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A Miniature Forward-imaging B-scan Optical Coherence Tomography Probe to Guide Real-time Laser Ablation

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Abstract

Background and Objective—Investigations have shown that pulsed lasers tuned to 6.1 μm in wavelength are capable of ablating ocular and neural tissue with minimal collateral damage. This study investigated whether a miniature B-scan forward-imaging optical coherence tomography (OCT) probe can be combined with the laser to provide real-time visual feedback during laser incisions.

Study Design/Methods and Materials—A miniature 25-gauge B-scan forward-imaging OCT probe was developed and combined with a 250 μm hollow-glass waveguide to permit delivery of 6.1 μm laser energy. A gelatin mixture and both porcine corneal and retinal tissues were simultaneously imaged and lased (6.1 μm , 10 Hz, 0.4–0.7 mJ) through air. The ablation studies were observed and recorded in real time. The crater dimensions were measured using OCT imaging software (Biotigen, Durham, NC). Histological analysis was performed on the ocular tissues.

Results—The combined miniature forward-imaging OCT and mid-infrared laser-delivery probe successfully imaged real-time tissue ablation in gelatin, corneal tissue, and retinal tissue. Application of a constant number of 60 pulses at 0.5 mJ/pulse to the gelatin resulted in a mean crater depth of $123 \pm 15 \mu\text{m}$. For the corneal tissue, there was a significant correlation between the number of pulses used and depth of the lased hole (Pearson correlation coefficient = 0.82; $P = 0.0002$). Histological analysis of the cornea and retina tissues showed discrete holes with minimal thermal damage.

Conclusions—A combined miniature OCT and laser -delivery probe can monitor real-time tissue laser ablation. With additional testing and improvements, this novel instrument has the future possibility of effectively guiding surgeries by simultaneously imaging and ablating tissue.

Keywords

ablation; cornea; eye; gelatin; mid-infrared; ophthalmology; retina; waveguide

INTRODUCTION

Optical coherence tomography (OCT) has emerged as an important medical imaging tool to evaluate structures within the eye, skin, heart, larynx, and teeth amongst other biological tissues before and after surgical ablation [1-9]. Optical coherence tomography imaging of laser ablation has been previously achieved through an external imaging system and through large probes [1-9], but not through a miniature forward-imaging probe 0.5 mm in diameter. Moreover, most studies have used OCT to evaluate the tissue after completion of the laser ablation and not continuously during the actual lasing process.

Real-time monitoring of an incising laser would be useful in retinal surgeries. Recurrent retinal detachments may lead to anterior proliferative vitreoretinopathy with subsequent shortening of the retina as glial remodeling occurs. Surgeons may resort to a procedure, known as a retinectomy, to incise the shortened and stiff scarred retina. This procedure achieves reattachment of the retina in those difficult cases. However, retinectomies are associated with complications and high failure rates [10-17]. This procedure is technically challenging with the instrumentation currently available. It is performed in severe anterior proliferative vitreoretinopathy associated with non-traumatic rhegmatogenous retinal detachments [10-12, 14-16], and tractional retinal detachments from diabetes mellitus [11, 14-15]. More recently, retinectomy is performed during subsequent retinal detachment repair in eyes which were ruptured by improvised explosive devices (IED) and other blasts [13, 17]. Complete incision of the retinal layer in the retinectomy procedure is continuously desired for up to 360 degrees without damaging underlying structures. This led to the search for an intraocular surgical instrument, which would bypass possible interface distortions caused by the ocular tissue structures of cornea, aqueous humor, and lens, to provide real-time z-directional guidance of the depth of the incision.

A novel intraocular surgical instrument combining an incising laser with special wavelengths, such as mid-infrared 6.1 μm , with real-time OCT-monitoring of the ablation depth is technically feasible. This novel miniature forward-imaging device will challenge current surgical paradigms to improve retinectomy outcomes in eyes with complex detachments as well as improve surgical efficiency. Successful real-time monitoring of laser incisions can be adapted to other procedures in ophthalmology as well as in other specialties. Such guidance may be valuable in the development of future robotic surgical techniques.

