Development of functional near-infrared optical coherence tomography

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Introduction
Chapter 1
Medical imaging is a discipline where the fields of mathematics, physics, biology, and medicine are combined in the acquisition, computation and interpretation of three-dimensional images of (human) tissue. A variety of imaging modalities that provide information about morphological structure and function of biological tissues has been developed. These modalities are based on electromagnetic waves (magnetic resonance imaging, X-ray computed tomography, confocal microscopy), sound (ultrasonography), or nuclear tracers (positron emission tomography, single photon emission computed tomography). In the last decades, these medical imaging modalities have become a key tool in disease diagnosis, treatment monitoring, and disease prevention.

Compared to other imaging techniques, optical imaging is low-cost, can be used in non-contact arrangements, has a small form factor, and does not use ionizing radiation. The main disadvantage is its limited imaging depth, which is caused by the strong scattering of light in biological tissues.

Optical coherence tomography (OCT) is a relatively new optical imaging technique. OCT is the optical analogue of ultrasound imaging, in which light (instead of acoustic waves) backscattered from tissue structures is detected. Due to the high speed of light, the path length that the light has travelled into the tissue is determined using low-coherence interferometry (instead of time of flight as for acoustic pulses) [1]. With OCT, high resolution (2-10 μm) cross-sectional images of biological tissues up to 1-2 mm deep can be acquired. Figure 1-1 shows an overview of many medical imaging modalities based on their imaging depth and spatial resolution. As can be seen, OCT fills up the gap that is present between confocal microscopy and high frequency ultrasound.
OCT has found many applications in medicine, e.g. in ophthalmology, where cross-sectional retinal imaging provides previously inaccessible information about the condition of the retina. OCT is also used for visualization of the anterior segment of the eye, which is particularly important in the diagnosis of glaucoma. Using a fiber optic probe, OCT can also be applied endoscopically. Imaging of (vulnerable) plaques, imaging of stent placement, and characterization of the structural integrity of the vasculature in the coronary artery provide valuable information for diagnosis and treatment of vascular diseases. Currently, OCT is being evaluated in many fields of medicine, including dentistry, dermatology, urology, and developmental biology [2-14].

The field of OCT is in a continuously developing cycle, in which clinical applications lead to more research, which leads to improved technology, which opens up new applications. Furthermore, besides imaging, different features of the backscattered light can be analyzed, for example its spectral content, polarization state, and Doppler shift, which can be used to determine functional parameters of the tissue as blood oxygen saturation, tissue birefringence and blood flow, respectively [15-21]. Consequently, next to the improvement of OCT system performance, also the extraction of more functional, i.e. morphological and/or physiological information, from the OCT signal is currently being investigated.
OCT PRINCIPLE

In its most basic form, OCT is based on low coherence interferometry with a Michelson interferometer. Figure 1-2 shows a schematic representation of a time-domain OCT (TD-OCT) setup. A collimated beam from a light source is split into a reference and sample arm. Light, back-reflected by the scanning mirror in the reference arm, and backscattered from the tissue under study in the sample arm, is combined and directed on the photo detector. The electric field at the detector is the sum of the sample $E_S$ and reference arm $E_R$ fields. The detector measures the intensity of the detected light $I_D$, which, in case of a single reflector in the sample arm and a monochromatic light source, is proportional to the square of the total field given by:

$$I_D \sim |E_R|^2 + |E_S|^2 + 2E_R E_S \cos(k\Delta L)$$  \hspace{1cm} (1-1)

