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Analysis of the influence of spherical aberration from focusing through a dielectric slab in quantitative nonlinear optical susceptibility measurements using third-harmonic generation

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Abstract: The third-order nonlinear susceptibility ($\chi^{(3)}$) can be measured quantitatively using third-harmonic generation (THG) from two different interfaces. For the first time it is demonstrated both in experiments and theory that the magnitude of the THG signals from the two interfaces is not only determined by material properties (refractive index and $\chi^{(3)}$), but also by optical aberrations. It is found that this method of $\chi^{(3)}$ determination can be applied without additional correction factors only for focusing conditions with a numerical aperture (NA) $\leq 0.35$. The implications for general application of THG in three-dimensional microscopy are discussed.

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References

1. Introduction

The contrast in THG microscopy is based on the generation of third-harmonic signals from tightly focused ultra-short laser pulses [1, 2]. Whereas in principle THG is allowed in any material with a non-zero $\chi^{(3)}$, it can be shown that for a homogenous, isotropic medium of infinite extension and with normal dispersion, no third-harmonic is generated by a tightly focused beam in case of perfect phase matching [3, 4]. This can be explained by considering the Gouy phase shift accumulated by a focused laser beam during its propagation across the focal plane [5]. Effectively, in this case, the third-harmonic radiation generated before the focal plane interferes destructively with that generated after the focal plane [6]. On the other hand, inhomogeneities, in either refractive index or in $\chi^{(3)}$, within the focal volume can lead to a measurable third-harmonic signal. This is the contrast generating mechanism in THG microscopy, which has been demonstrated to be a useful imaging tool in biology and the material sciences [7-16].

Spherical aberration that results from focusing through a dielectric slab, can significantly distort the focal field of a high NA microscope objective [17]. It causes primarily (i) a reduction of the peak intensity, (ii) a focal shift and (iii) an elongation of the focal field along the optical axis. This type of aberration is common in microscopic applications and leads to a loss in contrast and in a reduced axial resolution. Spherical aberration may result either from the use of cover glasses with a thickness and/or refractive index that deviates from that specified by the microscope objective's manufacturer or from focusing in a medium with a refractive index that does not match that of the immersion medium of the microscope objective [17, 18]. The influence of spherical aberration on the three-dimensional imaging performance of a THG microscope can be expected to be especially significant due to the third-order dependence of the signal on the fundamental laser field. Another area where spherical aberration may be important is that of the quantitative measurements of the sample's third-order nonlinear optical properties. Recently the use of THG has been proposed for the
measurement of the $\chi^{(3)}$ of solutions in a simple and accurate manner compared to conventional techniques [19], [20].

In this report, we analyse the influence of spherical aberration, induced by focusing through a dielectric slab, on quantitative measurements of $\chi^{(3)}$ using THG. We determine the experimental conditions for which such measurements can be done accurately. In addition we consider the implications of spherical aberration for general three-dimensional THG microscopy.

2. Materials and methods

Principle of the technique. Accurate determination of $\chi^{(3)}$ of solutions has been the subject of a number of recent discussions in experimental nonlinear optics. Among the techniques employed, the recently proposed ‘THG ratio method’ [19-21] is particularly simple and can be used even for samples that are highly scattering and/or absorbing at the third-harmonic wavelength. The experimental arrangement for the measurement is schematically depicted in Fig. 1(a). The solution for which the $\chi^{(3)}$ is to be measured is contained between two transparent cover glasses, separated by a spacer and sealed. The different interfaces are denoted by A (air-glass), B (glass-solution), C (solution-glass) and D (glass-air). The fundamental infrared beam is usually focused using a microscope objective and an axial scan of the focus across the interfaces A and B is performed. Due to the Gouy phase shift no THG signal is generated within the bulk of the glass or solution. A third-harmonic signal is generated only in the vicinity of an interface. In first approximation the peak of the THG signal coincides with the position of the interface and the full width at half maximum (FWHM) of the THG signal, as a function of axial position, equals the confocal parameter $b = k w_0^2$ [1], where $k = 2\pi n/\lambda$, $n$ is the refractive index at wavelength $\lambda$, and $w_0$ is the beam waist. The peak third-harmonic intensities at the interfaces A and B are denoted as $I_A$ and $I_B$ respectively. The ratio $I_B/I_A$ can be related to the $\chi^{(3)}$ of the solution kept in the region BC, provided that the $\chi^{(3)}$ of the region AB and the refractive indices of regions AB and BC are known. The accuracy of this technique especially depends on the accuracy with which these material constants are known. This method of $\chi^{(3)}$ measurement assumes that, if the media in the regions BC and the one preceding interface A are equal in both $\chi^{(3)}$ and refractive indices, the ratio $I_B/I_A$ will be unity (if proper corrections for reflections are incorporated). As pointed out in the previous section, focusing through a dielectric slab results in spherical aberrations. Thus in general the THG signals at A and B will differ. The magnitude of the primary spherical aberration depends strongly on the NA of focusing (proportional to $\sin^4(\alpha/2)$, where $\alpha$ is the semi-aperture angle of focusing).

