



AFRL-RH-FS-TR-2022-0018

**Investigation of NIR femtosecond Cornea
Damage Threshold in Dutch Belted Rabbits**

Xomalin G. Peralta
Joseph E. Clary
Amanda M. Peterson
Brian J. Lund
Gary Noojin
SAIC

Amanda J. Tijerina
Conceptual MindWorks, Inc.

Benjamin A. Rockwell
Morgan S. Schmidt
Francesco Echeverria
Wesley Kinerk
711th Human Performance Wing
Airman Systems Directorate
Bioeffects Division
Optical Radiation Branch

April 2022
Interim Report for January 2020 to April 2022

DESTRUCTION NOTICE – Destroy by any method that will prevent disclosure of contents or reconstruction of this document.

Distribution Statement A. Approved for public release; distribution is unlimited. Cleared: PA Case # AFRL-2022-5749. The opinions expressed on this document, electronic or otherwise, are solely those of the author(s). They do not represent an endorsement by or the views of the United States Air Force, the Department of Defense, or the United States Government

Air Force Research Laboratory
711th Human Performance Wing
Airman Systems Directorate
Bioeffects Division
Optical Radiation Bioeffects Branch
JBSA Fort Sam Houston, Texas
78234

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation; or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

This report was cleared for public release by the AFRL Public Affairs Office and is available to the general public, including foreign nationals. Copies may be obtained from the Defense Technical Information Center (DTIC) (<http://www.dtic.mil>).

"Investigation of NIR femtosecond Cornea Damage Threshold in Dutch Belted Rabbits"

(AFRL-RH-FS-TR- 2022 0018) has been reviewed and is approved for publication in accordance with assigned distribution statement.

FERRIS.LYNDSEY.
MARIE.1381070391

Digitally signed by
FERRIS.LYNDSEY.MARIE.1381070391
Date: 2022.05.02 15:40:46 -05'00'

LYNDSEY M. FERRIS, Maj, USAF, BSC
Chief, Optical Radiation Bioeffects Branch

MILLER.STEPHANIE
E.A.1230536283

Digitally signed by
MILLER.STEPHANIE.A.1230536283
Date: 2022.11.18 11:54:02 -06'00'

STEPHANIE A. MILLER, DR-IV, DAF
Chief, Bioeffects Division
Airman Systems Directorate
711th Human Performance Wing
Air Force Research Laboratory

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute an official position of the U.S. Government.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 19-04-2022		2. REPORT TYPE Interim Technical Report		3. DATES COVERED (From - To) January 2020 – March 2022	
4. TITLE AND SUBTITLE Investigation of NIR femtosecond Cornea Damage Threshold in Dutch Belted Rabbits				5a. CONTRACT NUMBER FA8650-19-C-6024	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Xomalin G. Peralta, Joseph E. Clary, Amanda M. Peterson, Brian J. Lund, Gary Noojin, Amanda J. Tijerina, Benjamin A. Rockwell, Morgan S. Schmidt, Francesco Echeverria, Wesley Kinerk				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER H14B	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) 711th Human Effectiveness Wing Airman Systems Directorate Bioeffects Division Optical Radiation Branch JBSA, Fort Sam Houston, Texas 78234				8. PERFORMING ORGANIZATION REPORT	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) 711th Human Performance Wing Airman Systems Directorate Bioeffects Division Optical Radiation Branch JBSA, Fort Sam Houston, Texas 78234				10. SPONSOR/MONITOR'S ACRONYM(S) 711 HPW/RHDO	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) AFRL-RH-FS-TR-2022-0018	
12. DISTRIBUTION / AVAILABILITY STATEMENT Distribution Statement A. Approved for public release; distribution is unlimited. CLEARED: PA Case # AFRL-2022-5749. The opinions expressed on this document, electronic or otherwise, are solely those of the author(s). They do not represent an endorsement by or the views of the United States Air Force, the Department of Defense, or the United States Government.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Pulsed lasers with ultrashort pulse durations have become ubiquitous in a variety of military and civilian applications. Although the upcoming revision to the ANSI laser safety standard provides exposure limits for wavelengths longer than 1400 nm and pulse durations as short as 100 fs, it is unclear how to assess the safety of femtosecond long pulse durations with much smaller beam diameters than the 1 mm limiting diameter aperture. In addition, there is not much biological data to support the safety standards for exposures to single femtosecond pulse durations. In this study we determined the median effective dose, or ED ₅₀ , that produces a minimum visible lesion (MVL) on the cornea upon exposure to a single, approximately 100 fs pulse at three different NIR wavelength; 1318 nm, 1540 nm, and 2000 nm. The resulting ED ₅₀ values were compared to the MPE limits directly and by considering a 1 mm diameter limiting aperture. Notably, the comparisons were not in agreement, highlighting the need for the ANSI standard to clarify how to assess small diameter beams for ultrashort pulses and to potentially review the MPE values for NIR sub-nanosecond exposures. More generally, this study contributes to the current understanding of laser ablation dynamics in cornea as well as the knowledge base used for setting laser safety standards in the NIR range for ocular exposure to ultrashort pulses.					
15. SUBJECT TERMS ultrashort, laser safety, MPE, femtosecond, damage threshold					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
Unclassified					19b. TELEPHONE NUMBER (include area code)
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	U	93	Francesco J. Echeverria 210-539-8205

This Page Intentionally Left Blank

TABLE OF CONTENTS

Section	Page
TABLE OF CONTENTS	i
LIST OF FIGURES	iv
LIST OF TABLES	viii
1.0 SUMMARY	1
2.0 INTRODUCTION	1
2.1 Background	1
2.2 Approach	3
3.0 METHODS	4
3.1 Animal Model	4
3.1.1. Sedation.....	4
3.1.2. Pre-exposure preparation and examination.....	5
3.1.3. Laser exposure.	5
3.1.4. 1 h Post-exposure examination.	6
3.1.5. Animal recovery.....	6
3.1.6. 24 h Post-exposure examination.	7
3.2 Exposure Setup	7
3.2.1. Optical setup.	7
3.2.2. Single shot capability and data acquisition.	9
3.2.3. Laser characterization, alignment and calibration.	9
3.3 Experimental Protocol	12
3.3.1. Data collected during baseline exams.....	13
3.3.2. Data collected during exposures.	13
3.3.3. Data collected during 1 h and 24 h post-exposure examinations.....	13
3.4 Data Analysis	14
3.4.1. Initial lesion identification and analysis.	14
3.4.2. Final lesion identification.	14
3.4.3. Peak radiant exposure calculations and uncertainty.	15
3.4.4. Probit analysis.....	19
3.4.5. Comparison to the current laser safety standards.....	20
4.0 RESULTS	20
4.1 Wavelength 1 – 1318 nm	20

4.1.1.	Representative images.	20
4.1.2.	Probit analysis.	25
4.2	Wavelength 2 – 1540 nm	25
4.2.1.	Representative images.	25
4.2.2.	Probit analysis.	28
4.3	Wavelength 3 – 2000 nm	29
4.3.1.	Representative images.	29
4.3.2.	Probit analysis.	31
5.0	DISCUSSION	32
5.1	Comparison of ED ₅₀ Values to Current Laser Safety Standards	33
5.2	Comparison of Injury Characteristics Between Wavelengths	34
5.3	Insights Gained	36
5.4	Conclusions	36
6.0	REFERENCES	37
	APPENDIX A – List of Instrumentation	41
	APPENDIX B – Laser Beam Profiles	44
	APPENDIX C – Subject Summary and Exposure Characteristics	47
	APPENDIX D – Cornea Exposures	48
D.1	Wavelength 1 – 1318 nm	48
D.1.1.	Subject Ra21-006 OD.	48
D.1.1.1.	<i>Pre-exposure examination.</i>	48
D.1.1.2.	<i>Post-exposure examination and evaluation.</i>	48
D.1.1.3.	<i>Data.</i>	49
D.1.2.	Subject Ra21-003 OS.	49
D.1.2.1.	<i>Pre-exposure examination.</i>	49
D.1.2.2.	<i>Post-exposure examination and evaluation.</i>	50
D.1.2.3.	<i>Data.</i>	51
D.1.3.	Subject Ra21-007 OD.	52
D.1.3.1.	<i>Pre-exposure examination.</i>	52
D.1.3.2.	<i>Post-exposure examination and evaluation.</i>	52
D.1.3.3.	<i>Data.</i>	53
D.1.4.	Subject Ra21-010 OS.	54
D.1.4.1.	<i>Pre-exposure examination.</i>	54
D.1.4.2.	<i>Post-exposure examination and evaluation.</i>	54

D.1.4.3.	<i>Data</i>	56
D.1.5.	Comparison of various probit analyses.....	58
D.1.6.	Cumulative probit analysis.	59
D.2	Wavelength 2 – 1540 nm	60
D.2.1.	Subject Ra21-009 OD.....	60
D.2.1.1.	<i>Pre-exposure examination</i>	60
D.2.1.2.	<i>Post-exposure examinations</i>	60
D.2.1.3.	<i>Data</i>	62
D.2.2.	Subject Ra21-007 OS.....	62
D.2.2.1.	<i>Pre-exposure examination</i>	62
D.2.2.2.	<i>Post-exposure examinations</i>	62
D.2.2.3.	<i>Data</i>	64
D.2.3.	Subject Ra21-010 OD.....	64
D.2.3.1.	<i>Pre-exposure examination</i>	64
D.2.3.2.	<i>Post-exposure examinations</i>	65
D.2.3.3.	<i>Data</i>	66
D.2.4.	Cumulative probit analysis.	66
D.3	Wavelength 3 – 2000 nm	68
D.3.1.	Subject Ra21-004 OD.....	68
D.3.1.1.	<i>Pre-exposure examination</i>	68
D.3.1.2.	<i>Post-exposure examinations</i>	68
D.3.1.3.	<i>Data</i>	69
D.3.2.	Subject Ra21-005 OS.....	70
D.3.2.1.	<i>Pre-exposure examination</i>	70
D.3.2.2.	<i>Post-exposure examinations</i>	70
D.3.2.3.	<i>Data</i>	71
D.3.3.	Subject Ra21-003 OD.....	72
D.3.3.1.	<i>Pre-exposure examination</i>	72
D.3.3.2.	<i>Post-exposure examinations</i>	72
D.3.3.3.	<i>Data</i>	73
D.3.4.	Subject Ra21-006 OS.....	73
D.3.4.1.	<i>Pre-exposure examination</i>	73
D.3.4.2.	<i>Post-exposure examinations</i>	73
D.3.4.3.	<i>Data</i>	75

D.3.5.	Cumulative probit analysis	75
APPENDIX E – Evaluation of the Supercontinuum Generated		78
E.1	Wavelength 1 – 1318 nm	78
E.2	Wavelength 2 – 1540 nm	79
E.3	Wavelength 3 – 2000 nm	80

