Polarization-sensitive optical coherence tomography on different tissues samples for tumor discrimination

F. Fleischhauer, H. Schulz-Hildebrandt, T. Bonin and G. Hüttmann

***Abstract*—Optical coherence tomography (OCT) is now state of the art in ophthalmology. Other medical departments, such as otolaryngology, could also benefit from this imaging modality. Polarization is a property which is a benefit to conventional OCT. It can be used in particular to detect birefringent tissue layers. To evaluate possible OCT applications in Ear, Nose and Throat (ENT) department, a fiber-based polarization-sensitive OCT (PS-OCT) is used to measure human ex-vivo tissue biopsies. A total of 58 samples from 20 different tissues were measured.**
The measurements show that small biopsies lose their birefringent property, this leads to the assumption that tissue-matrices lose tension and molecular order. Larger samples show phase retardation and are used to detect different birefringent layers of tissue. We also show a method of verifying cancer diagnosis by displaying the tissue layer structure.
As a conclusion, PS-OCT may improve cancer diagnosis in ENT.

I. Introduction

Cancer is the leading cause of death in the European Union. [1] Early detection of cancer leads to an improve of survival rate and an enhancement of the patients quality of life. [2]
Optical coherence tomography (OCT) can visualize early malign and benign changes in tissue and represents a tool for cancer detection. [2]
The destruction of tissue layers by epithelial tumors can be visualized with polarization sensitive techniques, such as polarization-sensitive optical coherence tomography (PS-OCT). [3] Collagen, a birefringent tissue, is destroyed by tumors and the polarizing effect is lost. It is formed in long fibrils of proteins, which make up the main part of connective tissue in humans. Collagen is mostly found in tendon, skin and ligament [4], in otolaryngology mostly in mucosa such as chorda or vocal cord tissue. [5]
The lamina propria is a layer of loose connective tissue found beneath the epithelium of mucosa. The lamina propria consists of a well-structured network of collagen fibrils, it embeds, among others, blood vessels and nerves. [6]
The vocal cord tissue is a stack of different layers. The first layer is the epithelium, which contains almost no collagen, beneath is the lamina propria found. [6] Fig. 1 gives an overview of vocal cord structure.

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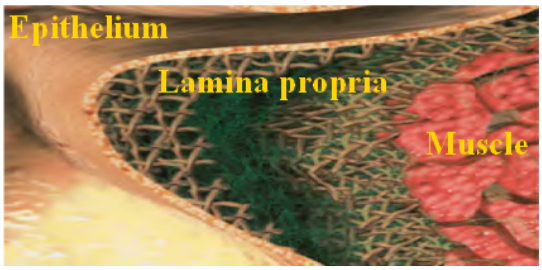

Fig. 1. Overview of vocal cord. Brown displays thick collagen fibrils, green thin ones. [6]

Optical coherence tomography, invented in 1991, is a noninvasive imaging modality based on white-light interferometry. The benefits in medicine are high scan speed, high axial and lateral resolution and a penetration depth of up to 3 mm. [7], [8] The main usage of OCT is to detect and visualize different tissue layers. [9] A conventional OCT uses the intensity of scattered light. Other information can be acquired from analyzing the phase or frequency information of the wave of light. [10]
A longitudinal wave, such as sound, can be completely described with amplitude, frequency and phase. Transversal waves, such as light, can oscillate in every orthogonal direction, so another parameter is needed to describe such a wave. This parameter is polarization.
Polarization can be composed in two different orthogonal directions, if the wave oscillates in one direction, it is called linear polarized light. If it oscillates in both orthogonal directions and not in phase, circular or elliptically polarized light results.
The different states of polarization can be described by the four Stokes parameters I, Q, U and V.

$$\begin{aligned} I &= \langle E_{\parallel} E_{\parallel}^{*} + E_{\perp} E_{\perp}^{*} \rangle \\ Q &= \langle E_{\parallel} E_{\parallel}^{*} - E_{\perp} E_{\perp}^{*} \rangle \\ U &= \langle E_{\parallel} E_{\perp}^{*} + E_{\perp} E_{\parallel}^{*} \rangle \\ V &= \langle i(E_{\parallel} E_{\perp}^{*} - E_{\perp} E_{\parallel}^{*}) \rangle \end{aligned} \tag{1}$$

$E_{\parallel}$ and $E_{\perp}$ are the over time averaged amplitudes of the wave vector in an orthogonal coordinate system, $E_{\parallel}^{*}$ and $E_{\perp}^{*}$ the complex conjugated. These time-averages can describe monochromatic light, when time-averaging, compared to the period, is much longer. [7], [11]
The optical property describing material with a refractive index depending on polarization and propagation direction of light is called birefringence. Birefringent materials influence light of two orthogonal polarization states diffract. These two states have unequal refractive indexes. [12]

