Functional optical coherence tomography: spatially resolved measurements of optical properties
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CHAPTER 3

Measurement of the axial point spread function in scattering media using single mode fiber based optical coherence tomography

We studied the axial point spread function of single-mode fiber-based optical coherence tomography systems for signals from scattering media. The determined Rayleigh length (half the depth of focus) of the axial point spread function was roughly twice the one measured from the reflection of a mirror. Using the measured point spread function in combination with the single backscatter model allowed determination of the attenuation coefficient of a suspension of calibrated scattering particles.

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3.1 introduction

Optical coherence tomography (OCT) is a recently developed technique for non-contact imaging of microscopic tissue structures [1-5]. This technique is analogous to ultrasound imaging but uses remitted light from tissue structures for image assembly. Whereas with ultrasound the location of reflecting objects is determined by measuring the time of flight, in OCT the optical depth of light scatterers is determined by interferometry using low coherence light sources. This so-called coherence gating is based on the fact that light backscattered from within the tissue (sample) will only interfere with light reflected from a reference mirror when the optical path length difference between photons in the reference arm and those in the sample arm is within the source coherence length. In addition, focusing optics in the sample arm suppress the detection of light backscattered from outside the volume of the focused beam, similar to confocal microscopy [6]. Lateral scanning in combination with the axial scanning (by the reference mirror) produces two or three-dimensional tomographic images that map the amplitude of light backscattered from the sample. The axial resolution is determined by the source coherence length (~2-20 μm), whereas the lateral resolution is determined by the beam waist (~5-30 μm) in the sample [7].

In vitro and in vivo studies have demonstrated that OCT reveals, non or minimally invasive and in non-contact mode, anatomical structures in superficial tissues (up to 2 mm depth) with unprecedented spatial resolution [1;8-16]. The identification of these structures is based on spatially resolved differences in the amount of penetrated and backscattered light from tissue structures (e.g. collagen and elastin fibers), cells (e.g. nuclei and membranes), and other constituents. These differences in backscattered signals, due to differences in the optical properties of the tissue constituents, however, are influenced by the optical components of the OCT system itself. To discriminate tissue structures based on quantitative determination of the attenuation of the light in the tissue, the effect of the axial point spread function (PSF) of the used optics has to be taken into account [6;17;18].

In these previous studies [6;17;18], the confocal gating of the OCT signal was either ignored (because of large depth of focus), implemented with theoretical point spread functions similar to those from confocal microscopy using pinholes, or described by Gaussian beam theory. As a result, most proposed axial PSF’s were complicated. The effect of using single mode fibers, which almost reduces the beam profiles to be perfectly Gaussian, influences the point spread functions. In this study we will deduce a simplified form for the axial PSF and compare it with the previously used PSF’s. Furthermore, we will determine the axial PSF for a high resolution OCT system from a mirror and in scattering media, and use it to measure the attenuation coefficient of the suspension of microspheres.

3.2 materials and methods

THEORY

Izatt et al. [6] used the axial point spread function \( T_{\text{Axial}} \) (equation 3-1) from a uniform planar object (e.g. a mirror) in air deduced by Min Gu et al.,[19] to describe more thoroughly the OCT signal for SMF based systems.

\[
T_{\text{Axial}} = \frac{\left| \alpha \right|^2}{\left[ 1 - \exp(-\alpha) \right]} \left[ 1 - \exp\left(-\alpha - j\mu\right) \right] \tag{3-1}
\]
here \( \alpha = (2\pi R_0 r_0 / \lambda_0)^2 \) and \( u = (8\pi / \lambda) d \sin^2(\varphi / 2) \) where \( R_0 \) is the objective lens pupil radius, \( \lambda \) is the center wavelength of the light source, \( r_0 \) is the beam spot radius of the single mode fiber, \( s \) is the distance between the collimating lens and the tip of the optical fiber, \( d \) is the axial distance to the focal plane of the objective (sample) lens and \( \sin \varphi \) is the numerical aperture of the objective lens. Due to the complexity of this formula, and possibly the fact that the index of refraction was not incorporated, a paraxial approximation was utilized by Schmitt et al. [20] and used by Izatt et al. [17], resulting in an axial PSF \( T_{\text{Schmitte}} \) given by:

\[
T_{\text{Schmitte}} = \frac{f^2}{(nd + f)^2 + (nd^2 \alpha_0^2 / \lambda^2)^2}
\]

in which \( \alpha_0 \) is the radius of the beam in front of the objective lens with focal length \( f \). In other publications for example on telecommunication applications, the coupling efficiency \( \eta_c \) of Gaussian intensity profiles launched from single mode fibers into single mode fibers has been calculated as the overlap integral of the two Gaussian fields (and not the overlap area as for multimode fibers) [21]. A Gaussian beam is characterized by the waist \( w \) and its position, the Rayleigh length \( Z_0 \) (half depth of focus), wavelength \( \lambda \) (in vacuum) and the index of refraction \( n \) of the medium. The efficiency of coupling the light from one SM fiber (with mode field diameter \( 2w_0 \)), via a lens (system) resulting in a new waist \( w_f \) and Rayleigh length \( Z_h \) to another SM fiber, again via a lens (system) with a different receiving mode field diameter \( 2w_r \), can be calculated as the overlap integral of the two Gaussian fields at any position in the optical system (see figure 3-1).