LIST OF FIGURES

Figure 1. Schematic diagram of the human eye. Inset shows the layers that comprise the cornea.	2
Figure 2. Optical setup for sub-ns cornea exposures. SM: silver mirror. NDF: Neutral density filter. PD: Photodiode. W: window. GM: gold mirror. Ref. Meter: Reference detectors (power meter). LPF: long-pass filter. L: lens. BSC: Beam splitter cube. Cam 1: Imperx CCD camera. Cam 2: Teledyne Nano GigE camera. Note: Cam 1 is located at a different height than the NIR beam and provides a straight-on view of the target. NDF* was mounted on a flip mount so it could be placed into the beam path when needed.	7
Figure 3. Schematic diagram showing the sequence and timing of events used to implement the single shot capability. Each line represents an oscilloscope trace.	9
Figure 4. Schematic diagram of the knife edge technique used to obtain the focal spot size diameter. Blue lines represent the locations where measurements were take, z is the direction of propagation.	11
Figure 5. Representative calibration graphs: a) and b) pre-exposure calibration; c) and d) post-calibration procedure. Different color dots correspond to the same half-wave plate and neutral density filter combinations. Graphs b) and d) correspond to exposure energies which required the use of the neutral density filter on the flip mount. Data shown here was obtained from the experiment conducted on 05/26/2021.	16
Figure 6. Slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-007 OD. Arrows indicate laser-induced lesions. Circles in panels e) and f) highlight a small abrasion unrelated to laser exposure. Panels b), d), and f) were taken after fluorescein was applied. One lesion identified in panel c) is also visible in panels d), g), and h). Panel h) is a magnified view of panel g).	21
Figure 7. Subject Ra21-006 OD $0.7 \times ED_{50}$. a) Slit lamp image showing the location of the lesion studied in the following panels as well as a large abrasion in the central region. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion.	22
Figure 8. Subject Ra21-007 OD $1.1 \times ED_{50}$. a) Slit lamp image showing the location of the lesion studied in the following panels. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion and possible damage to Descemet's membrane.	22
Figure 9. Subject Ra21-007 OD $7.1 \times ED_{50}$. a) Slit lamp image showing the location of the lesion studied in the following panels. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion and epithelial ablation.	23

Figure 10. Slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-003 OS. Arrows indicate laser-induced lesions. Panels b), d), and g) were taken after fluorescein was applied. Panel f) is a magnified view of panel e). Two lesions identified in panel c) are also visible in panel d). Lesions identified in panel e) are also visible in panel g).....	24
Figure 11. Subject Ra21-003 OS 3.3×ED ₅₀ . a) Slit lamp image showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scans shown in the following panel. c) B-scan showing a full-thickness corneal lesion and apparent disruption of the Descemet membrane.....	24
Figure 12. Probit analysis of exposures at 1318 nm for 3 subjects.....	25
Figure 13. Representative slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-010 OD. Circle highlights a feature resembling a laser-induced lesion identified in the baseline examination. Arrows indicate laser-induced lesions. Panels c), f), and g) were taken after fluorescein was applied. The two lesions identified in panel d) are also visible in panels e) and f).....	26
Figure 14. Subject Ra21-010 OD 0.9×ED ₅₀ . a) Slit lamp image showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing epithelial damage that possibly extends to Bowman's layer.....	27
Figure 15. Subject Ra21-010 OD 1.2×ED ₅₀ . a) Slit lamp image showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion with damage to Bowman's layer.....	27
Figure 16. Subject Ra21-009 OD 3.9×ED ₅₀ . a) Slit lamp image showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion with damage to the stroma below Bowman's layer.....	28
Figure 17. Probit analysis of exposures at 1540 nm.....	28
Figure 18. Representative slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-003 OD. Arrows indicate laser-induced lesions. Panels b), e), and g) were taken after fluorescein was applied. Panel d) corresponds to a magnified view of panel c).	29
Figure 19. Subject Ra21-005 OS 1.2×ED ₅₀ . a) Slit lamp image with fluorescein showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan presented in the following panel. c) B-scan of the lesion showing damage to the epithelium.....	30
Figure 20. Subject Ra21-006 OS 2.3×ED ₅₀ . a) Slit lamp image with fluorescein showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan presented in the following panel. c) B-scan of the lesion showing epithelial ablation.....	30
Figure 21. Subject Ra21-006 OS 3×ED ₅₀ . a) Slit lamp image with fluorescein showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow	

indicating the location of the B-scan presented in the following panel. c) B-scan of the lesion showing epithelial ablation.	31
Figure 22. Probit analysis of exposures at 2000 nm.	31
Figure 23. a) Comparison of MPE limits (black line), peak ED ₅₀ (crosses), and average ED ₅₀ values (exes). b) Cornea transmittance (black line) where vertical lines are located at 1318 nm (solid green line), 1540 nm (dotted red line), and 2000 nm (dashed yellow line).	34
Figure 24. Beam profiles at 1318 nm for exposures performed on a) 05/12/2021, b) 05/17/2021, c) 05/24/2021, and d) 06/14/2021.	44
Figure 25. Beam profiles at 1540 nm for exposures performed on a) 05/26/2021, b) 06/01/2021, and c) 06/21/2021.	45
Figure 26. Beam profiles at 2000 nm for exposures performed on a) 05/04/2021, b) 05/06/2021, c) 05/10/2021, and d) 06/03/2021.	46
Figure 27. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	48
Figure 28. Slit lamp images a) without and b) with fluorescein showing a large abrasion in the central region of the cornea.	49
Figure 29. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	50
Figure 30. Image showing the SC light generated during exposure 3 as captured by the front-viewing camera. The open white circle corresponds to the location of the alignment laser. The blueish comet shape is the SC generated on the surface of the cornea as viewed from the vantage point of the front-viewing camera.	50
Figure 31. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. Note that in exposures 1, 3, and 5 the eye was not adequately illuminated with the red LED light during the exposure. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure therefore that image is the corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	51
Figure 32. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	52
Figure 33. Slit lamp images a) without and b) with fluorescein showing a small abrasion in the upper right hand side of the cornea (white circle).	53
Figure 34. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. Note that in exposure 1 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	53
Figure 35. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations. During the first exposure, some pixels in the front-view camera got damage, appearing as a white crescent above and to the right of the solid dot representing the alignment laser.	54
Figure 36. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. The image for exposure 1 has a large number of pixels saturated, presumably due to the high energy incoming NIR beam. The corresponding pre-shot	

image is shown as an inset on the upper left for reference. Pixels that got damage during the first exposure form a white crescent shape above and to the right of the exposure location. Note that in exposures 2 and 6 the eye was not adequately illuminated with the red LED light during the exposure. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure therefore that image is the corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.....55

Figure 37. Slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-010 OS. Arrows indicate laser-induced lesions. Panels b), d), and g) were taken after fluorescein was applied. Panel f) is a magnified view of panel e). Several of the lesions identified in panel c) are also visible in the other panels. The elongated appearance of the lesions is more apparent in panels d), e), and f). 56

Figure 38. Probit analysis for 4 subjects exposed to 1318 nm. The area used to calculate the peak radiant exposure for Ra010-OS utilized a) the knife edge measurement for d_x from the day of the exposures and the ratio obtained from the slit lamp images of the injuries for d_y (Method 2) and b) the knife edge measurements for d_x and d_y from the day following the exposures (Method 3). 58

Figure 39. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Note that in exposure 4 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations. 61

Figure 40. Images showing the SC light generated during the exposure as captured by the front-viewing camera. Note that in exposures 1, 4, and 6 the eye was not adequately illuminated with the red LED light during the exposure. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations. 61

Figure 41. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Note that in exposure 4 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations. 63

Figure 42. Images showing the SC light generated during the exposure as captured by the front-viewing camera. In exposures 2, 3, 5, 6, and 7 the eye was not adequately illuminated with the red LED light during the exposure. In exposures 2, 4, and 6 the SC light generated is extremely weak so the brightness and contrast of the image was adjusted. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure so that image is the corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations. 63

Figure 43. Representative slit lamp images a) without and b) with fluorescein of subject Ra21-007 OS. Arrows indicate identified laser induced lesions. 64

Figure 44. Slit lamp examinations showing an apparent feature in the cornea of subject Ra21-010 OD during the baseline examination. Feature is highlighted by a black circle in panel b) but is not visible when fluorescein was applied, panel c). 65

Figure 45. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations. 65

Figure 46. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. Note that in exposure 1 the eye was not adequately illuminated with the red LED light during the exposure. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure therefore that image is the

corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	66
Figure 47. Hand drawn map showing the exposure locations for subject Ra21-004 OS.....	69
Figure 48. Slit lamp images with fluorescein of subject Ra21-004 OD 1 h post-exposure. Arrows indicate laser-induced lesions. One lesion identified in a) is also visible in b).....	69
Figure 49. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Note that in exposure 1, 2, 8, and 9 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	71
Figure 50. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Note that in exposure 6 and 9 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	72
Figure 51. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	74
Figure 52. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. Given that the SC light captured in the image corresponding to exposure 2 was extremely weak, the brightness and contrast was adjusted to aid in locating it. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure therefore that image is the corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.	74
Figure 53. Optical setup used to measure the spectra of the SC generated by a 1 mm thick glass slide.	78
Figure 54. a) Spectra of incoming NIR laser beam (orange) and SC spectra (black). b) Spectra of the SC generated with an incoming energy of 90 μ J at 1318 nm (black) and the 3 rd harmonic of 1318 nm (yellow).	78
Figure 55. Photograph of the SC generated by 1318 nm on a white piece of paper.	79
Figure 56. a) Spectra of incoming NIR laser beam (orange) and SC spectra (black). b) Spectra of the SC generated with an incoming energy of 90 μ J at 1540 nm (black) and the 3 rd harmonic of 1540 nm (yellow).	79
Figure 57. Photograph of the SC generated by 1540 nm on a white piece of paper.	79
Figure 58. a) Spectra of incoming NIR laser beam (orange) and SC spectra (black). b) Spectra of the SC generated with an incoming energy of 90 μ J at 2000 nm (black) and the 3 rd harmonic of 2000 nm (yellow).	80
Figure 59. Photograph of the SC generated by 2000 nm on a white piece of paper.	80

LIST OF TABLES

Table 1. Average laser beam parameters	11
Table 2. Parameters of the probit analysis for 1 h post-exposure damage thresholds	32
Table 3. Comparison of the ED ₅₀ to current MPE limits	33
Table 4. Lesion characteristics based on the radiant exposure relative to the ED ₅₀	35
Table 5. List of instrumentation for laser exposures.....	41
Table 6. List of instrumentation used during eye exams.....	41

Table 7. Instrument make, model, serial number, and calibration date	43
Table 8. Beam parameters for exposures at 1318 nm	44
Table 9. Beam parameters for exposures at 1540 nm	45
Table 10. Beam parameters for exposures at 2000 nm	45
Table 11. Summary of subjects and exposure parameters	47
Table 12. Exposure parameters and 1 h post-exposure scoring: Ra21-006 OD at 1318 nm.....	49
Table 13. 24 h post-exposure scoring for Ra21-006 OD at 1318 nm	49
Table 14. Exposure parameters and 1 h post-exposure scoring: Ra21-003 OS at 1318 nm	51
Table 15. Exposure energy and 24 h post-exposure scoring for Ra21-003 OS at 1318 nm	52
Table 16. Exposure parameters and 1 h post-exposure scoring: Ra21-007 OD at 1318 nm.....	53
Table 17. 24 h post-exposure scoring for Ra21-007 OD at 1318 nm	54
Table 18. Focal spot size diameter calculations	56
Table 19. Exposure parameters (Method 2) and 1 h post-exposure scoring: Ra21-010 OS	57
Table 20. Exposure parameters (Method 3) and 1 h post-exposure scoring: Ra21-010 OS	57
Table 21. 24 h post-exposure scoring for Ra21-010 OS at 1318 nm	57
Table 22. Summary of the probit analyses conducted	58
Table 23. Three subject raw probit data for exposures at 1318 nm	59
Table 24. Exposure parameters and 1 h post-exposure scoring: Ra21-009 OD at 1540 nm.....	62
Table 25. 24 h post-exposure scoring for Ra21-009 OD at 1540 nm	62
Table 26. Exposure parameters and 1 h post-exposure scoring: Ra21-007 OS at 1540 nm	64
Table 27. Exposure energy and 24 h post-exposure scoring for Ra21-007 OS at 1540 nm	64
Table 28. Exposure parameters and 1 h post-exposure scoring: Ra21-010 OD at 1540 nm.....	66
Table 29. Raw probit data for exposures at 1540 nm.....	66
Table 30. Exposure parameters and 1 h post-exposure scoring: Ra21-004 OD at 2000 nm.....	70
Table 31. 24 h post-exposure scoring for Ra21-004 OD at 2000 nm	70
Table 32. Exposure parameters and 1 h post-exposure scoring: Ra21-005 OS at 2000 nm	71
Table 33. 24 h post-exposure scoring for Ra21-005 OS at 2000 nm.....	72
Table 34. Exposure parameters and 1 h post-exposure scoring: Ra21-003 OD at 2000 nm.....	73
Table 35. 24 h post-exposure scoring for Ra21-003 OD at 2000 nm	73
Table 36. Exposure parameters and 1 h post-exposure scoring: Ra21-006 OS at 2000 nm	75
Table 37. Raw probit data for exposures at 2000 nm.....	75

