Multidisciplinary Ophthalmic Imaging

Time-Resolved Ultra–High Resolution Optical Coherence Tomography for Real-Time Monitoring of Selective Retina Therapy

Patrick Steiner,1,2 Andreas Ebneter,3 Lieselotte Erika Berger,3 Martin Zinkernagel,3 Boris Považay,2 Christoph Meier,2 Jens H. Kowal,1 Carsten Framme,4 Ralf Brinkmann,5,6 Sebastian Wolf,3 and Raphael Sznitman1,3

1ARTORG Center, University of Bern, Bern, Switzerland
2HuCE OptoLab, Berne University of Applied Sciences, Biel, Switzerland
3Department of Ophthalmology, Inselspital, Bern, Switzerland
4Department for Ophthalmology, Medizinische Hochschule Hannover, Hannover, Germany
5Institute of Biomedical Optics, University of Lübeck, Lübeck, Germany
6Medical Laser Center Lübeck GmbH, Lübeck, Germany

Correspondence: Patrick Steiner, University of Bern, Murtenstrasse 50, CH-3010 Bern, Switzerland; patrick.steiner@artorg.unibe.ch.
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PURPOSE. Selective retina therapy (SRT) is a novel treatment for retinal pathologies, solely targeting the RPE. During SRT, the detection of an immediate tissue reaction is challenging, as tissue effects remain limited to intracellular RPE photodisruption. Time-resolved ultra-high axial resolution optical coherence tomography (OCT) is thus evaluated for the monitoring of dynamic optical changes at and around the RPE during SRT.

METHODS. An experimental OCT system with an ultra-high axial resolution of 1.78 µm was combined with an SRT system and time-resolved OCT M-scans of the target area were recorded from four patients undergoing SRT. Optical coherence tomography scans were analyzed and OCT morphology was correlated with findings in fluorescein angiography, fundus photography, and cross-sectional OCT.

RESULTS. In cases in which the irradiation caused RPE damage proven by fluorescein angiography, the lesions were well discernible in time-resolved OCT images but remained invisible in fundus photography and cross-sectional OCT acquired after treatment. If RPE damage was introduced, all applied SRT pulses led to detectable signal changes in the time-resolved OCT images. The extent of optical signal variation seen in the OCT data appeared to scale with the applied SRT pulse energy.

CONCLUSIONS. The first clinical results proved that successful SRT irradiation induces detectable changes in the OCT M-scan signal while it remains invisible in conventional ophthalmoscopic imaging. Thus, real-time high-resolution OCT is a promising modality to monitor and analyze tissue effects introduced by selective retina therapy and may be used to guide SRT in an automatic feedback mode (www.swissmedic.ch number, 2011-MD-0006).

Keywords: selective retina therapy, optical coherence tomography, dosimetry, laser therapy, retina

Retinal photocoagulation, first introduced in the 1950s by Meyer-Schwickerath using sunlight,1 has become a clinical standard for the treatment of a variety of retinal pathologies, such as retinal tears or ischemic retinal diseases (e.g., proliferative diabetic retinopathy or ischemic retinal vein occlusion). In ischemic retina, retinal photocoagulation is used to destroy the retinal pigment epithelium and the photoreceptor layer so as to reduce the amount of hypoxia and the VEGF load. In the clinical setting, this is usually done by slit lamp delivery of frequency-doubled solid-state laser light, and the therapeutic effects are judged by the amount of whitening of the inner retina. Methods to reduce strong adverse effects associated with laser photocoagulation have recently been introduced using micropulsing2,3 and endpoint management,4 whereas more sophisticated approaches include temperature measurements either by optoacoustics5,6 or by optical coherence tomography (OCT).7 However, treatment of macular diseases (e.g., chorioretinopathy centralis serosa or diabetic edema) with conventional laser photocoagulation may induce laser scotoma and includes the denaturation of the photoreceptor layer, which is considered to be disproportionate, especially for RPE-linked pathologies.