METHODS AND MATERIALS

Gelatin and Tissue Preparation

The gelatin was prepared by mixing a solution of 10% gelatin by weight with boiling distilled water and coffee creamer (Coffee-Mate, approximately 2% by weight) and poured into Petri dishes with refrigeration overnight. A Petri dish was then mounted onto a stage that permitted translational movement.

Porcine cadaver eyes were dissected from the orbits and used within 24 hours. All animal procedures were performed in accordance with Association for Research in Vision and Ophthalmology (ARVO) and the National Institutes of Health guidelines. The cornea preparation involved removing the corneal epithelium and mounting the eye on a piece of Styrofoam. The retinal preparation involved removing cornea, lens, and vitreous. The remaining globe was bisected in half through the optic nerve head. Each half was then divided into 2 petals to enable the eye to lie flat on wet gauze within a Petri dish, thus exposing the retina for laser ablation.

Miniature B-scan OCT Probe

A miniature 25-gauge forward-imaging OCT intraocular probe was developed with a 0.35 mm gradient-index (GRIN) image lens within the tip of a 25-gauge stainless steel tube and a forward-imaging scanner within the handpiece [18, 19]. The hand-held probe was synchronized to an 870 nm spectral-domain optical coherence tomography (SDOCT) system (Bioptigen, Inc. Durham, NC). The scanning range was 2 mm with the probe tip positioned 3 to 4 mm from the tissue surface. Axial resolution was 4-6 μm and lateral resolution was 25-35 μm [19].

Mid-infrared Laser

The light source used for ablation in this study was a prototype Raman-shifted alexandrite (RSA) laser system (Light Age, Inc., Somerset, NJ) [20]. The system used a tunable pulsed alexandrite laser with a 10 Hz repetition rate that was frequency-converted into the mid-IR using stimulated Raman-shifting in pure gases. It passed first through deuterium and then through hydrogen. The output pulses were tunable from ~6.0 to 6.5 μm , (full width at half maximum [FWHM] ~15 nm), had a pulse duration of ~25 ns, and had pulse energies from 1 to 3 mJ, depending on wavelength. For this work, the laser was tuned to 6.1 μm and the output beam was coupled into a 250 μm internal diameter hollow-glass waveguide [21], using a CaF_2 focusing lens (focal length = 200 mm). The coupling efficiency was about 50%, and the overall transmission was 20-30% of the input pulse energy.

Combined OCT-Laser Probe

The miniature B-scanning OCT probe was combined with the mid-infrared laser waveguide into a single co-planar intraocular instrument (Fig. 1A – 1D). A plate (Fig. 1B, white arrow) with two openings to accommodate the OCT cannula (Fig. 1B, red arrow) and the laser cannula (Fig. 1B, blue arrow) was attached to the basic miniature OCT probe with 4 set screws. The waveguide with an inner diameter of 250 μm consisted of a glass tube with an outer plastic coating and an inner AgI film on top of an electrolysis plated Ag film [21]. The waveguide was positioned within the 25-gauge cannula that was aligned adjacent to the 25-gauge OCT scanning cannula (Figs. 1C-1F). A CaF_2 window polished to a calculated 15.2°, per the equation below, was sealed within the laser 25-gauge cannula tip to permit waterproofing of the hollow waveguide, and transmission of the 6.1 μm energy to overlap with the OCT imaging beam in a plane at a distance of 4 mm from the tip.