1.0 SUMMARY

Advances in ultrashort laser technology have motivated a steady increase in the availability and use of these systems at near infrared (NIR) wavelengths in both military and civilian applications. The 2022 revision of the American National Standard for Safe Use of Lasers (ANSI Z136.1-2022) includes ocular Maximum Permissible Exposure (MPE) limits for wavelengths from 400 nm up to 10,000 nm with 100 fs pulse durations, where the MPE limits are averaged over a 1 to 11 mm limiting diameter aperture. This study determined the minimum visible lesion (MVL) damage threshold in live Dutch-belted rabbit corneas from single femtosecond long pulses at three NIR wavelengths: 1318 nm, 1540 nm, and 2000 nm. The MVL damage thresholds are reported as the median effective dose (ED_{50}) and were obtained using probit analysis. The resulting ED_{50} values were compared with the current MPE limits using two different approaches. In the first approach, the peak ED_{50} was directly compared to the MPE as published in the ANSI Z136.1-2022. In this comparison, the MPE limits provide an ample safety margin for corneal exposures to focused, single pulse femtosecond exposures to 1318 nm and 2000 nm, and a moderate safety margin for corneal exposures to 1540 nm. In the second approach, the ED_{50} values were evaluated over a 1 mm limiting diameter aperture before comparing them to the current MPE limits. Using this approach, it was determined that the ANSI Z136.1-2022 MPE limits are fully protective of the cornea for focused, single pulse femtosecond exposures to 1318 nm. However, they are not sufficiently protective for exposures to 1540 nm, and are marginally protective for exposures to 2000 nm. The ambiguity in these comparisons highlights the need for the ANSI standard to clarify how to assess small diameter beams for ultrashort pulses and to potentially review the MPE values for NIR sub-nanosecond exposures. This study was also able to provide some preliminary observations concerning the appearance of the laser induced corneal alterations or lesions. The 1 h post-exposure slit lamp examinations showed that for all three wavelengths, the laser induced lesions appeared as small white spots on the cornea with clearly defined edges. However, the optical coherence tomography examinations revealed that there were wavelength dependent differences in the depth of the corneal alterations. Exposures to 1318 nm appeared to always involve the full corneal thickness regardless of the level of the radiant exposure. For 1540 nm, the depth of the lesions increased as the radiant exposure increased. In contrast, the alterations resulting from exposures to 2000 nm appeared to always remain confined to the epithelial layer regardless of the level of the radiant exposure. This study contributes to the current understanding of laser ablation in cornea as well as the knowledge base used for setting laser safety standards in the NIR range for ocular exposure to ultrashort pulses.

2.0 INTRODUCTION

2.1 Background

In recent years, pulsed lasers with ultrashort pulse durations (shorter than $10 \text{ ps} = 10 \times 10^{-12} \text{ s}$), have become ubiquitous in military and civilian applications [1–4]. Some of these applications include material processing [5], medical procedures [1,6], metrology [7], as well as advanced military applications. The upcoming revision to the American National Standard for Safe Use of Lasers (ANSI Z136.1-2022 [8]) includes Maximum Permissible Exposure (MPE) limits for wavelengths from 400 nm up to 1 mm with 100 fs pulse durations, where the MPE limits are averaged over a 1 to 11 mm limiting diameter aperture for laser hazard classification. In comparison, the International Commission for Non-Ionizing Radiation Protection (ICNIRP) guidelines include MPE limits for wavelengths ranging from 400 nm up to 1400 nm with pulse durations longer than 100 fs, and from 1400 nm to $10 \text{ }\mu\text{m}$ with pulse durations longer than 1 ns. However, the ICNIRP guidelines indicate that “for beam diameters less than 1 mm and pulse durations less than 0.35 s, the actual radiant exposure, *i.e.* not averaged over of the limiting aperture of 1 mm, should be compared to the exposure limit” [9]. The fact that these two laser

safety standards provide different approaches for evaluating the safety of lasers with small diameter beams, points to an outstanding question within the laser safety community. In addition, in spite of the increased use of ultrashort pulse lasers and the inclusion of 100 fs long pulse durations into the revised ANSI standards, there is limited biological data for this wavelength and pulse duration regime. Therefore, in order to safeguard all personnel that can come into contact with or utilize ultrashort pulse laser technologies, there is a pressing need to gather biological data for this wavelength and pulse duration regime in order to better inform the laser safety standards [10].

The human eye contains many different structures that work together to allow us to see. The two structures that are generally considered when establishing laser eye safety standards are the cornea and the retina. The retina is the eye's inner lining that, in essence, receives light and converts it to neural signals that are interpreted as vision by the brain, thus injuries or damage to the retina typically affect vision. The cornea is the clear, protective outer layer of the eye and can be considered as the first line of defense against pathogens and foreign bodies. It plays a role in vision as it refracts light that enters into the eye and, together with the lens, helps determine how well the eye can focus. Therefore, vision can also be affected if the cornea is injured or damaged [11]. The different optical properties and absorption characteristics of the tissues render them more sensitive to laser damage at different wavelengths [12,13] due to absorption of laser energy along the visual pathway. A region of particular interest is 1200 to 1400 nm, known as the transitional region. As the exposure wavelengths increase from 1200 to 1400 nm there is a gradual progression from the retina being the most sensitive tissue to laser damage (true for wavelengths in the visible range and up to ~1064 nm) to the cornea becoming the most sensitive tissue above 1400 nm. Consequently, the near-infrared (NIR) region of the electromagnetic spectrum is a region of particular interest from a laser safety standpoint.

Human corneas are comprised of five layers: the epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium (see Figure 1) [11,14]. However, not all layers are present in all animal species. Although porcine and rabbit corneas have no Bowman membrane [15], rabbits in particular have become a widely accepted model for measuring corneal damage thresholds [13,16–23].

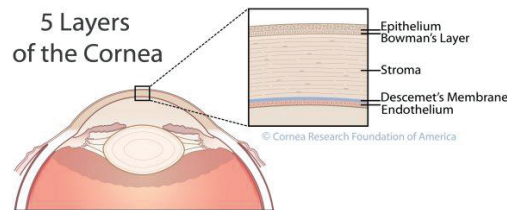


Figure 1. Schematic diagram of the human eye. Inset shows the layers that comprise the cornea.

The cornea increases in absorption below 380 nm and in the 1400 nm to 1 mm range [24,25], 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 [13,26–31] 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 [25,32]. Three particular wavelengths within the NIR region that have been the focus of several studies due to their potential applications, use CW or pulse durations down to the nanoseconds at 1318 - 1319 nm (Nd:YAG laser) [27,29,33,34], 1540 nm (Er:glass laser) [22,34–36], and 2000 nm (Tm:YAG laser) [16,34,37]. These three wavelengths fall into different regimes in terms of the penetration depth into the cornea and, consequently, the tissues that can be affected as the parameters of exposure change. Wavelengths between 1300 and 1400 nm lie within the transitional region, therefore changes in wavelength and/or the duration of exposure can change significantly the damage threshold for the cornea, lens, iris, and retina [38–41,13]. Furthermore, they can change which tissue or tissues are affected and which

tissue exhibits the lowest damage threshold. Wavelengths between 1500 and 1800 nm penetrate the cornea and can even penetrate into the aqueous. This results in a larger volume which absorbs the energy, raising the damage threshold [35] while the damage can involve the deeper corneal stroma [19]. Wavelengths between 1800 and 2600 nm have moderate absorption into the cornea, resulting in a lower damage threshold compared to that for wavelengths between 1500 and 1800 nm and the damage is confined to the epithelium [16,21,37].

Experimental threshold damage studies have identified thermal, thermomechanical, photochemical, photothermal, and photomechanical processes as the mechanisms of injury depending on the precise temporal characteristics of the exposure ranging from CW to nanosecond pulse exposure durations [42,43]. In particular, in the nanosecond pulse regime, ablation occurs as a result of chemical bond breaking at UV wavelengths, *i.e.* photochemical decomposition, and/or a combination of photomechanical and photothermal mechanisms which result in vaporization and ejection of tissue at visible and IR wavelengths [42]. These processes involve the formation of a free-electron plasma which, in transparent materials, essentially consists of the generation of quasi-free electrons in the conduction band, often referred to as free electrons, through an interplay between multiphoton ionization, the tunnel effect, impact ionization which results in avalanche ionization, and losses from electron recombination, collisions and diffusion [44–46]. When the free electron density exceeds the critical free-electron density at which the plasma absorbs a significant fraction of the incoming laser light, this process is referred to as optical breakdown or laser-induced breakdown. This results in highly localized energy deposition via nonlinear absorption which is responsible for tissue damage [47].

In the picosecond pulse regime, the deposition of energy into the tissues occurs on comparable timescales to the acoustic and thermal relaxation times. This makes the interplay between mechanical and thermal effects and the formation of the plasma dominate the response, resulting in a larger amount of energy coupling into the medium as compared to that in the nanosecond range [44]. In this regime, the free-electrons are generated by multiphoton absorption, which is a probabilistic process [44]. In order to reach the threshold for optical breakdown, the irradiance must be strong enough to cause rapid ionization so that losses do not suppress the subsequent electron avalanche, therefore the threshold for optical breakdown is higher than for nanosecond pulses [48].

Several groups have shown that the threshold radiant exposure for optical breakdown in transparent materials, including tissues, scales as the square root of the pulse duration for pulses longer than 10 ps [43,44,46,49–52]. However, for pulse durations shorter than 10 to 20 ps, electron recombination does not play a major role, therefore, the threshold radiant exposure depends weakly on the pulse duration while the irradiance threshold for optical breakdown increases as the pulse duration decreases [44,47]. For femtosecond pulse durations, there is a deterministic relationship between the free-electron density and the irradiance such that the maximum electron density during breakdown steadily increases with growing irradiance. This results in a large irradiance range below the optical breakdown threshold producing free electrons where the free electrons create spatially confined chemical, thermal, and mechanical effects [44,47]. The balance between these effects, and which one dominates the response, depends on the repetition rate of the laser. For high repetition rates, the ablation process is generally considered non-thermal given that there is a strong spatial and temporal confinement [47].

There have been a few studies focused on establishing the corneal damage thresholds for pulse durations between 7 fs and 200 ps at near-infrared (NIR) wavelengths [53–58]. All of these studies made use of excised porcine or human eyes exposed to a single ultrashort pulse, none exposed them to wavelengths above 1450 nm, and the majority defined the energy damage

threshold as the maximum pulse energy for which no tissue damage was observed. In particular, studies found that the corneal damage threshold was $\sim 1.5 \text{ J/cm}^2$ for 100 fs, 8 μm diameter spot ($1/e^2$), at 800 nm [55,56,58] while for 100 fs at 1450 nm, the corneal damage threshold increased to 2.2 J/cm^2 [56]. In [56], the authors measured the ablation threshold radiant exposure at wavelengths between 800 and 1450 nm for 100 fs laser pulse durations. They found that the ablation threshold increases rapidly for wavelengths shorter than 1000 nm, and reaches a plateau for wavelengths between ~ 1000 and 1400 nm. From models of optical breakdown in water at ultrashort pulse durations [44,47,45], it can be concluded that this wavelength dependence is related to variations in the relative importance of multiphoton ionization versus avalanche ionization during plasma formation although a consensus has not been reached on the details as summarized in [45].

