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**Ex-Vivo Porcine Cornea Suprathreshold Dose
Response to 10 ms and 100 ms Pulse Durations
at 1940 nm**

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14. ABSTRACT Advances in the development and increased use of high-energy lasers in military settings requires the development of corneal dose response models that relate the level of exposure to the probability and severity of an injury. This enables a comprehensive risk assessment as part of pre-employment reviews. To develop and validate those models, there is a need to document and characterize supra-threshold corneal damage, including ablation, resulting from laser exposures. There is a lack of data on supra-threshold corneal exposures for wavelength between 1400 nm and 2000 nm. This study focused on documenting and characterizing supra-threshold corneal damage in response to a strongly absorbing laser at 1940 nm for 10 ms and 100 ms single pulse exposures in excised porcine corneas. The tissue appears to be disrupted primarily through heating, resulting in melting and coagulation for 10 ms exposures. For 100 ms exposures, heating results in melting and vaporization of water within the cornea. The latter creates large trapped bubbles that disappear over time. This study contributes to the body of data needed to inform and validate dose response models that seek to describe and predict the effects of supra-threshold events in corneal tissues.					
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1.0 SUMMARY

Advances in the development and increased use of high-energy lasers in military settings requires the development of corneal dose response models that relate the level of exposure to the probability and severity of an injury. This enables a comprehensive risk assessment as part of pre-employment reviews. To develop and validate those models, there is a need to document and characterize supra-threshold corneal damage, including ablation, resulting from laser exposures. A previous report which reviewed the published literature on threshold and supra-threshold level laser-induced damage to skin and cornea noted that there are no corneal ablation thresholds documented for wavelengths between 1400 nm and 2000 nm although there is an increasing number of lasers operating in that range. Therefore, this study focused on documenting and characterizing supra-threshold corneal damage in response to a strongly absorbing laser at 1940 nm for 10 ms and 100 ms single pulse exposure durations in excised porcine corneas. After exposure, the disrupted areas were documented by collecting images using an optical microscope and an optical coherence tomography system. From the optical images it was found that for 10 ms and 100 ms single pulses, there is no clear ablation event where tissue is obviously removed from the exposed area. Instead, the tissue appears to be disrupted primarily through heating, resulting in melting and coagulation for 10 ms exposures. For 100 ms exposures, heating results in melting and vaporization of water within the cornea. The latter creates large, trapped bubbles that disappear over time. For both pulse durations, the depth of disruption and the area of disruption as a function of radiant exposure can be approximately described by linear relationships, with the 100 ms pulse duration having higher R^2 values than that for 10 ms. This study contributes to the body of data needed to inform and validate dose response models that seek to describe and predict the effects of supra-threshold events in corneal tissues.

2.0 INTRODUCTION

2.1 Background

In recent years, there has been an increase in the use of high-energy lasers for military applications, requiring a different approach to hazard analysis and risk assessments. For laboratory environments or where enclosed products are in use, ensuring the safety of the users is the objective of any type of hazard analysis. However, in more generalized, less restrictive situations that may involve unknown settings, a risk-based approach is more appropriate. In the case of laser hazard analyses, risk is a combination of the probability of exposure and the severity of an injury resulting from that exposure. Dose response models are being developed that incorporate hazard analysis by relating the level of exposure to the probability and severity of an injury.

The Optical Radiation Branch of the AFRL's Bioeffects Division has developed a laser exposure dose response model that incorporates analysis for both ocular and skin tissue obtained from onset-of-injury or threshold levels of laser-induced damage [1–4]. Currently, there is an effort to expand the dose response model to include higher levels of possible damage or supra-threshold effects. A previous report reviewed the published literature and provided an overview of existing data on skin and ocular laser-induced damage for strongly absorbing laser wavelengths (1400 nm and 2000 nm), as well as supra-threshold laser exposure in general [5]. The report exposed the fact that, while there are several corneal ablation threshold studies, there is no consensus on how to approach supra-threshold corneal damage in the injury region between the minimum visible lesion and ablation onset. In addition, the report stated that several studies indicated that the ablation threshold is proportional to the square root of the laser pulse duration for pulse durations longer than ~ 10 ps [6–12] and noted that there are no ablation thresholds documented for wavelengths between 1400 nm and 2000 nm. Therefore, the focus of the current project was to document and characterize supra-threshold corneal damage, including ablation, resulting from

exposure to a strongly absorbing laser at 1940 nm for 10 ms and 100 ms single pulse durations with the objective of informing the existing dose response model. The current maximum permissible exposure (MPE) limit for 10 ms exposures at 1940 nm is about 0.2 J/cm² where the limiting aperture (circular area over which exposure is averaged) is 1 mm. Analogously, the MPE for 100 ms exposures at 1940 nm is 0.3 J/cm². In this study, the focus was placed on corneal disruptions caused by radiant exposures at least 50 times the MPE as defined in the 2014 ANSI Z136.1 American National Standard for Safe Use of Lasers [13].

2.2 Cornea

The human cornea is a ~ 600 µm thick avascular tissue that forms the anterior surface of the eye. It is the clear, protective outer layer of the eye and can be considered as the first line of defense. It plays a role in vision as it refracts light that enters the eye and, together with the lens, helps determine how well the eye can focus. Starting from the anterior layer, the five layers that comprise the human cornea are: epithelium, Bowman's layer, stroma, Descemet's membrane, and the endothelium – see Figure 1a). The corneal epithelium is covered with an acellular tear film (3 - 8 µm thick), which consists of lipid, aqueous, and mucin layers although 98% of its total volume is water [14,15].

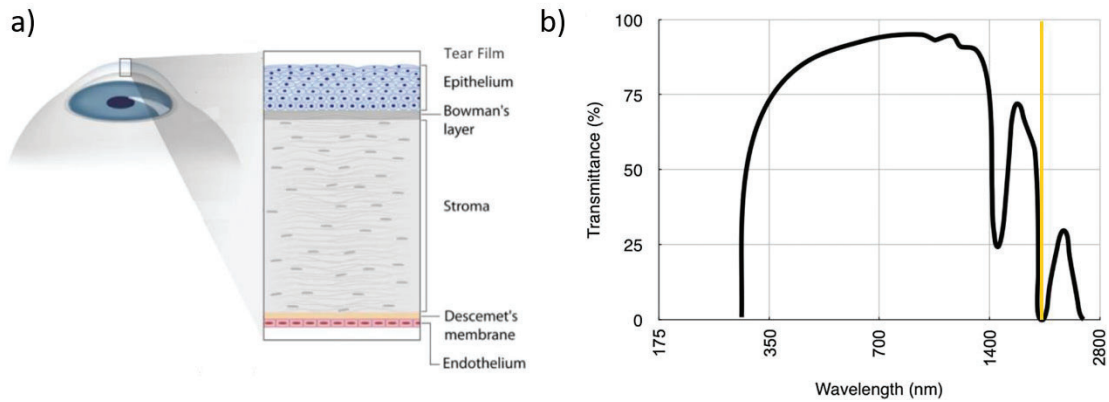


Figure 1. a) Schematic diagram of a human cornea showing the different layers with approximate dimensions (adapted from [16]). b) Transmittance of the cornea [17]. Yellow line located at 1940 nm.

Given that this study utilized porcine corneas, it is important to note that the porcine cornea contains the same layers as human cornea except for the Bowman's layer [18], but this difference should have a negligible impact on the suprathreshold injuries observed given that its composition and consistency is very similar to the surrounding layers and is very thin. In addition, the layer thicknesses differ from those in the human cornea as shown in Table 1.

Table 1. Thickness of human and porcine corneal layers

Layer	Human Thickness (µm)	Porcine Thickness (µm)
Epithelium	50	70 – 80
Bowman's layer	8 – 12	Absent
Stroma	500	665 – 870
Descemet's membrane	2 – 10	6 – 10
Endothelium	5	6 -8

The cornea absorbs light with wavelengths shorter than 380 nm and in the 1400 nm to 1 mm range [17,19] as shown in Figure 1b), therefore these wavelength regions pose a risk of injury as the laser's energy is increased. Since the early 1980s, there have been many studies to establish the laser induced damage thresholds at NIR wavelengths for CW and various pulse durations

down to the nanoseconds, see [20–26] and references within. At these wavelengths, the damage thresholds generally follow the wavelength dependence of the penetration depth into the cornea, which is dominated by the absorption coefficient of water [17,27], see Figure 1b). Given that wavelengths between 1800 and 2600 nm have moderate penetration into the cornea, it is expected that the damage for short pulse durations would be confined to the epithelium [28–30]. Furthermore, for millisecond long exposures, the primary mechanism of corneal damage is expected to be photo-thermal in nature [6,31].

2.3 Approach

An IPG Photonics TLR-200-1940 thulium CW fiber laser with a maximum power output of 200 W was used to deliver a single 10 ms or a 100 ms long pulse at a wavelength of 1940 nm onto an *ex-vivo* porcine cornea. Initially, a single cornea was exposed to a large range of radiant exposures. The radiant exposure level needed to cause clearly visible whitening or opacification of the cornea was nominally identified as threshold-level damage and was therefore considered as a lower bound. Given that the objective of the experiment was to produce supra-threshold level damage, subsequent experiments focused on radiant exposures well above the radiant exposure needed to cause threshold-level damage. The exposure sites were documented using an optical microscope and an optical coherent tomography system. The optical images were primarily used to measure the aspect ratio of the region that was altered by the laser exposure and to qualitatively describe the disruption to the tissue produced by the laser exposures. The OCT images were primarily used to quantify the extent of the damage. Damage in this context was defined as the region of tissue that was disrupted by the laser exposure.