$$\begin{aligned} \tan \theta'' &= ((\text{Hollow waveguide OD} + 25 \text{ gauge tube OD})/2)/4 = 0.425/4 = 0.14, \text{ so } \theta'' = 6^\circ. \\ \text{Because: } \theta + \alpha + \theta'' &= 90^\circ, \text{ and } \theta' + \alpha = 90^\circ, \text{ then } \theta + \theta'' = \theta' \text{ and } \theta'' = \theta' - \theta = 6^\circ. \\ \text{Because: } \sin \theta' &= n \sin \theta = 1.38 \times \sin \theta, \text{ (} n = 1.38 \text{ as the refractive index of } \text{CaF}_2 \text{ at } 6.1 \mu\text{m}), \\ \text{therefore: } \theta' &= 6^\circ + \theta, \text{ and } \sin \theta' = \sin (6^\circ + \theta) = \sin 6^\circ \times \cos \theta + \cos 6^\circ \times \sin \theta \\ &= 1.38 \times \sin \theta, \text{ and therefore: } \sin 6^\circ = 1.38 \times \tan \theta - \cos 6^\circ \times \tan \theta \text{ and } (1.38 - \cos 6^\circ) \times \tan \theta = \sin 6^\circ. \\ \text{Because } \tan \theta &= \sin 6^\circ / (1.38 - \cos 6^\circ) = 0.2716, \text{ then } \theta = 15.2^\circ. \end{aligned}$$

The laser energy passed through this 15.2° CaF_2 window at the probe tip and then through air to form a 200 μm diameter spot on the gelatin or tissue (Fig. 1C). The tips were tested to ensure they were co-planar at 4 mm by using infrared viewing paper (VRC5, Thorlabs, NJ) (Fig. 1D). A characteristic of the hollow waveguide is that the laser beam will maintain its spot size over several centimeters [21]. For ablating the samples, the individual laser pulse energies varied from 0.2 – 1.1 mJ/pulse, with an average pulse energy of 0.7 mJ/pulse (Model JD500, Molelectron Detector, Inc., Portland, OR) at a repetition rate of 10 Hz. The tip was positioned 4 mm above the samples. Holes, and lines several centimeters in length, were incised in the gelatin and tissues. A predetermined number of laser pulses was delivered through the waveguide using a computer-controlled shutter for the gelatin and corneal studies since there was no underlying layer to avoid ablating. The real-time image was

monitored during lasing of the retina to avoid ablating the choroid. The feedback for the decision to stop lasing the retina was the presence in the real-time image of increased scattering (density) reaching the retinal pigment epithelium. In addition, when a retinectomy would be performed to repair a tractional retinal detachment with proliferative vitreoretinopathy, there would be an additional protective fluid buffer between the detached retina and the choroid.

Imaging

Real-time imaging was performed with the Biotigen Spectral Domain Ophthalmic Imaging System (840 nm) to visualize lasing of gelatin, cornea, and retina. Imaging was recorded for the duration of tissue ablation and analysis was performed on the depth of the final individual craters. Using InVivoVue Clinic (Biotigen, Durham, NC), markers were placed on the images to measure the depth of incision for each specimen (Figs. 2F, 3E, and 5F).

Histology and Immunohistochemistry

Representative corneas and retinas were placed in Pen-Fix (Richard-Allan Scientific, Kalamazoo, MI) overnight. The samples were dehydrated in serial alcohols, embedded in paraffin, and sectioned at 5 – 10 μm . Serial sections of the ablated craters were deparaffinized and stained with hematoxylin and eosin (H&E). These sections were qualitatively examined and compared to the Biotigen images.

Statistical Analysis

The correlation coefficient between the number of pulses and ablation depth for cornea was calculated and tested using Spearman's rank-order correlation method. The analysis was conducted using R software version 2.15 (R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>).

RESULTS

This study demonstrated that an OCT probe and laser hollow waveguide could be combined into a small 20-gauge (0.9 mm diameter) co-planar device capable of simultaneously delivering laser energy and imaging in real time. With the laser probe CaF_2 window set at 15.2° , a co-planar spot 4 mm from the tip was produced and confirmed with an infrared viewing card (VRC5, Thorlabs, NJ; Fig. 1D). The OCT/laser probe can be handheld to direct the laser energy to the area of interest. The 4 mm distance was found not to be absolutely critical, since the laser spot size remains constant over a relatively large distance [21], and OCT imaging works over a range of distances as demonstrated during tissue ablation. The combined probe functioned as long as the surface being ablated was within the scanned volume approximately 3 to 5 mm from the probe tip.