where $\Delta L$ is the optical path length difference between the reference and sample arms of the interferometer, and $k$ is the wave number [22]. For a broadband light source as used in OCT, equation 1-1 is integrated over the source spectrum. If the reference arm is scanned, interference fringes are generated as a function of time as long as the optical path length difference $\Delta L$ is within the coherence length of the light source. The frequency of the interference signal is determined by the reference mirror velocity and the wavelength of the light, the amplitude of the interference signal is determined by the backscattering properties of the sample. Note that the first two terms in Equation 1-1 describe the DC offset of the signal, which in general is rejected by band-pass filtering and/or lock-in detection at the modulation frequency. The third term is the interferometric signal, containing information about the depth-dependent reflectivity of the sample. In a time-domain OCT system without focus tracking, the OCT detector
current $i_d(\Delta L)$ is equal to the field-backscatter profile of the sample $r(z)$ as function of depth $z (z=\Delta L/2n)$ convoluted with the complex coherence function $\gamma(\Delta L/c)$ [23]. The OCT detector current signal as a function of $z$ is given by

$$i_d(z) = \eta \text{Re} \left\{ \gamma \left( \frac{2\pi n}{c} \right) \right\} \otimes h(z)r(z)\sqrt{P_r P_s}$$ (1-2)

where $\eta$ is the detector conversion factor from the incident light power to the electric current, $Re\{\}$ is the real part of the complex coherence function, $n$ is the refractive index of the medium, $c$ is the speed of light in vacuum, $h(z)$ is the confocal point spread function describing the change of the OCT signal as a function of distance between the probed location $z$ in the sample and the focus position $z_0$ [24]. The powers $P_r$ and $P_s$ are the powers from the reference and sample arm, respectively. Since the reference mirror position at each moment is known, i.e. $\Delta L$ is controlled, the in-depth image of a sample can be reconstructed as a function of $z$ by longitudinal scanning of the reference arm. Finally, a three-dimensional image of the sample can be obtained by lateral scanning of the sample to acquire a 2D array of depth scans.

The axial resolution in medium with refractive index $n$ is determined by the central wavelength $\lambda_0$ of the light source. Assuming a Gaussian shaped spectrum with a full width at half maximum spectral bandwidth $\Delta \lambda$, the axial resolution $\delta z$ can be calculated as:

$$\delta z = \frac{2 \ln 2 \lambda_0^2}{n \pi \Delta \lambda}$$ (1-3)

The lateral resolution of the OCT system is decoupled from the axial resolution and is determined by the focusing optics of the sample arm. Assuming a Gaussian beam with diameter $D$ (1/e$^2$ width), focused by the lens with focal length $f$, diameter of the focal spot is calculated as:

$$\delta x = \frac{4 \lambda_0 f}{\pi D}$$ (1-4)

FOURIER-DOMAIN OCT

A superior approach to the depth ranging using a moving reference mirror is the acquisition of the interferometric signal as a function of the optical wavenumber with a fixed group delay [25]. This method, called Fourier-domain OCT, has two forms. Spectral-domain OCT (SD-OCT) uses a broadband light source and a spectrometer in the detection arm to detect the interference spectrum [26]. The second approach, swept-source OCT (SS-OCT), employs a single detector in the detector arm (similar to TD-OCT), but instead of a broadband light source, a wavelength sweeping laser is used [27-29]. The swept laser has a narrow instantaneous linewidth, which is rapidly tuned through a broad optical bandwidth.
Introduction

In Fourier-domain OCT, a single reflector in the sample arm results in a periodic fringe in the $k$-space domain whose frequency and amplitude are directly proportional to the depth and reflectance of the sample, respectively. In the case of multiple reflectors, a multitude of sinusoids are superimposed. The reflection coefficient from all depths can be simultaneously determined by Fourier transformation of the measured interference spectrum.

The maximum imaging depth $z_{\text{max}}$ in Fourier-domain OCT is determined as:

$$z_{\text{max}} = \frac{\lambda_0^2}{4n\delta\lambda}$$  \hspace{1cm} (1-5)

where $\delta\lambda$ is the wavelength sampling interval.