All of the studies focused on establishing the corneal damage thresholds for ultrashort pulses at NIR wavelengths reviewed, show that the corneal damage threshold does not vary more than a few percent between porcine and human tissues, or between the epithelium, the stroma and the Bowman layer [55,57,58]. However, studies exposing excised rabbit eyes and *in vitro* cornea models to 1540 nm with 800 μs pulse durations found differences of up to 45% in the ED_{50} values [59]. In addition, they found that the *in vivo* ED_{50} was approximately 2.64 and 1.81 times that for the *in vitro* and *ex vivo* corneal models respectively [19,59]. Factors that may contribute to these differences include the presence of the tear film layer, the hydration level and temperature of the tissues [60]. Although the studies discussed above were performed utilizing microsecond-long pulse durations, the factors that may be responsible for the differences between *in vivo*, *in vitro* and *ex vivo* ED_{50} values may also play a role in exposures to ultrashort pulses.

From the discussions above, it can be seen that the physical mechanisms involved and the dynamics of the interaction between the structures of the human eye with femtosecond pulse durations are different from longer pulse durations. Given the limited biological data of exposures to femtosecond pulse durations at NIR wavelength, there is a need to obtain experimental data to better inform the laser safety standards in this wavelength and pulse duration regime. And, given the differences observed between *in vivo*, *in vitro* and *ex vivo* ED_{50} values for microsecond-long pulse durations, support the need for obtaining *in vivo* ED_{50} for exposures to NIR femtosecond long pulses.

2.2 Approach

In this study we anesthetized rabbits and the corneas were exposed to single femtosecond laser pulses of varying energies at 1318 nm, 1540 nm and 2000 nm and determined the minimal pulse energy that had a 50% probability of causing a minimum visible lesion (MVL) using probit analysis [61]. We report the MVL thresholds as the median effective dose (ED_{50}) based on individual pulse energies. The range of energies used for exposure were guided by preliminary experiments performed to determine the damage threshold of collagen gels, which have been shown to be a good corneal model at other wavelengths. However, at the wavelengths under consideration for this study, they only served as a rough guide therefore the results from those preliminary experiments are not included in this report. We used anesthetized live subjects, eliminating the potentially confounding effects introduced by *in vitro* and *ex vivo* corneal models and allowing us to align with modern practices for setting cornea laser safety standards which take into account 1 h post-exposure results while monitoring the injury at 24 h post-exposure to ensure there are no delayed effects [16]. The median effective doses found were compared to the current maximum permissible exposure (MPE) limits in order to determine if they are sufficiently protective of the cornea for these wavelengths and pulse durations. The results of this study will contribute to the current understanding of laser ablation in cornea as well as the knowledge base used for setting laser safety standards in the NIR range for ocular exposure to ultrashort pulses.

3.0 METHODS

3.1 Animal Model

Mature female Dutch Belted rabbits (*Oryctolagus cuniculus*) were used for this study. The animals were procured, maintained and used according to an IACUC-approved Animal Use Protocol and established animal welfare standards, compliant with: DoD Instruction 3216.01 [62]; US Department of Agriculture Animal Welfare Regulations [63]; The Guide for the Care and Use of Laboratory Animals, 8th Edition, National Research Council [64]; and AFMAN 40-401(1) [65]. The Air Force Research Laboratory at JBSA-Fort Sam Houston, Texas has been accredited by AAALAC, International since 1967.

3.1.1. Sedation.

Prior to sedation, a Hot Dog Multifunctional Warming Controller System (WC52, S/N 11882) was turned on to begin warming the large pad (V104) wrapped in a fleece blanket and a rabbit snuggle (Lomir Biomedical, BS01 small). The date, species and subject number were entered into the SurgiVet Monitor (Smith's Medical, #92V667335, S/N AM08090101) used to monitor the vital signs of the subject during the entire procedure. Subject monitoring consisted of temperature, SpO₂, heart rate (ECG). Respiratory rate and non-invasive blood pressure was monitored manually. The level of isoflurane, (Patterson Veterinary, 07-893-1389) in the anesthesia system (Gas Anesthesia System, Ohmeda, Isotech 4) was checked and refilled as needed. In addition, the F/air Canister (Bickford, #80120) was weighed, the weight was recorded on the canister and was replaced if it showed a difference of over 50 grams relative to its initial weight or if nearing 12 hours of time used. The Sodasorb LF CO₂ Absorbent (Henkle Corp, SS66) was replaced if a noticeable amount of purple coloration was observed. The oxygen tank levels were also checked and replaced as needed.

Subjects were sedated with 20 – 40 mg/kg Ketamine (Ketaset, Patterson Veterinary, #07-803-6637, [100mg/mL]) and 2 mg/kg Xylazine (ShopMedVet, #RXANASED-20), [20mg/ml]) using a 23-gauge winged infusion set (Jorgenson Labs Inc., #JO454C, blue) with a 3 mL syringe (Monoject, 8881250305). Buprenorphine SR LAB (ZooPharm, 0.03-0.05mg/kg) was administered to the subject to control any potential pain. Injections were administered intramuscularly (IM) at either rear thigh muscle directed away from the femur, avoiding the sciatic nerve. Injections were placed at alternating sites when sedated for subsequent procedures to minimize pain and/or swelling. An anesthesia mask was placed to cover the subject's nose and mouth and was secured using a surgical string tied around the base of the anesthesia mask and behind subject ears. The subject was then wrapped in the warmed rabbit snuggle and preoxygenated for 2-3 minutes at 2 - 4 L/min via the secured anesthesia mask, prior to the induction of anesthesia. The subject was placed under anesthesia using ~3 - 4% isoflurane, ~2 – 4 L/min oxygen via anesthesia mask. The sensors from the SurgiVet monitor were attached to the subject to collect the vitals. The SpO₂ sensor (Digicare Animal Health, #PO725) was attached to one ear with surgical tape. A temperature probe (Mindray DS USA Inc, #0011-30-90440) was inserted into the rectum and secured with surgical tape (Patterson Veterinary, 07-850-1249). To monitor EtCO₂ and respiratory rate values, an EMMA capnograph with infant adapter (Maisimo, #3639, #17449) was placed in between the anesthesia mask and the anesthesia airway. Due to the added weight of the capnograph, an adjustable clamp was placed on the stage in order to hold the airway tube in place.

Both ears were examined to identify the one most suitable for the placement of an ear catheter. The hair on the designated ear was shaved over the marginal ear vein and the site was cleaned with alcohol wipes (Webcol, #6818). A 24G IV catheter (Patterson Veterinary, 07-891-5695) was inserted into the marginal vein. The catheter was then flushed with a 3 mL syringe filled with 9% normal saline to ensure a good placement. Once flushed properly, the catheter was

secured with surgical tape (Patterson Veterinary, 07-850-1249) with the use of an ear roll. If the optimal ear was located on the same ear as the SPO₂ sensor, the sensor was relocated to the subject's other ear.

3.1.2. Pre-exposure preparation and examination.

In preparation for laser exposures, the eyelashes of the eye to be exposed were clipped to remove any obstructions during exposure. This eye was rinsed with 9% normal saline solution and administered two drops of each of the following: Proparacaine HCl 0.5% Ophthalmic Solution, USP (Bausch Health US, NDC24208-730-06), Tropicamide Ophthalmic Solution 1%, USP (Sandoz, NDC61314-355-02), Phenylephrine HCl Ophthalmic Solution 2.5%, USP (Paragon, NDC42702-102-15). GenTeal severe strength gel (Alcon Laboratories Inc., NDC0078-0429-47) was generously applied to the eye before closing and securing it with gauze (Dermacea, #441217) and surgical tape to keep it from drying out during the procedure. Petrolatum ophthalmic ointment (Paralube, Dechra, 221-38) was administered to the unexposed eye prior to closing it and securing it with gauze and surgical tape. Peribulbar injections were administered to reduce extraocular muscular movement if significant eye movement was present during exam. A 3 mL syringe with a 25G needle containing 1.5 mL Lidocaine HCl 2% (Phoenix, NDC57319-533-06) was prepared and administered as follows: 0.75 mL on top of eye and 0.75 mL below eye. Every 30 minutes throughout the experimental procedure, the unexposed eye was uncovered, rinsed with normal saline, petrolatum was reapplied generously, and the eye was covered again with gauze and surgical tape. Throughout the experiment, a 60 mL syringe (Monoject, #0388815601255) with a catheter tip attached, containing 0.9% normal saline with sodium chloride (Hospira, NDC0409-7983-03) was used to rinse/irrigate the eye. In the case that an exposure was to be conducted at the 30 minute time point, the exposure took priority and the unexposed eye was irrigated immediately after exposure.

In order to perform the pre-exposure examination, the subject was placed in an adjustable 5-axis positional animal stage to facilitate the placement of the eye for examination with multiple instruments. The eye to receive laser exposures was uncovered and completely rinsed of GenTeal using 0.9% normal saline with sodium chloride (Hospira, NDC0409-7983-03). Once the GenTeal gel was rinsed off, a 0.9 cm infant Barraquer wire speculum (Fisher Scientific, NC1405443) was put in place to keep the eye open. If needed for better viewing, the subject's eye was held open by hand. The cornea was irrigated with normal saline every 15 – 20 seconds throughout the examination. A slit lamp biomicroscope (Topcon, SL-7E) and/or an OCT (Heidelberg Eng., Spectralis HRA+OCT) were used to examine the cornea and record images. The slit-lamp was used immediately before and after a fluorescein stain had been administered to highlight epithelial injuries of the cornea. Fluorescein was introduced to the surface of the cornea by wetting a fluorescein strip (1 mg/strip, Patterson Veterinary, 07-892-7782) with a small amount of saline and gently touching the bulbar conjunctiva with the strip. The eye was closed and opened a few times to evenly distribute the fluorescein before continuing with the eye exam. A fundus camera (Topcon, TRC-50 DX) was used to examine the retina and record images. Upon completion of the examination, the cornea was irrigated with normal saline, the wire speculum was removed, GenTeal gel was applied and the eye was securely covered with gauze and surgical tape. In some cases, both eyes were examined. After the exam, the subject was transported to the laser laboratory.

3.1.3. Laser exposure.

This section summarizes the refined procedure after performing laser exposures on 2 to 3 eyes. During those initial exposure sessions we identified some minor flaws in the optical setup as well as in the experimental procedure which we subsequently adjusted. The final exposure procedure is detailed below while the final optical setup is described in section 3.2.1.

The anesthetized subject, with the complete warming snuggle system, was secured in a sternal position in the adjustable 5-axis positional animal stage to facilitate the precise placement of the exposures. Room lights were turned off during exposure procedures. Four desk lamps distributed around the experimental area were turned on. The subject was then positioned, the eye to be exposed was uncovered and rinsed of GenTeal. The eye was kept open using a wire speculum and the cornea was rinsed with normal saline every 15 – 20 seconds while the speculum was in place. The alignment laser and the view from the front camera (see section 3.2.1 and Figure 2) were used as a guide to position the subject for the exposure. Then, by looking at the two low-power alignment laser beams from above using the overhead camera, the side-to-side position of the subject was finely adjusted by bringing the crossing point onto the surface of the cornea. During this procedure, a red hand-held LED light was used to illuminate the eye when positioning the subject. Approximately 30 seconds prior to exposure, the cornea was irrigated with normal saline and excess liquid was dabbed with eye spears (Fisher Scientific, NC9342323) at the corner of the eye. Immediately before the laser exposure, an image of the eye illuminated with the LED was recorded to provide a reference for the location of the exposure. During the exposure, the eye was illuminated with the LED light while a series of images were taken with both the straight-on front facing camera and the top-down camera intended to visualize the exposure event. Immediately following exposure, the cornea was irrigated with normal saline.

If repeated and persistent eye movement occurred, proparacaine drops were re-administered when necessary and after 1 minute, the peribulbar injections were repeated as follows: 0.25 mL on top and 0.25 mL below eye as to not exceed the maximum dose of 2 mL of lidocaine for a single eye per day. When all 9 exposures were complete, the wire speculum was removed, GenTeal was generously applied to the exposed eye and the eye was securely covered with gauze and surgical tape. The subject was transported back to the exam room for post-exposure examination.