With selective retina therapy (SRT), an irradiation method completely avoiding thermal tissue damage was introduced. The SRT limits the introduced tissue damage to the RPE layer while neural retinal layers are left unaffected, facilitating reduced side effects and better healing of the introduced retinal lesions.8,9 Various studies have identified the influence of SRT parameters on short- and long-term therapy success in animal and patient studies,8,10-11 and results showed that for a reliable and safe therapy, the treatment pulse energy must be kept within a therapeutic irradiation window, limited by the...
threshold pulse energies for angiographic and ophthalmoscop-
ic visibility. An overview on SRT methods, techniques, and
preclinical and clinical results can be found in Reference 9. The
increased selectivity of the treatment was presented both experi-
mentally and clinically while successful treatments for chorioretinopathy centralis serosa (CSR), diabetic macular edema (DME), and chronic subfoveal fluid have been shown.15-17

The use of SRT, however, is challenging in terms of therapy
monitoring and dosage because an immediate tissue reaction is
not biomicroscopically discernible, as treatment effects are
limited to the outer retinal layers. The a priori determination of
the appropriate treatment pulse energy is further hindered by
the patient-specific concentration of melanin in the RPE,18
influencing the rate of conversion from laser energy to heat.
Thus, a feedback technique for real-time monitoring of selective
RPE damage is highly demanded.

In SRT, RPE damage is introduced by short living micro-
bubbles forming around the intracellular melanosomes that
nucleate after the vaporization threshold is exceeded and the
increased cell volume subsequently leads to a disruption of the
cellular membrane.29 To monitor SRT-induced RPE damage, the
detection of the ultrasonic pressure generated by microbubble
formation19 and the detection of the light reflection changes
during bubble lifetime20 may be used. For the latter, a feedback
technique has been developed in which the SRT pulse energy
is increased stepwise until bubble formation is detected and
the laser emission is ceased.21

Optical coherence tomography,22 with its volumetric
representation of optical sample properties, represents anoth-
er promising modality to examine the retina during laser
treatment. as it enables the display and monitoring of the
position and orientation of retinal layers as well as the tissue-
scattering structure.23 To this date, various experiments have
been published discussing OCT imaging of retinal laser
lesions,24-26 including polarization-sensitive OCT27 and
a proposed lesion classifier.28 Recently, ultra-high axial resolu-
tion OCT imaging during retinal photocoagulation has shown
promising results for detection of optical changes in the retinal
layers on ex vivo samples29 and on primates.30 Follow-up OCT
also provides important information about long-term lesion
healing and therapy progression31; however, no persistent
structural tissue changes that directly influence the OCT signal
by changing the tissue-scattering potential were detectable in
standard cross-sectional retinal OCT recorded immediately after
SRT.31-33 Structural changes included thickening of the
RPE layer, first detectable only hours to days after treatment.
The optically monitored short-time effects are assumed to be
linked to biochemical, subresolution structural or intracellular
changes. It thus becomes obvious that phase-sensitive OCT
imaging techniques for simultaneous lesion analysis need to
focus on dynamic optical changes occurring during the SRT
laser application and have to build a model that links the
immediate physical to the slower biological response.

In this article, a proprietary ultra-high resolution OCT
system was introduced into an existing SRT system to facilitate
the simultaneous acquisition of OCT data during application of
the treatment laser energy. We investigated real-time, ultra-
high resolution OCT in a clinical setting and assessed its
capability of mapping immediate and dynamic optical changes
in retinal tissue during treatment. Time-resolved OCT A-scans
(M-scans in OCT terminology) were recorded during SRT on
patients. Optical coherence tomography data were analyzed
for optical tissue effects introduced by the laser application
and clinical data were correlated with fundus fluorescein
angiography, color fundus images, and conventional cross-
sectional OCT.