![Figure 3-1: In general, light from the SMF is collimated by lens 1, and after a given distance focused by the objective (sample) lens, resulting in a certain waist and Rayleigh length. The transfer of this light into the receiving fiber is determined by the ratio of the waists of the receiving and emitting optical system and the distance \( \delta \) between their positions and their lateral offset \( x \).](image)

Then, \( \eta_c \) depends on any losses due to separation, offset \( \delta \) between the waist positions, angular misalignments and mode field diameter mismatch between the two beams. The axial transfer function \( T \), defined as \( T = \eta_c \eta_e \) can be calculated theoretically [21]. For confocal setups, the effect of angular and lateral offset are not present, and the power transmission can be derived from equation 2-23

\[
T = \frac{4 \left( \frac{w_r}{w_f} \right)^2}{\left( \frac{\delta}{Z_i} \right)^2 + \left( \frac{w_r}{w_f} \right)^2 + 1}
\]

with \( Z_i = \frac{n \pi w_f^2}{\lambda} \)

and \( n \) the index of refraction of the medium. From this transfer function, two cases for the axial PSF \( T \) of a confocal optical system, can then be deduced. For specular reflection, which
can be measured as the power transmission by axially translating a mirror through the focus of the beam, using \( w_f = w_r \), the axial PSF reduces to eq. 2-25:

\[
T_s(d) = \frac{1}{\left( \frac{d}{Z'} \right)^2 + 1}
\]  

(3-4)

with \( d \) the distance of the reflecting object to the waist position of the beam (figure 3-2).

\[
\text{figure 3-2: for specular reflection, i.e. a mirror positioned at distance } d \text{ from the focal plane, only the distance between the waist positions (2d) determines the axial PSFs.}
\]

In the case of diffuse reflection, e.g. scattering from tissues or as for signals in fluorescence and Raman confocal microscopes, in analogy of the paper of de Grauw et al. [22], illumination of an object at distance \( d \) from the waist position of the incoming beam will form a secondary source. This source will be treated as a new beam with waist \( w'_f \) and Rayleigh length \( Z'_f \), which will then overlap with the incoming beam (figure 3-3).

\[
\text{figure 3-3: for diffuse reflection such as scattering in tissues, a new waist at } d \text{ can be considered for calculating the axial PSF.}
\]

The scattering axial PSF can then be described as equation 2-27:

\[
T_s(d) = \frac{1}{\left( \frac{d}{2Z'_f} \right)^2 + 1}
\]  

(3-5)

The validity of equation 3-5 will be subject of investigation in the following experiments. Using the known values of SM field diameter and focus distance of the used lenses of our setup (described in the materials and methods section below), we plotted the expected axial PSF's given by equations 3-1, 3-2, 3-4 and 3-5 (figure 3-4). The curves demonstrate the similarity of the equations 3-1, 3-2 and 3-4, in contrast to equation 3-5.
OCT SYSTEM

OCT imaging was performed by a Michelson interferometer using a commercially available FemtoSource Ti:Sapphire laser source, operating at 800 nm with a bandwidth of 120 nm FWHM, and SM fibers with a mode field diameter of 5.3 μm. Light from the fiber was focused into the sample by means of two identical lenses with a focal length of 25 mm. By knife-edge measurements, the beam-radius just in front of the second lens was determined to be 1.75 mm, which indicated an expected Rayleigh length in air of 51 μm. The sample lens and the double-pass (reference) mirror were positioned on voice coil translation stages that facilitated scanning up to 6 mm with a resolution of 0.15 μm. Dispersion was compensated for by a ‘static’ rapid scanning optical delay line described by Rollins et al. [23] Precise coherence gating was performed by scanning the double pass mirror in the reference arm. For OCT measurements, the interferometric signal was detected by a photodiode, band pass filtered, amplified and demodulated by a Lock-In amplifier. The axial resolution of this system was 3.5 μm and the dynamic range was 115 dB.

MEASUREMENTS

The mean heterodyne signal photo-detector current $i_\delta$ is proportional to the field back reflected from the sample. Like Schmitt [20] and Izatt [17], we assume that the single back-scattering model is valid (eq. 2-33):

$$i_\delta(z) \propto \sqrt{T(z)} \exp(-\mu z)$$ (3-6)

Where the square root accounts for the fact that $T$ was calculated as the power transmission function whereas the OCT signal is proportional to the field.