Generally, microscope objectives are designed for focusing through a cover glass of specified thickness and refractive index. When used under optimal conditions, microscope objectives thus produce aberration free focusing at interface B for a specified cover glass, while at interface A, significant spherical aberrations may be present. Thus, even if the material properties are identical for the regions BC and the one preceding A, the ratio $I_B/I_A$ depends on the NA of the focusing microscope objective, and the thickness and refractive index of the cover glass (i.e. the medium in region AB).

Experimental Setup. The schematic of the experimental setup used for the THG measurements is shown in Fig. 1(c). A laser (FemtoTrain, High-Q Laser GMBH, Austria) produces 1062 nm pulses with a duration of 113 fs with a repetition rate of 72.3 MHz. After collimation, the laser beam is focused onto the sample using an infinity corrected microscope objective. This microscope objective is mounted on a piezo (Physik Instruments, Germany) for axial scanning. The signal is collected in the forward direction using a second microscope objective and imaged onto a spectrometer equipped with a low temperature CCD camera (spectral resolution ~0.15 nm at 351 nm). To ensure full collection of the generated signal, the
NA of the collection objective was chosen to be always larger than the required one-third of the numerical aperture of the focusing objective [22].

Figure 1(a) shows the sample configuration used in quantitative $\chi^{(3)}$ measurements using THG. To investigate the influence of aberration, glass types with different dispersion and thickness have been used (see table 1).

![Figure 1](image)

**Fig. 1.** Experimental configuration for (a) quantitative $\chi^{(3)}$ measurements and (b) evaluation of the THG signal depth dependence in a refractive index mismatched sample. Regions AB and CD are cover glasses, region BC contains the sample of interest. (c) Schematic of the experimental set-up for the THG measurements. Symbols used: L: lens; v.a.: variable aperture; S: sample; O: microscope objective; C: collection lens; F: filter. L4 can be translated along the optical axis, as can the microscope objective (O), which is mounted on a piezo scanner.

Table 1. Properties of glass types used.

<table>
<thead>
<tr>
<th>Glass Type</th>
<th>$n_0(*)$</th>
<th>$n_{3\omega(*)}$</th>
<th>$d$ (μm)</th>
<th>Vendor</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Duran</td>
<td>1.4625</td>
<td>1.491</td>
<td>200</td>
<td>VitroCom Inc., USA</td>
</tr>
<tr>
<td>G2 D 263 M</td>
<td>1.5158</td>
<td>1.5481</td>
<td>150</td>
<td>Menzel Glasser GmbH, Germany</td>
</tr>
<tr>
<td>G3 Duran</td>
<td>1.4625</td>
<td>1.491</td>
<td>50</td>
<td>VitroCom Inc., USA</td>
</tr>
</tbody>
</table>

(*): Refractive indices were obtained from Schott GmbH (Germany) for the Duran glass and from Menzel Glasser GmbH (Germany) for the D 263 M glass.

In order to demonstrate the dependence of the magnitude of spherical aberration on the focusing NA, the effective NA of the focusing objective was adjusted using a variable aperture. A detailed description of this procedure can be found in [22]. The experiment consists of an axial scan of the focal volume across the two different interfaces labeled A and B with air in the region BC. To measure the effect a refractive index mismatch on THG efficiency, a sample configuration as shown in Fig. 1(b) is used. It consists of a wedge shaped container formed by two G2 glasses at a small angle (~ 7·10^-3 rad). The container is filled with water. Measurements done at different transverse locations at interface C, correspond to measurements at different depths.