**Materials and Methods**

**Treatment and Measurement System**

In this work, SRT was executed using a frequency-doubled
Nd:YLF laser (SRT Vario; Medical Laser Center Lübeck, Lübeck,
Germany).35 Treatment parameters included a pulse width of
250 ns and a pulse repetition rate of 100 Hz for trains of 30
pulses. Pulse energies were varied from 40 μJ to 140 μJ for
patient treatment. The treatment laser radiation was coupled to
the delivery optics using a standard 90-μm fiber with a
numerical aperture of 0.22 and guided onto the retina using
the optical system of a scanning digital ophthalmoscope (SDO)
(Wild Medscop, Wien, Austria), leading to a full-width at half-
maximum treatment spot diameter of approximately 170 μm.

The experimental system for simultaneous OCT data acquisi-
tion during SRT was specifically designed to feature an axial
resolution in the range of a few micrometers so as to facilitate
imaging of small retinal structures while providing an A-scan
rate suitable for in vivo measurements. The system featured a
line scan frequency of 70 kHz and a spectral bandwidth of 170
nm centered at 830 nm (EBS8C10; Exalos AG, Schlieren,
Switzerland), leading to an axial resolution of 1.78 μm in air
and is described in detail elsewhere.29 The system was
combined with the laser treatment system using a dichroic
mirror as shown in Figure 1.

The OCT sample beam was positioned on the retina using
the optical system of the SDO, resulting in a retinal spot size of
approximately 40 μm, centered to the treatment spot. The
integration time of the OCT scans was set to the minimum
value of 14 μs to achieve the highest temporal resolution with
an incident power of the OCT probing beam of 0.7 mW, which
is below the maximum permissible exposure.34

During the application of the treatment laser pulses, time-
resolved M-scans were recorded. Thereto, the OCT and
treatment laser beams were carefully aligned using a RetiEye
ophthalmoscope eye model (Gulden Ophthalmics, Elkins Park,
PA, USA) and optical landmarks. Alignment was verified by
visual inspection of cross-sectional OCT images of RetiEye test
lesions. Slight misalignment of OCT and treatment laser were
considered acceptable because of the significantly smaller OCT
spot size compared with the lesion diameter and the top-hat-
shaped energy profile of the treatment beam. Experiments also
showed that the A-scan measurement position within the treatment
spot to be of no detectable influence on obtained data. The
recorded OCT M-scans underwent standard OCT postproces-
sing, including remapping, dispersion correction, and mapping
to an intensity false color map.

To detect the single SRT pulses of 250 ns pulse width,
which are much shorter than the minimal OCT integration
time, a standard photodetector was placed within the SDO and
sampled with 1 MS/s leading to a temporal resolution of 1 μs.
The photodetector was synchronized with the OCT line trigger
and processing software allowed an overlay of the two signals
to detect the start and stop of the laser application.

**Patient Treatment**

Ethics approval (KEK-Nr. 003/10, Swissmedic Clearance 2011-
MD-0006) to conduct this study was obtained from the local
ethics committee, which is working in accordance with ICH-GCP
guidelines. Patients were chosen and informed about the study
according to the approved study protocol and patient consent
was obtained beforehand. Before laser application, patient pupils
were dilated using tropicamide 0.5% and phenylephrine HCl
2.5%. Two patients suffering from DME, one patient with
chorioretinopathy centralis serosa, and one patient suffering
from branch vein occlusion were included in this pilot study.
The position of the laser lesions was chosen using the built-in beam deflection mirror, operated manually by the ophthalmologist. The OCT system was adjusted using a live cross-section feedback with a reduced frame rate of 4 Hz and by manual alignment of the reference arm position. The OCT software was manually switched to 70 kHz acquisition mode on feedback from the ophthalmologist. According to the clinical study protocol, first lesions were positioned at the retinal periphery outside the vessel arcs with pulse energy starting at 40 μJ. Pulse energy was increased in steps of 20 μJ until slight whitening of the inner retina became visible. Spots at treatment positions were subsequently set with a pulse energy 20 to 40 μJ below the determined visibility threshold. After therapy, the patients underwent fluorescein fundus angiography (FFA) and fundus photography as well as conventional volumetric OCT imaging using a commercial OCT system (Spectralis HRA; Heidelberg Engineering, Heidelberg, Germany).

