Quantitative Raman spectroscopy in turbid media

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1 Introduction

Raman spectroscopy is a powerful analytical method due to its chemical bond specificity. The molecular vibrations of each substance produce a characteristic fingerprint spectrum that can be used to determine the chemical and structural composition of the sample. Applications in biomedical research are wide-ranging, including the diagnosis of cancer, 1-3 atherosclerosis, 4 and blood analysis. 5 The major advantages of Raman spectroscopy compared to fluorescence applications are that Raman signals do not bleach, and that Raman bands are spectrally narrow, which improves the separability of the signals.

A basic challenge for Raman spectroscopic measurements in turbid media is the signal quantification. The spectral line shape and overall intensity of the measured spectra depend on the absorption and scattering properties of the sample. In the case of biological tissue, absorption arises from chromophores such as hemoglobin and melanin. Light scattering in tissue occurs due to the refractive index inhomogeneities of the cell nuclei, cell organelles, or collagen fibers. Due to different biochemical tissue compositions and microstructures, the optical properties of tissues may vary strongly between tissues, tissue sites, and individuals. Since a contrast in optical properties can, on its own, indicate pathological changes such as tumor angiogenesis or dysplasia, extensive research has been carried out on the measurement of optical properties for diagnostic purposes. 6-9 Nevertheless, most Raman spectroscopic tissue measurements are not corrected for these influences, which hinders the quantitative comparison of the measured Raman spectra.

Since the measured Raman scattered light and purely elastically scattered light are both influenced by variations in the optical properties, correction methods have been suggested based on diffuse reflectance measurements. Some approaches have been developed for applications where either absorption or scattering is assumed to change 10,11 and are therefore not suitable for in vivo applications, where independent and simultaneous variations of the optical properties are common. The first theoretical and experimental studies on the correction of Raman signals in biological tissue were published recently 12-14 for a parameter range of absorption coefficient \( \mu_s \) and scattering coefficient \( \mu_t \), which are typical for measurements in the near infrared wavelength range (NIR). The correction functions utilize the elastic diffuse reflectance, which is measured in the same excitation-detection geometry as the Raman spectrum. However, knowledge of the total attenuation coefficient given by \( \mu_t = \mu_s + \mu_a \) is required, 12,13 or the reduced scattering coefficient \( \mu_t' \) is used. 14 However, the determination of optical properties from a single elastic diffuse reflectance spect-

Abstract. Intrinsic Raman spectra of biological tissue are distorted by the influences of tissue absorption and scattering, which significantly challenge signal quantification. A combined Raman and spatially resolved reflectance setup is introduced to measure the absorption coefficient \( \mu_s \) and the reduced scattering coefficient \( \mu_t' \) of the tissue, together with the Raman signals. The influence of \( \mu_s \) and \( \mu_t' \) on the resonance Raman signal of \( \beta \)-carotene is measured at 1524 cm\(^{-1}\) by tissue phantom measurements and Monte Carlo simulations for \( \mu_s = 0.01 \) to 10 mm\(^{-1}\) and \( \mu_t' = 0.1 \) to 10 mm\(^{-1}\). Both methods show that the Raman signal drops roughly proportional to \( 1/\mu_s \) for \( \mu_s > 0.2 \) mm\(^{-1}\) in the measurement geometry and that the influence of \( \mu_t' \) is weaker, but not negligible. Possible correction functions dependent on the elastic diffuse reflectance are investigated to correct the Raman signal for the influence of \( \mu_s \) and \( \mu_t' \), provided that \( \mu_s \) and \( \mu_t' \) are measured as well. A correction function based on the Monte Carlo simulation of Raman signals is suggested as an alternative. Both approaches strongly reduce the turbidity-induced variation of the Raman signals and allow absolute Raman scattering coefficients to be determined. © 2010 Society of Photo-Optical Instrumentation Engineers.

Keywords: Raman spectroscopy; intrinsic Raman spectroscopy; quantitative Raman spectroscopy; spatially resolved reflectance; tissue diagnostics; lookup table.

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trum, together with sample-specific assumptions regarding the wavelength dependency of the absorption and scattering coefficients,\textsuperscript{15} which reduces the method's flexibility.

