Functional optical coherence tomography: spatially resolved measurements of optical properties
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CHAPTER 1

General introduction

Optical Coherence Tomography (OCT) is a relatively new diagnostic modality for micrometer scale imaging of superficial tissues. As such, it has potential as an \textit{in vivo} optical biopsy tool in many fields of medicine. In contrast to other optical techniques, in OCT the path length of the light that is used for image formation is known, with an accuracy of only a few micrometers. Two key factors determine the clinical relevance of OCT. First, OCT is able to measure local tissue properties (reflectivity, flow, birefringence and other optical properties). Secondly, these measurements can be performed quantitatively. In this thesis, two applications of functional measurements with OCT are investigated: measurement of the attenuation coefficient to distinguish different tissue types; and spectroscopic OCT measurements to determine tissue oxygen saturation. This chapter places OCT in the field of biomedical optics and provides a rationale for the work presented in this thesis.
1.1 biomedical optics

The field of biomedical optics has grown rapidly since the introduction of the laser in 1960. In the broadest sense it covers the application of light in biology and medicine, including the physics of the interaction of light with tissues or drugs. Both diagnostic and therapeutic use of light is possible.

The latter is well known from various fields in medicine. In some fields, lasers are already used as a standard tool. In general surgery, various laser systems are used to cut, coagulate or vaporize tissues. In ophthalmology for example, laser treatment is used in glaucoma patients to improve drainage of the eye, for photocoagulation of blood vessels in diabetic retinopathy, and for corneal reshaping in LASIK procedures. Photodynamic therapy (PDT, in which drugs are activated upon illumination) is used to treat choroidal neovascularization. In dermatology, lasers are used to coagulate blood vessels in the treatment of port wine stains or other disorders of blood vessels, as well as in the treatment of numerous pigmented disorders.

Other laser treatments are less established today. In trans-myocardial laser re-vascularization laser light is used to acupuncture the heart in patients with severe chest pain. Although the working mechanism has not yet been completely elucidated, clinical improvement is reported. Laser light is also used to coagulate placental anastomoses in a pathological condition called twin-twin transfusion syndrome, in which twins share a circulation and a net flow exists from one fetus to the other. In oncology, PDT is used in treatment of superficial malignancies (e.g. in skin, the throat, and Barrett’s esophagus). Research on antimicrobial PDT is motivated by the growing resistance of bacteria to antibiotics. In these specific examples, laser treatment is the optimal, if not the only treatment of choice.

Imaging of (pathological) tissue is part of many clinical disciplines such as ophthalmology, dermatology, urology, gastro-enterology and cardiology. A variety of non-optical imaging methods ranging from MRI to ultrasonography are being used at different stages of the process of diagnosis, treatment and follow up of various diseases. Despite their advantages, each of these methods has some inherent limitations related to image resolution, acquisition time, specificity, accuracy, safety or cost, which motivates the search for new, more precise and less invasive imaging techniques. Many of the limitations can (at least in principle) be overcome by optical techniques which allow miniaturization, can be cheap and pose no health risks because they use non-ionizing radiation. Unfortunately, some important clinical parameters still cannot be measured by currently available optical techniques. In ophthalmology for example, fluorescein angiography is used to visualize the retinal vasculature, to evaluate vascular leakage and macular edema in diseases such as diabetic retinopathy. However, the retinal thickness, another important parameter, cannot be measured by this technique. In dermatology, the choice of treatment of burn wounds is highly dependent on the grade and depth of the wound. Ideally, an optical technique assesses both aspects, and also provides follow up information, for example by quantifying perfusion in transplanted skin sections. In gastro-enterology the identification of the various layers of the esophageal wall is indispensable for in vivo recognition and staging of tumors. The current diagnostic method of choice is to take white-light endoscopy guided biopsies, which has low sensitivity since only limited information can be extracted from the endoscopic images. In cardiology, unstable atherosclerotic plaques in the coronary arteries are important targets to identify. These plaques consist of a thin fibrotic cap (60-150 μm) covering a large lipid lesion. Cap rupture causes
exposure of the lipids to the blood flow which leads to the formation of a thrombus, which, depending on the shear stress caused by the flowing blood, may grow or shrink in time. This causes patients to experience chest pain that is unrelated to exercise. In more unfortunate circumstances, the thrombus can block the blood stream causing an acute infarction. Presently, intravascular ultrasound (IVUS) is used to image the vascular wall. Unfortunately, it is not possible to distinguish lipid pools from caps in IVUS images. Although IVUS is capable of measuring mechanical properties (elastography), which enables discrimination, the resolution is not sufficient to accurately measure cap thickness, which is an important risk factor.

