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Laser Spot Size as a Function of Tissue Depth and Laser Wavelength in Human Sclera

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ABSTRACT

We determined the wavelength dependence of the minimum spot size of a laser beam focused through human sclera to evaluate the potential for transcleral glaucoma surgical techniques using ultrashort-pulsed lasers. The spectrum of the forward scattered light was measured by collimating the incident and transmitted beam in a spectrophotometer. This spectrum shows that sclera is highly scattering until 1100 nm, after which, the transmission spectrum is similar to water. To measure the minimal spot size, a laser beam was focused on the back surface of sclera of differing thickness. The minimum spot at 800 nm, 1060 nm, 1301 nm, and 1557 nm was imaged. At 800 nm, the spot size was invariant upon focal lens position, being a thousand fold larger than the incident beam spot size. As the wavelength increased, the area of the spot decreased, so that at 1557 nm, the minimal spot size was on the order of the incident beam spot size.

Keywords: sclera, scattering, spot size, wavelength dependence, surgery, glaucoma

2. INTRODUCTION

Glaucoma affects approximately two million Americans while another ten million are at risk due to statistically significant elevated intraocular pressure (IOP). Medications can lower IOP, although they are only partially effective, with an approximately 40% failure rate over 3 years. Laser and incisional surgical procedures, which increase fluid drainage from the eye, are generally reserved for medication failures. Argon laser trabeculoplasty, the most common laser glaucoma procedure, may stretch the normal channels in the trabecular meshwork, thereby increasing outflow of aqueous humor. The effectiveness of this procedure is short lived however, averaging about two years, after which incisional surgery is often tried. Filtering surgery, the cutting of a drainage channel (fistula) directly into the sclera, is the most common incisional surgery for the treatment of glaucoma. The various techniques all share a host of complications, including very low intraocular pressure (hypotony), cataract formation, and infection. In addition, surgical failure often occurs over the course of months to years due to scarring and closure of the fistula. Despite these problems, glaucoma specialists are moving towards earlier surgical intervention due to its better IOP control in most patients, increasing the need for a safer and more effective surgical alternative.

The majority of post-operative scarring responsible for fistula closure occurs due to wound healing in the tissues overlying the sclera, the conjunctiva and episclera, which are damaged during incisional procedures. A number of laser surgical procedures to improve surgical outcomes have been proposed and tested. These generally fall into two approaches:
1. Ab intemo procedures, which ablate tissue from the internal surface of the sclera, either by directing the light through the cornea with a contact lens or directly via a probe introduced into the anterior chamber of the eye.

2. Ab externo procedures, which ablate tissue from the external surface of the sclera, either by directing the light through the conjunctiva or directly via a probe introduced under the conjunctiva.

Despite these attempts, no laser-based method has delivered enough clinical or economic advantages to displace traditional surgical methods. Ultrashort-pulsed lasers, operating at appropriate wavelengths, may permit unique transcleral procedures that could offer advantages of each of these methods.

Femtosecond laser pulses used for cornea surgery have been demonstrated to cause little collateral damage, both due to the low average power -- no burning -- and to their low pulse energy -- small shock waves and bubble oscillations. In addition, subsurface tissue photodisruption is possible and has been demonstrated in transparent corneal tissue using near-infrared and infrared wavelengths. By using a wavelength that is transmitted by the sclera, and is able to be focused at its back surface, it may be possible to create an ab intemo fistula without the technical problems associated with contact lens or probe delivery methods. Such a method would avoid disruption of the overlying conjunctiva and episclera, something current ab externo techniques cannot offer.

As a first step, this paper examines the minimum spot size attainable as a function of wavelength when the laser is focussed through the sclera itself to its back surface. Our first experiment yielded forward transmission for the wavelengths between 500 nm and 2500 nm using a spectrophotometer to obtain an initial indicator of scattering and minimum spot size. For the second experiment, we imaged the spot on the back surface of the sclera using a vidicon camera with a microscope objective, comparing wavelengths from 800 nm to 1557 nm.

3. METHODS

3.1 Tissue Samples

Human scleral sections (not suitable for transplantation ) from regions adjacent to the cornea were cut to a thickness ranging from 0.30 mm to 0.77 mm using a vibratome. Four different thickness sections from each of three globes were cut for a total of twelve samples. The sample thickness was measured before each experiment using a calipers and all measurements were made within two weeks of globe donation.

3.2 Experiment 1: Spectrophotometer Measurements

The forward scattered light as a function of wavelength was measured using a spectrophotometer (Perkin-Elmer Lambda 9 UV/VIS/NIR Spectrophotometer) by placing the apparatus shown in Fig. 1 inside the sample chamber. The apparatus collimates the incident incoherent broadband light using two irises with a 2.16 mm diameter opening separated by approximately 3.0 cm. The tissue was mounted to a slide (1mm thick) using a drop of saline solution. The forward scattered light was collected using two irises separated by 3 cm with 2.16 mm opening. The tissue was centered between the two sets of irises separated by approximately 2.3 cm.