In our setup, the sample was at a fixed position and $T$ was determined by moving the sample lens. Using a mirror, we verified that in our sample arm geometry this produced the same results compared to moving the sample through a fixed focus. Furthermore, for low NA, the change in the position of the focus in the sample with (phase) refractive index $n$ is then given by $n$ times the changed position of the sample lens ($n=1.33$).
Using OCT, we determined $T(z)$ for the reflection by a mirror in air and submerged in water (specular reflection), and for a scattering object in a scattering medium (diffuse reflection). We extract the Rayleigh $Z_0$ length by fitting the data to $\sqrt{T}$, given by equation 3-4 in all cases. Note that if $\sqrt{\tau}$ given by equation 3-5 is indeed valid for diffuse reflections from scattering media, the extracted $Z_0$ using $\sqrt{\tau}$ will be twice the value extracted from the mirrors. In these latter experiments, to avoid any specular reflection of a target, $T(z)$ was determined from and in a suspension of polystyrene beads (1 μm diameter, 0.06%, polyciences) with an anticipated scattering coefficient of 2.0 mm$^{-1}$. Using the coherence gate a ‘fixed diffuse reflector’ is selected inside the sample. $T(z)$ is then determined by measuring the amplitude of the OCT signal as a function of focus position in the suspension. This procedure was repeated for several depths inside the sample. Finally, the averaged A-scans obtained from the suspension were correlated with the expected heterodyne signal given by equation 3-6 using both dynamic focusing [i.e. $T(z)$] and static focusing [$T(z)$] given by equation 3-4).

All depths are physical depths, thus corrected for the index of refraction of the medium. The suspension was contained in a glass cuvette which was positioned under an angle to avoid direct back-reflection of the OCT light.

### 3.3 results

In figure 3-5, the measured axial PSF of a mirror in air and submerged in water is plotted. The data were fitted to the square root of equation 3-4 (i.e. to $\sqrt{T}$), which resulted in a Rayleigh length $Z_0=47$ μm for the mirror in air, which was close to the expected 50 μm, and $Z_0=65$ μm for the mirror in water. Please note that the ratio of the two measurements (65/47 = 1.38) should correspond to the index of refraction of water (1.33).

![figure 3-5: normalized OCT signals reflected from a mirror in air (circles) and in water (dots) as a function of the focus shift, which is the distance $d$ (mm) between waist position and mirror. The lines are the fits of axial PSF given by equation 4 ($\sqrt{T}$) resulting in $Z_0$ to be 47 μm in air and 65 μm in water.](image)

The axial response curves of backscattered light in the micro-spheres suspension were measured at various depths in the solution (figure 3-6A). Fitting the data to square root of equation 3-4 (i.e. to $\sqrt{T}$) resulted in increased values of $Z_0$ (approximately 100 to 140 μm) up to a depth of 1.2 mm into the scattering medium (figure 3-6B).
The fitted amplitude of the measured axial response curves can be used to determine the attenuation coefficient of the light. In that case, the amplitudes as a function of depth obtained in the suspension were fitted with respect to $\mu_t$ using equation 3-6 with the axial PSF constant ($T=1$) and resulted in $\mu_t = 1.5 \pm 0.1 \text{ mm}^{-1}$ (figure 3-7). For the static focus position of 649 $\mu$m in the suspension, and $Z_0$ taken from the fit in figure 3-4A to be 116 $\mu$m, we fitted the average A-scan to equation 3-6 ($T$ given by equation 3-4, using $Z_0 = 116 \mu$m) which resulted in an attenuation coefficient to be $\mu_t = 2.1 \pm 0.1 \text{ mm}^{-1}$ (figure 3-5).
3.4 discussion

In this study, we investigated the axial PSF in non-scattering and in scattering media of known optical properties, and compared these measurements to the theoretical known axial PSF's. The major results of our study are that, for the utilized setup and scattering medium, the measured depth of focus of our OCT system measured from a perfect reflector in non-scattering media can be described by the known PSF's, whereas the depth of focus in scattering media is increased. Furthermore, the measured axial PSF in combination with the single backscattering model seems to be valid to determine the attenuation coefficient of the light in the scattering suspension.