3.0 METHODS

The procedures described below for sample handling and laser exposures were developed over the course of several experiments. The driving forces behind those developments were the reduction of disruptions to the corneal surface, including epithelial cell detachment and/or dehydration, and ensuring the results were reproducible. Below is a brief description of the final procedures.

3.1 Experimental Model

Fresh porcine corneas were obtained from Animal Technologies, Inc., a provider of raw animal biological products to research and veterinary institutions. The corneas were transported and preserved in plastic bags at 4 °C and arrived at the experimental site within, at most, 72 h after being excised. The corneas were delivered with the sclera ring attached. Immediately after arrival, they were placed in a water bath (Bibby Scientific Limited) at 37 °C within the plastic bag. Corneas appear milky at 4 °C but become clear as their temperature increases. The water bath was also used to pre-warm the rest of the materials and supplies used for the experiment. After 10 min, the corneas were removed from the plastic bags one at a time and rinsed with pre-warmed balanced salt solution (Gibco DPBS). In order to avoid damaging the epithelium, the corneas were held with tweezers by the scleral ring. A drop of DPBS was placed on the apex of a pre-warmed plastic or glass sphere, *i.e.* a 2.54 cm diameter marble, mounted in a holding ring, see Figure 3a), immediately before placing the cornea onto the sphere, endothelial side down. A few drops of DPBS were placed on the epithelial side in order to keep the cornea from drying out. Whenever the corneas were not actively being used for imaging or exposure, they were placed within the water bath enclosure mounted on the sphere and holding ring to keep them warm and moist, ensuring they were not directly in contact with the water. During imaging procedures, a few drops of DPBS were frequently placed on the epithelium in order to keep the cornea hydrated.

3.2 Experimental Procedures

3.2.1 Exposure setup.

An IPG Photonics (Oxford, MA) TLR-200-1940 thulium fiber CW laser with a maximum power output of 200 W was used to produce either a 10 ms or a 100 ms long pulse at 1940 nm. The laser's beam profile at the output was quasi-Gaussian with a $D4\sigma$ diameter collimated beam of 5.90 ± 0.06 mm obtained with a pyrocam (Spiricon PY-111 h-C-A), see Figure 2.

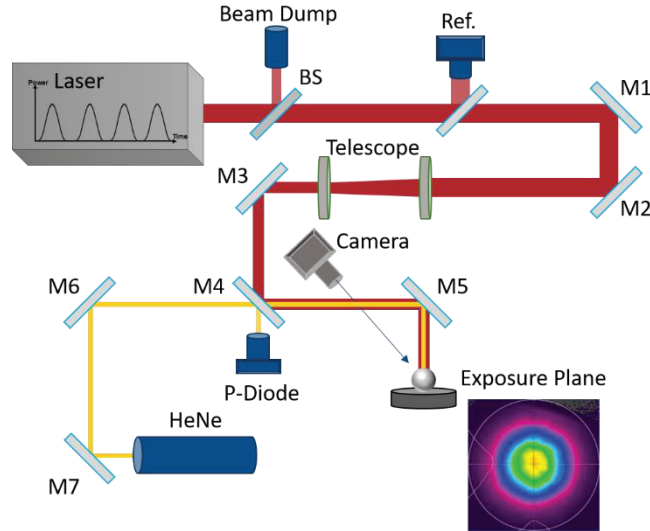


Figure 2. Optical setup for suprathreshold *ex-vivo* cornea exposures. BS: beam splitter. W: glass window. M: silver mirrors. L1: plano convex lens $f = +300$ mm. L2: $f = -75$ mm. P-Diode: photodiode. Telescope: Camera: Imperx CCD camera. Inset: Beam profile.

Figure 2 is a schematic diagram of the setup used for the exposures. Initially, the output of the laser passed through a beam splitter (BS; LEO BS-2000-R50-45-UF-2038) which sent 50% of the incident light to a beam dump (Kentek ABD-2). After the beam splitter, the laser beam passed through a pick-off window (Thorlabs WG42012) which sent a fraction of the beam to a reference detector (Ref.; Ophir 919P-003-10) connected to a meter (Ophir Centauri Model 7Z01701). The laser beam was reflected off two mirrors (LEO RX-2000-45) before being contracted to a ~ 1 mm diameter collimated beam using a telescope consisting of a plano-convex 2" CaF₂ lens with a $f = +300$ mm focal length (L1; CVI PLCX-50.8-154.5-UV-2020) and a plano-concave 1" CaF₂ lens with a $f = -75$ mm focal length (L2; CVI PLCC-25.4-38.6UV-2020). Three more mirrors (M3; CVI TLM1-2010-45UNP-2037, M4; CVI BS1-2010-90-2037-45UNP, M5; Edmund R5000793709-21132) directed the laser beam to the cornea with M4 also serving as a beam combiner for a visible diode laser beam and the NIR beam. The collinear diode laser beam served to position the NIR beam at the desired location for exposure. In addition, the NIR light that leaked through M4 illuminated a photodiode (P-Diode; Thorlabs DET10D InGaAs Biased Detector). The output of P-Diode was sent to an oscilloscope (Tektronix, MDO3024) for visual confirmation of a single pulse being delivered.

A high speed, mirrorless camera (Cam 1; Imperx, IGV-B0610C-KC000) imaged the exposure event in real time. It is a high speed, low noise camera, with electronic shutter that captured full quality images at a frame rate of 100 fps.

A custom written LabView program was used to control the laser's power, the pulse duration, and number of pulses delivered while simultaneously saving the oscilloscope trace, the energy recorded by the reference, and triggering the camera to collect a series of images during the exposure.

3.2.2 Energy calibration and radiant exposure calculation.

For the energy calibration procedure, an energy detector (Ophir 919E-10-20-250) was placed underneath a 2.54 cm opening in the center of the heating stage such that it collected all the incoming light. The energy measured by the meter (Coherent LabMaxTOP) connected to the detector will be referred to as energy at target. Using the custom LabView program, single pulses were delivered to the detector at target for varying laser powers while recording the laser's power setting, the energy measured by the reference detector, and the energy at target. The parameters of the line (slope and intercept) relating the energy at target (y) to the reference energy (x) were obtained using linear regression and used to calculate the energy delivered given a measured reference energy during the exposures.

The energy delivered at target was determined from the energy measured by the reference detector (Q_{ref}) at the time of exposure as

$$Q = R_{avg} * Q_{ref} + b_{avg} \quad (1)$$

where R_{avg} and b_{avg} were the average of the slopes or the intercepts obtained during the pre and post calibration procedures when available, or the slope and intercept of the single available calibration procedure.

During the calibration procedure, the power setting of the laser, the energy measured at the target, and the energy measured by the reference detector were collected. Graphs of the energy measured at the target versus the energy measured by the reference detector were generated showing a clear linear relationship. A linear regression algorithm in Excel was used to find the slope (R) and intercept (b) of the line and the standard error of the slope (SE_R) and of the intercept (SE_b). The standard error was related to the standard deviation by the number of data points used in the linear regression (n) such that $u(R) = \sqrt{n} * SE_R$ and $u(b) = \sqrt{n} * SE_b$.

In the cases when the slope of the pre and post calibration procedures were available, then they were averaged as $R_{avg} = \frac{1}{2}(R_{pre} + R_{post})$ and $b_{avg} = \frac{1}{2}(b_{pre} + b_{post})$. The uncertainties in R_{avg} and b_{avg} were found from

$$u(R_{avg}) = \frac{1}{2} \sqrt{u^2(R_{pre}) + u^2(R_{post})} \quad \text{and} \quad u(b_{avg}) = \frac{1}{2} \sqrt{u^2(b_{pre}) + u^2(b_{post})}. \quad (2)$$

In order to determine the uncertainty in the energy measured by the reference detector, we considered that the reference detector had a calibration uncertainty of 3% and the power/energy meter had a 1.5% uncertainty. The laser noise was 3% therefore the combined relative uncertainty for Q_{ref} was

$$\frac{u(Q_{ref})}{Q_{ref}} = \sqrt{(0.03)^2 + (0.015)^2 + (0.03)^2} = 0.045. \quad (3)$$

Therefore, the combined uncertainty in the delivered energy was found from

$$u(Q) = \sqrt{(R_{avg} * Q_{ref})^2 * \left(\frac{u(R_{avg})}{R_{avg}}\right)^2 + (R_{avg} * Q_{ref})^2 * \left(\frac{u(Q_{ref})}{Q_{ref}}\right)^2 + u^2(b_{avg})}. \quad (4)$$

The expression above was not simplified in order to make it easier to compare to the formulas used to calculate the uncertainties in the Excel spreadsheets.

The peak radiant exposure was defined as the total energy received by a surface (Q) per unit area (A). For a Gaussian beam it was given by:

$$H = \frac{Q}{A} = \frac{8Q}{\pi * d^2} \quad (5)$$

where the expression on the right is for a circular spot size with a $1/e^2$ diameter d . In the current study, Q was the energy delivered, obtained from the measured reference energy during an exposure. The uncertainty associated with the radiant exposure was obtained as

$$u(H) = H * \sqrt{\left(\frac{u(Q)}{Q}\right)^2 + 4 * \left(\frac{u_B(d)}{d}\right)^2} \quad (6)$$

where the first term was the relative uncertainty of the energy delivered calculated using equation 4, and the second one is the relative uncertainty in the beam diameters' determination.

The collimated beam diameter was measured using the knife edge technique at the sample location. The results are listed in Table 2 for each day when exposures were performed.