The aim of our investigation was to develop a method that could correct Raman signals for the influence of the optical properties in a large parameter range without a priori assumptions of the absorption and scattering properties. Therefore, we combined Raman spectroscopy with spatially resolved reflectance measurements.\textsuperscript{16} By performing measurements on various homogenous tissue phantoms as well as Monte Carlo simulations, we investigated how the Raman signal depends on the optical properties. To include parameters typical for measurements in the NIR as well as in the visible wavelength range (VIS), $\mu_a$ was varied from 0.01 to 10 mm$^{-1}$ and $\mu'_s$ from 0.1 to 10 mm$^{-1}$. These investigations exceed the parameter range of previous studies.\textsuperscript{12–14} To correct the Raman signals for the influence of the optical properties, we investigated possible correction functions that are dependent on the elastic diffuse reflectance. We also implemented a correction function based directly on the Monte Carlo simulation of the Raman signals.

2 Experimental Setup and Monte Carlo Simulation

In this section, we explain the Monte Carlo code that was used to simulate the Raman signal and describe our combined Raman and spatially resolved reflectance setup as well as the production of the tissue phantoms.

2.1 Monte Carlo Model for the Simulation of Raman and Reflectance Signals

In accordance with our experiments, we assumed that the absorption coefficient $\mu_a$ and the coefficient for Raman scattering $\mu_{Raman}$ were constant throughout the sample. Because $\mu_{Raman}$ was very small, multiple Raman scattering was neglected and the Raman signal was assumed to be proportional to $\mu_{Raman}$. We further assumed a Henyey-Greenstein phase function\textsuperscript{12} and an anisotropy factor $g=0.8$ for the elastic scattering processes.

We defined a flux density $\Phi_{ex}$ of photons that were not Raman scattered and a flux density $\Phi_{Raman}$ of Raman-scattered photons. Since multiple Raman scattering was neglected, both densities could be calculated with the conventional Monte Carlo method described in Ref. 17, which takes into account elastic scattering and absorption. Only the source term was different for both flux densities. For $\Phi_{ex}$, the source term was a boundary condition at the sample surface that corresponded to the excitation light entering the sample. For $\Phi_{Raman}$, the source term was the number density of Raman scattering events given by $n_{Raman}=\Phi_{ex} \times \mu_{Raman}$, which was proportional to the number density of the absorbed excitation photons $n_{abs} = \Phi_{ex} \mu_a = (\mu_a/\mu_{Raman}) \times n_{Raman}$. Therefore, $n_{abs}$ was used as the source density to calculate $\Phi_{Raman}$ and scale the results with $\mu_{Raman}/\mu_a$. In the Monte Carlo calculation for $\Phi_{ex}$, the density $n_{abs}$ was given by the ensemble of absorption process positions. Thus, the following scheme was used to calculate $\Phi_{Raman}$: At each position where an excitation photon was absorbed, a Raman scattered photon was launched with an isotropic initial direction. The photon propagated with optical parameters that corresponded to the Stokes shifted wavelength. A fraction $N_{Raman}$ of these photons was remitted at the sample surface and reached a Raman detector, which was defined as a circular area on the sample surface. Normalization with the number of incident excitation photons $N_{ex}$ and scaling led to the detected Raman signal $R_{Raman}=(N_{Raman}/N_{ex}) \times (\mu_{Raman}/\mu_a)$. In this paper, $R_{Raman}$ corresponds to the calculated or measured relative Raman signal of a Raman scatterer embedded in a turbid matrix. The elastic diffuse reflectance of the sample was determined by the number of excitation photons $N_{elastic}$ detected at the sample surface. In the results shown below, the relative elastic diffuse reflectance signal is given by $N_{elastic}/N_{ex}$.

The size and position of the excitation and detector area corresponds to the images of the fiber endfaces on the sample surface in Fig. 1. For simplicity, the fiber bundle for the Raman detection in Fig. 2 was assumed to correspond to one fiber with a 0.7-mm radius, and the slant of excitation and receiving optics were neglected. The propagation direction of the remitted photons were analyzed by taking into account the finite numerical aperture (NA) of the imaging optics. For our application, we used NA=1 and scaled the results by a constant.
stant factor accordingly. We used $10^5$ photons for $\mu_a=1.5$ mm$^{-1}$, leading to a computation time of a few seconds for each set of parameters but $10^6$ photons for $\mu_a<0.1$ mm$^{-1}$.