Fourier-Domain OCT has several advantages over TD-OCT. First of all, the absence of a mechanically scanned mirror in the reference arm makes higher data acquisition rates possible. Second, Fourier-domain OCT measures all depths simultaneously. Thirdly, the sensitivity of FD-OCT is higher than TD-OCT. Typically FD-OCT has 20-30 dB better sensitivity than TD-OCT (for the same data acquisition rate) [30-32]. In practice, the sensitivity advantage of FD-OCT is used to increase the imaging speed, which is especially useful for measurements on humans.

For SS-OCT the development of rapid swept lasers with large bandwidths and high output powers is required. Although the stationary mirror in the reference arm and single photo detector in the detector arm simplify the optical design of the OCT system, all complexity is moved to the design of the swept light source. To achieve high axial resolution in OCT applications, the swept laser should have a broad spectral output. For large imaging depths a narrow instantaneous linewidth is required which preferably is much smaller than the sampling wavelength interval (equation 1-5), otherwise the fringe visibility becomes very small at the maximum imaging depth. Finally, for in-vivo imaging, high sweep rates are needed. Still, comparing SD-OCT and SS-OCT, swept-source OCT is a more advantageous method due to the larger depth range (smaller instantaneous linewidths are easily achieved), the possibility to do balanced detection for a suppression of the relative intensity noise, and compact OCT layout.

To achieve good sweeping performance, different lasers and different tuning techniques are demonstrated. In general, OCT applications require lasers with a broad emission spectrum (e.g. dye, semiconductors, Ti:Sapphire gain material). Tuning can be performed mechanically or electronically, for which the latter is preferred due to a more stable performance. However, the time necessary to build-up lasing from the spontaneous emission is a fundamental limitation in the maximum achievable sweeping rate [33]. Recently, significant advantage in the field of the swept laser is achieved by the demonstration of Fourier-domain mode-locking (FDML) lasers with sweeping rates up to 5.2 MHz [34-36]. In this technique, lasers are based upon a cavity with a long optical delay.
line (~km) and synchronously tune a narrowband filter with the round-trip time of the cavity. Although FDML lasers demonstrate advanced sweeping performance, the development of new types of swept lasers continues [37, 38], and issues related to the optimization of such lasers in terms of prize, form factor, and performance for OCT applications still need to be addressed.

LIGHT-TISSUE INTERACTION

The interaction of light with tissue occurs through scattering and absorption. The overall effect of these processes is the attenuation of ballistic light in depth, which can be described by the attenuation coefficient $\mu_t$:

$$\mu_t = \mu_s + \mu_a$$

(1-6)

where $\mu_s$ is the scattering coefficient, and $\mu_a$ is the absorption coefficient. In the near infrared (NIR) part of the optical spectrum, the majority of biological tissues are highly scattering media with the scattering coefficients larger than the absorption coefficients. However, for longer near infrared wavelengths the increased light absorption by water also becomes a significant factor of light attenuation (Figure 1-3).

![Figure 1-3. The light absorption coefficient of water as a function of wavelength.](image)

Due to the low coherence path length selection and the confocal gating by the sample arm optics, OCT is mostly based on light that has scattered once (in the backward direction). Consequently, for superficial tissue layers with low scattering coefficients, the single scattering model is appropriate to describe the light attenuation. In this case the ballistic light intensity is described by the law of Lambert-Beer and attenuates exponentially with depth:

$$I(z) = I_0 \exp(-\mu_t z)$$

(1-7)
where $I_0$ is the intensity of the light at the tissue surface, and $I(z)$ is the intensity at depth $z$. For OCT, the single scattering model results in a detected OCT signal that is described as [39]:

$$i_d(z) \propto \sqrt{\exp(-2\mu z)} \quad (1-8)$$

The factor 2 in the exponent is caused by the double path length the detected light travels through the scattering medium, and the square root is because the detector current is proportional to the sample field rather than sample intensity (Equation 1-2).