Table 2. Collimated beam diameter at exposure site and corresponding area of exposure

Pulse duration (ms)	Sample	Collimated beam diameter (mm)	Area of exposure (mm ²)
10	10202021-C4	1.64	2.11
10	11032021-C2	1.74	2.38
100	11042021-S4	1.60	2.01
100	11172021-C2	1.52	1.81
100	11172021-C4	1.52	1.81

The $1/e^2$ beam diameter was obtained using the knife edge technique therefore it was found by taking the difference between the two positions at which the signal's intensity became 16% or 84% of the unobstructed signal: $d = 2(x_{16} - x_{84})$. The uncertainty in the determination of the beam diameter was obtained as

$$u_B(d) = \sqrt{\left(\frac{\partial d}{\partial x_{16}}\right)^2 u^2(x_{16}) + \left(\frac{\partial d}{\partial x_{84}}\right)^2 u^2(x_{84})} \quad (7)$$

so

$$u_B(d) = \sqrt{(2)^2 u^2(x_{16}) + (2)^2 u^2(x_{84})} = 2\sqrt{2}u(x) \quad (8)$$

were the last identity comes from assuming the uncertainties were the same. Given that the uncertainty in position of the micrometer stage used during the knife edge measurement was 10 μ m, the reference detector had an uncertainty of 1% and the power/energy meter had an uncertainty of 1.5%, the uncertainty in the determination of the collimated beam diameter became

$$u_B(d) = 2\sqrt{2 * [(10\mu m)^2 + d^2 * [(0.01)^2 + (0.015)^2]]}. \quad (9)$$

All the radiant exposures and associated uncertainties reported in the tables and spreadsheets in the rest of this report were calculated using the relationships presented above.

3.2.3 Initial sample characterization and selection.

The corneas mounted on spheres were imaged with an optical coherence tomography system (OCT; Thorlabs, Telesto-II SD-OCT, LSM04 OCTG head, EFL = 54 mm objective OCT-LK4) while on the holding ring, prior to any laser exposure. The OCT was used to visualize the topography of the cornea and locate any ridges and valleys that were present. It is speculated that the ridges and valleys were a result of wrinkles that developed when the corneas were removed from the ocular globe. Given that the excised corneas were not stretched, they were simply placed on the spheres, this initial characterization was used to select the corneas with a smoother surface, *i.e.* fewer wrinkles, for laser exposures and to create a map of the regions to target with the laser.

3.2.4 Exposure procedure.

While the corneas were initially being characterized, the computer-controlled microscope heating stage (Linkam Scientific Instruments LTD, PE100-OI Thermoelectric Cooling System, T96-P LinkPad controller, ECP water circulation pump) was set to 60 °C. A 100 mm diameter glass Petri dish filled with 47.5 ml of pre-warmed water was placed on the heating stage with a 3D printed custom designed sphere holder straddling it – blue piece in Figure 3b). The sphere holder was designed to partly submerge the sphere into the surrounding water in order to keep it at a stable and uniform temperature. It was determined experimentally that when the heating stage was set to 60 °C and the water, Petri dish, sphere holder, and sphere had reached thermal equilibrium, the apex of the glass sphere was at 37.20 ± 0.21 °C. Therefore, the heating stage remained set to 60 °C throughout the experiments. Immediately before an exposure, a cornea mounted on a sphere was transferred from the water bath on to the pre-warmed heating stage and sample holder setup shown in Figure 3. Subsequently, the epithelial surface of the cornea was thoroughly wetted with DPBS prior to tilting the spherical mount to let the excess DPBS slide off. A 1 min counter was started, the alignment laser was turned on, and the cornea was rotated to the desired exposure location. The alignment laser was turned off and, at the 1 min time point, the cornea was exposed to a single 1940 nm pulse. Immediately after the first exposure, the laser parameters were set for the next exposure while the alignment laser was turned on and the cornea was rotated to the next desired exposure location in order to keep the laser beam normal to the surface of the cornea. This sequence was repeated until all exposures in the exposure plan were completed, up to 10 in some cases. Note that it took less than 5 min to expose up to 10 sites, which was not enough time for the corneal surface to dehydrate.

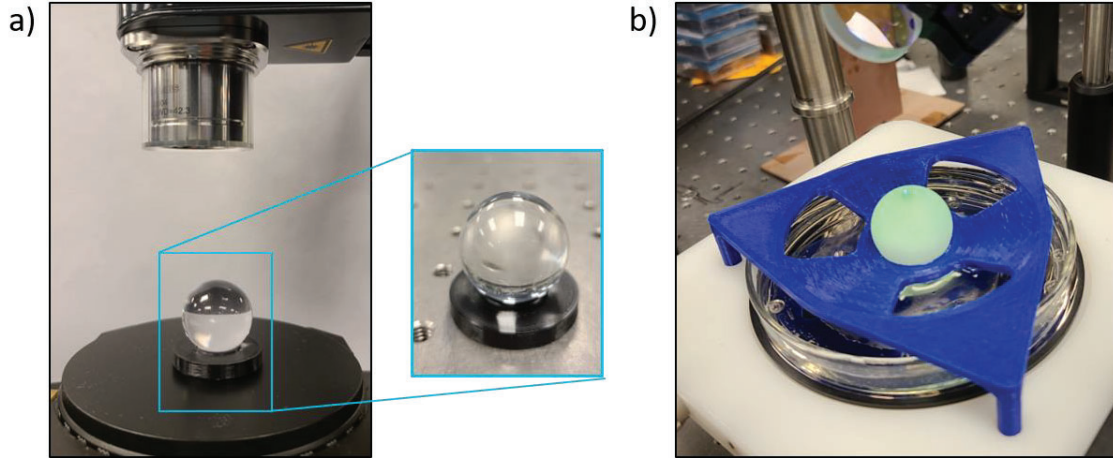


Figure 3. a) Photographs of glass sphere mounted on the holding ring for imaging with the OCT. Close up of the glass sphere and holding ring on the right. b) Sphere placed on the heating stage. For illustration purposes, a frosted sphere was placed on the sphere holder such that the (green) spot from the alignment laser can be seen.

3.3 Post-exposure characterization and uncertainty determination

After all the exposures in the exposure plan were completed, the exposure sites were documented with the optical microscope and the OCT. Visual inspection of the optical images was used to qualitatively describe the laser induced disruption. Optical images were also analyzed using ImageJ (NIH ImageJ v1.53a) to obtain more quantitative results [32]. At each exposure site, the elliptical selections tool was used to select the outer edge of the disrupted tissue by hand up to 5 different times, see Figure 4 a). After each one, the measurement tool reported the length of the major and minor axes in pixels. Converting measurements from pixels to millimeters was not possible in this case due to lack of information about the zoom setting on the microscope used to take the optical images. The average and standard deviation of the 5 independent measurements were calculated and used to obtain the ratio of the major axis (A) to the minor axis (B), denoted as *aspect ratio*, with the corresponding uncertainty. The aspect ratio was used as a measure of how circular the disrupted area was, where 1 denoted a perfect circle. The uncertainty in the aspect ratio was obtained from

$$u(\text{aspect ratio}) = \text{aspect ratio} * \left(\frac{u(A)}{A} + \frac{u(B)}{B} \right) \quad (10)$$

where $u(A)$ and $u(B)$ are the standard deviations of the 5 independent measurements of the best fitting ellipses. This relationship was chosen since independent random errors are not present when performing the measurement of the major and minor axes [30].

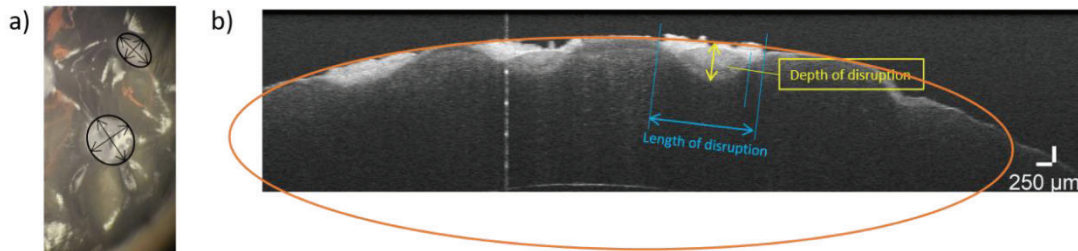


Figure 4. Analysis of laser induced disruptions. a) Optical image of two exposed regions indicating ellipses defining the disrupted tissues. b) OCT image. B-stack with overlay

showing the ellipse fit to the surface of the cornea, depth and length measurements of the disruption.

The analysis software from the OCT (Thorlabs, ThorImage OCT v. 4.4.8.0) was used to determine the extent of the disruptions to the tissue directly from the B-stacks. The spatial separation between the first and the last images where a disruption was observed for a particular exposure site was obtained from the scan parameters window. This length was denoted as the *width of disruption* in the tables and spreadsheets (YZ in the OCT software) and the uncertainty associated with it was the spatial separation between the frames, *i.e.* ± 0.04 mm in all cases. The length of the disruption (XZ in the OCT software) was obtained by measuring the linear extent of the disruption shown in the B-stack corresponding to the midpoint of the width of disruption. The marker tool was used to select the linear extent of the disruption and automatically obtain its length. The uncertainty in these measurements was ± 0.07 mm and was determined by measuring the same length several times and noting the variability between the measurements. The average of those measurements was reported in the tables and spreadsheets as the *length of disruption*. Note that the major and minor axes obtained from the optical images do not necessarily correspond to the width and length of disruption obtained from the OCT images due to variations in the orientation of the cornea when imaging in the two modalities.