3.1.4. 1 hour Post-exposure examination.

The anesthetized subject was placed in the adjustable 5-axis positional stage for post-exposure examinations. Nominally at one-hour post exposure, the eye was uncovered, the GenTeal gel was rinsed off and the eye was kept open using either the wire speculum or fingers. The exposed eye was examined using a slit-lamp biomicroscope, an optical coherence tomography (OCT) device and a fundus camera in that order. The slit lamp examination was performed before and after the fluorescein stain had been administered where the fluorescein dye was applied in a similar manner as described in section 3.1.2. In the event that the eye examination had to be cut short the examination with the OCT was skipped.

After completing the one-hour post exposure examination, Puralube Petrolatum Vet Ointment (Leedstone, 103-8484) was generously applied before covering the eye with gauze and surgical tape. The subject was taken back to the preparation room for recovery from anesthesia.

3.1.5. Animal recovery.

Upon arrival in the recovery room, Isoflurane was turned off and the subject was manually stimulated to initiate recovery. The SPO₂ sensor, temperature probe, ECG leads, IV catheter, and anesthesia mask were removed. Both eyes were rinsed with normal saline to remove GenTeal or petrolatum. The subject was then transferred to its cage and kept warm with a warming blankets. Full recovery was logged when the subject was able to walk/hop around its cage twice without assistance.

3.1.6. 24 hour Post-exposure examination.

For 24 h reads of the first exposed eye, the same preparation/sedation/recovery procedures occurred as for the baseline examination and laser exposure. The 24 h examination was nominally the same time of day as the 1 h post exposure examination.

For 24 h reads of the second eye followed directly by euthanasia of the subject, the same preparation/sedation procedures occurred with the following modifications: after the 24 h post-exposure examination, the subject remained deeply anesthetized and was administered Euthasol via IV catheter. Once death was determined from vital readings and verified by the absence of a heartbeat, the exposed eye was immediately exenterated. A suture was placed superior and nasal to the pupil to mark orientation of the eye for histological sectioning. The enucleated eyes were fixed in 10% Neutral Buffered Formalin (NBF) with 0.01% glutaraldehyde using a fixative infusion technique.

3.2 Exposure Setup

3.2.1. Optical setup.

Figure 2 is a schematic diagram of the exposure setup used to deliver single pulses to the target location at the exposure plane. An optical parametric amplifier (OPA; HE-TOPAS, Light Conversion Technologies) was pumped by a Ti:Sapphire regenerative amplifier (Spitfire Ace SPTFPA-100F-1K-ACE, Spectra Physics) to produce a 15 mJ pulse with a 1 kHz repetition rate and a nominal pulse duration of 100 fs. These pulses were injected to an OPA amplifier that was capable of producing a wide range of wavelengths for study. The three wavelengths of interest used in this research were: 1318 nm ($735 \pm 30 \mu\text{J}$), 1540 nm ($700 \pm 41 \mu\text{J}$), and 2000 nm ($647 \pm 54 \mu\text{J}$) where the numbers in parenthesis correspond to the (average $\pm$ standard deviation) maximum pulse energy measured on the different days of exposure. This system produced an approximately Gaussian beam profile at the output of the OPA with a $D4\sigma$ diameter collimated beam of about 7 mm depending on the wavelength (see Table 1).

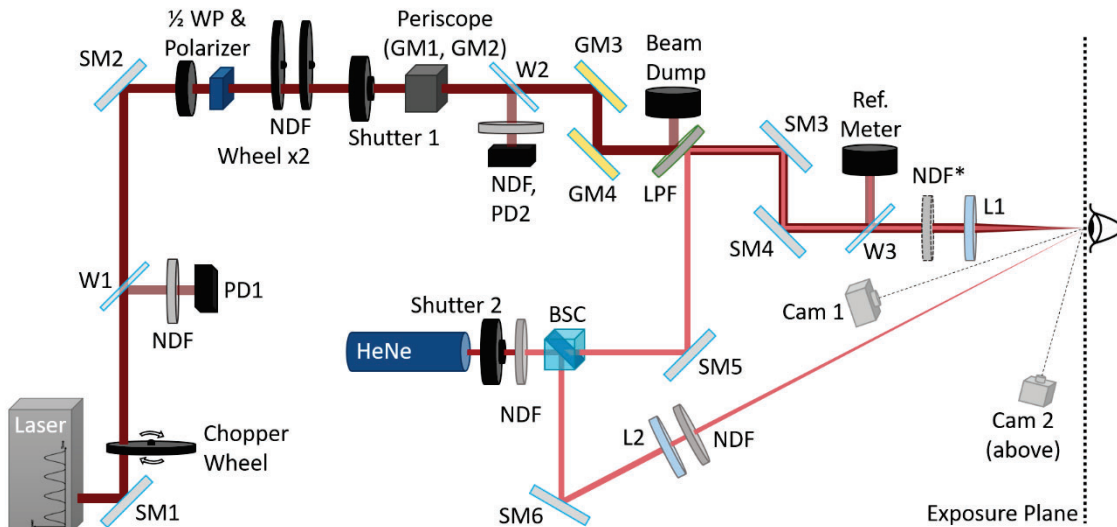


Figure 2. Optical setup for sub-ns cornea exposures. SM: silver mirror. NDF: Neutral density filter. PD: Photodiode. W: window. GM: gold mirror. Ref. Meter: Reference detectors (power meter). LPF: long-pass filter. L: lens. BSC: Beam splitter cube. Cam 1: Imperx CCD camera. Cam 2: Teledyne Nano GigE camera. Note: Cam 1 is located at a

different height than the NIR beam and provides a straight-on view of the target. NDF* was mounted on a flip mount so it could be placed into the beam path when needed.

The output from the OPA passed through a set of optics after going through a 10:1 chopper wheel (custom machined part) synchronized with the regenerative amplifier's native 1 kHz frequency, in order to reduce the 1 kHz pulse repetition rate to 100 Hz. A pick off CaF₂ window (Thorlabs WG51050) was used to send a fraction of the beam to a photodiode (PD 1; Thorlabs PDA10CF) which was part of the electrical circuit used to implement the single shot capability (see below). The 100 Hz signal was sent to a delay generator (SRS, DG535) which controls the timing of the shutter. The main beam traveled through a half-wave plate (CVI, AWCP-1000-1600-06-2) and a linear wire grid polarizer (Thorlabs, WP25M-UB) set to allow only vertical or horizontal polarizations through depending on the polarization of the beam at a particular wavelength. The half-wave plate and linear polarizer combination operated as a fine power adjustment over 90% of the range of the energy available. A set of neutral density filters mounted on wheels (NDF) operated as a rough power adjustment. The NIR beam subsequently passed through a computer controlled mechanical shutter (25 mm Uniblitz Shutter/Driver T132) that only allowed individual pulses to continue along the defined optical path. A second pick off window (CaF₂, Thorlabs) sent a fraction of the beam to another photodiode (PD 2; Thorlabs, PDA20CS), which triggered the reference meter externally. The output of PD 2 was sent to an oscilloscope (Tektronix, MDO3054) for visual confirmation of a single pulse being delivered. The signal from PD 2 also triggered the reference detector (Ref. Meter in Figure 2). A long-pass filter (LPF; Thorlabs FEL1000) served to block any residual light from the 800 nm pump beam and any visible light components produced by the NIR light generation in the OPA. It also combined the NIR beam with a visible low-power HeNe (635 nm, Spectra Physics Model 905) beam for co-alignment and targeting purposes. A window (CaF₂, Thorlabs) was utilized at the end of the optical path to send a fraction of the beam to the reference detector, which was externally triggered by PD 2. At the end of the optical path, a 25.4 mm diameter, 250 mm effective focal length plano-convex lens (Newport Corp. KPX109) focused the incident beam to an approximately 100-200 μ m diameter spot where the exact dimensions depended on the specific wavelength. A lens with a long focal length was used in order to have a longer Rayleigh range and, consequently, more room to place the target within the waist of the NIR beam. In order to achieve certain output energies, it was necessary to attenuate the beam even more. To accomplish this, an additional neutral density filter was placed on a flip mount immediately before the lens (NDF*) and could be placed in the beam path when needed.

A HeNe laser was used to help locate the exposure plane during the alignment procedure detailed in section 3.2.3.2 below. A beam splitter cube (BSC) split the beam of a HeNe laser into two paths. One beam was injected into the NIR beam path by reflecting it off the LPF, allowing the visualization of the direction of travel of the NIR beam, *i.e.* co-aligned HeNe. The other beam traveled diagonally towards the target through a plano-convex lens 2.54 mm in diameter with a 25 mm effective focal length (L2; 1000 mm Newport), labeled as cross HeNe. The two HeNe beams were aligned to cross at the position of the waist of the NIR exposure beam to aid in positioning the subject. Neutral density filters reduced the intensity of both beams to $\sim$ 0.5 mW in order ensure that, even when they overlapped, the target was not saturated with red light and allowed for a more precise location to be determined.

A high speed, mirrorless camera (Cam 1; Imperx, IGV-B0610C-KC000) imaged the exposure event in real time at a shallow viewing angle (14° above the optical axis of the NIR laser beam). This camera will be referred to as the front-view camera. It was a high speed, low noise camera, with electronic shutter that captured full quality images at a frame rate of 100 fps. The location of the camera was optimized to have the subject's cornea fill most of the field of view.

A GigE camera (Cam 2; Teledyne Genie Nano, 66 fps) visualized the target from a top-down perspective and helped to ensure the location of the crossed HeNe beams coincided with the target location.

3.2.2. Single shot capability and data acquisition.

In order to perform single pulse exposures, an optoelectronic circuit was built such that every time the trigger was pressed, a single pulse traveled to the target. The timing and sequence of events described below is schematically represented in Figure 3. The 1 kHz output of the femtosecond laser was sent to the controller of a 10:1 chopper wheel in order to synchronize them. The chopper wheel reduced the 1 kHz repetition rate to $1 \text{ kHz}/10 = 100 \text{ Hz}$ such that the pulses traveling beyond the chopper wheel were 10 ms apart. The train of pulses at a 100 Hz repetition rate was detected by a photodiode (PD 1). The output from PD 1 was sent to a delay generator connected to the computer-controlled shutter. When the trigger button (handheld push button switch) was pressed, the delay generator waited until it received a signal from PD 1 indicating it had detected a pulse. 2.5 ms after detecting that pulse, the delay generator sent a signal to the computer-controlled shutter to open for 8 ms. However, given the reaction time of the shutter and other delays in the electronics, this resulted in keeping the shutter open for 9.8 ms, allowing only a single pulse through. In order to corroborate that a single pulse was directed towards the target, the output from the second photodiode (PD 2) was connected to an oscilloscope.

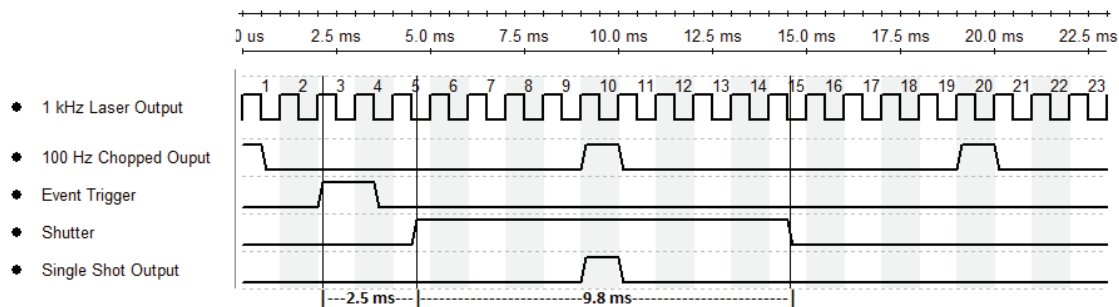


Figure 3. Schematic diagram showing the sequence and timing of events used to implement the single shot capability. Each line represents an oscilloscope trace.