Scattering by a single particle is characterized by the scattering cross-section $\sigma_s$, which describes the light scattering capability in units of cross sectional area. The larger the cross-section, the more likely the scattering occurs. The scattering coefficient of a sample containing many scatterers is the product of the scattering cross-section $\sigma_s$ and the number of scatterers $N$ per unit volume:

$$\mu_s = N\sigma_s \quad (1-9)$$

In case of high concentration of scatterers the scattering coefficient typically is lower and is described in more complicated way, which will be discussed in Chapter 7.

Classical light scattering theory for small particles was derived by Rayleigh. For spherical particles with a diameter much smaller than the wavelength of the incident light, the scattering cross-section is:

$$\sigma_s = \frac{2\pi^5}{3} \frac{d^6}{\lambda^2} \left(\frac{m^2-1}{m^2+2}\right)^2 \quad (1-10)$$

where $d$ is the particle diameter, and $m$ is the relative refractive index, i.e. the ratio of the (complex) refractive index of the particle to the refractive index of a medium. As can be seen, Rayleigh scattering is strongly dependent on the wavelength of light: the scattering cross-section is inversely proportional to fourth power of wavelength.

For scatterers comparable to or larger than the wavelength of light, the Rayleigh light scattering theory breaks down. In this case, light scattering can be described by solving Maxwell’s equations, for which the exact solution for light scattering by a single sphere was given by Mie. Mie theory is applicable for the scattering from spherical, homogeneous, isotropic and non-magnetic particles in a non-absorbing medium. The wavelength-dependence of Mie scattering is more complex than Rayleigh scattering. In general, the scattering decreases with increasing wavelength of the incident light at a lower rate than for Rayleigh scattering.

The scattering process induces a change in the direction of light propagation that can be described by the angle $\theta$, which is the angle between directions of propagation of incident and scattered light. Depending on the particle size and wavelength of the
incident light, a particle has its own scattering profile, which is called the scattering phase function \( p(\theta) \). The phase function is a normalized probability distribution as a function of the scattering angle. Plotted in polar coordinates as a function of \( \theta \), it represents the scattering diagram of the particle (Figure 1-4).

![Figure 1-4](image)

To characterize the angle-dependent scattering properties of scatterers in a more simple way, the scattering anisotropy parameter \( g \) is used. By definition, the scattering anisotropy parameter \( g \) is the average of the cosine of the scattering angle over the phase function:

\[
g = \frac{1}{\pi} \int_{0}^{\pi} p(\theta) \cos(\theta) 2\pi \sin(\theta) d\theta = \langle \cos \theta \rangle
\]

The scattering anisotropy \( g \) provides information about the dominant direction of scattering. The value of \( g \) can be in the range from -1 to 1. If forward scattering dominates, then \( g \) approaches 1; \( g \) approaching 0 represents equal scattering in forward and backward direction, e. g. for Rayleigh scattering.

The scattering coefficient \( \mu_s \) gives information about the number of scatterers, the size of the scatterer, and differences in the refractive index between the scatterer and its environment. The scattering anisotropy parameter \( g \) gives information about the size of the scatterer. Thus, the scattering properties contain diagnostically valuable information on the tissue structure and composition. For example, changes in the tissue morphology can lead to changes in the scattering coefficient, and changes in the tissue composition can lead to changes in the tissue refractive index and the absorption coefficient. Consequently, using carefully calibrated OCT systems, we can measure changes in the
optical properties and relate these changes to functional processes in biological tissues, which can be used for tissue diagnostic purposes. As an example, development of cancer involves morphological transformation of the cell architecture, i.e. increase of the nuclei size and increase of the amount of cells, which leads to changes in optical properties [5, 13, 40-42].