The single back-scatter model was proposed by Schmitt et al., and was deduced from atmospheric lidar and from ultrasonic reflectometry studies [20]. In these studies no confocal properties were utilized, but more or less implemented by treating the sample as a secondary source with a radial extent given by the spot size of the focused incident beam, and by the interference of the light reflected from the sample with the Gaussian reference beam. Still, the effect of the focusing optics can be calculated (equation 3-2) and does not differ, as depicted in figure 3-2, from the axial PSF's calculated from SMF and Gaussian beam theory (equation 3-2 and 3-4). The measurement of the axial PSF from a mirror, both in air and in water (figure 3-5), and the comparison with the theoretical values given by equation 3-1, 3-2 and 3-4 (figure 3-2), demonstrate that the model given by equation 3-4 is valid as well. For the single back-scatter model it is assumed that the light in the sample is only back-scattered once and that the axial PSF is undisturbed. In that case, knowing the axial PSF from reflection measurements on a mirror, the OCT signal can be described by equation 3-6. Indeed, Schmitt et al. [18], which also simplified the axial PSF conform the theory given by Gaussian beams given by equation 3-4, demonstrated that the exponential attenuation of the light in the tissue can be determined. Discrepancies were thoroughly (both experimentally and theoretically) investigated and attributed to the contribution of multiple scattering to the OCT signal.

Determining the axial response of the OCT light in scattering media, as depicted in figure 3-6, demonstrates that the axial PSF is disturbed. Consequently, the axial response is lengthened, both for superficial and larger depths (Figure 3-6B). The Rayleigh length is, given the errors in the fit, almost constant as a function of depth, and is roughly twice the theoretical expected one, in accordance with the prediction of equation 3-5. Using the larger $Z_0$ for fitting the OCT data and for the fixed focus position, the fitted $\mu_t$ gives a realistic value close to the theoretically predicted one (figure 3-7). Furthermore, using the fitted amplitudes of the axial PSF's measured and plotting these as a function of depth mimics the results of focus tracking OCT. In that case, the confocal gate is perfectly matched with the coherence gate, the axial PSF can be set to 1, and the attenuation of the light can be directly determined by fitting the signal to the exponential attenuation ($\mu_t = 1.5 \text{ mm}^{-1}$). Still, some discrepancies between expected and measured coefficients are present. We speculate that this can be partly attributed to the relatively large index of refraction mismatch between the glass-liquid boundaries. This mismatch might induce some more reflections of back-scattered light in the sample, which might be more prominent for lower optical depths. Furthermore, the effect of multiple scattering will still be present.

LIMITATIONS AND IMPLICATIONS

In this study, we measured the axial PSF in scattering media with an attenuation coefficient of approximately $2 \text{ mm}^{-1}$, using sample arm optics with $\text{NA} = 0.08$, and interpreted the results in terms of equations 3-4 and 3-5. The derivation of equations 3-4 and 3-5 is based on Gaussian
beam theory and therefore has the same range of validity. Moreover, since in OCT usually relatively low numerical apertures are used, any assumptions in Gaussian beam theory relying on propagation in the paraxial region are valid. High NA, as used in for example Optical Coherence Microscopy were outside the scope of this study, but will be addressed in future studies. Also the effect of other scattering properties of the medium on the PSF will be addressed in future experiments. The model presented in equation 3-6 using the point spread function of equation 3-4 or 3-5 in combination with the single backscattering model, may still suffice to retrieve the sample’s scattering coefficient. Further investigations also have to clarify whether, for scattering media, simply doubling the depth of focus measured from a mirror (and thus using equation 3-5), may be sufficient for precise determination of the attenuation coefficient in tissue. Furthermore, the effect of the position of the focus on the accuracy of the retrieved scattering coefficient of the tissue has to be determined. A major result is that the axial PSF given by equations 3-4 or 3-5 may be easily implemented for quantifying these tissue properties and, consequently, help to identify tissue components, which is one of the key factors in the clinical usefulness of OCT. The imaging of the morphology of (pathological) tissue is currently performed many specialities like ophthalmology [24], gastroenterology [25], dermatology [5] and cardiology [26]. For example in gastro-enterology, the identification of the epithelium, lamina propria and muscularus mucosae in the esophageal wall is indispensable for the recognition and staging of tumors. In cardiology, unstable plaques, which are typically characterized by a thin fibrotic cap covering a large thrombogenic lipid core, are the targets to identify. For all clinical applications of morphological imaging by OCT, the axial PSF can be easily implemented, and therefore may improve the identification of the characteristic tissue constituents, which is crucial to have a significant impact on the diagnosis, monitoring, choice of treatment and follow up in the various clinical disciplines.

3.5 conclusions

Using the known coupling efficiency functions of SM fibers, we were able to deduce a simple formula for the axial response function $T(z)$ for OCT, which correlated very well with measured data on mirrors. Furthermore, we measured axial response in a scattering medium to correlate well with the axial PSF $T(z)$, albeit that the measured depth of focus was increased. Consequently, the axial PSF $T(z)$ in combination with the single backscatter model might be used for determining attenuation coefficients in scattering media, and may profit the clinical usefulness of OCT.

REFERENCES


