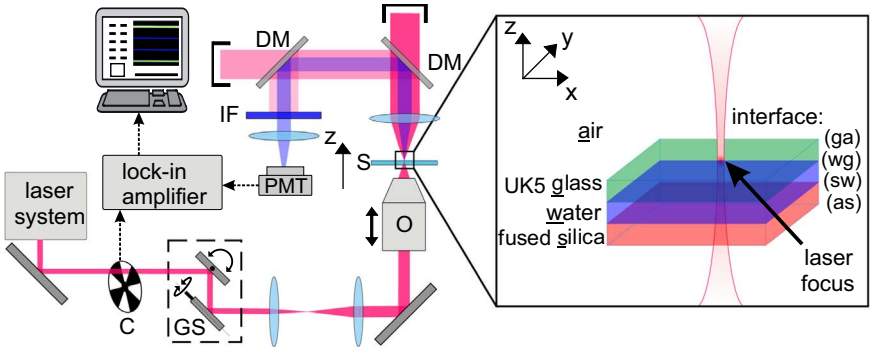

Fig. 1. Schematic setup of the nonlinear microscope and enlarged view of the laser focus intersecting a water-filled cuvette as a demonstration sample for dispersion-enhanced THG microscopy.