OCT IMAGING DEPTH IMPROVEMENT

Besides spatial resolution and imaging speed, the OCT imaging depth is an important characteristic of OCT performance. OCT is a superficial imaging technique with a depth range of 1-2 mm in scattering tissue. For many applications, improvements in the imaging depth can open new possibilities and application areas. As an example, in ophthalmology, imaging of the anterior segment of the eye is important for early diagnosis of eye diseases, in particular glaucoma. Glaucoma is the disease that is associated with elevated intraocular pressure. Elevation of the intraocular pressure results from an imbalance between the production and drainage of aqueous fluid [43-45] and is caused by the closure of the angle between iris and cornea. As a result, Schlemm’s drainage channel becomes blocked and results in an increase of the intraocular pressure. Visualization of this area of the anterior segment is difficult due to the highly scattering sclera. OCT systems with improved imaging depth can be successful in imaging of this part of the eye.

One of the possibilities to increase the OCT imaging depth is to use longer imaging wavelengths, for which scattering is lower. The advantage of using longer wavelengths was recognized, and, after the first OCT systems operating at wavelengths around 800 nm, OCT at wavelengths around 1300 nm and 1050 nm were introduced [46-49]. Clear improvement in the OCT imaging depth was demonstrated, and currently 1300 nm OCT systems are routinely used in the clinic. Further improvement of the imaging depth can be achieved with longer wavelengths. However, with increasing wavelength the light absorption by water increases, see Figure 1-3 [50]. Until approximately 1360 nm the absorption is relatively low and has negligible influence on the light attenuation. However, the water absorption peak at 1460 nm results into an increase in absorption to 2.9 mm$^{-1}$. Consequently, for wavelengths larger than 1360 nm the light penetration is governed by both absorption and scattering, and the optimal wavelength depends on the relative contribution of the two effects.

The 1600-1800 nm spectral range presents an opportunity for increased OCT imaging depth as the absorption is relatively low (only 0.67 mm$^{-1}$ at 1600 nm compared to 0.11 mm$^{-1}$ at 1300 nm) and scattering is much lower compared to the 1300 nm window. Consequently, for some biological tissues, the decrease in scattering can be larger than the increase in the absorption by water, which can result in an enhanced OCT imaging depth. The reported optical properties of some biological tissues in the NIR spectral range suggest that such enhancement is possible [51-54], which was supported by comparative
Chapter 1

studies of the OCT light imaging depth at different wavelengths [55, 56]. However, quantification of the imaging depth is difficult because the OCT system performance has to be well calibrated for the imaging wavelengths that are used in the comparison.

SCOPE OF THIS THESIS

In this thesis, entitled “Development of functional near-infrared optical coherence tomography”, the potential for improved OCT imaging depth and the extraction of more functional information from OCT data is explored. In Chapter 2, we investigated potential wavelength bands for OCT imaging depth improvement by performing an analysis of the wavelength-dependent NIR light penetration depth. The proposed imaging depth quantification formalism is tested using Intralipid optical transmission measurements, and is applied to three kinds of biological tissues. In Chapter 3, the OCT imaging depth is quantified and measured around 1300 nm and 1600 nm using a TD-OCT setup of which the technical characteristics at the two wavelength regions are matched. The next two chapters focus on methods to extract functional information from the OCT signal. In particular, we focused on measurements of light backscattering characteristics of tissue phantoms (Chapter 4) and cells (Chapter 5). In Chapter 4 we determined the relation between the OCT signal amplitude and the scattering anisotropy \( g \). Chapter 5 shows OCT measurements of the optical properties of the thin samples: absolute measurements of the scattering coefficient in thin phantoms, and measurements of relative changes in the backscattering in a single layer of retinal pigment epithelium (RPE) cells during the development of apoptosis. Chapter 6 describes the construction of a swept laser and its fundamental performance characteristics. A swept Ti:Sapphire laser with intracavity acousto-optic tunable filter that we developed is investigated, mainly focusing on the possibilities for optimization of the sweeping performance for application to SS-OCT. Finally, Chapter 7 provides a general discussion and conclusions.
REFERENCES

Chapter 1

Introduction

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