**Figure 1.** Schematic setup of the OCT system combined with the SRT setup. The OCT setup includes light source, fiber coupler, dichroic mirror, and spectrometer. The therapy system includes the treatment laser source and the scanning digital ophthalmoscope (SDO). SLED, superluminescent light-emitting diode; ND, neutral density filter. Reprinted with permission from Steiner P, Enzmann V, Meier C, Považay B, Kowal JH. Retinal laser lesion visibility in simultaneous ultra-high axial resolution optical coherence tomography. *IEEE Photonics J*. 2014;6:1–11. Copyright 2014 IEEE.

**Figure 2.** Fluorescein fundus angiography (a) and fundus photography (b) of SRT lesions set with 80 μJ/pulse. (c, d) Conventional OCT B-scans of the lesions indicated with yellow arrows, acquired within 1 hour after SRT. Optical coherence tomography B-scans were taken at the position indicated by the dotted yellow lines in (a). No persisting optical or structural retinal tissue changes can be detected in the acquired B-scans.
RESULTS

Clinical SRT

For the clinical trials, a total of 15 OCT M-scans from selective retina treatment with pulse energies of 60 µJ to 130 µJ at both the center and the periphery of four patients’ eyes was recorded. All captured lesions were applied with pulse energies below the empirically determined individual ophthalmoscopic visibility threshold and remained biomicroscopically invisible. Twelve OCT M-scans included data from successful therapy causing RPE damage as proved by FFA leakage while the remaining three scans covered treatment with laser energies below the therapy threshold. In OCT cross-sections...
recorded using a Heidelberg Spectralis HRA OCT system within 1 hour after therapy, all lesions remained invisible even if FFA indicated RPE damage. Figure 2 shows two examples of OCT B-scans of SRT lesions applied with 80 µJ recorded at the position indicated by the yellow dotted lines. Although FFA clearly confirms RPE damage, the lesions remain invisible in fundus photography and conventional cross-sectional OCT. In the OCT M-scans, large axial scale motion artifacts caused by relative movement between the patient and the treatment device were corrected offline by registration of the single A-scans to a reference scan position by pointwise shifting of the A-scan and maximizing the cross-correlation function. Lateral movement could not be corrected as no eye-tracking system was available for the presented measurement setup.

Figure 3 shows an example of an OCT M-scan from successful SRT with 120 µJ pulse energy. For the recorded dataset, leakage visible in FFA confirmed the RPE lesion, whereas in visual inspection of fundus photography the treatment spot remained invisible. Figure 4 shows an example of an OCT M-scan from a treatment position where the pulse energy of 80 µJ was not sufficient to cause a retinal lesion. In the corresponding OCT data, no significant optical changes at the time of laser application could be found and FFA confirmed the absence of RPE defects.

Figure 5 illustrates the variation in the extent of optical signal changes depending on the applied energy on a patient treated for chorioretinopathy centralis serosa. Figure 5 shows OCT M-scans of three test lesions applied at the periphery of the fundus.
Time-Resolved Ultra–High Resolution OCT During SRT

**TABLE.** Tabulated Data for the Acquired Datasets From Four Patients Undergoing SRT

<table>
<thead>
<tr>
<th>Pulse Energy, μJ</th>
<th>Detectable OCT Effects</th>
<th>FFA positive</th>
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<tbody>
<tr>
<td>65</td>
<td>No</td>
<td>No</td>
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<tr>
<td>65</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>75</td>
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<td>81</td>
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<td>84</td>
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<tr>
<td>125</td>
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</tr>
</tbody>
</table>

Optical coherence tomography and FFA visibility were evaluated by two independent observers. Optical coherence tomography visibility was assessed positive if at least signal changes at the RPE were visible in the OCT M-scans. False-positive and false-negative datasets are bolded.