**Theory.** The paraxial equations describing third-harmonic generation have an analytical solution when the amplitude of the excitation electric field has a Gaussian profile [3]. This approach has been extensively used in a number of theoretical and experimental investigations of third-harmonic generation with both high and low NA focusing [1], [20], [22]. Recently a Green’s function formulation of THG was proposed [23], where the third-harmonic signal is obtained as a superposition of third-harmonic fields generated by the...
induced dipoles within the sample. Also, instead of a paraxial approximation, an angular spectrum representation for a homogeneous medium is used to represent the focused laser beam. This representation is considered to be a better approximation in the case of high NA focusing.

The numerical calculations of the THG signal as a function of NA and axial position of the focal field with respect to an interface, closely follow the method of [23]. The sample is assumed to have linear optical properties exactly equal to that of the surrounding medium. The only way the sample differs from the surrounding medium is in its $\chi^{(3)}$. This means that in the calculations of the focal field, it is assumed that the focusing is in a uniform medium and that the generated third-harmonic is also propagating through a homogeneous medium towards the detector. Another simplification used in the calculation is to neglect a possible wave vector mismatch ($\Delta k = k_{3\omega} - 3 k_\omega$), which is usually permitted for tight focusing conditions [23]. In order to incorporate the effect of spherical aberration in the focal field distribution, a phase error $\phi(\alpha)$ is used. The phase error corresponding to primary spherical aberration introduced by a dielectric slab of thickness $t$ in a converging wavefront is given by [18]

$$\phi = \frac{1}{2} \frac{1}{n_1^2} \left[ n_2 \left( 1 - \frac{4 n_1^2 s^2 (1 - s^2)}{n_2^2} \right)^{1/2} - n_1 (1 - 2 s^2) \right]$$

where $n_2$ is the refractive index of the slab, $n_1$ that of the medium and $s = \sin(\alpha/2)$. Hence, for a microscope objective designed for a cover glass of thickness 170 $\mu$m, the phase error introduced by focusing through a cover glass with an actual thickness $t$ can be written as:

$$\phi = k(t - 170) \left[ n_2 \left( 1 - \frac{4 n_1^2 s^2 (1 - s^2)}{n_2^2} \right)^{1/2} - n_1 (1 - 2 s^2) \right]$$

To simplify calculations the THG intensity was evaluated only on-axis at the detector, while using the full three-dimensional shape of the focal field. Since reflection at the glass-solution interface never exceeds 0.06%, its contribution was neglected. For the amplitude across the aperture of the microscope objective, either a flat field or Gaussian distribution was used.

In the ‘THG ratio method’ [19], the $\chi^{(3)}$ of a material in the BC region (Fig. 1(a)) can be determined from the measured ratio $I_B/I_A$ in an air-glass-material configuration. Using the known $\chi^{(3)}$ of the glass and the refractive indices of both glass and the material at the fundamental and the third-harmonic, the $\chi^{(3)}$ of the material is given by:

$$\chi^{(3)}_{\text{mat}} = \left( \frac{\chi^{(3)}_{\text{glass}}}{J_{\text{glass}}} \right) \left( \frac{I_B}{I_A} \right) \frac{J_{\text{mat}}}{J} \tag{3}$$

where $J$ represents the modulus of the J integral given by

$$J = \int_0^\infty \exp(i \Delta k z) dz$$

The sign ambiguity in Eq. (3) is overcome by comparing different measurements and the known trend of increase of $\chi^{(3)}$ from water to 2-propanol.

3. Results

**Spherical aberration from the cover glass.** In this measurement the sample consists of a single air-glass-air configuration. For a given sample configuration the optimal microscope objective illumination conditions are determined by measuring the FWHM of the THG $z$-response across interface B as function of the position of lens L4 (Fig. 2). The position of L4
that provides a minimum in the FWHM is taken as the configuration with minimal spherical aberration. Indeed, the minimal FWHM $L_4$ lens position coincides with a flip in the asymmetry of the z-response (see insets Fig. 2) which corresponds to a flip in the sign of the induced spherical aberration. The effective z-position is corrected for the focal shift that results from the refraction at the air-glass interface. The FWHM values are obtained by fitting Voigt profiles to both the left-hand and right-hand side of the z-response. The choice for fitting with a Voigt line profile is prompted by the observation from experiments and numerical simulations, that the shape of the z-response varies smoothly between purely Lorentzian for $\Delta k=0$, to Gaussian in case of large $\Delta k$ values or the presence of significant spherical aberration.