2.2 Combined Raman and Spatially Resolved Reflectance Setup

A schematic overview of our setup is shown in Fig. 2. For the alternating measurement of Raman spectra and spatially resolved reflectance, we combined a Raman excitation channel, a Raman detection channel, and one channel for the spatially resolved reflectance measurement. The three channels were imaged onto the sample with the optics described in Fig. 2. The laser power for Raman and spatially resolved reflectance measurements was $P=2$ mW/mm$^2$.

2.2.1 Raman spectroscopy

The resonance Raman scattering of carotenoids was excited at 488 nm using an argon laser with a cleanup filter (max line, AHF Analysentechnik). For the detection, we used a CCD-based spectrometer (Triax 320, Jobin Yvon) with the CCD chip cooled to $-20$ °C. We used a 1200-1/mm grating and an exposure time of 10 s for each spectrum. For the following analysis, we evaluated the Raman peak (1524 cm$^{-1}$) at 527 nm, which originates from the C=C stretch vibrations of $\beta$-carotene. In addition, we evaluated the fraction of the elastically scattered laser light at 488 nm, which passed the long pass filter (edge basic, AHF Analysentechnik). To obtain the Raman peak values from the fluorescence background, we performed a nonlinear least square fit of a Lorentzian-shaped curve to the spectra (gnuplot).

2.2.2 Spatially resolved reflectance measurement

The spatially resolved reflectance measurement was based on a customized fiber bundle, which consisted of 58 multimode silica fibers, each 200 μm in diameter with NA=0.22 (see Fig. 2, Fiberguide). One central illumination fiber was surrounded by three concentric rings of detection fibers ($n=8$, 19, and 30). The fiber bundle was imaged onto the sample surface at an angle of 20 deg to avoid detection of specular reflection and to permit the arrangement of the three channels in one plane. The resulting source-detector distances were 0.4, 0.8, and 1.3 mm, which were the average distances of the slightly distorted image. The fibers of each detection ring were bundled and fixed in close proximity to photodiodes (PD-B160SM, Laser Components). The amplification was varied automatically for each readout to optimize analog-to-digital (A/D) conversion. The background signal was acquired automatically before each measurement and subtracted. The reflectance signals were normalized to the illumination power, which was measured simultaneously (see Fig. 2). The optical properties of the sample were measured at the Raman excitation wavelength with the argon laser at 488 nm and close to the Raman peak with a frequency-doubled Nd:YAG laser at 532 nm. Using lasers instead of white light was motivated by the fact that simple photodiodes could be used for the measurement without spectral filtering of the reflected light.

2.2.3 Lookup table-based determination of $\mu_a$ and $\mu_s'$ from spatially resolved reflectance

In order to infer the optical properties of the samples, we used a lookup table (LUT), which is a table with forward simulations of the reflectance for various combinations of $\mu_a$ and $\mu_s'$, for each of the source-detector distances given in Fig. 2. The data were produced with the Monte Carlo code described in Sec. 2.1 for the purely elastically scattered photons using a 200-μm detector at the source-detector distances given above. The sample geometry, anisotropy factor, and refractive index ($n_r=1.42$) were constant within the LUT. The calibration factors for the measured reflectance values, which accounted for the transmission properties of the optics, differences in alignment, and the detector response, were obtained by measuring a set of three calibration phantoms with known optical properties. The measured reflectance values were then compared to the LUT by a customized program. The program output was $\mu_a$ and $\mu_s'$ of the best-fitting LUT data.

When the method was evaluated for 15 phantoms with $\mu_a=0.15$ to 0.7 mm$^{-1}$ and $\mu_s'=1.5$ to 6 mm$^{-1}$, the optical properties differed $\approx20\%$ from the values obtained by integrating sphere measurements (Sec. 2.3). The average deviation was $+3\%$ ($\mu_a$) and $-5\%$ ($\mu_s'$), and the average absolute deviation was $11\%$ ($\mu_a$) and $10\%$ ($\mu_s'$). This range of optical properties corresponds to the results of preliminary in vivo measurements of skin. Since the setup was designed for in vivo skin measurements, the illumination power was limited to 2 mW/mm$^2$. Phantoms with a $\mu_a$ higher than 0.7 mm$^{-1}$
could not be measured with the current implementation of our setup because there was no reflectance signal detectable at the second distance (r=0.8 mm) using 488 nm.