The same frame corresponding to the midpoint of the width of disruption was used to determine the *depth of disruption*. In that case the elliptical selections tool in ImageJ (NIH ImageJ v1.53a) [32] was used to fit the surface of the cornea, see Figure 4 b). The depth was measured from the surface determined by the ellipse to the lowest point in the disrupted region 5 different times. The average of these 5 measurements was reported in the tables and spreadsheets as the depth of disruption. The standard deviation of these 5 measurements $u_A(\text{depth})$ was combined with the variability of the radius of curvature at the vertex on the minor axis of the unexposed corneas $u_A(b)$ to obtain the uncertainty in the depth determination reported in the tables and spreadsheets as

$$u(\text{depth}) = \text{depth} * \sqrt{\left(\frac{u_A(\text{depth})}{\text{depth}}\right)^2 + \left(\frac{u_A(b)}{b}\right)^2} \quad (11)$$

The variability in the radius of curvature of unexposed corneas was obtained by fitting an ellipse to the surface of 8 different unexposed corneas, 5 different times each. The radius of curvature at the vertex on the minor axis was obtained as $R_b = a^2/b$ where $2a$ was the major axis and $2b$ was the minor axis for each measurement. The average radius of curvature at the vertex on the minor axis, the standard deviation and the relative error were calculated for each of the 8 unexposed corneas. We chose the maximal relative error ($\sim 5\%$) of all unexposed corneas as the uncertainty in the radius of curvature at the vertex on the minor axis of the unexposed corneas, therefore

$$\frac{u_A(b)}{b} \cong 5\% \quad (12)$$

which we consider as a measure of the biological variability.

The length and width of the disruption were used to obtain the *area of disruption* by assuming that they correspond to the major and minor axes of an ellipse. Although this assumption is known to be false, it is still a useful parameter to calculate in order to compare the area of disrupted tissue with the area of the laser spot causing the disruption. The uncertainty associated with the area of disruption was obtained as

$$u(\text{area of disruption}) = \text{area of disruption} * \left(\frac{0.040}{\text{width of disruption}} + \frac{0.070}{\text{length of disruption}} \right) \quad (13)$$

where 0.04 mm and 0.07 mm were the uncertainties in the width and length determinations as describe above.

4.0 RESULTS AND ANALYSIS

The data is organized by pulse duration and date of exposure. Within the folder with the date, there are multiple folders which correspond to the optical images collected, the OCT, the oscilloscope traces, and the movies taken during the exposures. There is a digital master spreadsheet for the corneas whose exposure sites were analyzed in detail. The master spreadsheet contains information about the day the data was taken, the sample number, the order of exposures, the energy measured on the reference detector, the corresponding energy at target, the radiant exposure, the aspect ratio and area of disruption obtained from the optical images, the width, length, depth and area of the disruption obtained from the OCT, the ratio of the area of the disruption to the area of the laser's spot size, and the corresponding uncertainties among other parameters. However, a subsection of the most relevant parameters are included in the tables below and APPENDIX A where each sample and radiant exposure combination was assigned an identifying letter.

4.1 Corneal exposures to 10 ms single pulses

4.1.1 Results of 10 ms single pulse exposure durations.

Two corneas, both with seven representative exposure sites, were imaged using the optical microscope and OCT. The optical images of these corneas are shown in Figure 5. Both the optical and OCT images were used to analyze the exposure sites and to describe them qualitatively when appropriate. Results were compiled into a spreadsheet and included as a table below. Four other corneas were exposed and imaged using only OCT, *i.e.* no optical images were collected. Two additional corneas were exposed and imaged using the optical microscope and OCT, however, they were not included in the analysis due to resource constrains.

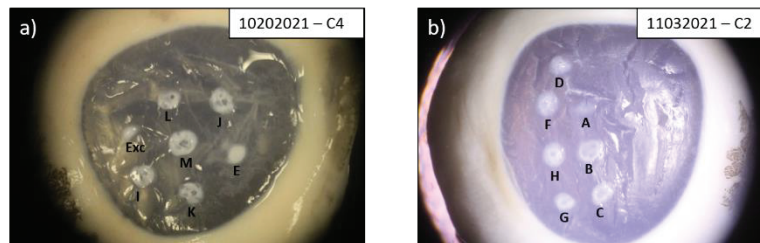


Figure 5. Optical images of the excised corneas analyzed after exposure to a single 10 ms pulse with varying radiant exposures. Letters identify the exposures referenced in Tables 1, 2 and 3. The abbreviation Exc in panel a) means that data point was excluded from the analysis.

The results of the aspect ratio obtained from the optical images of the laser induced disruptions on two different corneas are tabulated in Table 3 together with the radiant exposure and a brief description of the visual appearance of the disruption. The optical images used were taken immediately post-exposure for 10202021-C4 but 24 h post-exposure for 11032021-C2. However, there were no significant differences in the overall appearance. One data point (labeled Exc in Figure 5) was excluded from the quantitative analysis given that we deemed it an outlier. It corresponded to a radiant exposure close to threshold, it was extremely elongated (aspect ratio

= 1.88) compared to the other exposures and, given that there was only one other exposure at that radiant exposure, we considered it could bias the quantitative results if included. However, we did include a brief description of the disruption's appearance in Table 3 for completeness.

Table 3. Optical characterization and description of 10 ms single pulse disruptions

ID	Sample	Radiant exposure (J/cm ²)	Aspect ratio	Notes on disruption
A	11032021-C2	19.15 ± 1.90	1.372 ± 0.091	Uniformly white hazy area. No clearly defined edges.
B	11032021-C2	20.09 ± 1.99	1.228 ± 0.023	Opaque white area with four thinned areas inside. Clearly defined edges. Slightly elevated ridge at edges.
C	11032021-C2	21.06 ± 2.08	1.484 ± 0.107	Opaque white area with thinned area inside. Elevated ridge at edges.
D	11032021-C2	21.06 ± 2.08	1.082 ± 0.042	Uniformly opaque white area. No clearly defined edges.
E	10202021-C4	21.75 ± 2.85	1.372 ± 0.046	Uniformly white hazy area. No clearly defined edges.
F	11032021-C2	22.03 ± 2.16	1.082 ± 0.036	Uniformly opaque white area. No clearly defined edges. Thinned area inside.
G	11032021-C2	22.07 ± 2.17	1.294 ± 0.081	Opaque white area with thinned area inside, centered. No clearly defined edges. Wide ridge at edges.
H	11032021-C2	23.07 ± 2.26	1.096 ± 0.035	Opaque white area with thinned area inside, centered. No clearly defined edges. Wide ridge at edges.
I	10202021-C4	25.90 ± 3.32	1.120 ± 0.054	Opaque white area with small depression inside. Clearly defined edges.
J	10202021-C4	25.90 ± 3.32	1.159 ± 0.037	White, translucent area. Elliptical open area and 2 smaller thinned areas inside. Clearly defined edges.
K	10202021-C4	30.18 ± 3.82	1.301 ± 0.024	Opaque white area with small circular depression inside. Tapered edges.
L	10202021-C4	30.18 ± 3.82	1.361 ± 0.043	White area with circular open area inside. Tapered edges.
M	10202021-C4	34.30 ± 4.31	1.236 ± 0.039	Opaque white area with thinned circular area inside, centered. Clearly defined edges.
Exc	10202021-C4	21.75 ± 2.85	1.833 ± 0.148	Uniformly opaque white hazy area. No clearly defined edges.

The radiant exposures and associated uncertainties listed in Table 3 and Table 4 were calculated using the equations presented in Section 3.2.2, where the corresponding beam diameters were listed in Table 2. The aspect ratios and associated uncertainties listed in Table 3 and Table 4 were calculated using the relationships presented in Section 3.3.

The results obtained from the measurements on the OCT images of the 10 ms single pulse laser induced disruptions on two different corneas are tabulated in Table 4. The length and width of the disruptions were used to calculate the area of disruption as described in Section 3.3. Section 3.3 also details the procedure used to obtain the depth of disruption measurement, its associated uncertainty, and the uncertainty in the area of disruption calculation.

Table 4. Exposure parameters and disruption characterization from OCT images exposed to a 10 ms single pulse

ID	Sample	Radiant exposure (J/cm ²)	Width of disruption [†] (mm)	Length of disruption [‡] (mm)	Depth of disruption (mm)	Area of disruption (mm ²)
A	11032021-C2	19.15 ± 1.90	0.571	1.325	0.417 ± 0.070	0.594 ± 0.073
B	11032021-C2	20.09 ± 1.99	1.111	1.427	0.372 ± 0.027	1.245 ± 0.106
C	11032021-C2	21.06 ± 2.08	1.292	1.327	0.448 ± 0.023	1.347 ± 0.113
D	11032021-C2	21.06 ± 2.08	1.111	1.418	0.403 ± 0.043	1.237 ± 0.106
E	10202021-C4	21.75 ± 2.85	1.209	1.275	0.601 ± 0.036	1.211 ± 0.107

F	11032021-C2	22.03 ± 2.16	1.411	1.384	0.442 ± 0.035	1.534 ± 0.121
G	11032021-C2	22.07 ± 2.17	1.081	1.300	0.478 ± 0.030	1.104 ± 0.100
H	11032021-C2	23.07 ± 2.26	1.442	1.425	0.557 ± 0.033	1.614 ± 0.124
I	10202021-C4	25.90 ± 3.32	1.397	1.716	0.611 ± 0.045	1.883 ± 0.131
J	10202021-C4	25.90 ± 3.32	1.549	1.453	0.536 ± 0.044	1.768 ± 0.131
K	10202021-C4	30.18 ± 3.82	1.284	1.584	0.494 ± 0.029	1.597 ± 0.120
L	10202021-C4	30.18 ± 3.82	1.095	1.605	0.641 ± 0.035	1.380 ± 0.111
M	10202021-C4	34.30 ± 4.31	1.625	1.791	0.692 ± 0.039	2.286 ± 0.146

† The uncertainty in the width was 0.04 mm for all data.