For each of the twelve samples, three measurements of the absorption spectrum, 500-2500 nm, were taken, then converted to total transmission. This measurement was taken to identify trends in forward scattering to narrow the search for optimal wavelengths to penetrate the sclera.
3.3 Experiment 2: Spot Size Measurements

Figure 2 shows the experimental setup used to measure the minimum spot size. The laser light is incident from the left onto a variable attenuator to prevent camera saturation. The beam then passes through a soft aperture (about 2 m of Corning Flexcor 1060 or Newport F-SV 620 for 800 nm optical fiber) to insure that the beam from each source is fundamental Gaussian mode. The emergent beam was collimated (Newport 1015LD, f = 7.4 mm), then focused using a 6.24 mm focal length lens with a NA of 0.40 (Thor Labs 350110-C). The front surface of a 0.15 mm thick microscope cover slip was placed at the focal spot. This surface corresponds to the back surface of the sclera. The vidicon camera (Electrophysics Model 7290A) was positioned such that the object plane corresponded to the back surface of the tissue while using a 20X 0.40 NA microscope objective. The minimum spot size was recorded as a 640 X 480 24 Bit RGB bitmap.

The tissue was placed on the cover with a drop of saline. The lens position that produced the minimum spot size at the back surface of the sclera was determined by shaking the tissue horizontally in the focal plane and moving the lens until the smallest spot occurred. The Electrophysics Model 7290A camera has a slow response time so that shaking the sample allowed us to average over a portion of the tissue. The shaker was DC motor with an off center circular cam that provided about 1 mm of motion. This image was recorded, then the shaker was stopped and several stationary images of the speckle were taken. The above procedure was preformed using four different lasers: a 800 nm Ti:Sapphire oscillator operating at 100 MHz and 50 fs pulses, a 1060 nm Nd:Glass oscillator at 100 MHz and 300 fs pulses, a 1301 nm continuous wave laser diode, and a Er:Glass fiber laser operating with 100 fs pulses at 100 MHz.
3.4 Image Analysis

The camera images collected were corrected for the nonlinear camera response and normalized such that the peak intensity was the same for all images. The full-area half-maximum (FAHM) sizes, as defined as the area of the spot that is greater than or equal to the maximum pixel intensity, were calculated for each image as a comparison between the different wavelengths. The FAHM is the relevant measure since the area of the spot is directly proportional to the energy for photodisruption.

4. RESULTS

4.1 Forward Scattering of Sclera

Twelve absorption spectra of half thickness sclera (average 0.34 mm) were averaged and are shown in Fig. 3. Very little forward transmission occurs below 1100 nm. The graph shows transmission dips that follow the water dips at 1450 nm and 1900 nm. Several wavelengths of interest are listed in Table 1.

![Transmission Spectrum of Half Thickness Sclera](image_url)

**Figure 3:** The forward transmission of sclera is the jagged curve. The smooth curve is the percent transmission through 0.78 mm of saline solution. See Table 1 for the numbers noted in the graph.
Table 1: Forward transmission of half thickness of sclera.

<table>
<thead>
<tr>
<th>Number</th>
<th>Wavelength (nm)</th>
<th>Laser</th>
<th>Forward Transmission (%)</th>
<th>Saline Transmission (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>800</td>
<td>Ti:Sapphire</td>
<td>2.15</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>1060</td>
<td>Nd:Glass</td>
<td>4.02</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>1301</td>
<td>Laser Diode</td>
<td>6.22</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>1557</td>
<td>Er:Glass</td>
<td>6.77</td>
<td>59.5</td>
</tr>
<tr>
<td>5</td>
<td>1639</td>
<td>NA</td>
<td>8.96</td>
<td>76.3</td>
</tr>
<tr>
<td>6</td>
<td>1695</td>
<td>NA</td>
<td>11.02</td>
<td>77.9</td>
</tr>
<tr>
<td>7</td>
<td>1777</td>
<td>NA</td>
<td>12.11</td>
<td>63.5</td>
</tr>
<tr>
<td>8</td>
<td>1845</td>
<td>NA</td>
<td>12.14</td>
<td>55.3</td>
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<tr>
<td>9</td>
<td>2229</td>
<td>NA</td>
<td>9.01</td>
<td>31.8</td>
</tr>
</tbody>
</table>

The trends in spectrophotometer measurements were consistent between samples. Sclera displayed the same transmission minima as saline at 1450 nm and 1900 nm (as can be expected since sclera is 80% water). However, at wavelengths below 1100 nm, where water is transparent at this thickness, the forward transmission of sclera increased linearly, in contrast to expected results and implying that significant scattering occurs for these shorter wavelengths. At longer wavelengths, the forward transmission increased except for the absorption peaks of water at 1450 nm and 1900 nm. Local maxima occur at 1695 nm, 1777 nm, 1845 nm, and 2229 nm. The first and last of these peaks correspond to the water transmission peaks as shown in Fig. 3.