2.3 Phantom Preparation and Characterization

Tissue phantoms with $\mu_a=0.2$ to 4 mm$^{-1}$ and $\mu'_s=0.1$ to 8 mm$^{-1}$ were produced based on silicone (Wacker, RT 601) using red silopren color paste (GE Bayer Silicones) as an absorber and titanium dioxide as a scatterer. To ensure that the highly lipophilic $\beta$-carotene (Fluka) was completely dissolved, it was first dissolved in methylene chloride and paraffin (Vaseline, Unilever) and then mixed with the silicone. The final $\beta$-carotene concentration was 10 $\mu$g/ml in all phantoms. The contribution of 10 $\mu$g/ml $\beta$-carotene to the $\mu_a$ of the silicone phantom was 0.07 mm$^{-1}$ at 488 nm, as measured in a transparent phantom. The Raman spectra were measured on the day of preparation. A thin layer from each phantom mixture was used to determine the optical properties with an integrating sphere spectrometer (Lambda 900, Perkin Elmer). The total transmission, diffuse transmission, and diffuse reflectance were measured and used as the input parameters for an inverse Monte Carlo algorithm to yield $\mu_a$, $\mu_s$, and $g$. For comparison with the spatially resolved reflectance measurements, $\mu'_s$ was calculated from $\mu_a$ and $g$.

3 Dependency of the Raman Signal on the Macroscopic Optical Properties

First we investigated how the Raman peak at 1524 cm$^{-1}$ [C=C bonds, see Fig. 3(a)] depends on the optical properties of the silicone phantom, which correspond to typical values for skin in the VIS. The Raman signal dropped roughly proportional to $1/\mu_a$, while the influence of $\mu'_s$ was fairly small, see Fig. 3(b). The optical properties of the phantoms are the mean optical coefficients at excitation and Stokes wavelength. These coefficients were measured with the integrating sphere spectrometer, since the whole parameter range was not accessible using our implementation of the spatially resolved reflectance setup. Since the silicone color paste contributed to the scattering coefficient of the phantom, $\mu'_s$ of the phantoms was not constant within one series of increasing absorber concentration.

Next we compared our phantom measurements with simulations [Fig. 3(b)]. The simulation results of the Raman signal were all scaled with the same factor to fit the experimental results. The factor includes the unknown in vivo Raman cross section as well as setup-specific constants. The trends of the experimental values corresponded well to the simulations. However, the experimental and simulated values did not match for all phantoms within the given errors, which could be explained by errors incurred by variations in the sample preparation.

We further performed Monte Carlo simulations to investigate the dependency of the Raman signal on $\mu_a$ and $\mu'_s$ in a larger parameter range, as with the phantom measurements (Fig. 4). For our measurement geometry, $Ram_T$ was roughly proportional to $1/\mu_a$ in the VIS ($\mu_a > 0.1$ mm$^{-1}$) and $\mu'_s > 1$ mm$^{-1}$). For smaller $\mu_a$ (typical for the NIR), the curves deviated significantly from this behavior. When the same simulation was performed for a pencil-beam excitation and a semi-infinite detection geometry, which is a typical imaging geometry, the $1/\mu_a$ behavior continued into the NIR parameter range (data not shown). When the excitation and detection geometry corresponded to a 200-$\mu$m fiber, the $\mu_a$ dependency was weaker, as for the measurement geometry used here (data not shown). This can be explained by the influence of the measurement geometry on the pathlength distribution of the detected Raman photons, which determines the influence of the optical properties on the Raman scattered light. For our measurement geometry, the influence of $\mu'_s$ in the NIR is larger than the VIS.

In addition to the Raman signal, we also analyzed the elastic diffuse reflectance of the laser light, which was extracted from the same spectra as the Raman signals (Fig. 5). The elastic signal was much more sensitive to variations of $\mu'_s$ and less sensitive to $\mu_a$. As a consequence, a simple ratio of Raman and elastic diffuse reflectance signal still depended on $\mu_a$ and $\mu'_s$ and therefore was not sufficient for correction in this...
parameter range, which covered tissue optical properties in the VIS.