‡ The uncertainty in the length was 0.07 mm for all data.

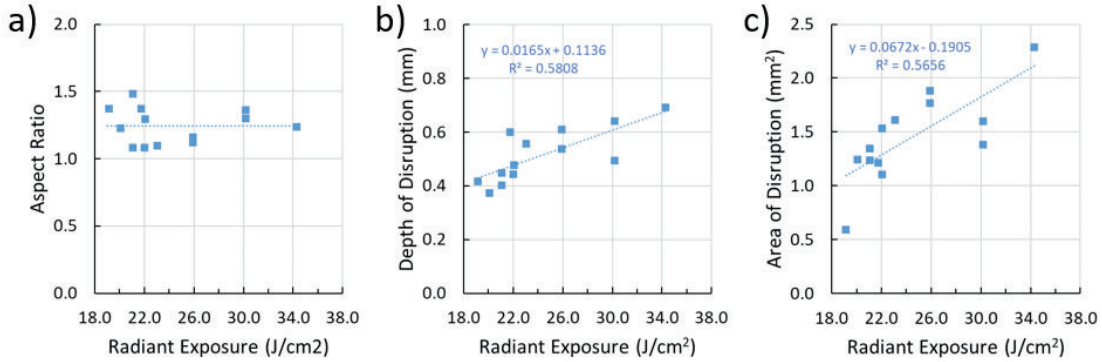


Figure 6. a) Aspect ratio obtained from the optical images of all 10 ms exposures. The b) depth and c) area of disruption of all 10 ms exposures. Dotted line in a) marks the average aspect ratio while in b) and c) they are linear fits to the data. The equation of the linear fit and R^2 value are shown in b) and c).

Figure 6 shows plots of the aspect ratio, the depth, and area of disruption as a function of radiant exposure for the data in Table 4, *i.e.* all individual exposures at 10 ms. The aspect ratio of the disruptions varied between 1.082 to 1.484 but had an average value of 1.245 ± 0.125 (average $\pm$ standard deviation), dotted line in panel a). An aspect ratio different than 1 indicated that the disruptions were not entirely circular. This may be due to the local curvature of the excised corneas, the angle of incidence of the laser beam, and/or the actual shape of the beam after the collimating optics. If we consider that the stroma begins $\sim 70 \mu\text{m}$ below the surface of the cornea and extends about $870 \mu\text{m}$ into the cornea, then we can say that the disruption extended into the stroma for all exposures studied. The depth and area of disruption increase roughly linearly as a function of radiant exposure. However, there was a large variability in the measurements extracted from different exposures, even at the same radiant exposure. This variability was reflected in the poor R^2 values, *i.e.* ~ 0.6 , for the depth and area of disruption.

4.1.2 Analysis of 10 ms single pulse exposure duration results.

As a means to draw out the relationships between the parameters describing the disruption caused by the laser exposure and the radiant exposure, data was averaged from different corneas with similar radiant exposures, see Table 5. The ID column states which exposures were considered for the averages. For the average radiant exposure, the uncertainty was calculated using the square root of the sum of the squares divided by the number of data points included in the average. For the length, depth and width of disruption, the uncertainty associated with the average of N numbers α_i was given by the uncertainty of the individual numbers divided by the square root of N when the uncertainties were the same and by

$$u(\text{average}) = \text{average} * \left(\sum_{i=1}^N \frac{u(\alpha_i)}{\alpha_i} \right) \quad (14)$$

when the uncertainties were different. The latter is an upper bound on the uncertainty. Using the averaged width and length of disruption, the averaged area of disruption was calculated. In order to elucidate if there was a clear relationship between the area of exposure and the area of disruption, the area of disruption was compared to the area exposed to the laser, *i.e.* the area of the laser spot size, where

$$\text{ratio of areas} = \frac{\text{area of disruption}}{\text{area of exposure}}. \quad (15)$$

For the 10 ms single pulse exposures, from Table 2 it can be seen that the average area of exposure from the 2 different exposure days was $2.245 \pm 0.133 \text{ mm}^2$ (average $\pm$ standard deviation). A *ratio of areas* < 1 implies that the area of the cornea disrupted by the laser exposure is smaller than the area covered by the laser's spot size. This relationship was a measure of the radial extent of the photothermal damage caused by the laser.

Table 5. Aggregated data for 10 ms single pulse exposures

ID	Average radiant exposure (J/cm ²)	Average width of disruption (mm)	Average length of disruption (mm)	Average depth of disruption (mm)	Average area of disruption (mm ²)	Ratio of areas
A	19.15 $\pm$ 1.90	0.571 $\pm$ 0.040	1.325 $\pm$ 0.070	0.417 $\pm$ 0.070	0.594 $\pm$ 0.073	0.25
B	20.09 $\pm$ 1.99	1.111 $\pm$ 0.040	1.427 $\pm$ 0.070	0.372 $\pm$ 0.027	1.245 $\pm$ 0.106	0.52
C, D, E	21.29 $\pm$ 1.36	1.204 $\pm$ 0.023	1.340 $\pm$ 0.040	0.484 $\pm$ 0.105	1.265 $\pm$ 0.325	0.56
F, G	22.05 $\pm$ 1.53	1.246 $\pm$ 0.028	1.342 $\pm$ 0.049	0.460 $\pm$ 0.066	1.319 $\pm$ 0.224	0.55
H	23.07 $\pm$ 2.26	1.442 $\pm$ 0.040	1.425 $\pm$ 0.070	0.557 $\pm$ 0.033	1.614 $\pm$ 0.124	0.68
I, J	25.90 $\pm$ 2.35	1.473 $\pm$ 0.028	1.585 $\pm$ 0.049	0.574 $\pm$ 0.089	1.825 $\pm$ 0.262	0.86
K, L	30.18 $\pm$ 2.70	1.190 $\pm$ 0.028	1.595 $\pm$ 0.049	0.568 $\pm$ 0.065	1.489 $\pm$ 0.231	0.70
M	34.30 $\pm$ 4.31	1.625 $\pm$ 0.040	1.791 $\pm$ 0.070	0.692 $\pm$ 0.039	2.286 $\pm$ 0.146	1.08

Figure 7 shows plots of the average depth of disruption, the average area of disruption, and the ratio of the areas as a function of radiant exposure for the data in Table 5. The process of averaging moderately reduced the variability in the data such that the R^2 value of the linear fits increased up to ~ 0.8 for the depth of disruption and up to ~ 0.7 for the area of disruption relative to the original data. The ratio of the areas increased approximately monotonically as the radiant exposure increased, resulting in an R^2 value of ~ 0.8 for the linear fit. The data for the exposure labeled A corresponds to the lowest radiant exposure and has an elongated shape. The elongated shape renders it an outlier in the area of disruption versus radiant exposure graph and, consequently, in the ratio of the areas versus radiant exposure graph. However, the variability in the data is such that, even if the data point for exposure A was excluded from the analysis, the R^2 value for the ratio of the areas would only increase from 0.76 to 0.77.

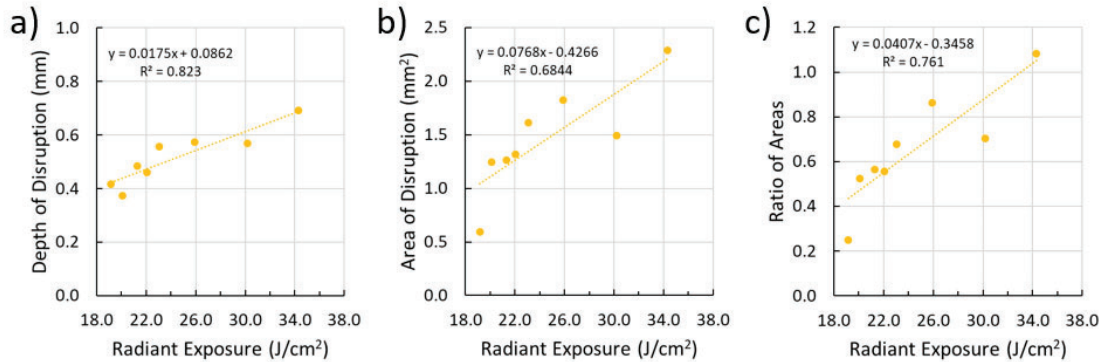


Figure 7. Plots of the a) average depth of disruption and b) average area of disruption. c) ratio of the average area of disruption to the average area of exposure. Dotted lines are

linear fits to the data. The equations of the linear fits and R^2 values are shown in each panel.

The ratio of the areas remained below one for all radiant exposure doses except the highest one, *i.e.* 34.3 J/cm². This indicated that the disruptions observed on the corneal tissue were only caused by the central region of the laser beam which contained enough energy to affect the tissue. In addition, it appeared to indicate that radial thermal diffusion effects did not play a significant role during the exposures.

4.2 Corneal exposures to 100 ms single pulses

4.2.1 Results of 100 ms single pulse exposure durations.

Three corneas with representative exposure sites, two with ten exposure sites and one with seven, were imaged using the optical microscope and OCT. The optical images of these corneas are shown in Figure 8. Both, the optical and OCT images were used to analyze the exposure sites and to describe them qualitatively when appropriate. Results were compiled into a spreadsheet and included as a table below. Four other corneas were exposed and imaged using OCT; however, no optical images were collected. Four additional corneas were exposed and imaged using the optical microscope and OCT but were not included in the analysis due to resource constraints.