A LabView program was used for data acquisition during exposures. When the trigger button was pressed, the program directed the delay generator to wait for a signal from PD 1 before activating the mechanical shutter to open and close allowing a single shot to reach the cornea. In addition, it collected a series of images with Cam 1 and Cam 2, recorded the energy of the incident beam measured on the reference detector and calculated the energy delivered to the target from the ratios obtained during the energy calibration procedure.

3.2.3. Laser characterization, alignment and calibration.

The sensitivity of the laser system required a daily beam characterization consisting of measuring the spectra, beam profile, collimated beam diameter, focal spot diameter, and pulse width. In addition, an energy calibration was performed daily as described below.

3.2.3.1. Beam characterization.

A) Spectral profile.

After turning on the laser and setting it to the desired wavelength of operation, the spectra was measured close to its output as detailed in the SOP for the laser. In brief, a beam block was

placed after the shutter and the tip of the optical fiber was placed so as to collect the scattered NIR beam at that location. The wavelength was adjusted by tuning the crystals in the OPA while monitoring the spectra prior to optimizing the output power.

In order to measure the spectral profile of the NIR beam reaching the target, a beam block was placed after the beam sampling window W3 and the tip of the optical fiber from a spectrometer (Ocean Optics NIR 512-2.5) was positioned to collect part of the scattered NIR beam. Once the signal was stable, the technician corroborated that the wavelength was centered on the target wavelength and maximized its power before saving the spectrum using the NIR spectrometer's software (Ocean View 1.6.7).

B) Beam profile and collimated beam diameter

To obtain the beam profile of the collimated NIR beam, an infrared camera (Spiricon, Pyrocam-III, PY-111 h-C-A) was placed after the linear polarizer. The energy of the incoming beam was reduced to $< 200 \mu\text{J}$ for a ~ 7 mm diameter beam in order to avoid damaging the CCD element in the infrared camera. The camera was aligned to center the beam within the CCD before saving the image. The camera's software (Beam Gage Professional 6.3) processed the image and calculated the collimated beam's $D4\sigma$ diameter in two perpendicular directions, referred to as D_x and D_y throughout the remainder of this document.

C) Focal spot diameter

In order to measure the focal spot size, a knife-edge technique was used, see Figure 4. An apparatus was set up to hold a razor blade at a constant height above the optical table and enable its motion in two perpendicular directions, along the direction of beam propagation and into the laser beam's path, defining the xz plane. The apparatus consisted of a linear stage with a manual 25 mm travel micrometer control in the direction of propagation (z -axis). This manually controlled stage served as a mount for a computer controlled linear stage with a piezo motor (Newport CONEX-AG-LS25-27P) for precise motion control of the razor blade in the x direction, *i.e.* into the laser beam's path. The energy of the beam was reduced to less than $1 \mu\text{J}$ to prevent damaging the razor blade due to the high peak powers which was validated by visual inspection. The incoming beam's total energy was measured by placing an energy meter after the stage. We calculated 84% and 16% of the total energy and, by moving the razor beam into the path of the laser beam in the x -direction, located the stage position that rendered 84% (x_{84}) and 16% (x_{16}) of the total energy at multiple locations along the direction of propagation. An array of beam diameters was obtained by doing measurements at multiple locations along the direction of propagation, where the beam diameters were found using the equation $d_{x,1/e^2} = 2|x_{16} - x_{84}|$. The beam diameter measured in this way corresponds to the $1/e^2$ diameter. However, in an effort to be consistent with the laser safety standards guidelines, we have chosen to use the $1/e$ diameter for all calculations which is found from $d_x \equiv d_{x,1/e} = (d_{x,1/e^2})/\sqrt{2}$.

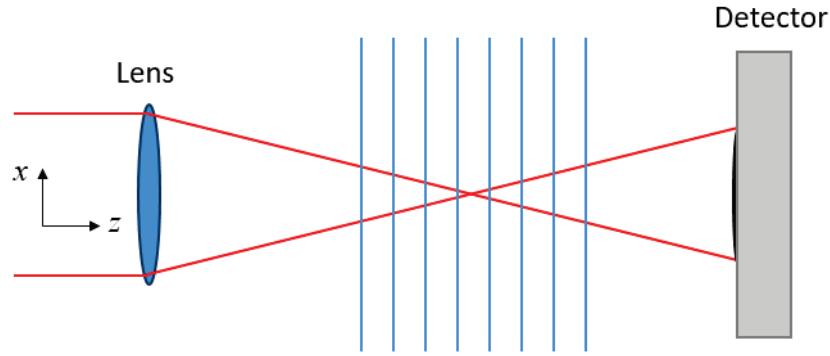


Figure 4. Schematic diagram of the knife edge technique used to obtain the focal spot size diameter. Blue lines represent the locations where measurements were taken, z is the direction of propagation.

D) Pulse width

In order to measure the pulse width, the beam was intercepted immediately before the beam sampling window W3 using a mirror on a magnetic mount in order to send the beam into the auto correlator (Mini Optic Technology Inc. Delta Autocorrelator). The output of the auto correlator was sent to a digitizing oscilloscope (Tektronix, MDO3054). The signal in the oscilloscope's screen was maximized in order to maximize the resolution and, using the oscilloscope's built-in functions, the full width half max (FWHM) for that particular trace was measured. The micrometer on the auto correlator 100 μm was moved in both directions, recording the temporal location of the peak in each case and ensuring the FWHM stayed the same. We refer to the separation between the peak locations as the path and it is measured in milliseconds. The pulse width was calculated as

$$\tau = \frac{0.66 * \sqrt{2} * \text{FWHM}}{\text{path}}$$

where the $\sqrt{2}$ is for a Gaussian beam. For 1318 nm and 1540 nm the pulse width was measured after the exposures on most experimental days. The upper working limit of the autocorrelator is 2000 nm which makes it more challenging to use to obtain the pulse width at that wavelength, therefore, the technician only measured it once after one of the experiments.

Table 1 lists the laser beam parameters at each of the wavelengths of interest obtained by averaging over the data obtained on each exposure day. The measured wavelengths were obtained from the peak in the measured spectra, the collimated beam diameters in X and Y were obtained from the beam profiles, the pulse durations were obtained from the autocorrelator, and the average focal spot diameters listed were obtained by averaging the $1/e$ focal spot diameters obtained from the knife edge measurements ($1/e^2$). The itemized lists of the parameters measured each day are in APPENDIX B – and APPENDIX C – .

Table 1. Average laser beam parameters

Nominal Wavelength (nm)	Average Measured Wavelength (nm)	Average Beam Diameter in X (mm)	Average Beam Diameter in Y (mm)	Average Pulse Duration (fs)	Average $1/e$ Focal Spot Diameter in X (μm)	Average $1/e$ Focal Spot Diameter in Y (μm)
1318	1319 $\pm$ 4	7.3 $\pm$ 0.5	8.0 $\pm$ 0.2	93 $\pm$ 6	78 $\pm$ 12	87 $\pm$ 16
1540	1534 $\pm$ 4	7.1 $\pm$ 0.7	7.6 $\pm$ 1.1	136 $\pm$ 9	87 $\pm$ 21	106 $\pm$ 39

2000	2000 ± 3	7.4 ± 0.9	7.6 ± 0.9	241 ± 14	105 ± 11	107 ± 12
------	----------	-----------	-----------	----------	----------	----------

3.2.3.2. *Alignment procedure.*

Once the beam waist location was determined from the knife edge measurement, the z -axis stage was moved to the waist location z_0 and the razor blade was replaced with a card. Using mirror SM6, the focal spot of the cross HeNe was moved to overlap the co-aligned HeNe at z_0 . The plane where the two HeNe beams crossed determined the exposure plane. The Rayleigh range of the lens used to focus the NIR beam was long, therefore, in order to ensure the exposure plane was close to the middle of the Rayleigh range, the crossing point of the two HeNe beams was fine-tuned via the knife edge technique.

3.2.3.3. *Energy calibration.*

The energy calibration procedure on the day of the experiment and prior to any exposures consisted of two steps. First, an energy meter was placed beyond the plane of exposure ensuring that the NIR beam was not focused on it and that the detector collected all the incoming light. The energy measured by this meter will be referred to as energy at target. Without using the single shot capability, the half-wave plate was rotated and the combination of neutral density filters in the laser's path was varied until the desired energy noted in the exposure plan was measured at the target detector. For the lower energies, the neutral density filter on the flip mount was put in place. The half-wave plate's angle, and the combination of neutral density filters, including the one on the flip mount, used to obtain the desired energy were recorded. In addition, the range set on the meters, the energy measured at the reference detector and the energy at target were recorded. The ratio of the energy at target to the reference energy was calculated and recorded for each energy in the exposure plan.

The second step of the procedure consisted in setting the power control components to the positions recorded for a particular energy in the exposure plan. Using the trigger button to deliver single shots, the reference energy was recorded and the ratio was used to calculate the delivered energy. The delivered energy was compared to the energy at target to ensure the settings were correct. This was repeated multiple times for each desired energy within the exposure plan to ensure reproducibility. During exposures, the ratio recorded for each energy in the exposure plan was used to corroborate that the delivered energy was close to that listed on the exposure plan. A second check was to compare the delivered energy calculated using the ratio to the energy measured at target. Given the pulse duration and response time of the detector, it can be considered that the energy delivered is the total energy within the pulse. The procedure to find the relationship between the energy measured by the reference detector and the delivered energy used for the analysis was a generalized version of this procedure as described in section 3.4.3.

3.2.3.4. *Post-exposure calibration.*

Immediately after exposures, the second step of the calibration procedure detailed above was repeated, enabling us to document and subsequently account for variations in the laser's output power over time and variations in the angular positioning of the half-wave plate as described in section 3.4.3.

3.3 Experimental Protocol

The corneas of anesthetized rabbits were exposed to single femtosecond laser pulses of varying energies at one of three infrared wavelengths to determine the damage threshold energy that caused any detectable change in the cornea. The three wavelengths used were 1318 nm, 1540 nm, and 2000 nm. Each cornea received exposures from only a single wavelength. An array of up to 9 exposures were placed on each cornea in an approximately periodic square grid pattern to facilitate the identification of sub-threshold exposures. A large range of pulse energies were

used on the first exposed cornea at each wavelength in order to get a gross estimate of the ED₅₀. For the second and third corneas, the measurement was refined by changing the energy in smaller steps around the estimated ED₅₀. In order to account for biological variability, exposures at a specific wavelength were placed on different subjects and on both, right and left eyes.

3.3.1. Data collected during baseline exams.

During the baseline examination, one or two readers used the slit lamp microscope, with and without fluorescein, to determine if there were any abnormalities on the cornea. The readers took pictures to document the condition of the cornea before the laser exposures. It should be noted that the photographic images taken with the slit lamp microscope were not optimal. The field of view and focal point of the external camera that is mounted on the slit lamp do not perfectly coincide with that of the eye piece, resulting in pictures that are slightly blurry and out of focus that do not necessarily represent what was seen more clearly during the visual exam. A fundus exam was also conducted, usually by just a single reader, sometimes two. The fundus camera exam was focused on establishing the condition of the retina and document it with pictures.

3.3.2. Data collected during exposures.

The first step before performing any exposure was to set the waveplate and the neutral density filter to the position determined for a specific exposure listed in the exposure plan during the pre-exposure calibration procedure (section 3.2.3.3). Once set, the waveplate's position (angle), the neutral density filters used, and the range of the reference meters were recorded manually. In addition, before exposure, a pre-shot reference image with the alignment laser on was recorded using the front-view camera to aid in locating the exposure site. The alignment laser was turned off during exposures. The data collection for an individual exposure event was set to initiate when the handheld push button switch (trigger) was pressed. This triggered the LabView program to run and start the collection of a burst of images with Cam 1 and Cam 2, during which the mechanical shutter opened and closed, a single shot reached the cornea, and the energy of the incident beam was measured with the reference detector. The program also calculated the energy delivered to the target from the ratios obtained during the energy calibration procedure. Independently, the energy measured by the reference detector was manually recorded for verification as well as the ratio that was input into the LabView program. The relative location of the shots were also recorded in a hand drawn map of exposure locations which was subsequently provided to the readers during the 1 h and 24 h post-exposure examination.