4 Determination of $\mu_{\text{Raman}}$ by Correction Functions

To link Raman signals from turbid samples directly to Raman scattering coefficients, we had to correct the Raman signal for the influence of the macroscopic optical properties. The measured or calculated relative Raman signal, which depended on $\mu_s$, $\mu_s'$, and $\mu_{\text{Raman}}$, but also on the sample geometry, measurement geometry, and sensitivity of the setup, is called $R_{\text{Ram}}$. We assumed that the application of a dimensionless correction function $F(\mu_s, \mu_s')$, which was sample and measurement geometry-dependent, led to

$$R_{\text{Ram}}(\mu_s, \mu_s') \times F(\mu_s, \mu_s') = s \times l_0 \times \mu_{\text{Raman}}.$$  \hspace{1cm} (1)

The right-hand side of Eq. (1) is independent from $\mu_s$ and $\mu_s'$ of the sample and can be interpreted as the signal that could be measured if the sample were transparent, similar to that defined in Refs. 12–14. The factor $s$, in units of $R_{\text{Ram}}$, depends only on the geometry and the sensitivity of the setup. The constant $l_0$ is the average pathlength of excitation photons in the transparent sample for the given sample and measurement geometry. In the case of a simulation (data not shown) where a transparent layer of Raman scatterers with refractive index $n = 1$ was measured in backscattering geometry using an ideal detector (NA=1), $s \rightarrow \frac{1}{2}$ was obtained and $l_0$ corresponded to the layer thickness. The factor $s \times l_0$ can be obtained by measuring a calibration sample of the same geometry and known $F(\mu_s, \mu_s')$ and $\mu_{\text{Raman}}$. For many applications, it might be sufficient to correct $R_{\text{Ram}}$ relative to a reference sample of the same geometry with $\mu_{s,\text{ref}}$ and $\mu_{s,\text{ref}}'$ by

$$R_{\text{Ram}}(\mu_s, \mu_s') \times \frac{F(\mu_s, \mu_s')}{F(\mu_{s,\text{ref}}, \mu_{s,\text{ref}}')} = R_{\text{Ram}}(\mu_{s,\text{ref}}, \mu_{s,\text{ref}}').$$  \hspace{1cm} (2)

Next we will describe two types of the correction function $F$. Motivated by the existing literature, we investigated correction functions that utilize the elastic diffuse reflectance, which

Fig. 4  Dependency of the simulated Raman signal $R_{\text{Ram}}$ on the optical properties of the tissue matrix for our measurement geometry, where $\mu_s$ and $\mu_s'$ correspond to the mean of the optical properties at Raman and Stokes or wavelength in units of 1/mm. (a) $\mu_s$ dependency; $\mu_s'$ is increasing from bottom to top. (b) $\mu_s'$ dependency; $\mu_s$ is increasing from top to bottom. $R_{\text{Ram}}$ is defined as $N_{\text{Raman}}/N_{\text{ins}}$ and is normalized by the unknown $\mu_{\text{Raman}}$. In the VIS, $R_{\text{Ram}}$ is roughly proportional to $1/\mu_s$. The influence of $\mu_s'$ is smaller but not negligible.

Fig. 5 (a) Comparison of experimental (filled symbols) and calculated results (lines, open symbols) for the dependency of the elastic diffuse reflectance signal ($R_e$) on $\mu_s$ for various series on $\mu_s'$, where $\mu_s$ and $\mu_s'$ correspond to the mean of the optical properties at the Raman and Stokes or wavelength in units of 1/mm. The data for $\mu_s'=4$ mm$^{-1}$ and $\mu_s'=6$ mm$^{-1}$ are not included in the graph for clarity. (b) Comparison of experimental and simulated results (fitted to experiments) for the dependency of the Raman signal ($R_{\text{Ram}}$) and elastic diffuse reflectance signal ($R_e$) on $\mu_s$ for $\mu_s'=0.25$ mm$^{-1}$. The elastic scattering signal was attenuated by a long-pass filter ($\sim$-OD6).
is measured in the same geometry as the Raman signal (Sec. 4.1). We also suggest an alternative correction function that is based only on the simulations of the Raman signal (Sec. 4.2).

### 4.1 Correction Utilizing the Elastic Diffuse Reflectance Signal

It would be very convenient if a simple ratio of $R_{\text{Ref}}$ and $R$ could completely remove the influence of the optical properties. However, as shown for our measurement geometry in Fig. 6, the ratio only decreases the influence of $\mu_s$. The influence of $\mu'_s$ increases except for the parameter range $\mu_a = 0.1 \text{ mm}^{-1}$ and $\mu'_s > 0.4 \text{ mm}^{-1}$, where the influence of $\mu'_s$ decreases as well. This explains why the ratio improved the results in Ref. 14, where a measurement spot of similar size was used ($r_{\text{ex}} = r_{\text{det}} \approx 1 \text{ mm}$).