The ablation experiments showed that the combined miniature B-scan forward-imaging OCT and hollow-glass waveguide probe was capable of delivering pulsed mid-infrared energy from a prototype table-top Raman-shifted alexandrite laser system. Material from the plume only gradually degraded the image quality after multiple holes or retinal lines with image quality restored following a surgical lint-free wipe of the tip. It is likely that material on the window (both water and tissue) was ablated by successive pulses, which tended to 'self-clean' the window. We were able to control the number of pulses emitted to correlate with real-time imaging of the ablation in both gelatin and in ocular tissues.

The gelatin experiments demonstrated that cross-sectional ablation with crater formation could be observed and monitored over time in a gel of uniform consistency. As the selected

frames from a gelatin ablation demonstrate in Figs. 2A-2F, the crater becomes deeper over time. The laser (6.1 μm , 10 Hz, 0.45-0.6 mJ from the probe) produced consistent appearing craters with 60 pulses (Fig. 2G). By measuring the craters with the OCT software (InVivoVue Clinic), the data were determined to be normally distributed with the Shapiro-Wilk normality test ($W = 0.9404$, P value = 0.2946). A mean depth of $122.6 \pm 15 \mu\text{m}$ (CI 95%: 116 – 129 μm) was measured (Fig. 2H).

The corneal experiments showed that the laser combined with the OCT probe was capable of visualizing the corneal stromal tissue ablation. Epithelium was mechanically removed prior to the ablation to provide a uniform tissue structure. The laser (6.1 μm , 10 Hz, 0.45-0.6 mJ from the probe) produced corneal craters with a consistent external appearance within the 4 groups of 30, 60, 90, and 120 pulses. As the selected frames from a cornea ablation video demonstrate in Figs. 3A-3E, the crater became deeper with additional laser pulses. Histology (Fig. 3F) showed ablation craters similar in appearance to those visualized with the real-time imaging. The amount of thermal damage on the edges of the incision was $< 15 \mu\text{m}$. This was similar to the thermal damage previously described in corneal ablation with this laser [20]. There was a significant correlation between the number of pulses and the measured depth of the craters from the OCT images for each cornea (Pearson correlation coefficient = 0.82; $P = 0.0002$) (Fig. 4). However, there was significant variability among the different cadaver corneas. Hydration of the cornea, which has a great effect upon the ablation rate, can occur rapidly after enucleation. However, as the number of pulses increased, real-time monitoring of the increasing depth of the craters was successfully accomplished in spite of the variability among laser pulses and corneal hydration.

Retinal experiments showed that the laser combined with the OCT probe was able to consistently ablate the retinal tissue with minimal damage to the surrounding tissue. The laser (6.1 μm , 10 Hz, 0.45-0.6 mJ from the probe) produced holes with the cross-sectional ablation easily imaged in real time as demonstrated by selected images (Figs. 5A-5F) from a retinal ablation video. An *en face* photo of a retinal incisional line is shown in Fig. 5G. Histological examples (Figs. 5H, 5I) of the laser's effect upon the attached porcine retinas are illustrated. The partial-thickness retinal incision in the histological section (Fig. 5H) correlated with the OCT partial-thickness ablation images (Figs. 5E, 5F). Thermal damage was measured to be less than 10 μm . This was considerably less than the thermal damage delivered by the standard intraocular argon or diode lasers [22]. The laser ablation can be halted prior to incision of the underlying choroid (Fig. 5I).