In diagnostic optical methods, light is directed towards the tissue under investigation. Through interaction with the tissue, (subtle) changes in the properties of the light occur. The light returning from the tissue is collected and from this, images are formed and/or functional parameters (such as oxygenation) are assessed. Light interacts with tissue in many ways. Light that has entered the tissue is scattered by tissue structures such as collagen and elastin fibers, cells, membranes and organelles. The amount of scattering depends on size, orientation and refractive index of the involved structures. In addition, light scattered by moving objects, e.g. red blood cells, gets Doppler shifted. From the Doppler spectrum flow and perfusion can be determined. The polarization of the light is changed by interaction with birefringent structures such as collagen fibers.

Apart from scattering, light can be absorbed inside the tissue. Figure 1-1 shows the absorption spectra of some important tissue constituents. Specific absorption peaks may be used as indicator of the presence of certain chromophores inside the tissue. The absorbed light can also be remitted as light of different frequency, which can happen at a variety of energy shifts and timescales, e.g. fluorescence or Raman scattering. By choosing light sources of suitable wavelengths, different tissue constituents can be probed, for instance to determine the ratio of hemoglobin and oxygenated hemoglobin, or to determine glucose concentration (which is one of the 'holy grails' of biomedical optics, because a device for non-invasive measurement of glucose levels would have enormous marketing potential).
Optical imaging techniques will generally use wavelengths between 650 nm and 1300 nm where absorption in tissue is low (figure 1-1), to allow reasonable imaging depths. Roughly, scattering decreases monotonically with wavelength in this region. A trade-off exists between the depth resolution and probing depth of currently available techniques, as is shown in figure 1-2.

![Figure 1-2: Imaging ranges of optical diagnostic techniques and the corresponding targets.](image)

Microscopy (on the lower left side of figure 1-2) detects non-scattered light transmitted through excised tissue sections. In conventional microscopy, contrast is provided for by absorption differences in the stained section. Image resolution can be very high (laterally approximately 0.2 micron, in depth 0.7 micron) but imaging depth is very small. Imaging in scattering media is possible with (scanning) confocal microscopy, since light returning from outside the focal volume is sufficiently suppressed. Resolutions are comparable to conventional microscopy and depth resolved imaging up to 500 μm is possible.

In optical tomography methods such as time-of-flight measurements or frequency modulated optical tomography (upper right side) multiple scattered light is used for image formation and measurement of optical properties. This yields higher imaging depths at the expense of resolution. The underlying theory and models are very complex, and consequently so are reconstruction algorithms. An active research area is optical mammography, which may ultimately lead to the replacement of ionizing X-ray diagnostics with non-ionizing optical imaging for breast cancer screening.

### 1.2 Optical coherence tomography

The gap in the spectrum of techniques in figure 1-2 is partly filled by Optical Coherence Tomography (OCT) [1], which permits in vivo cross-sectional imaging of superficial tissues. Compared to microscopy, OCT provides morphological details at significantly greater depths in dense tissue because it detects only light that has been scattered once or a few times. OCT is analogous to ultrasound imaging yielding cross-sectional images of tissue reflectivity. In ultrasound (figure 1-3, left), the location of reflecting objects is determined by measuring echo delay times. This is not possible with optical techniques given the high speed of light compared to detector response times. Instead, the location of reflecting objects is determined by using a classical optical measurement technique: white light or low coherence interferometry (figure 1-3, right).
In a Michelson interferometer, light emitted from a source is divided by a beam splitter in two directions ('arms') towards two mirrors R and S. The back reflected beams recombine at the beam splitter, and are guided to a detector. When R is moved (while S is fixed), an interference pattern is measured by the detector. Interference will only be detected when the difference in path lengths traveled by the light in both arms is less than the so-called coherence length of the light source. A monochromatic light source (for example a Helium-Neon laser), has a large coherence length and consequently the interference fringes will be seen over a large range of path length differences. If a low coherent (broad bandwidth, i.e. many wavelengths) light source is used, the coherence length can be very small: for sources used in OCT typically from 20 μm down to 1 μm. Figure 1-4 shows a schematic of a fiber-based OCT system, here used to image a retina.

In this setup, the retina 'replaces' the fixed mirror S above, and the mirror in the reference arm is moved. Again, interference only occurs if the difference in path lengths traveled by the light reflected from the sample and that returning from the reference mirror is within the coherence length. Therefore, when interference is observed at the detector, and the path length in the reference arm is known, the path length in the sample arm and thus the position of the reflecting structure can be calculated within the accuracy of the coherence length. Following the terminology of ultrasound imaging, a measurement of reflectivity vs. depth is called an A-scan; the OCT image, or B-scan is constructed from adjacent A-scans with reflectivity now plotted on a color scale. For example, figure 1-5 shows an (ex-vivo) OCT image (left) and corresponding histology section (right) of the aorta of a rat.
The lateral resolution in the OCT image is determined by the focusing optics in the sample arm, similar to confocal microscopy. OCT can therefore be regarded as an extension of confocal microscopy [2] because the axial resolution in OCT is determined only by the coherence length of the light source. The confocal measurement technique suppresses the detection of light scattered from outside the focus. The difference between high numerical aperture (NA) optics and low NA optics is shown in figure 1-6: for high NA optics the lateral resolution is high, but the depth of focus is small (compared to low NA optics, where the situation is reversed).