In Fig. 7(a), $R_{\text{Ref}}$ is plotted versus $R$ for various combinations of $\mu_a$ and $\mu'_s$ and for three different measurement geometries. Obviously, $R_{\text{Ref}}$ cannot be described as a function of $R$ only without knowledge of the other variables.

However, it was shown recently for measurements in the NIR that $R_{\text{Ref}} \times \mu_a$, with $\mu_d = \mu_a + \mu_s$, can be described by a function of $R$ only; see Refs. 12 and 13. This leads to the correction function $F_1 = \mu_d \times l_1 / f_1(R)$, where $f_1(R)$ is a geometry-dependent function of the diffuse reflectance ($R$), and $l_1$ is a length to make $F$ dimensionless as defined in Eq. (1). Using our Monte Carlo model, we searched for similar empirical correction functions and evaluated their validity with respect to the range of optical properties and the measurement geometry. While we found some correction functions that were only valid in the semi-infinite measurement geometry or the VIS parameter range, only $F_1$ and $F_2 = \mu'_s \times l_2 / f_2(R)$ could be used for our measurement geometry in the whole parameter range investigated here. Since $F_1$ and $F_2$ performed very similarly, we present only the application of $F_2$ in Fig. 7(b). It is obvious that $F_2$ works in the semi-infinite as well as in our measurement geometry, but not for a measurement spot with a diameter of 200 $\mu$m. In order to find a function $f_2(R)$ for the whole parameter range, we applied a 4th-order polynomial to

Fig. 6 Dependency of the ratio of simulated $R_{\text{Ref}}$ to elastic diffuse reflectance $R$ on the optical properties (given in units of $1/\text{mm}$) for our measurement geometry. (a) $\mu_s$ dependency: $\mu'_s$ is increasing from bottom to top. (b) $\mu'_s$ dependency: $\mu_s$ is increasing from top to bottom.
the log of the data. We accounted for different optical proper-
ties at the excitation and Stokes wavelength by using the mean optical properties. A wavelength-dependent variation of
R can be accounted for by using the geometric mean \( \sqrt{R_x R_s} \)
instead.

When \( F_2 \) was applied to the simulated data in Fig. 4, the
variation of the data decreased from 109% to 6%. This means
that the accuracy of the \( \mu_{\text{Raman}} \) to be determined is strongly improved. When \( F_2 \) was applied to our silicone phantom data
(Sec. 2.3), the variation of the data decreased from 88% to
21%. The remaining variability of the signal could result from
variations in the sample preparation and from the fact that the
diffuse reflectance was only measured at the excitation wave-
length. Therefore, an experimental investigation of the correc-
tion effect was limited with this setup.

4.2 Correction Based on Raman Signal Simulations
Since our Monte Carlo model for Raman measurements in
turbid media corresponded sufficiently well with our experi-
ments, we also used directly the forward simulation of
\( R_{\text{MC}}(\mu_x, \mu_s')/\mu_{\text{Raman}} \) to obtain the correction function \( F_{\text{MC}} \)
according to Eq. (1). For a relative correction according to Eq.
(2), we simulated

\[
F(\mu_x, \mu'_s)/F(\mu_{x,\text{ref}}, \mu'_{s,\text{ref}}) = Ram_1(\mu_{x,\text{ref}}, \mu'_{s,\text{ref}})/Ram_1(\mu_x, \mu'_s).
\]

To minimize the computational effort during the measure-
ment, the correction factors were simulated once for various
combinations of \( \mu_x \) and \( \mu'_s \) and stored in a table. To account
for the different optical properties at the excitation and Stokes
wavelength, \( Ram_1(\mu_x, \mu'_s) \) can be replaced by the geometric
mean of \( Ram_1(\mu_x, \mu'_s) \) at both wavelengths. However, if the
values are simulated \( a \text{ priori} \), using four variables would drastically
increase the size of this table. Therefore, it is therefore
convenient to use the Raman signal at the mean optical
properties of excitation and Stokes wavelength, which we found to
be equal to the letter approach in the case of \( \mu_x = \mu'_s \).

The effect of this correction procedure on the simulated
data led to a straight line (not shown). When applied to the
phantom data, the variation of the data decreased from 88% to
16%. The remaining variability of the signal could be due to
variations in the sample preparation.