Polarization-sensitive OCT (PS-OCT) uses light of known polarization. Is this state changed in a birefringent sample, it gets detected. [13]
Earlier work in 2011 by Just et al. shows that conventional OCT can improve the precision of surgical interventions. Furthermore an improvement in detection of the basal membrane with PS-OCT is shown. [2] In 2012 Kim et al. shows that determination of malignant invasion and submucosal spread into adjacent healthy structures can be quantified by PS-OCT. [14]

The polarization states are calculated by measuring the interference in different channels of the optical setup, so that the state of polarization can be calculated quickly. The Stokes parameters can be calculated in a PS-OCT with a set of polarizers and wave plates. It is possible to measure all four Stokes parameters with one measurement regarding the phase relation between interference fringes in polarization channels. This phase relation is displayed as phase retardation. If a birefringent sample changes the Stokes parameters, the phase, after Fourier transformation is delayed. This retardation is displayed with the phase retardation. Phase retardation is a fixed value depending on a physical property and should always provide the same result.

II. Material and Methods

The measurements were done at the Otto Körner Ear, Nose and Throat (ENT) clinic in Rostock. A total of 58 biopsies are measured. The samples came from surgery and were directly measured. Special interest is on vocal cord (15 samples) and tympanum (10 samples), because of the lamina propria.

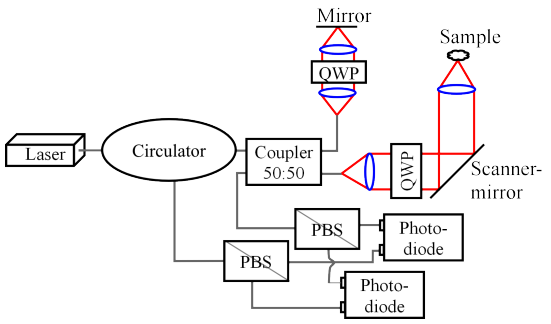

Fig. 2. Sketch of used PS-OCT. The light emitted from the laser is split into reference and sample arm, both consists of a quarter wave-plate (QWP). After reflection and scattering the beams are directed onto polarized beam-splitters (PBS), which split the beams in linear polarized light. This light is measured with photo-diodes.

The used light source has a central wavelength of $\lambda = 1310$ nm and a spectral bandwidth $\Delta\lambda$ of > 100 nm, a repetition rate of 50 kHz and a coherence length of > 12 mm. [15] The sensitivity of the used setup is 80 dB.
A fiber-based PS-OCT was used to measure different tissues from otolaryngology. Fig. 2 shows a sketch of the used PS-OCT. Light is emitted out of a Axsun (Axsun Technologies Inc., Billerica, USA) swept-source laser. After passing through a circulator the light is split by a 50:50 coupler. One beam goes into the reference arm, consisting of a collimator, a quarter wave-plate (QWP) orientated at 22.5° and a mirror. The beam passes the QWP twice so that the oscillation is 45°rotated. The light is reflected on the mirror and passes through the coupler onto two polarized beam-splitters (PBS) from Opto-Link (Opto-Link Corperation Ltd., Hong Kong). The other beam from the 50:50 coupler is directed into the sample arm, which consists of a collimator, a quarter wave-plate orientated at 45°, so circular polarized light falls on the sample, but also obtains scanning components and a focusing lens. The backscattered light, elliptically polarized, of the sample passes through the 50:50 coupler onto two PBS. The two PBS split the beams according to the state of polarization in two linear polarized beams. Each state of polarization is pointed at a balanced detection and a Thorlabs PDB430C photo-diode (ThorLabs Inc. Newton, USA).
Balanced detection is used to suppress noise from the light source, that is split in both arms evenly.

The A-scan in Fourier-domain OCT is calculated by the inverse Fourier transformation of the photo-diode signal.
A linear in λ-swept laser creates a chirped signal of the wave number k. This chirped signal is sent into reference and sample arm and interferes at the photo-diode. If the sample signal is shifted, an interference pattern is created. This means that in Fourier-domain OCT, interference of each spectral component is measured and a depth profile is generated.
The logarithm of the Stokes parameter I represents the OCT image. The arctangent of dividing the phase-retarded signal by the non-retarded signal, calculates the retardation image.

There is special interest for the thickness of tissue layers, because it is an indicator of diseases and its progress. These information could be provided by the retardation images, because borders between epithelium and birefringent tissue can be detected and distinguished better.
To reduce noise, adjacent A-scans were averaged after alignment to a horizontal surface. This can be used to measure the thickness of the aligned B-scan or it can be displayed as a function showing the retardation value over depth.