In the clinical data, SRT success was indicated by the RPE damage to increase with pulse energy, whereas the color fundus photography confirms that all lesions remained ophthalmoscopically invisible. The detectable changes in OCT signal pattern appear to scale with the applied pulse energy and remain strictly limited to the RPE/Bruch’s membrane complex for low pulse energies.

For the data acquired during the clinical study, the FFA and the OCT showed close agreement when assessing the presence of RPE damage except for one false-positive and one false-negative dataset. The Table shows the tabulated data for all acquired scans with highlighted false-positive and false-negative datasets.

**DISCUSSION**

Selective retina therapy is a method targeting solely the RPE cells without affecting the connecting photoreceptors and the adjacent choroidea. Owing to these minimal effects, the cell damage remains invisible in white light examination.

In this article, we present for the first time real-time, time-resolved ultra–high resolution OCT scans with 1.78 μm axial resolution during SRT to uncover SRT effects on the retinal tissue. OCT M-scans provided a time series of 70 KHz A-scans from a central 40-μm area of a 170-μm treatment spot were investigated. We show that tissue effects secondary to SRT were detectable as small-scale optical changes for every individual SRT pulse.

All examined lesions remained invisible for visual inspection, whereas OCT data showed detectable optical effects in the treated tissue only in the case of FFA visibility except for one data sample. Optical coherence tomography data furthermore showed the amount of detectable signal changes to qualitatively scale with the applied treatment energy. Although algorithms to quantitatively measure and describe the observed signal pattern changes are currently not available, such a method may provide the means to confirm or to grade RPE damage in the absence of FFA imaging. A detailed description of the origin of the OCT signal variations is thus of crucial value for future algorithmic approaches.

In this work, we found FFA positive effects and corresponding OCT effects already with pulse energies of 60 μJ, which is a radiant exposure of 264 mJ/cm². Using the same pulse duration and a retinal spot diameter of 200 nm, Framme et al. found median effective dose damage thresholds of 99.6 μJ corresponding to a radiant exposure of 320 mJ/cm² when analyzing 122 lesions.

Close examination of the detected signal changes revealed contrast loss in OCT imaging coincident and directly after laser pulse application. Optical coherence tomography cross-sections after SRT did not confirm large-scale retinal tissue damage, a finding that is supported by the reversibility of the observed changes in time-resolved OCT. It is thus reasonable to assume, that the detected signal changes do not represent macroscopic inner retinal tissue damage but originate from SRT-induced and OCT-specific phenomena.

The question arises about the origin of the observed reflective changes and contrast loss after individual SRT pulses. When irradiating the fundus with green light, most of the energy is absorbed by the melanosomes of the RPE cells, which consequently leads to tissue heating and thermoelastic expansion. This effect was thoroughly investigated and can be used to guide conventional photocoagulation by detecting the emitted pressure waves at the cornea. However, with respect to SRT, the expected tissue expansion in the nm-range is too small to be directly detectable even by very high-resolution OCT. Additionally, such an expansion should be proportional to the pulse energy and reversible. Thus, the detected signals should not show a threshold behavior as it is observed.

Another explanation may include microbubble formation as in SRT, RPE damage has been proven to be caused by microvaporization at the individual melanosomes within the RPE cells. The microbubbles with microseconds lifetime range transiently increase the cell volume, eventually leading to mechanical cell disruption, as the cell membrane tolerates overstretching of only a few percent. This photodisruption leads to an immediate cell collapse and diffusion of cellular
fluid to the environment. This collapse significantly reduces the cells’ thickness. We can thus assume a thickness reduction of the RPE monolayer of several micrometers, which is possibly detectable with this high-resolution OCT. The fact that reflective changes were detected in vivo only after FFA, proving the RPE damage, strongly supports this hypothesis.