5 Determination of Molecular Raman Cross
Section or Raman Scattering Concentration
In Sec. 4 we described how \( \mu_{\text{Raman}} \) could be determined once
the calibration factors were measured. Another way to deter-
mine \( \mu_{\text{Raman}} \) independent from a calibration measurement is
by comparing the simulated ratio of the Raman to elastic sig-

\[
\sigma = \frac{\mu_{\text{Raman}}}{4\pi \times c}.
\]

We obtained \( \sigma = 2 \times 10^{-23} \text{ cm}^2 \text{ molecule}^{-1} \text{ Sr}^{-1} \) for our sili-
cone phantoms. Compared to the results of Berdyugin et al.\textsuperscript{21}
\( (3.2 \times 10^{-23} \text{ cm}^2 \text{ molecule}^{-1} \text{ Sr}^{-1} \), measured in cyclohexane
at 514 nm excitation and concentration of \( 2 \times 10^{-6} \) M)}, our
result was one order of magnitude larger. This can be at least
partially explained by the enhanced resonance excitation at
488 nm. Surprisingly, the results of Tian et al.\textsuperscript{22} varied up to 3
orders of magnitude (\( 10^{-24} \) to \( 10^{-21} \text{ cm}^2 \text{ molecule}^{-1} \text{ Sr}^{-1} \))
depending on the \( \beta \)-carotene concentration (\( 10^{-6} \) to \( 10^{-10} \) M), which was dissolved with pyridine and
diluted with water. Thus, for the absolute quantification of
\( \beta \)-carotene in biomedical applications, more investigations
of the molecular resonance Raman cross-section in different bio-
logical matrices and for different concentrations should be
performed.

Assuming the molecular Raman scattering cross-section \( \sigma \)
can be found in the literature, the concentration \( c \) of the Raman
scatterer can be calculated by Eq. (3) using the measured
\( \mu_{\text{Raman}} \).

6 Summary and Discussion
We introduced a method to combine Raman and spatially re-
solved reflectance spectroscopy that allows the measurement of
the optical properties together with the Raman spectrum. To determine the absolute Raman scatterer coefficients \( \mu_{\text{Raman}} \)
independent from the optical properties, we compared to dif-
ferent methods to correct for the turbidity-induced signal
variation. One correction method utilized empirical functions
that are dependent on the elastic diffuse reflectance but also
require knowledge of the optical properties. An alternative
method used a Monte Carlo model to generate and propagate
Raman signals in turbid media to directly calculate the cor-
rection factors, regardless of any functional relationship.

Both correction methods reduced the turbidity-induced sig-
nal variation by 95% or more for our simulated data, and by
76% or more for our phantom data. Both correction methods
need to be adjusted once to the excitation and the detection
using phantom measurements or Monte Carlo simulations.
Since the empirical equations still require knowledge of the
optical properties, we see no advantage to this method. The
correction method based on the Monte Carlo simulation, how-
ever, does not require the measurement of elastic reflectance
in the Raman measurement geometry and can even be used in
geometries such as a 200-\( \mu \text{m} \) measurement spot, where an
empirical correction function has not been described yet. For
the data presented here, we used correction functions obtained
for samples that were homogenous within the sampling vol-
ume. However, this simplification may be critical if a sam-
pling volume includes different layers, like the epidermis and
the dermis in skin measurements. The influence of the sample
structure on the correction function is the subject of a current
investigation.

From the determined \( \mu_{\text{Raman}} \), we calculated the molecular
Raman scattering cross-section \( \sigma \) of a given \( \beta \)-carotene
concentration in our silicone phantoms. For a known \( \sigma \), the ab-
solute Raman scatterer concentration can be determined from
\( \mu_{\text{Raman}} \). For unknown molecular Raman cross-sections, which
is a possible scenario especially in complex biological matrices, the methods can also correct Raman signal variations induced by sample-to-sample turbidity variation, which allows the quantitative comparison of results obtained from different individuals, tissues, or tissue sites.

Although we took advantage of the outstanding high-resolution Raman scattering cross-section of β-carotene, the correction methods are transferable to any other Raman scatterer. For the correction of multiple Raman peaks at different wavelengths, the optical property measurement can be easily performed using white light instead of lasers. In addition to biomedical applications, the correction method might also be beneficial in the food industry or environmental research, where quantitative measurements of analytes independent of turbidity variations are necessary. Furthermore, we suggest that the correction procedures may be applied similarly to fluorescence measurements.

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