III. Results and Discussion

A total of ten ex-vivo tympanum were measured. Fig. 3 gives an overview of all measurements on the tympanum.
The mainly small biopsies show almost no phase retardation. Fig. 4 shows one tympanum biopsy as a photograph and the corresponding OCT image with the retardation as overlay.
The size of the samples leads to the assumption that small biopsies lose their birefringent property due to loss of tension and organization of the tissue matrix, especially the collagen fibrils, or dehydration.

Another measured tissue is vocal cord. Fig. 5 gives an overview of 12 small measured vocal cords.

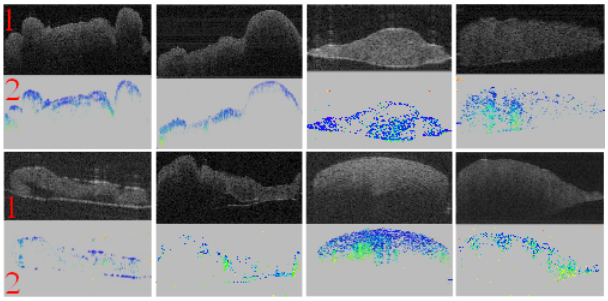

Fig. 3. Overview of tympanum samples: Row 1 shows the OCT images, row 2 the corresponding retardation images.

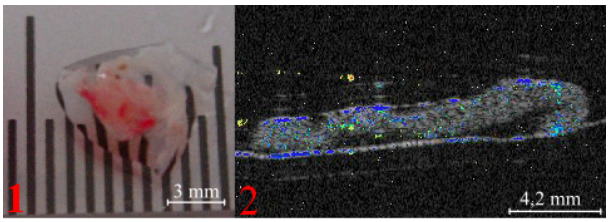

Fig. 4. Small human tympanum tissue from surgery; A: Photography of tympanum sample; B: OCT image with retardation image as overlay.

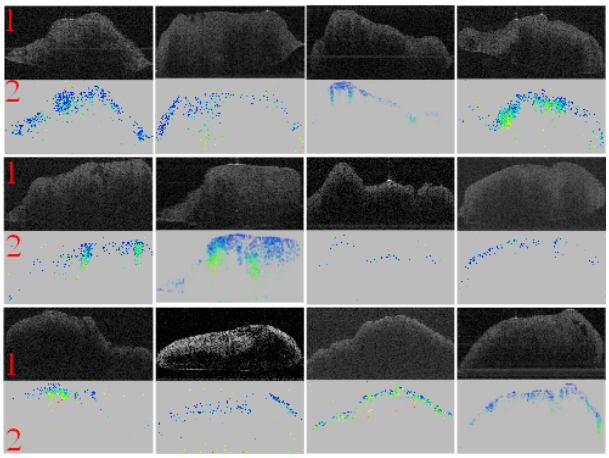

Fig. 5. Overview of vocal cord samples: Row 1 shows OCT images, row 2 the corresponding retardation images.

The same lack of retardation can be observed as in tympanum. Again, the size of biopsies is thought as the main reason.
In contrary healthy vocal cord from a bigger biopsy, here a whole larynx, shows the expected phase retardation. The result can be seen in Fig. 6.
To distinguish tissue layers, the border between layers has to be detected. To achieve this distinguishing the surface of the biopsy has to be found and flatten on a horizontal position. The mean retardation of a region of interest can be used to discriminate tissue. As a single A-scan is too noisy, average A-scans were calculated.
A software was programmed to display the retardation image, a region of interest and a function of phase retardation value over depth. The user can choose a region of interest, in which the surface is detected and pulled on a horizontal position. The different thicknesses of the layers can be seen

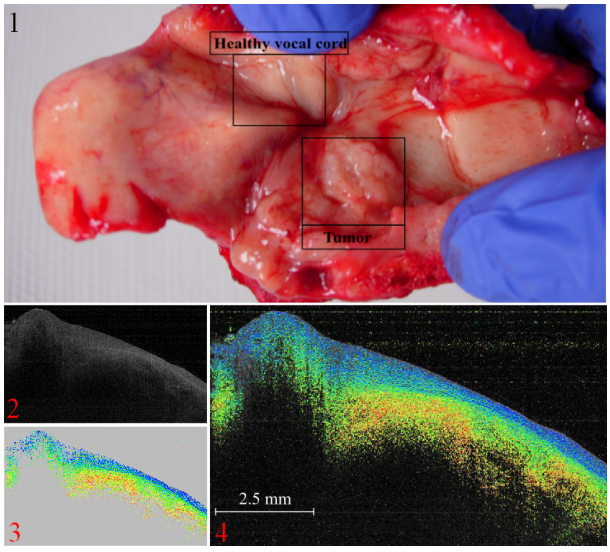

Fig. 6. Healthy vocal cord sample. Picture 1 shows a macroscopic photography of the larynx with tumor and healthy vocal cord, picture 2 shows the OCT image of the healthy vocal cord, picture 3 the corresponding retardation image and picture 4 the OCT with the retardation as overlay.

in each A-scan already. Fig. 7 shows the retardation image, the chosen region of interest and the mean retardation value as a function of depth.