The fact that (i) the minimum FWHM values are identical for all three glass types within the experimental error margin, and (ii) that the optimal $L_4$ positions vary consistently with the thickness and refractive index of the glass used (data not shown), indicates that this procedure provides comparable minimum aberration conditions at interface B for all experimental conditions.

![Figure 2: FWHM of the THG z-response as a function of the $L_4$ position. Error bars represent one standard deviation of error and the solid line is a guide to the eye. Representative z-responses - open circles and corresponding fits to the left- and right-hand side (red and blue curves respectively) – are shown for $L_4 = 11.5, 14$ and $16.5$.](image)

Once the optimal position for lens $L_4$ - and thus the microscope objective illumination conditions that provides minimal aberrations at interface B - is determined for the different glasses (G1, G2 and G3), the effect of aberrations can be determined from a measurement of $I_B/I_A$ as a function of NA (Fig. 3). The sample configuration is identical to that used for Fig. 2. To permit comparison with earlier work on the ‘THG ratio method’ for the quantitative determination of $\chi^{(3)}$, peak intensities at the interfaces A and B are used. By definition, spherical aberration is zero at interface B and nonzero at the interface A. The deviation of the ratio $I_B/I_A$ from unity is a direct indication for the magnitude of the aberrations. The ratio $I_B/I_A$ increases sharply with NA and with the thickness of the cover glass (G3 = 50 $\mu$m $\rightarrow$ G1 = 200 $\mu$m). The solid line in Fig. 3 represents a numerical calculation for the G2 case.

The effect of spherical aberration on the determination of $\chi^{(3)}$. In order to demonstrate the effect of spherical aberration on quantitative $\chi^{(3)}$ measurements using THG, the ratio $I_B/I_A$ is measured for methanol, ethanol and 2-propanol using objectives with NA = 0.35 and NA = 0.65, and G1 glass. The $\chi^{(3)}$ values are determined from the ratio $I_B/I_A$ using the method described above. As a reference point, the $\chi^{(3)}$ value of water ($2.8 \times 10^{-14}$ e.s.u) [24] was used

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to obtain $\chi^{(3)}$ of Duran glass ($2.87 \times 10^{-14}$ e.s.u) in a separate measurement. Using this $\chi^{(3)}$ of Duran glass as the reference, the $\chi^{(3)}$ of the liquids (methanol, ethanol and 2-propanol) was determined. The required refractive indices of the different liquids for the J integral evaluation, were obtained using the Cauchy parameters as determined by Kozma et al. [25]. The measured $\chi^{(3)}$ values are shown in Fig. 4. From Fig. 3 it follows that for NA = 0.35 the effect of aberrations can be neglected, whereas for NA = 0.65 aberrations significantly affect the measured $I_B/I_A$ ratio. For the high NA case the effect of aberrations can be corrected for as follows. Since aberrations are appearing at interface A alone, only the THG signal from that interface need to be corrected. This correction factor is obviously independent of the material medium in the region BC, provided that the confocal parameter is significantly smaller than the axial extent of region AB, to ensure that the signal from interface A is entirely due to AB. In that case, the correction factor $r = (I_B/I_A)_{air-glass-air}$ as measured using air-glass-air interface and the NA of interest. The resulting $\chi^{(3)}$ value for NA = 0.65 after correction for the effect of aberrations is also shown in Fig. 4. For comparison, $\chi^{(3)}$ values from literature [24, 26] for these three liquids are also shown in Fig. 4. It should be noted, however, that the literature value for 2-propanol corresponds to a different wavelength (1910 nm) than used in these experiments (1062 nm).