4.2 Minimum Spot Size Measurements

The minimum size (defined as the spot size without the tissue) are shown in Figure 4. The difference in sizes is most likely due to slightly different beam divergences and the wavelength. The size of the speckle image at 800 nm was invariant with translation of the lens. Figure 5 shows the minimum spot size obtainable at different wavelengths through full thickness of sclera. As expected in highly scattering tissue, the pattern is dominated by speckle. At 800 nm, the average spot size is roughly 1000 times the size of the minimum spot. The average sizes appear to decrease for 1301 nm and 1557 nm, respectively. Figures 6 display the moving averaged images of the spot size through the full thickness of the sclera.

Figure 4: The minimum spot size at the tested wavelengths are shown. The minimum spot size is the size of the beam when focused on to the front surface of the cover slip.
Figure 5: Typical speckle patterns through full thickness of sclera.
Figure 6: The image obtained when the tissue was shaken over 1 mm.
Figure 7: The FAHM for the averaged measurements (shaken) and the speckle (stationary) patterns are shown. The FAHM were normalized to the FAHM of the minimum spot size without the tissue.

Figure 7 shows the FAHM of the shaken tissue. The FAHM of the speckle can be less than the minimum spot size. The data at 800 nm suggests severely peaked and separated speckle since the FAHM of the speckle is up to two orders of magnitude less than the average (Figure 5). As the wavelength increases, the average FAHM becomes more predictable, i.e. increases with increasing tissue thickness, and the average FAMH and speckle FAHM begin to converge.

4.3 Prototype Trans-tissue Procedure

To demonstrate how a transocular channel might be created using subsurface photodisruption, we next performed a transcorneal fistula using a 1060 nm 450 fs laser. The method is schematized in Figure 8a. In this study, we left the channel partial thickness to show that no damage to the external surface was created.
A similar experiment in sclera using the same laser was able to obtain consistent photodisruption at depths of 250 \( \mu \text{m} \) or less. This corresponds with the average FAHM of Figure 7.

![Focused Beam](image)

Figure 8: (a) Procedure used to cut the cornea fistula. (b) A hole cut in cornea from the back surface to the front surface using 1060 nm femtosecond pulses. Note that the top (external surface) layer is intact.

5. DISCUSSION

Analogous to recent work using ultrashort-pulsed lasers for subsurface corneal photodisruption, we have begun to characterize laser parameters for transcleral surgical procedures. Beginning with laser wavelength, we first defined the scattering properties of the scleral tissue. In contrast to the cornea, which transmits up to 90% of visible wavelengths, the white sclera is an excellent scatterer of visible light. Surprisingly, the cornea and sclera are similar in structure. Both are composed of collagen fibers (optical index \( n = 1.47 \)) embedded in a mucopolysaccharide ground substance (\( n = 1.345 \)). The main differences are the average center to center fibril spacing and the regularity of the structure. Fourier analysis reveals that the center-to-center spacing in cornea and sclera are 59 nm and 285 nm, respectively, with the variations being greater in sclera.\(^{14,15} \) Bragg diffraction theory predicts that scattering begins once the half wavelength of light in the tissue is on the order of the index variations in the medium.\(^{16} \) For cornea and sclera, scattering is expected to be significant for wavelengths shorter than 166 nm and 800 nm, respectively, using this theory. Since the peaks of the spatial frequency spectrum of sclera are quite broad,\(^{15} \) significant scattering at longer wavelengths may also occur.

Since the average spot size decreases by about two orders of magnitude from 800 nm to 1557 nm, longer wavelengths may be preferable for transcleral applications. Considering only scattering, subsurface photodisruption at 800 nm would require at least energies 1000 times greater than surface photodisruption. Pulses of such high energy may reach threshold above the focal spot, resulting in photodisruption near the surface of the tissue. At 1557 nm, the pulse energy needs to be only about four times greater than at the surface. These results require expansion in ex vivo and in vivo models to determine laser energy, repetition rate and pulse width parameter specifications. The preliminary data in this paper suggests that transcleral glaucoma surgery may be possible at longer wavelengths and ultrashort pulse widths.
6. ACKNOWLEDGMENTS

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7. REFERENCES