Figure 8. Optical images of the excised corneas analyzed after exposure to a single 100 ms pulse with varying radiant exposures. Letters identify the exposures referenced in Tables 4, 5 and 6. The * indicates that there was no apparent damage to the cornea for that exposure. The abbreviation Exc in panel c) means that data point was excluded from the analysis.

The results of the aspect ratio obtained from the 24 hr post-exposure optical images of the laser induced disruptions on three different corneas are tabulated in Table 6 together with the radiant exposure and a brief description of the visual appearance of the disruption and plotted in Figure 9 a) below. One data point (labeled Exc in panel c)) was excluded from the analysis given that no clear depth data could be obtained from the OCT images. However, a brief description of the disruption's appearance was included in Table 6 for completeness. Although another point had an elongated shape (ID = L; aspect ratio = 2.233), given that the radiant exposure was similar to others and it was in the middle range of the radiant exposure doses, it was not excluded from the analysis.

It should be noted that during the exposures, there was enough energy transferred to the tissue that there was noticeable movement of the tissue akin to being poked. This movement can be observed in the movies recorded during the exposures. Immediately after exposure, the disruptions on sample 11172021-C2 and 11172021-C4 presented bubbles within the area exposed to the laser. However, the descriptions included in Table 6 refer to 24 h post-exposure observations where there were no bubbles remaining for any of the exposures on 11172021-C2 and a few in some of the exposures on 11172021-C4.

The radiant exposures and associated uncertainties listed in Table 6 and Table 7 were calculated using the equations presented in Section 3.2.2, where the corresponding beam diameters were

listed in Table 2. The aspect ratios and associated uncertainties listed in Table 6 and Table 7 were calculated using the relationships presented in Section 3.3.

Table 6. Optical characterization and description of 100 ms single pulse disruptions

ID	Sample	Radiant exposure (J/cm ²)	Aspect ratio	Notes on disruption
A	11042021-S4	15.35 ± 4.18	1.249 ± 0.047	White hazy area within thinned area.
B	11042021-S4	15.91 ± 4.22	1.082 ± 0.035	White opaque external edge with translucent area inside. Radial striations.
C	11042021-S4	26.57 ± 5.02	1.324 ± 0.081	White area. Clearly defined edges. Slightly pear shaped. Radial striations on one side.
D	11042021-S4	26.57 ± 5.02	1.251 ± 0.030	White opaque elevated ridge surrounding thinned central area. Radial striations.
E	11042021-S4	36.49 ± 5.94	1.186 ± 0.119	Opaque white area with slightly thinned area off-center inside. Clearly defined edges. Pear shaped. Rough surface.
F	11042021-S4	36.68 ± 5.96	1.075 ± 0.041	Opaque white with irregular shaped slightly thinned area inside. Clearly defined edges. Elevated external edge. Radial striations. Rough surface.
G	11172021-C2	46.18 ± 7.25	1.192 ± 0.074	Opaque white central area surrounded by a light grayish, thinner area. Rough surface. No clear edges.
H	11172021-C4	46.39 ± 7.27	1.307 ± 0.049	Variegated opaque white and light grayish, thinned areas. Rough surface. Clear edges.
I	11172021-C2	46.60 ± 7.29	1.256 ± 0.055	Opaque white raised central area surrounded by a light grayish, thinner area. No clear edges.
J	11042021-S4	46.78 ± 7.00	1.075 ± 0.013	Donut shaped. Outer ring half opaque white, half slightly thinned out. Central circular open region. Clearly defined edges. Elevated external edge. Radial striations
K	11172021-C4	46.82 ± 7.31	1.258 ± 0.077	Opaque white outer rim. Large circular thinned area inside. Clearly defined edges. Slight radial striations.
L	11172021-C4	46.82 ± 7.31	2.233 ± 0.242	White hazy area with opaque white area inside. Pear shaped. No clear edges.
M	11042021-S4	47.15 ± 7.04	1.078 ± 0.041	Opaque white with multiple slightly thinned areas inside. Clearly defined edges. Elevated external edge. Radial striations
N	11042021-S4	57.63 ± 8.18	1.078 ± 0.041	Opaque white area with multiple slightly thinned areas inside. Clearly defined edges. Radial striations. Rough surface.
O	11172021-C2	95.58 ± 12.73	1.146 ± 0.058	Opaque white area. Clearly defined edges. Small radial striations. Rough surface.
P	11172021-C2	95.79 ± 12.76	1.113 ± 0.071	Variegated opaque white and light grayish, thinned areas. Rough surface. No clear edges.
Q	11172021-C4	96.22 ± 12.81	1.224 ± 0.049	Variegated opaque white and light grayish, thinned areas. Clearly defined edges. Some disruption external to the main area.
R	11172021-C4	96.85 ± 12.88	1.134 ± 0.033	Variegated opaque white and light grayish, thinned areas. Clearly defined edges.
S	11172021-C4	96.85 ± 12.88	1.099 ± 0.037	Variegated opaque white and light grayish, thinned areas. Clearly defined edges.
T	11172021-C2	150.07 ± 19.25	1.102 ± 0.063	Opaque white area. Clearly defined edges. Slightly elevated external edge. Small radial striations. Rough surface.
U	11172021-C2	150.28 ± 19.27	1.368 ± 0.054	Frosted glass appearance. Rough surface. No clear edges.
V	11172021-C4	150.71 ± 19.33	1.224 ± 0.027	Variegated opaque white and light grayish, thinned areas. Raised external edge. Clearly defined edges.
W	11172021-C4	151.77 ± 19.46	1.144 ± 0.028	Opaque white outer edge with uneven thinned area within and some bubbles. Clearly defined edges.
X	11172021-C4	151.77 ± 19.46	1.059 ± 0.021	Variegated opaque white and light grayish, thinned areas. Raised external edge. Clearly defined edges.
Y	11172021-C2	204.56 ± 25.90	1.106 ± 0.054	Frosted glass appearance. Brown (charred) region inside. Rough surface. No clear edges.
Exc	11172021-C4	46.604 ± 7.292	1.092 ± 0.042	Opaque white outer rim. Large circular thinned area inside. Clearly defined edges. Slight radial striations.

The results obtained from the measurements on the OCT images of the 100 ms single pulse laser induced disruptions on three different corneas were tabulated in Table 7. The length and width

of the disruptions were used to calculate the area of disruption as described in Section 3.3. Section 3.3 also details the procedure used to obtain the depth of disruption measurement, its associated uncertainty, and the uncertainty in the area of disruption calculation.

Table 7. Exposure parameters and disruption characterization from OCT images exposed to a 100 ms single pulse

ID	Sample	Radiant exposure (J/cm ²)	Width of disruption [†] (mm)	Length of disruption [‡] (mm)	Depth of disruption (mm)	Area of disruption (mm ²)
A	11042021-S4	15.35 ± 4.18	1.141	1.379	0.426 ± 0.038	1.236 ± 0.11
B	11042021-S4	15.91 ± 4.22	1.291	1.353	0.495 ± 0.033	1.372 ± 0.11
C	11042021-S4	26.57 ± 5.02	1.321	1.538	0.517 ± 0.043	1.596 ± 0.12
D	11042021-S4	26.57 ± 5.02	1.261	1.443	0.595 ± 0.040	1.429 ± 0.11
E	11042021-S4	36.49 ± 5.94	1.561	1.534	0.212 ± 0.016	1.881 ± 0.13
F	11042021-S4	36.68 ± 5.96	1.591	1.778	0.629 ± 0.036	2.222 ± 0.14
G	11172021-C2	46.18 ± 7.25	2.053	2.295	0.515 ± 0.043	3.701 ± 0.18
H	11172021-C4	46.39 ± 7.27	1.802	2.102	0.571 ± 0.035	2.975 ± 0.17
I	11172021-C2	46.60 ± 7.29	2.178	2.256	0.600 ± 0.034	3.859 ± 0.19
J	11042021-S4	46.78 ± 7.00	1.651	1.822	0.583 ± 0.039	2.363 ± 0.15
K	11172021-C4	46.82 ± 7.31	2.203	1.880	0.489 ± 0.036	3.253 ± 0.18
L	11172021-C4	46.82 ± 7.31	1.252	1.069	0.327 ± 0.030	1.051 ± 0.10
M	11042021-S4	47.15 ± 7.04	1.771	1.793	0.678 ± 0.049	2.494 ± 0.15
N	11042021-S4	57.63 ± 8.18	1.921	1.859	0.673 ± 0.044	2.805 ± 0.16
O	11172021-C2	95.58 ± 12.73	2.203	2.244	0.522 ± 0.039	3.883 ± 0.19
P	11172021-C2	95.79 ± 12.76	2.328	2.752	0.674 ± 0.043	5.032 ± 0.21
Q	11172021-C4	96.22 ± 12.81	2.002	2.636	0.659 ± 0.042	4.145 ± 0.19
R	11172021-C4	96.85 ± 12.88	2.178	2.194	0.636 ± 0.054	3.753 ± 0.19
S	11172021-C4	96.85 ± 12.88	2.178	2.346	0.668 ± 0.038	4.013 ± 0.19
T	11172021-C2	150.07 ± 19.25	2.428	2.379	0.639 ± 0.038	4.537 ± 0.21
U	11172021-C2	150.28 ± 19.27	2.353	3.182	0.579 ± 0.040	5.880 ± 0.23
V	11172021-C4	150.71 ± 19.33	2.103	2.365	0.602 ± 0.057	3.906 ± 0.19
W	11172021-C4	151.77 ± 19.46	2.228	2.643	0.637 ± 0.055	4.625 ± 0.21
X	11172021-C4	151.77 ± 19.46	2.453	2.349	0.719 ± 0.044	4.526 ± 0.21
Y	11172021-C2	204.56 ± 25.90	3.505	2.929	0.840 ± 0.064	8.063 ± 0.28

[†] The uncertainty in the width was 0.04 mm for all data.