DISCUSSION

A miniature forward-imaging B-scan OCT probe was combined with a mid-infrared Raman-shifted alexandrite laser probe to permit co-planar imaging of ablation in real time. The size of the handheld combined probe tip was compatible for use with 20-gauge vitrectomy ports. If photocoagulation was desired rather than incising, a 60 μm diameter multi-mode laser fiber for delivery of visible radiation inserted into a 36-gauge -thin-wall cannula (0.102 mm outer diameter, 0.076 mm inner diameter) could be substituted in the future for a combined 0.61 mm diameter probe to permit access through the recently popular vitrectomy ports permitting 23-gauge (0.635 mm) instruments. Alternatively, a 125 μm diameter multi-mode laser fiber could be directly attached to the 25-gauge cannula for the greatest total diameter of 0.635 mm permitting access through the 23-gauge ports. The imaging distance of 3-5 mm from the tissue was appropriate for retinal procedures with the laser beam co-planar at 4 mm distance from the tissue. The desired imaging distance also can be altered by changing the thickness of the GRIN imaging lens. After further improvements and testing, this miniature forward-imaging OCT probe may have the potential for guiding laser surgery. Previous studies have shown that real-time OCT imaging of laser ablation is possible through an

external imaging system and through large probes [1-9], but not with a 0.5 mm diameter hand-held probe.

We were able to use the gelatin to observe the precision of the laser ablation with real-time imaging of the ablation with a preset number of pulses. Subsequent measurement of crater depth demonstrated slight variations, which could be attributed to: 1) small variations in the distance in which the probe is positioned above the gelatin surface and 2) the energy fluctuation of individual pulses from the Raman-shifted alexandrite laser. A handheld probe would have slight variations in positioning due to human estimation of positioning as well as slight hand tremors. Therefore, real-time imaging of the ablation would improve precision by adding the visible cue to halt ablation at the desired level. Improvement of the laser's pulse energy consistency also would be expected to increase crater depth uniformity.

The ability to image both corneal and retinal ablation demonstrated that the miniature hand-held probe could be utilized for different types of biological tissues. Several groups, including ours, have identified a wavelength at 6.1 μm as capable of ablating tissue with a minimal amount of collateral damage [23-30]. This is desirable for precise incisions of tissue. This laser wavelength is at a water-absorption peak and is coincident with the Amide I protein peak in the collagen spectrum. Tissues that have been treated with this wavelength include articular cartilage, fibro-cartilage, skin, cornea, and optic nerve sheath [23-30]. Previous studies with the RSA laser have indicated that the pulse energies currently available for this work are near the threshold for ablation of corneal tissue so a large variation in the resulting ablation rate was expected [20]. However, ablation of the corneal samples at 6.1 μm in the current study with the combined probe produced increasing crater depths as the preset number of pulses increased. The penetration of the ablation was observed as it occurred with the real-time OCT imaging. The crater sizes could then be measured from the final images to quantitate the correlation between pulse number and crater depth. Rather than using pre-set pulses, ablation of the retinal samples was observed in real time as the layers within the retina were incised. The visible feedback to stop the laser ablating the retina was the presence in the real-time image of increased scattering (density) reaching the retinal pigment epithelium. After full-thickness ablation of the retinal layer, the sides of the incision were observed to usually collapse together in this pliable tissue, making quantification of the final crater depth in complete holes impossible. Depths could be measured in several partial-thickness incisions. From the histological analysis, the minimal thermal damage from the RSA laser 6.1 μm energy in both corneal and retinal tissues suggested that the combined imaging and laser hand-held miniature probe has promise for guiding laser surgery, even when ablation rates are variable due to low or fluctuating pulse energies.

Successful real-time monitoring of laser incisions would be valuable for surgical procedures in ophthalmology as well as in other specialties. Boppart, *et al.*, described the feasibility of using OCT to monitor laser ablation in brain, liver, kidney, and muscle tissues in 1999 [31]. Currently, OCT and laser ablation are used at least experimentally in several specialties [1-9, 32-41]. In ophthalmology, OCT has been used clinically for imaging before and immediately following several procedures. Accurate measurement of corneal thickness is extremely important in laser refractive procedures such as laser *in situ* keratomileusis (LASIK). Anterior segment OCT has been utilized to provide these measurements in preoperative surgical planning to improve refractive outcomes [7]. Besides being a tool to evaluate a patient peri-operatively, OCT has been incorporated in several femtosecond laser cataract surgery systems with the possibility to increase surgical precision [8, 38]. Preoperative OCT has guided focal laser photocoagulation selection of microaneurysms in diabetic macular edema with changes in the microaneurysms observed by OCT at intervals following successful treatment [34,39]. Optical coherence tomography has also been used in