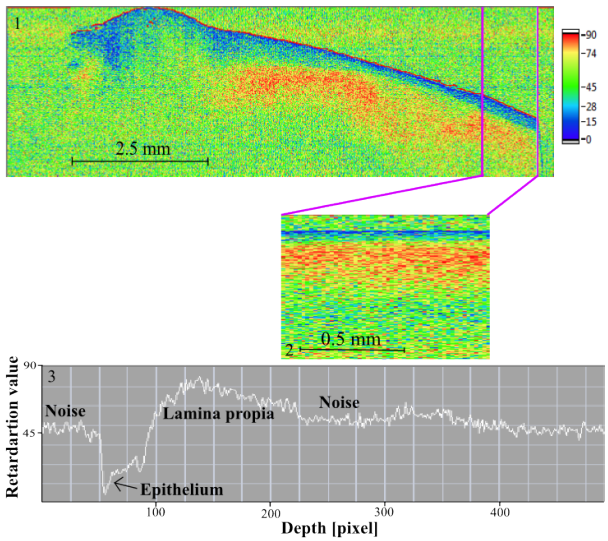

Fig. 7. Retardation image of healthy vocal cord. Picture 1 shows the retardation image, picture 2 the region of interest and picture 3 a graph displaying the changes of the retardation value.

Fig. 7 displays first a flat plateau, where only noise causes a retardation of 45. Afterwards the decrease of retardation shows the surface of the epithelium. Here no retardation is detected because the epithelium lacks of collagen and other birefringent structures. The following, almost flat, plateau describes the thickness of the epithelium. The epithelium is followed by the lamina propria with a well-structured network of collagen and an increase of the phase retardation. This rise is visualized by the increase in the graph. The highest point describes the middle of the lamina propria. Afterwards no

OCT signal is gathered and noise is displayed.
Another choice of the region of interest shows the lamina propria as aa almost flat plateau. The chosen region of interest and the change of retardation values can be seen in Fig. 8.

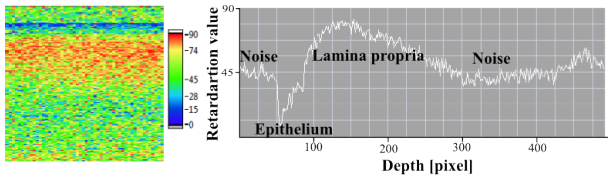

Fig. 8. A region of interest with lamina propria and its mean retardation value changes.

Tumors destroy the structure of the epithelium with its cell growth. This effects also the lamina propria, which is destroyed and the collagen destructed.
This effect can also be seen in Fig. 9 which shows a tumorous vocal cord.

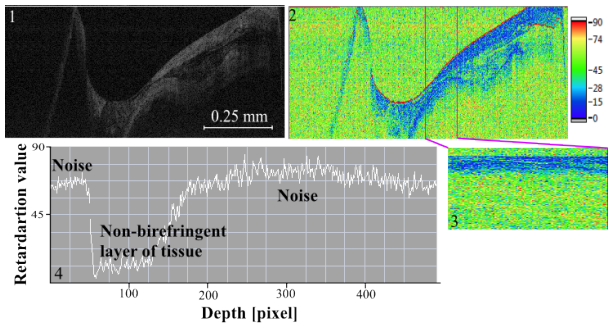

Fig. 9. Tumorous vocal cord. Picture 1 displays the OCT image, picture 2 displays the retardation image, picture 3 the region of interest and picture 4 the changes of retardation value over depth.

The tumor destroyed the tissue structure and no birefringent tissue was visible. Both effects can be seen in the retardation image as well as in the retardation value as a function of depth. That shows only one thick layer with no birefringence.

IV. Conclusions

Polarization-sensitive OCT can support clinical diagnosis of cancer in ENT.
Conventional OCT cannot distinguish between layers with similar intensity, PS-OCT can detect those with phase retardation, if the birefringent properties are different. The thickness of these different layers can be a hint for diseases and its advances. It also gives an opportunity to distinguish between healthy and tumorous tissue, because the birefringent property gets lost in it.
On the other hand, the measurements of ex-vivo samples showed, that small biopsies quickly lose their birefringence, which can depend on dehydration or the loss of tension, especially of the collagen fibrils.
The phase retardation is an advancement to conventional OCT, because a fixed value is produced which can be compared to other measurements, OCT images are qualitative and difficult to compare in intensity, because to many outer parameter influence the image.
The displacement of the phase retardation as a function over depth is an extension to the images. Measuring the thickness of layer is simplified.

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