![Fig. 3. (a) Measured ratio $I_B/I_A$ as a function of NA for G1 (triangles), G2 (circles) and G3 (squares) in a air-glass-air configuration. The solid line represents a numerical calculation for G2. (b) Measured (open symbols) and calculated (solid line) THG z-responses at interfaces A (red) and B (blue) for G2. The measurement error is approximately ±2%.](image)

![Fig. 4. Measured $\chi^{(3)}$ values for methanol, ethanol and 2-propanol. Red and light blue bars represent direct evaluation of $\chi^{(3)}$ based on $I_B/I_A$ measurements with NA = 0.35 and NA = 0.65 respectively. Dark blue bars represent $\chi^{(3)}$ value obtained from the NA = 0.65 measurement after correcting for the effect of aberration. Violet bars denote literature $\chi^{(3)}$ values obtained at 1062 nm for methanol and ethanol and at 1910 nm for 2-propanol [24, 26]](image)
Spherical aberration from refractive index mismatch. Equivalent effects resulting from induced aberrations, as observed in quantitative $\chi^{(3)}$ measurements using THG, are encountered in three-dimensional THG microscopy, when a refractive index mismatch is present between the immersion of the microscope objective and the mounting medium of the sample. Again, spherical aberration results from focusing through a dielectric slab. The magnitude of this effect is investigated by measuring the ratio $I_C/I_B$ in a sample configuration as depicted in Fig. 1(b), as a function of the lateral position and hence the depth inside the sample region $BC$. The medium in region $BC$ is water, while focusing is realised either with an oil immersion (NA = 1.25) or air spaced (NA = 0.65) microscope objective. Thus in both cases there is a refractive index mismatch between either oil ($n = 1.5158$) or air ($n = 1$) and water ($n = 1.33$). Figure 5 shows the ratio $I_C/I_B$ as a function of the thickness of the water layer for two different microscope objectives. The same ratio was calculated theoretically for NA = 0.65 using a uniform profile, and 1.25 using a Gaussian profile [23].

![Graph showing the ratio $I_C/I_B$ as a function of depth in a refractive index mismatched medium (water) for two different microscope objectives: 0.65 NA/40x air-spaced (blue circles) and 1.25 NA/63x oil immersion. Solid lines represent theoretical calculations for NA = 0.65 (blue) with a uniform profile and NA = 1.25 (red) with a Gaussian profile. All measurements and calculations are for G2 glass.]

4. Discussion

The 'THG ratio method' to determine $\chi^{(3)}$ of a medium is based on the measurement of the ratio ($I_B/I_A$) of the THG signal at the glass-medium interface and the glass-air interface. This simple and elegant method has been applied to the $\chi^{(3)}$ measurement of various liquids [19] and solutions [20]. In these measurements it is implicitly assumed that the $I_B/I_A$ ratio is unity when an identical medium is used at both interfaces. In the measurements presented here, it was found that this assumption is true only for low NA focusing (NA ≤ 0.35) conditions, in contrast to those used in these initial studies (NA = 0.65 and 0.55 respectively). It is well known that focusing through a dielectric slab results primarily in spherical aberration [18], and that this effect becomes more severe with increasing NA and optical path length through the material. Microscope objectives are generally pre-compensated for this effect to yield minimal aberration conditions at the backside (interface B) of the cover glass of a certain specified thickness and refractive index. Slight changes in collimation of the microscope objective's illumination can be used to correct for different glass types and cover glass thicknesses.

The procedure used to locate the optimum $L_4$ lens position provided us with the key initial settings required to quantify the effect of aberration as a function of numerical aperture,
focusing depth, and the refractive index and thickness of the cover glass used. A clear minimum in the THG z-response across interface B can be identified as a function of the $L_4$ position (Fig. 2), which is taken to coincide with minimum aberration conditions at focus. It should be noted that this method of optimising the microscope objective's illumination conditions can be used as a quick and effective way to control the effect of aberration and hence the signal level in quantitative as well as qualitative THG microscopy. Especially in quantitative applications, such as in the measurement of material properties as described in this report, it is useful to ensure that the focal field at, at least, one of the interfaces is aberration free. The effect of aberrations at the second interface can then readily be obtained either experimentally or theoretically. Once the effect of induced aberrations is determined, the measured $I_B/I_A$ ratio can be corrected to yield $\chi^{(3)}$ independent of the experimental conditions. Thus, although low NA focusing conditions are generally preferred for quantitative $\chi^{(3)}$ measurement, high NA focusing -with proper correction for the induced aberrations- can be used in specific cases (e.g. for thin samples).