the operating room to monitor pre- and post-diode laser ablation of retinal vascular lesions in Coats' disease [41]. A microscope-mounted OCT has also been used in the operating room to evaluate the retina in patients before and after macular surgeries such as macular hole repair and retinal detachment repair [42,43]. The experimental microscope-mounted vitreoretinal OCT systems have difficulty efficiently locating and tracking the intraocular surgical instruments within the vitreous cavity for real-time imaging and surgical manipulations [44]. Also, when external OCT infrared light contacts a metallic intraocular instrument, it casts an absolute shadow upon the underlying tissues of interest [44]. This would not be an issue with a miniature handheld intraocular OCT probe that can either point at the desired area to image at an angle to the instrument [18,19], or be potentially combined directly with various intraocular instruments such as a combined OCT/laser probe to treat the desired area with real-time imaging of the surgical manipulation. The combined miniature forward-imaging OCT and mid-infrared laser-delivery probe was developed for vitreoretinal surgery rather than for lens or corneal anterior segment surgery which is accessible for direct ablation by the femtosecond laser. Besides through air or gas media, the combined OCT/laser probe can image and lase through perfluorocarbon [45].

Perfluorocarbon is used clinically during vitrectomy surgery. If the probe is immersed in a liquid such as perfluorocarbon, the laser beam will still be coplanar with the OCT scanning surface. The laser ablation spot will still overlap with the OCT scanning line, but will be shifted slightly to one side compared to the overlap in air due to the difference in index of refraction. For instance, recurrent retinal detachments, such as from trauma or diabetes mellitus, may lead to severe scarring with subsequent shortening of the retina. A complete incision of the retinal layer, without damage to underlying structures, in the retinectomy procedure is desired for up to 360 degrees to permit retinal reattachment [10-17]. An intraocular OCT probe would permit real-time imaging of the tissues to identify the structures that need incision. When combined with an incising laser probe, real-time imaging of the process could occur to ensure completion without damaging underlying structures.

Other specialties have also embraced OCT-monitored procedures. Early pathologies within biopsies or *in situ* tissues of the GI tract, liver, bladder, and cervix that requires intervention, have been successfully diagnosed with OCT [32,33,36,40]. Additionally, OCT has the potential of guiding biopsies of targeted early pathological lesions [40]. In dermatology, *in vivo* OCT has been proposed for evaluation of the excisional margins obtained during Mohs surgery [9]. Optical coherence tomography has several applications in gastroenterology including imaging of dysplastic epithelium in the esophagus, stomach, and intestines. Tsai, *et al.* investigated OCT-guided radiofrequency ablation treatment for Barrett's esophagus [6]. Otolaryngology has used OCT for assessing architectural changes in the vocal cords [1] and in the trachea [5] following laser therapy. Cardiology has embraced OCT for imaging ablation of cardiac tissues to treat cardiac arrhythmias, open in-stent restenosis, and permit revascularization of chronic occlusions [4, 33]. In addition, OCT has proven effective in image-guided near-infrared laser ablation of caries in dentistry [2-3]. Finally, a 2.7 mm diameter OCT probe was used to confirm successful experimental non-incisional vasectomy performed by thermal coagulation with an Ytterbium fiber laser at a wavelength of 1075 nm [37].

Therefore, it is clear that OCT has a wide variety of biological applications and will continue to expand its utilization with laser surgery in the future. However, despite the success of OCT imaging, most current techniques only utilize OCT imaging before and after laser ablation for evaluation. As such, having a combined forward-imaging OCT with a laser probe would allow imaging in real-time during ablation. This would permit more precise and accurate surgical ablation leading to better outcomes for patients and surgical efficiency.