The mechanical shutter opened 1 ms after the trigger was pressed and it stayed open for a duration of 9.8 ms before closing. The front facing camera (Cam 1) was set to collect 45 images at a rate of 100 frames/sec and it initiated the collection ~20 ms after the trigger was pressed. The top down camera (Cam 2) was set to collect 24 images at a rate of 66 frames/sec.

On most experimental days, images were taken with the front viewing camera before and after the exposure session to record the position of the co-aligned laser on a target placed at the exposure plane. This image was subsequently used to extract the location of the NIR beam and overlay it on the pre-shot images taken with the front-viewing camera immediately before an exposure. This composite image was provided to the readers as a guide for the location of the exposure sites.

3.3.3. Data collected during 1 hour and 24 hour post-exposure examinations.

For the 1 h and 24 h post-exposure examinations, readers were provided with a hand drawn map of exposure locations and a power point slide of the pre-shot images with a white dot marking the location of the alignment laser, when available, to aid in finding the exposure locations. In

addition, readers were provided with a schematic diagram of the eye where they could mark the locations of any abnormalities they observed on the cornea.

During the 1 h and 24 h post-exposure examinations, one or two readers used the slit lamp microscope, with and without fluorescein, to identify any detectable alteration of the cornea at the exposure location. The readers took pictures to document the alterations they observed. For most subjects, the readers used the pre-shot images provided and drew a rough map of the alterations on the schematic diagram of the eye provided in order to assign them a tentative exposure number. The map drawn by the readers and verbal communications were used to fill in the scoring sheet. The other reader(s) based their assessment on the slit lamp images recorded.

The OCT examinations were primarily focused on confirming, when possible, the presence of the alterations identified during the slit lamp examination. The alterations that were confirmed were documented with OCT images for examination at a later date. The fundus camera exam was focused on determining if there were any visible lesions in the retina from laser exposure, however, none were found during this study.

3.4 Data Analysis

The data analysis was broken up into two main sections based on when it was performed. The first section took place while the exposure sessions were taking place. The second section took place after all the exposures had been completed and took a more holistic approach to the identification of the alterations, or lesions, as described below in 3.4.2. In addition, the second section utilized the pre- and post-calibration data, the beam profiles, and the $1/e$ beam diameters to obtain the peak radiant exposures, which were used in the probit analysis as detailed below.

3.4.1. Initial lesion identification and analysis.

During the 1 h post-exposure examination, one or two readers used the slit lamp microscope, with and without fluorescein, to identify any detectable alteration of the cornea. The readers took pictures to document the alterations they observed. For most subjects, the readers used the pre-shot images provided and drew a rough map of the alterations on the schematic diagram of the eye provided in order to assign them a tentative exposure number. The map drawn by the readers and verbal communications were used to fill in the scoring sheet. After each exposure session, a third reader looked at the slit lamp camera pictures and filled in the scoring sheet. The scoring data from two or three readers was used to do an initial probit analysis with the available data: energy delivered obtained from the energy calibration procedure (3.2.3.3) and 0/1 scores for 2 readers. The results of that initial probit analysis guided the development of the exposure plan for the next exposure session at that wavelength and, at the end, helped decide if a 4th eye was needed or not.

3.4.2. Final lesion identification.

After all the exposure sessions had been completed, the knife edge data was used to obtain the $1/e$ focal spot diameters which, when combined with the delivered energy, resulted in the peak radiant exposure for each exposure. One, two or three readers revisited the slit lamp images and revised their scores when needed, based on the peak radiant exposures calculated for each exposure location. This last step was necessary because there were several sources of uncertainty that contributed to misidentifying the location of the putative lesions, see section 3.4.2.1. The criteria that was used to redefine the location of a lesion and/or revise the score associated with a location, considered three items 1) was the lesion observed during the 1 h slit lamp examination, 2) was the peak radiant exposure associated with the lesion within the range where you would expect to see a lesion for that particular exposure session based on other lesions observed and, to a lesser extent, 3) was the lesion observed in the slit lamp images recorded. This second analysis provided an opportunity to examine the slit lamp images while noting the orientation of the eye,

the location of the exposure sites relative to each other and their relative peak radiant exposure. However, given that the slit lamp recorded images were not optimal (see section 3.3.1), not being able to confirm a lesion in the slit lamp images recorded was not weighted as much as having had two readers call a lesion during the actual slit lamp examination. After the readers finalized their scores, probit analysis was used on the 1 h post-exposure data to determine the ED₅₀, the probit slope, and the upper and lower fiducial limits when available. The results of the analyses are presented in section 4.0.

3.4.2.1. Sources of uncertainty in placement of the exposures and lesion identification.

There were several sources of uncertainty that played a role when locating the exposure sites and when identifying the lesion location during the post-exposure examinations. Two factors which affected both were the direction in which the subject's eye was looking and the orientation of the eye relative to the horizontal (which can be related to the position of the head). Both factors, independently or in combination, sometimes prevented the researchers from accessing a certain area of the cornea to expose or to examine. And although the slit lamp images at lower magnification could sometimes provide some information about the direction in which the subject's eye was looking or eye orientation during the examinations, the absence of that information during the exposures, at times prevented the researchers from unambiguously identifying the lesion location. The pre-shot images and videos taken during the exposure were taken in low light conditions. Therefore, they did not contain enough detail to identify the iris in order to know in which direction the subject was looking. This prevented the researchers from knowing precisely if the array of lesions was centered on the cornea or not. However, the pre-shot images did provide some sense of where within the cornea the lesions were placed.

Another factor which played a role in lesion placement was eye movement. When placing the lesions, there is a possibility that the eye moved between the pre-shot image and the actual exposure. However, from the videos taken during exposures, there appear to have been only minor movements. Another source of uncertainty in lesion placement was associated with the co-alignment of the alignment laser and the NIR beam. During some of the exposures, the front-facing camera recorded a bright light in one or two frames. Our analysis indicated that the bright light was a supercontinuum generated by the nonlinear interaction of an ultrashort pulse with the cornea (see APPENDIX F). If we assume that the supercontinuum generated during the exposures localizes the NIR beam at the surface of the cornea, then the two beams were collinear to within 1 - 2 mm.

The researcher performing the slit lamp examinations looked for visible lesions all over the region of the cornea they could access given the orientation of the eye. Once they identified a lesion, they tried to match its location to the pre-shot images. However, given that in some cases the lesions were close together or the eye was rotated relative to the pre-shot images, there was some guesswork involved in correlating a lesion with a specific exposure condition.

3.4.3. Peak radiant exposure calculations and uncertainty.

The objective of this study was to determine the median effective dose or ED₅₀ that produces a minimum visible lesion (MVL) on the cornea upon exposure to a single femtosecond pulse at three different NIR wavelengths. This particular ultrashort laser system can generate the same wavelength in several different ways which results in a different beam profile and output beam diameter each day. In order to eliminate the differences in beam profile and beam diameter from the different experimental days, probit analysis was conducted on the peak radiant exposure instead of the delivered energy where peak radiant exposure is defined as the total energy received by a surface (Q) per unit area (A). For a Gaussian beam it is given by:

$$H = \frac{Q}{A} = \frac{4Q}{\pi * d^2}$$

where the expression on the right is for a circular spot with a $1/e$ diameter d . In the following sections, we will discuss in detail how we obtained the peak radiant exposure for each exposure condition and the uncertainty associated with the quantities involved in these calculations.

3.4.3.1. Uncertainty in the delivered (total) energy.

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

$$Q = R_{avg} * Q_{ref}$$

where R_{avg} is the average of the slopes obtained from the pre and post calibration procedures when available, or the slope of the single available calibration procedure. Note that, given the pulse duration and response time of the detector, the energy measured by the reference detector is the total energy within the fraction of the pulse that reaches it. Therefore, the energy delivered at the exposure site can be considered as the total energy received by the cornea.

The slopes of the pre and post exposure calibration procedures were obtained as follows. The data collected during the calibration procedure for the different energies listed in the exposure plan was aggregated before separating it into two groups. One group contained the data collected with the neutral density filter on a flip mount placed in the optical beam path while the other group did not. Both groups were analyzed separately, using the same procedure. Graphs of the energy measured at the target versus the energy measured by the reference detector were generated showing a clear linear relationship, see Figure 5. A linear regression algorithm in Excel was used to find the slope (R) of the line and the standard error (SE_R) of the slope. The standard error of the slope was related to the standard deviation of the slope ($u(R)$) by the number of data points used in the linear regression (n) as $u(R) = \sqrt{n} * SE_R$.

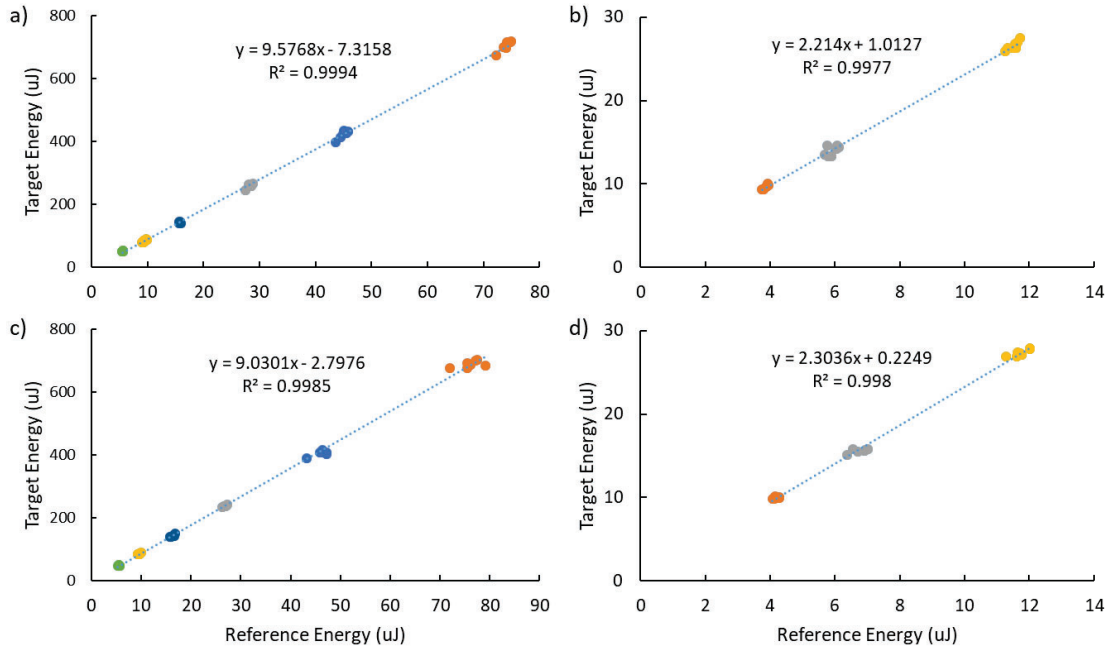


Figure 5. Representative calibration graphs: a) and b) pre-exposure calibration; c) and d) post-calibration procedure. Different color dots correspond to the same half-wave plate and neutral density filter combinations. Graphs b) and d) correspond to exposure

energies which required the use of the neutral density filter on the flip mount. Data shown here was obtained from the experiment conducted on 05/26/2021.

The slopes of the pre (R_{pre}) and post (R_{post}) calibration procedures were averaged as $R_{avg} = \frac{1}{2}(R_{pre} + R_{post})$ therefore the uncertainty in R_{avg} was found from

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

The procedure used to determine the pre and post calibration slopes was designed to account for any shot to shot variability. Averaging the slopes of the pre and post calibration allowed to account for changes in the laser's output power over time and variations in the angular positioning of the half-wave plate relative to the pre-exposure calibration.