The data shown in Fig. 3 emphasises the importance of knowing the extent of aberration: the $I_B/I_A$ ratio converges to unity and becomes independent of NA and glass type only for NA $\leq 0.35$. For a moderate NA = 0.65 and G1 glass combination, the $I_B/I_A$ ratio increases by a factor as high as $\sim 8.5$ as a result of induced aberrations. Figure 3(b) also shows the close agreement - both in absolute value and in the shape of the THG z-response - that is obtained between the experimental data and those calculated using the theory as described in section 2. In fact, the theoretical calculation of $I_B/I_A$ slightly underestimates the experimental value. This slight difference is most likely due to the assumptions employed in the calculation. The fundamental focal field is calculated under the assumption of a uniform medium in linear optical properties, where as in the actual experimental situation, the focusing is done at an interface of two media of relatively large refractive index difference. In this manner the fundamental focal field is not rigorously calculated. In addition to this assumption, we neglected the dispersion of the material medium and the ensuing wave vector mismatch, even though this is justified only in the case of a tight focusing condition [23].

Figure 4 summarises the main results in a $\chi^{(3)}$ measurement of three liquids for two different focusing conditions. At low NA (0.35) the $\chi^{(3)}$ can be determined directly from the $I_B/I_A$ ratio. At high NA (0.65) the effect of aberrations result in a much larger $I_B/I_A$ value and hence in an overestimate of $\chi^{(3)}$. Using the results of Fig. 3, however, this value can be corrected for the effect of aberration. The measured $\chi^{(3)}$ values correspond well with those reported in the literature, measured using the Maker-fringes technique [24]. Since the laser output shows very little power fluctuations, the $I_B/I_A$ ratio can be measured with high accuracy ($\pm 2\%$). The error in the measurement of $\chi^{(3)}$ then results from the uncertainty in the refractive index values($\pm 0.5\%$) of the liquid at the fundamental and third-harmonic wavelength and in the precision with which the initial reference $\chi^{(3)}$ value of water is known ($\pm 5\%$). This translates in an uncertainty in the measured $\chi^{(3)}$ value of $\pm 7\%$

The effect of spherical aberration on the THG signal strength in case of focusing in a refractive index mismatched sample medium is demonstrated in Fig. 5. This situation is often encountered in three-dimensional THG microscopy. Being a third-order nonlinear optical process, the third-harmonic signal level drops with the third power of incident signal level. Hence, the effect of aberrations is expected to be more significant in THG microscopy than in other microscopy techniques employing lower order processes such as second-harmonic generation and two-photon absorption fluorescence. It follows from Fig. 4 that, especially for high NA focusing, the THG signal is significantly reduced even for moderate penetration depths in case of a refractive index mismatch. On the other hand, reduced scattering at near IR wavelengths, provides THG microscopy with the opportunity to penetrate deeply in scattering media. This potential can be fully utilised by matching the refractive index of immersion medium to that of the sample, as has recently been demonstrated for \textit{in vivo}
imaging in extremely thick samples (~1 mm) [15]. In this particular case a water immersion microscope objective was used to match the refractive indices of the immersion and mounting medium.

The theoretical calculations (solid lines in Fig. 5) again show good agreement with the experimental results. For NA = 0.65, the calculated ratio $I_c/I_B$ slightly overestimates the experimental measurements, for the same reason as it underestimated $I_B/I_A$ (see above). A better agreement between the calculated and measured ratios is found for the 1.25 NA oil immersion objective, where the approximations used in the calculation are in better agreement with the experimental conditions compared to the case of 0.65 NA air objective. We also found that the input beam profile also influences the effect of aberration. A Gaussian beam profile was found to be less susceptible to aberrations compared to a flat profile. We have used a flat profile and a Gaussian profile for the low NA and the high NA cases respectively.

In conclusion, we have shown the importance of accounting for NA dependent, specimen induced, optical aberrations for accurate $\chi^{(3)}$ measurements using the ‘THG ratio method’. A theoretical analysis, based on a Green's function formulation, has been presented that permits quantitative calculation of both the THG peak intensity and the functional shape of the THG $z$-response in the presence of specimen induced aberrations. The close agreement between experiment and theory indicates that spherical aberration is the prime factor to account for in these measurements. We have shown that measurements at low NA ($\leq 0.35$) do not require additional correction for aberration. For the case of high NA focusing (NA > 0.35) - required e.g. to measure the $\chi^{(3)}$ of thin samples - a procedure has been developed and demonstrated that can correct for the effects of specimen induced aberrations.

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