The presented hypothesis, however, raises further questions, such as why later SRT pulses cause the same effects when already a previous one had led to cell collapse. It has been shown that microvaporization dynamics show a chaotic behavior in SRT. For every applied pulse, the nucleation occurs slightly differently. In consequence, the microbubble nucleation, growing and coalescing, shows a different behavior for each pulse. It is self-evident that the microbubbles also move and rearrange the melanosomes as the main light-scattering particles at the RPE, which leads to a slightly different scatter and reflection pattern after each pulse. Using high pulse energies, the large coalescing bubbles most likely also cause an upward shift of the whole retina of a few micrometers.

The motion-sensitive sampling range of the current OCT-spectrometer allows following local linear thermal expansion in the range of micrometers per second up to multiple meters per second, which does cover the expected changes, but does not cover the direct visualization of acoustic shockwaves. Henceforth, the M-scans of the presented system can provide information on the expansion process, but need sophisticated analysis to discriminate the linear motion from the overcasting vibration. The latter is most probably the cause of the detected signal contrast loss at stronger stimulation.

The axial stripe, which can be seen expanding over the whole neural retina, may thus be caused by rapid phase changes caused by microbubble formation and short time shifts of retinal layers due to local heating and acoustic shockwaves as also detected by acoustic measurements. Optical coherence tomography as a phase-resolving technique is sensitive to such high-frequency variations of the scattering structure. Sufficiently fast shifts can lead to large-scale phase changes that result in fringe washout where the spectral modulations fluctuate faster than the OCT spectrometer integration time. Subsequently, the phase contrast, which is associated with the spatial information, is reduced or lost, leading first to attenuation and to seemingly dark stripes. The regime of signal reduction might even unveil a possibility to

**FIGURE 6.** Scatter plot of the acquired SRT data showing the expected distribution (a) and the data for each patient individually (b–e). Optical coherence tomography M-scans were classified according to two observers by the visibility of detectable effects in the RPE and inner retina.
detect changes that cannot be picked up by solely acoustic therapy monitoring.

To fully monitor these opto-acoustic shockwaves detection delays beyond 0.25 \( \mu s \) would be needed that possibly can be achieved by ultra-high speed OCT-systems, shorter exposure times, via synchronous pulsed illumination, or by time-gated sampling of the individual treatment laser pulses. These promising modifications are under investigation, but are currently out of the scope of this work.

For OCT-based SRT feedback, it is obvious, that the parameters of the OCT measurement system play a crucial role in the way in which the RPE tissue damage is represented in the acquired signal. For microbubble formation with a lifetime of only up to 5 \( \mu s \),\(^{14}\) it appears consequent to assume that OCT imaging would benefit from a higher acquisition frame rate. Although the OCT system used for this work featured ultra-high axial resolution to facilitate the imaging of the thin retinal layers, the temporal resolution of 14 \( \mu s \) may be only barely sufficient to adequately represent the high-frequency changes in tissue scattering. When applying a A-scan rate of several MHz enabled by experimental systems,\(^{36}\) the RPE-related microbubbles may be measured directly. Ultra-fast OCT can thus be used to resolve the microbubbles during their lifetime and might give valuable information about the damage range of SRT. The exact influence of the temporal and axial resolution as well as the center wavelength on the detectable signal changes remains yet unclear and will be the focus of a future study.

CONCLUSIONS

In conclusion, we have shown that selective RPE lesions can be detected with simultaneous time-resolved OCT measurements. Based on the presented data, it appears likely that with the introduction of algorithms extracting quantitative parameters from OCT data, time-resolved M-scans can be used to determine whether RPE lesions were introduced by SRT.

A real-time classification of RPE lesions and thus therapy success would lead to a more reliable and repeatable treatment and help to promote this efficient and cost-effective treatment for retinal diseases. Optical coherence tomography imaging therefore may be used as a dosimetry control either in a standalone configuration or combined with other imaging modalities. As each individual successful SRT pulse provides local changes in OCT, the ultimate goal could be an automatic dosage control making use of a pulse train with stepwise increased pulse energy. As soon as microbubbles are detected, the laser emission can be ceased, as it is proposed\(^{20}\) and already demonstrated with a pure reflectometry device for SRT on rabbits.\(^{21}\)

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