[‡] The uncertainty in the length was 0.07 mm for all data.

Figure 9 shows plots of the aspect ratio, the depth, and area of disruption as a function of radiant exposure for the data in Table 7, *i.e.* all individual exposures at 100 ms. The aspect ratio of the disruptions varied between 1.075 to 2.233 but had an average value of 1.215 ± 0.226 (average ± standard deviation), dotted line in panel a). If we consider that the stroma begins ~70 μm below the surface of the cornea and extends about 870 μm into the cornea, then we can say that the disruption extended into the stroma for all exposures studied but none were full thickness. The depth and area of disruption increase roughly linearly as a function of radiant exposure. However, there was a large variability in the measurements extracted from different exposures, even at the same radiant exposure, more so in the depth of disruption. This variability was reflected in the R² values; 0.35 for the depth of disruption and 0.78 for the area of disruption.

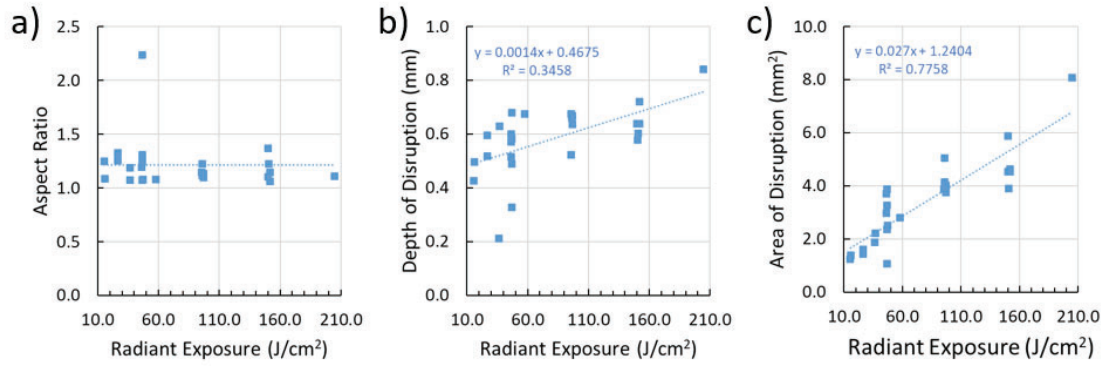


Figure 9. a) Aspect ratio obtained from the optical images of all 100 ms exposures. The b) depth and c) area of disruption of all 100 ms exposures. Dotted line in a) marks the average aspect ratio while in b) and c) they are linear fits to the data. The equation of the linear fit and R^2 value are shown in b) and c).

4.2.2 Analysis of 100 ms single pulse exposure duration results.

As a means to draw out to relationships between the parameters describing the disruption caused by the laser exposure and the radiant exposure, we averaged the data from different corneas with similar radiant exposures, see Table 8. The ID column states which exposures were considered for the averages. The uncertainty associated with the averages were obtained in a similar manner as explained in section 4.1.2. Using the average width and length of disruption, the average area of disruption and the ratio of areas was calculated, where for the 100 ms single pulse exposures, the average area of exposure from the 2 different exposure days was $1.913 \pm 0.098 \text{ mm}^2$ (average $\pm$ standard deviation), see Table 2.

Table 8. Aggregated data for 100 ms single pulse exposures

ID	Average radiant exposure (J/cm ²)	Average width of disruption (mm)	Average length of disruption (mm)	Average depth of disruption (mm)	Average area of disruption (mm ²)	Ratio of areas
A, B	15.631 $\pm$ 2.97	1.216 $\pm$ 0.028	1.366 $\pm$ 0.049	0.461 $\pm$ 0.035	1.304 $\pm$ 0.220	0.65
C, D	26.575 $\pm$ 3.55	1.291 $\pm$ 0.028	1.491 $\pm$ 0.049	0.556 $\pm$ 0.039	1.512 $\pm$ 0.236	0.75
E, F	36.583 $\pm$ 4.21	1.576 $\pm$ 0.028	1.656 $\pm$ 0.049	0.420 $\pm$ 0.209	2.051 $\pm$ 0.278	1.02
G – L	46.677 $\pm$ 2.73	1.844 $\pm$ 0.016	1.888 $\pm$ 0.029	0.538 $\pm$ 0.271	2.814 $\pm$ 1.215	1.47
N	57.63 $\pm$ 8.18	1.921 $\pm$ 0.040	1.859 $\pm$ 0.070	0.673 $\pm$ 0.044	2.805 $\pm$ 0.164	1.39
O – S	96.259 $\pm$ 5.729	2.178 $\pm$ 0.018	2.434 $\pm$ 0.029	0.632 $\pm$ 0.218	4.165 $\pm$ 0.987	2.18
T – X	150.918 $\pm$ 8.655	2.313 $\pm$ 0.018	2.584 $\pm$ 0.029	0.635 $\pm$ 0.235	4.695 $\pm$ 1.052	2.45
Y	204.56 $\pm$ 25.90	3.505 $\pm$ 0.040	2.929 $\pm$ 0.070	0.840 $\pm$ 0.064	8.063 $\pm$ 0.285	4.44

Figure 10 shows plots of the depth of disruption, the area of disruption, and the ratio of the areas as a function of radiant exposure for the data in Table 8, *i.e.* the average of exposures with similar radiant exposures at 100 ms. The process of averaging greatly reduced the variability in the data such that the R^2 value of the linear fits increased up to 0.72 for the depth of disruption and up to 0.95 for the area of disruption relative to the original data. The ratio of the areas increased directly proportional to the increase in radiant exposure, resulting in an R^2 value of 0.94 for the linear fit.

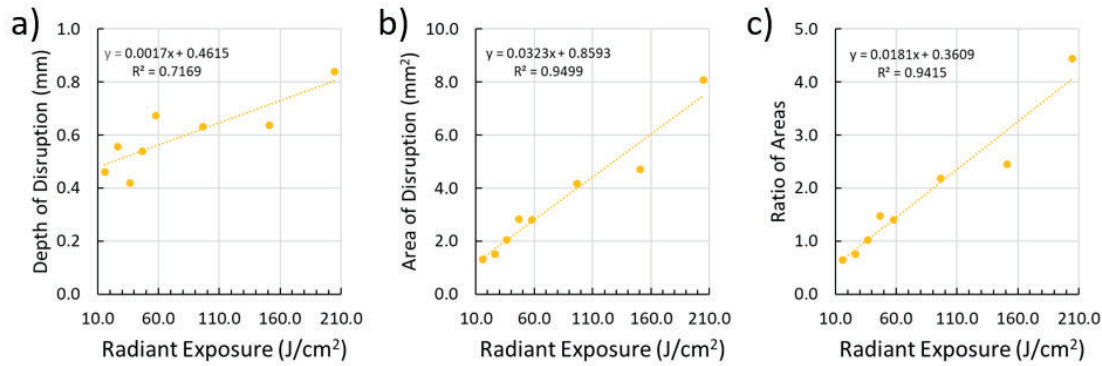


Figure 10. Plots of the a) average depth of disruption and b) average area of disruption. c) ratio of the average area of disruption to the average area of exposure. Dotted lines are linear fits to the data. The equations of the linear fits and R^2 values are shown in each panel.

The ratio of areas is larger than one for all radiant exposure doses larger than $\sim 36 \text{ J/cm}^2$, indicating that the laser exposure affected a larger area than that directly illuminated by the laser. This was due to thermal effects related to the length of the pulse and the high absorption coefficient of water at 1940 nm. We speculate that there are also thermomechanical effects, *i.e.* contraction, that result in the appearance of a radial striation pattern around the disruption for most radiant exposure doses with 100 ms pulse durations.

5.0 DISCUSSION

The objective of this study was to document and characterize supra-threshold corneal damage in response to 10 ms and 100 ms single pulse exposure durations at a strongly absorbing wavelength of 1940 nm in excised porcine corneas. For 10 ms exposure durations, the radiant exposures ranged from ~ 20 to 34 J/cm^2 , which corresponded to about 100 to 200 times the MPE limit [13]. For 100 ms exposures, the radiant exposures ranged from ~ 15 to 200 J/cm^2 , which corresponded to around 50 to 650 times the MPE limit. The appearance of corneal disruptions at a range of radiant exposures was documented using optical images and an OCT system. The optical images were used to describe the disruption characteristics and extract their aspect ratio or circularity. The OCT images were used to measure the length, width, and depth of the disruptions as well as confirm the interpretation of the optical images.

Optical images showed that for 10 ms single pulse exposure durations, the tissue disruption evolved from a white hazy area with undefined edges, to an opaque white area with larger, amorphously shaped translucent areas inside and an elevated ridge at the edge, to an opaque white area with small depressions inside for higher radiant exposures. The tissue appeared to be disrupted primarily by heat, having a melted or coagulated appearance which was confirmed by the OCT images. From the images collected, it could not be verified that there was removal of tissue as is typically described by the terms ablation or vaporization. Both the optical images and the OCT images were analyzed to extract quantitative parameters to describe the disruption. Averaging over multiple exposure in different corneas but with similar radiant exposures enabled us to determine that the average depth of disruption and the average area of disruption as a function of radiant exposure both followed linear relationships with R^2 values of ~ 0.8 and ~ 0.7 respectively. From comparing the area of disruption to the area of the laser spot, it was determined that for most radiant exposures used, the central region of the Gaussian laser beam profile caused the disruptions in the corneal tissue, *i.e.* the affected area was smaller than the area of the laser spot size. This also appeared to indicate that radial thermal diffusion effects did not play a significant role during the 10 ms duration exposures.