Moreover, the system may enable novel minimally invasive approaches to surgery not previously possible to reduce surgical morbidity and mortality.

Real-time imaging of laser ablation with a miniature combined OCT-laser probe was technically achievable and permitted reproducible and consistent results, even with marginal laser pulse energy and stability. Future studies would include introducing the probe through the pars plana to the vitreous cavity of an anesthetized animal model, and investigating other organs that would be compatible with this system. Tissues located on the surface of small cavities or in difficult to reach locations may be amenable to real-time OCT-guided laser ablation therapy. Additionally, enhancing the probe with robotic capabilities may add additional capabilities to robotic surgery.

CONCLUSIONS

These results demonstrate the capability of the 25-gauge forward-imaging OCT probe to display real-time mid-IR laser ablation in gelatin and ocular tissues. The real-time imaging correlates well with the delivery of laser pulses. Histological sections of the tissues showed minimal tissue damage at 6.1 μm . With additional testing and improvements, a combined laser and surgical intraocular OCT probe has the future potential to efficiently and effectively guide ocular surgery with delivery of laser energy.

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Fig.1.

A: Photograph of the entire combined miniature B-scan OCT and laser intraocular probe. **B:** Photograph of the combined OCT (red arrow) and laser (blue arrow) probe tip with the plate (white arrow) attaching the 2 cannulas. **C:** Diagram of the polished CaF_2 prism placed in front of the hollow waveguide to permit co-planar ablation and imaging. **D:** Diagram of the combined OCT and laser probe tip showing the scanning optical fiber encased within a 25xx-gauge stainless steel tube with a 0.35 mm GRIN imaging lens within the OCT probe. The Raman-shifted alexandrite mid-infrared laser was introduced into a 250 μm hollow-glass waveguide encased within a 25xx-gauge stainless steel tube with a 15.2° CaF_2 window. The miniature OCT probe and the laser were combined into a single co-planar device as confirmed with infrared paper and a power meter. The combined probe tip was held 4 mm from the intended tissue during ablation.

Fig. 2.

A: An OCT probe image of gelatin before ablation. **B-E:** Selected OCT images from a real-time video illustrating the cross-sectional gelatin crater formation as the laser ablates. **F:** Measurement of the crater depth was performed with BiopTigen software. **G:** An *en face* view of the craters made by the combined miniature OCT and laser probe in gelatin. **H:** The box plot shows the distribution of the crater sizes with an $n = 18$ and a range between 97 and 143 μm in depth.

Fig. 3.

A: An OCT probe image of cornea before ablation. **B-E:** Selected OCT images from a real-time video illustrating the cross-sectional crater formation in the cornea as the laser ablates. **E:** Measurement of the crater depth was performed with BiopTigen software. **F:** Histology showing a crater made by the combined miniature OCT and laser probe in cornea with minimal thermal damage.

Fig. 4.

A graph comparing a predetermined number of pulses emitted versus depth of the corneal craters. Despite a fluctuation of energy output, there was a significant correlation between pulse number and depth of the lased hole (Pearson correlation coefficient = 0.82; $P = 0.0002$).

Fig. 5.

A: An OCT probe image of retina before ablation. The layers of the retina are above the asterisk (*) which is placed at the level of the retinal pigment epithelium. **B-E:** OCT images from a real-time video illustrating the cross-sectional crater formation in the retina as the laser ablates. The folded area is the photoreceptor layer portion of the retina. The layers of the retina are above the asterisk (*) which is placed at the level of the retinal pigment epithelium. **F:** Measurement of the crater depth was performed with BiopTigen software. The layers of the retina are above the asterisk (*) which is placed at the level of the retinal pigment epithelium. **G:** An *en face* view of retinal ablation. **H.** Histology showing a crater corresponding to the OCT image in (E). The folded area is the photoreceptor layer portion of

the retina. **I:** Histology showing a crater in retina without ablation of the underlying retinal pigment epithelium and choroid.