![Figure 1-6: high NA focusing with high lateral resolution but limited depth of focus (left) and low NA focusing, with large lateral resolution and large depth of view.](image)

Ideally, to achieve high axial and lateral resolution, high NA optics are used and the measurement position and the position of the focus are matched throughout the depth scan (dynamic focusing). For clinical OCT systems, real time imaging is essential, with typical line scan rates in the order of several kHz [3]. Because it is currently not possible to mechanically move focusing optics at this rate, usually optics with relatively low numerical aperture are used, with a large depth of focus and modest lateral resolution (~20 μm). For most tissues imaging up to 1-2 mm deep is possible. This places OCT approximately in the center of figure 1-2.

To date, OCT imaging is routinely used in the clinic in ophthalmology, but has great potential as an 'optical biopsy' tool in other fields of medicine e.g. gastro-enterology and cardiology. There are two key factors determining the clinical relevance of OCT. The first is the ability to do 'point-like' measurements, i.e. measure tissue parameters in a highly localized volume. Basic OCT measurements record local tissue reflectivity, but localized measurement of flow/perfusion [4], index of refraction [5], birefringence [6], and absorption [7] and scattering coefficient [8] is also feasible. The second key factor is the ability to measure these properties quantitatively. This is possible because (contrary to other optical techniques) the path length of the detected light is controlled (or known).
1.3 scope of this thesis

The work described in this thesis explores two applications of quantitative measurement of local optical properties with OCT. First, the attenuation coefficient of tissue structures can be used for additional identification of tissues (or pathologies) in the OCT image. Second, localized tissue oxygenation can be derived when the attenuation coefficient is measured at two different wavelengths.

The attenuation coefficient \( \mu \), describes the decrease of signal strength with depth due to scattering and absorption. It is inherently different for different tissues and may therefore be used as a marker in an OCT image. Specifically, in images of atherosclerotic plaques, both lipid rich and calcified lesions show up as darker areas, whereas only the lipid lesion is the thrombogenic part of the vulnerable plaque.

As discussed earlier, dynamic focusing is usually not implemented in clinical OCT systems. Consequently the measured signal is influenced by the optical components of the OCT system itself. To quantitatively measure the attenuation coefficient, the effect of the axial point spread function (PSF) of the used optics has to be taken into account.

In chapter 3 a simplified form for the axial PSF is deduced and compared with previously published PSF's. The axial PSF for a high resolution OCT system from a mirror and in scattering media is calculated. Use of this PSF in combination with the single backscattering model allows determination of the attenuation coefficient of a suspension of calibrated scattering particles.

Next, the attenuation coefficient of calibrated, weakly scattering tissue phantoms is extracted in chapter 4. The data are analyzed using a single backscattering model and the PSF described in chapter 3. The validity of the single backscattering model for these samples is verified by a quantitative comparison with a multiple scattering model.

The combination of optical coherence tomography and spectroscopy may allow for highly localized, quantitative measurements of tissue spectral properties. Using the differences in absorption of hemoglobin and oxygenated hemoglobin (see figure 1-1), assessment of local tissue oxygenation may be feasible which could be helpful in understanding the basic mechanisms of oxygen transport and consumption in tissues. Moreover, non-invasive continuous oxygenation monitoring can be of importance in fields such as neonatology (to replace the invasive heel prick) and intensive care medicine, because maintaining adequate tissue oxygenation is a primary objective.

In chapter 5 quantitative measurements of the absorption coefficient of phantoms and of hemoglobin and oxygenated hemoglobin with spectroscopic optical coherence tomography (SOCT) are presented.

Blood is a highly (forward) scattering medium. Therefore, the influence of scattering on the SOCT signal should be investigated. Chapter 6 reports on the scattering properties of oxygenated and de-oxygenated whole blood in the range 250-1000 nm. The complex refractive index of oxygenated and de-oxygenated hemoglobin is determined using Kramers Kronig analysis and OCT measurements. Combining these data with Mie theory, the oxygen
saturation dependent scattering properties are calculated. Furthermore, the presented method provides a template for retrieving the optical properties of dense, highly (forward) scattering media (such as blood) where direct measurement is virtually impossible.

In CHAPTER 7 data is presented that correlate spectroscopic OCT measurements with oxygen saturation of whole blood samples. Contributions from both scattering and absorption are present in the OCT signal. However, to facilitate quantitative oxygen saturation measurements with OCT, theoretical descriptions taking into account multiple, concentration dependent scattering have to be developed.

Finally, CHAPTER 8 provides an additional analysis of multiple scattering and discusses the implications of the results described in this thesis.

The next chapter discusses OCT in formulas and numbers, to serve as a theoretical starting point for the other chapters.

REFERENCES