For 100 ms single pulse exposure durations, the optical images showed that the tissue disruption for lower radiant exposures were similar to the 10 ms exposures in the sense that there was a white hazy area with undefined edges. However, as the radiant exposure increased, the inner portion consisted of large amorphously shaped, thinned areas surrounded by an opaque, white, raised edge. It was speculated that the laser heats up the water within the tissue, vaporizing it and generating bubbles. Simultaneously, the remaining tissue melts and re-solidifies as it cools down. The bubbles disappear over time and what remains appears to be thinned out compared to the edges. The OCT images show a very rugged surface that extends above the unexposed corneal surface consistent with the formation of bubbles underneath the surface. However, from the images collected, it could not be unambiguously verified that there was removal of tissue as is typically described by the terms ablation or vaporization. Both the optical images and the OCT images were analyzed to extract quantitative parameters to describe the disruption. Averaging over multiple exposures in different corneas but with similar radiant energies enabled us to determine that the average depth of disruption and the average area of disruption as a function of radiant exposure both followed a linear relationship with R^2 values of ~ 0.7 and ~ 0.9 respectively. For the majority of the radiant exposures used, the area of disruption was larger than the area directly illuminated by the laser, so the ratio of areas were larger than 1. This is due to thermal effects related to the length of the pulse and the high absorption coefficient of water at 1940 nm. In addition, there is potentially a thermomechanical effect, *i.e.* contraction of the tissue that gives rise to the radial striations observed in the images around the disruptions.

The current study has concluded that 10 ms and 100 ms single pulses at a wavelength of 1940 nm produce corneal tissue disruption primarily through photothermal effects. There was no clear evidence of tissue removal through ablation. Instead, there was apparent melting and coagulation of the tissue. For 100 ms single pulse durations, in addition to a photothermal effect, there appeared to be a thermomechanical effect manifested as radial striations around the disruption. For all exposure conditions tested, the disruptions extended into the stroma, therefore it can be inferred that the collagen lamella that form the stroma were melted due to the laser exposure. The fact that the melting point of fibrous collagen is only around 60°C, while that of cross linked assemblies like those found in tissue is around 70°C [34], supports the notion that photothermal effects are primarily responsible for the corneal disruptions observed from 10 ms and 100 ms exposure durations at 1940 nm.

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APPENDIX A. DETAILED DATA COLLECTED

The delivered energy used to calculate the radiant exposures as well as the major and minor axis measurements for 10 ms exposure durations are tabulated in Table 9. The area used to obtain the radiant exposure are found in Table 2.

Table 9. Detailed exposure parameters and disruption characterization obtained from optical images for 10 ms exposures

ID	Sample	Energy (J)	Radiant exposure (J/cm ²)	Major axis (pixels)	Minor axis (pixels)	Major axis / minor axis
A	11032021-C2	0.228 ± 0.015	19.15 ± 1.90	90.938 ± 3.012	66.272 ± 2.194	1.372 ± 0.091
B	11032021-C2	0.239 ± 0.015	20.09 ± 1.99	117.654 ± 1.088	95.848 ± 0.887	1.228 ± 0.023
C	11032021-C2	0.250 ± 0.016	21.06 ± 2.08	122.558 ± 3.295	82.584 ± 3.715	1.484 ± 0.107
D	11032021-C2	0.250 ± 0.016	21.06 ± 2.08	96.640 ± 1.873	89.348 ± 1.731	1.082 ± 0.042
E	10202021-C4	0.230 ± 0.017	21.75 ± 2.85	103.882 ± 1.753	75.704 ± 1.276	1.372 ± 0.046
F	11032021-C2	0.262 ± 0.016	22.03 ± 2.16	119.110 ± 2.005	110.122 ± 1.855	1.082 ± 0.036
G	11032021-C2	0.262 ± 0.016	22.07 ± 2.17	101.660 ± 3.201	78.568 ± 2.473	1.294 ± 0.081
H	11032021-C2	0.274 ± 0.017	23.07 ± 2.26	122.004 ± 1.476	111.326 ± 2.241	1.096 ± 0.035
I	10202021-C4	0.274 ± 0.019	25.90 ± 3.32	114.200 ± 2.270	101.958 ± 2.882	1.120 ± 0.054
J	10202021-C4	0.274 ± 0.019	25.90 ± 3.32	125.380 ± 1.400	108.178 ± 2.216	1.159 ± 0.037
K	10202021-C4	0.319 ± 0.021	30.18 ± 3.82	120.430 ± 1.106	92.586 ± 0.853	1.301 ± 0.024
L	10202021-C4	0.319 ± 0.021	30.18 ± 3.82	116.984 ± 1.868	85.974 ± 1.368	1.361 ± 0.043
M	10202021-C4	0.362 ± 0.023	34.30 ± 4.31	142.560 ± 2.268	115.334 ± 1.834	1.236 ± 0.039
Exc	10202021-C4	0.230 ± 0.017	21.75 ± 2.85	88.398 ± 2.760	48.226 ± 2.381	1.833 ± 0.148

The delivered energy used to calculate the radiant exposures as well as the major and minor axis measurements for 100 ms exposure durations are tabulated in Table 10. The areas used to obtain the radiant exposure are found in Table 2.

Table 10. Detailed exposure parameters and disruption characterization obtained from optical images for 100 ms exposures

ID	Sample	Energy (J)	Radiant exposure (J/cm ²)	Major axis (pixels)	Minor axis (pixels)	Major axis / minor axis
A	11042021-S4	0.154 ± 0.039	15.35 ± 4.18	86.37 ± 1.64	69.17 ± 1.31	1.249 ± 0.047
B	11042021-S4	0.160 ± 0.039	15.91 ± 4.22	89.84 ± 1.44	83.06 ± 1.33	1.082 ± 0.035
C	11042021-S4	0.267 ± 0.041	26.57 ± 5.02	104.11 ± 2.65	78.65 ± 2.83	1.324 ± 0.081
D	11042021-S4	0.267 ± 0.041	26.57 ± 5.02	104.15 ± 0.86	83.22 ± 1.34	1.251 ± 0.030
E	11042021-S4	0.367 ± 0.045	36.49 ± 5.94	113.11 ± 6.43	95.41 ± 4.16	1.186 ± 0.119
F	11042021-S4	0.369 ± 0.045	36.68 ± 5.96	134.01 ± 2.54	124.68 ± 2.37	1.075 ± 0.041
G	11172021-C2	0.419 ± 0.048	46.18 ± 7.25	164.43 ± 4.60	137.99 ± 4.73	1.192 ± 0.074
H	11172021-C4	0.421 ± 0.048	46.39 ± 7.27	141.88 ± 1.70	108.56 ± 2.76	1.307 ± 0.049
I	11172021-C2	0.423 ± 0.048	46.60 ± 7.29	176.25 ± 3.87	140.30 ± 3.08	1.256 ± 0.055
J	11042021-S4	0.470 ± 0.049	46.78 ± 7.00	138.49 ± 0.85	128.85 ± 0.79	1.075 ± 0.013
K	11172021-C4	0.425 ± 0.048	46.82 ± 7.31	172.95 ± 7.08	137.46 ± 2.83	1.258 ± 0.077
L	11172021-C4	0.425 ± 0.048	46.82 ± 7.31	104.09 ± 2.02	46.61 ± 4.13	2.233 ± 0.242
M	11042021-S4	0.474 ± 0.049	47.15 ± 7.04	134.06 ± 2.68	124.34 ± 2.29	1.078 ± 0.041
N	11042021-S4	0.579 ± 0.053	57.63 ± 8.18	134.06 ± 2.68	124.34 ± 2.29	1.078 ± 0.041
O	11172021-C2	0.867 ± 0.067	95.58 ± 12.73	171.39 ± 3.18	149.59 ± 4.75	1.146 ± 0.058
P	11172021-C2	0.869 ± 0.067	95.79 ± 12.76	213.71 ± 8.23	191.97 ± 4.78	1.113 ± 0.071
Q	11172021-C4	0.873 ± 0.067	96.22 ± 12.81	166.15 ± 2.53	135.77 ± 3.38	1.224 ± 0.049
R	11172021-C4	0.879 ± 0.068	96.85 ± 12.88	166.63 ± 1.53	146.99 ± 2.88	1.134 ± 0.033
S	11172021-C4	0.879 ± 0.068	96.85 ± 12.88	176.77 ± 1.80	160.83 ± 3.78	1.099 ± 0.037
T	11172021-C2	1.362 ± 0.093	150.07 ± 19.25	184.92 ± 1.59	167.75 ± 8.21	1.102 ± 0.063
U	11172021-C2	1.363 ± 0.093	150.28 ± 19.27	215.04 ± 3.57	157.17 ± 3.59	1.368 ± 0.054
V	11172021-C4	1.367 ± 0.093	150.71 ± 19.33	177.36 ± 1.36	144.85 ± 2.10	1.224 ± 0.027
W	11172021-C4	1.377 ± 0.094	151.77 ± 19.46	183.00 ± 3.47	159.90 ± 0.83	1.144 ± 0.028
X	11172021-C4	1.377 ± 0.094	151.77 ± 19.46	188.98 ± 1.28	178.41 ± 2.35	1.059 ± 0.021
Y	11172021-C2	1.856 ± 0.121	204.56 ± 25.90	217.46 ± 3.30	196.67 ± 6.60	1.106 ± 0.054
Exc	11172021-C4	0.423 ± 0.048	46.604 ± 7.292	127.63 ± 1.37	116.90 ± 3.25	1.092 ± 0.042