In order to determine the uncertainty in the energy measured by the reference, we considered that the reference detector had a manufacturer specified calibration uncertainty of 2% and 20 nJ noise equivalent, the power/energy meter had a 1.5% uncertainty. The laser noise was 3% therefore the combined uncertainty for Q_{ref} was calculated as

$$u(Q_{ref}) = \sqrt{(20\text{nJ})^2 + Q_{ref}^2 * ((0.02)^2 + (0.015)^2 + (0.03)^2)}$$

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

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

Eight out of ninety-nine exposures presented technical difficulties that prevented the researchers from validating the delivered energy. These technical difficulties were manifested in two different ways: the oscilloscope indicated that more than one shot had been delivered to the target and/or the reference energy recorded differed from the intended energy for a particular exposure by more than 30%. The origin of these unusual readings could be attributed to a glitch in the oscilloscope or reference meter trigger, a variation in the laser's energy output, problems in the operation of the mechanical shutter, or a variation in the timing which disrupted the single shot capability. Exposures where these technical difficulties were observed were disqualified and the corresponding data was excluded from further analysis.

3.4.3.2. Uncertainty in the focused beam diameter determination.

As mentioned before, in an effort to be consistent with the laser safety standards guidelines, we have chosen to use the $1/e$ diameter for all calculations and comparisons to the laser safety standards. However, the focused beam diameter measured using the knife edge technique corresponds to the $1/e^2$ diameter. Therefore, in this section we will first elaborate on the procedure used to obtain the uncertainty in the measured $1/e^2$ focused beam diameter ($d_{x,1/e^2}$). Once obtained, we will propagate the uncertainty to the derived $1/e$ focused beam diameter.

The $1/e^2$ focused beam diameter in the x -direction ($d_{x,1/e^2}$) 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: $d_{x,1/e^2} = 2(x_{16} - x_{84})$. The uncertainty in the determination of the focused beam diameter was

$$u_B(d_{x,1/e^2}) = \sqrt{\left(\frac{\partial d_{x,1/e^2}}{\partial x_{16}}\right)^2 u^2(x_{16}) + \left(\frac{\partial d_{x,1/e^2}}{\partial x_{84}}\right)^2 u^2(x_{84})}$$

$$u_B(d_{x,1/e^2}) = \sqrt{(2)^2 u^2(x_{16}) + (2)^2 u^2(x_{84})}.$$

Assuming the uncertainties were the same, then

$$u_B(d_{x,1/e^2}) = 2\sqrt{2}u(x).$$

The uncertainty in determining the x position was related to the resolution of the piezoelectric linear stage, which had a 0.2 μm resolution, and how repeatable it was to find the positions at which the signal's intensity became 16% or 84% of the original, reported to be 1 μm . Given that the focusing lens used had a long Rayleigh length, the focused beam diameter reported was obtained by averaging over several measurements within the Rayleigh length, typically 5 to 7 data points. Therefore, the combined uncertainty in the beam diameter determination from the knife edge technique was

$$u(d_{x,1/e^2}) = \sqrt{u_A^2(d_{x,1/e^2}) + u_B^2(d_{x,1/e^2})} = \sqrt{u_A^2(d_{x,1/e^2}) + 8 * [(0.2)^2 + (1)^2]}$$

where $u_A(d_{x,1/e^2})$ is the standard deviation of the averaged measurements.

For the majority of the experiments, the knife edge technique was used to obtain the $1/e^2$ focal spot diameter in only the x -direction. However, during the 1 h post-exposure examination of the last subject exposed to 1318 nm (Ra21-010-OS on 06/14/2021), it was noted that the lesions were elongated vertically. This observation prompted us to perform the knife edge measurement in the perpendicular direction (y) on that and the subsequent experimental day, *i.e.* subject Ra21-010-OD exposed to 1540 nm on 06/21/2021. In addition it prompted us to modify the calculation of the peak radiant exposure. Instead of assuming a circular focal spot, we used the area of an ellipse considering the focal spot diameter in both x and y . In the cases when there was no experimental data for the focal spot diameter in the y -direction, which was the majority of the experiments, we calculated the aspect ratio of the collimated beam as $r = D_x/D_y$ where D_x and D_y were the collimated beam diameters in both directions. The ratio was used to obtain the $1/e^2$ focal spot diameter in the y -direction as $d_{y,1/e^2} = d_{x,1/e^2}/r$ with an uncertainty given by $u(d_{y,1/e^2}) = u(d_{x,1/e^2})/r$.

The $1/e$ focused beam diameters (d_x, d_y) were obtained from the $1/e^2$ focused beam diameters as

$$d_x = \frac{d_{x,1/e^2}}{\sqrt{2}} \quad \text{and} \quad d_y = \frac{d_{y,1/e^2}}{\sqrt{2}} = \frac{1}{\sqrt{2}} \frac{d_{x,1/e^2}}{r},$$

and the respective uncertainties were found from

$$u(d_x) = \frac{u(d_{x,1/e^2})}{\sqrt{2}} \quad \text{and} \quad u(d_y) = \frac{u(d_{y,1/e^2})}{\sqrt{2}} = \frac{1}{\sqrt{2}} \frac{u(d_{x,1/e^2})}{r}.$$

3.4.3.3. Uncertainty in the peak radiant exposure.

The peak radiant exposure for an elliptical spot from a Gaussian beam is given by

$$H = \frac{Q}{\pi \left(\frac{d_x}{2}\right) \left(\frac{d_y}{2}\right)} = \frac{4Q}{\pi d_x d_y}.$$

where Q is the total energy received by the cornea, and d_x and d_y are the 1/e focal spot diameters in each direction. Therefore the uncertainty in H is given by

$$u^2(H) = \left(\frac{\partial H}{\partial Q}\right)^2 u^2(Q) + \left(\frac{\partial H}{\partial d_x}\right)^2 u^2(d_x) + \left(\frac{\partial H}{\partial d_y}\right)^2 u^2(d_y)$$

where

$$\frac{\partial H}{\partial Q} = \frac{4}{\pi d_x d_y}, \quad \frac{\partial H}{\partial d_x} = -\frac{1}{d_x} \left(\frac{4Q}{\pi d_x d_y} \right), \text{ and } \quad \frac{\partial H}{\partial d_y} = -\frac{1}{d_y} \left(\frac{4Q}{\pi d_x d_y} \right).$$

Substituting and simplifying, it can be shown that

$$\frac{u^2(H)}{H^2} = \frac{u^2(Q)}{Q^2} + \frac{u^2(d_x)}{d_x^2} + \frac{u^2(d_y)}{d_y^2}$$

and

$$\frac{u(H)}{H} = \sqrt{\left(\frac{u(Q)}{Q}\right)^2 + \left(\frac{u(d_x)}{d_x}\right)^2 + \left(\frac{u(d_y)}{d_y}\right)^2}$$

where $u(Q)$ was calculated using the equation in section 3.4.3.1, $u(d_x)$ and $u(d_y)$ were calculated following section 3.4.3.2 above. The maximum uncertainty in the peak radiant exposure obtained for any exposure condition at 1318 nm was 10.4%, at 1540 nm it was 11.0%, and at 2000 nm it was 10.5%.

3.4.4. Probit analysis.

A computer program developed in-house (probit ver 2_1_3) [66] was used to analyze the data, *i.e.* radiant exposures and corresponding 0/1 scores, using the probit procedure [61]. It allowed the determination of the median effective dose, or peak ED₅₀, that produces a minimum visible lesion (MVL) on the cornea in 50% of the exposures upon exposure to a single femtosecond pulse. It was also used to determine the lower and upper fiducial limits (95% confidence interval) as well as the slope of the dose response curve at the peak ED₅₀ point. In addition, we used the results of the analysis to determine the standard deviation as $\sigma = (ED_{84} - ED_{16})/2$ where ED₈₄ and ED₁₆ represented the dose for 84% and 16% probability of damage respectively [67]. Note that the ED₅₀ values obtained in this manner were actually peak ED₅₀ values given that the energy delivered corresponded to the total energy received by the cornea.

After the readers inspected the corneas and assigned observable alterations to an exposure location by considering the relative location of the alterations observed, a subsequent analysis was used to refine these assignments while considering the actual radiant exposure used at each site. Another additional refinement of the data used for the probit analysis, was to remove any data points where the exposure energy could not be verified as described in 3.4.3.1. A total of 5 data points were disqualified for 1318 nm, 1 for 1540 nm, and 2 for 2000 nm.

Probit analysis was only performed on the 1 h post-exposure data as these are what is typically considered for establishing cornea safety standards. Enough data were collected to obtain a slope of the dose response curve greater than two. This resulted in exposing four subjects at 1318 nm, three at 1540 nm, and four at 2000 nm. However, one of the subjects exposed to 1318 nm (Ra21-010 OS) was excluded from the probit analysis given that there was a large uncertainty in the determination of the focal spot size on that day, see section 4.1 and D.1.4.

3.4.5. Comparison to the current laser safety standards.

This study had two main goals: 1) obtain biological data in the form of median effective doses for single femtosecond pulses at NIR wavelengths and 2) determine if the laser safety limits recommended by the current ANSI standards are sufficiently protective of the cornea for single femtosecond pulses at NIR wavelengths. Two different approaches were used to evaluate if the current laser safety standards for ~100 fs single pulse exposures at 1540 nm and 2000 nm provide adequate ocular protection. In the first approach the ED₅₀ obtained from the peak radiant exposure data using probit analysis, or peak ED₅₀, was directly compared to the MPE limits from the ANSI laser safety standards. This approach was aligned with the ICNIRP guidelines for visible beam diameters smaller than 1 mm and pulse durations less than 0.35 s. [9]

The second approach followed the recommendation of the ANSI standards to average the radiant exposure over a 1 mm limiting diameter aperture as the current MPE values are referred to a 1 mm limiting diameter aperture. [8] Therefore, for the second approach we utilized the mean 1/e focal spot diameter to obtain the ED₅₀ expressed as the average radiant exposure over a 1 mm diameter limiting aperture, or average ED₅₀ as prescribed in the ANSI standards. The average radiant exposure for a Gaussian beam is related to the peak radiant exposure by

$$H_{avg} = H \left(\frac{d_M}{d_C} \right)^2 \left[1 - \exp \left(- \left(\frac{d_C}{d_M} \right)^2 \right) \right]$$

where H is the peak radiant exposure (peak ED₅₀), d_M is the mean 1/e focal spot diameter from the experiments, and d_C is the 1 mm limiting diameter aperture. The resulting average radiant exposures were then compared to the MPE limits for 100 fs pulse durations listed in the ANSI laser safety standards.

4.0 RESULTS

4.1 Wavelength 1 – 1318 nm

Four anesthetized subjects were exposed to 1318 nm, and although we aggregated the data for three of them for the probit analysis, they presented some differences that are worth noting. As mentioned before, for one subject the researchers could not unambiguously determine what the focal spot size was during exposures. Consequently, that subject was excluded from the final probit analysis performed on the data (for more details see section D.1.4). In the following, we will address the differences we observed during the 1 h post-exposure examination of the three subjects used for the probit analysis.

4.1.1. Representative images.

Figure 6 shows slit lamp images extracted from the baseline, 1 h, and 24 h examinations of subject Ra21-007 OD. There was nothing notable during the baseline exam, panels a) and b). However, during the 1 h examination this particular subject presented a small abrasion on the upper right hand side of the cornea which is clearly visible in panels d), e) and f). It was determined that this

abrasion was unrelated to the laser exposures and the readers were able to identify 6 laser induced lesions. In the slit lamp images without fluorescein, the laser induced lesions appeared as small white spots on the surface of the cornea, at the location of the laser exposure, panels c), g) and h). After fluorescein was applied, the lesions appeared as bright white spots on a blue background, panel d).

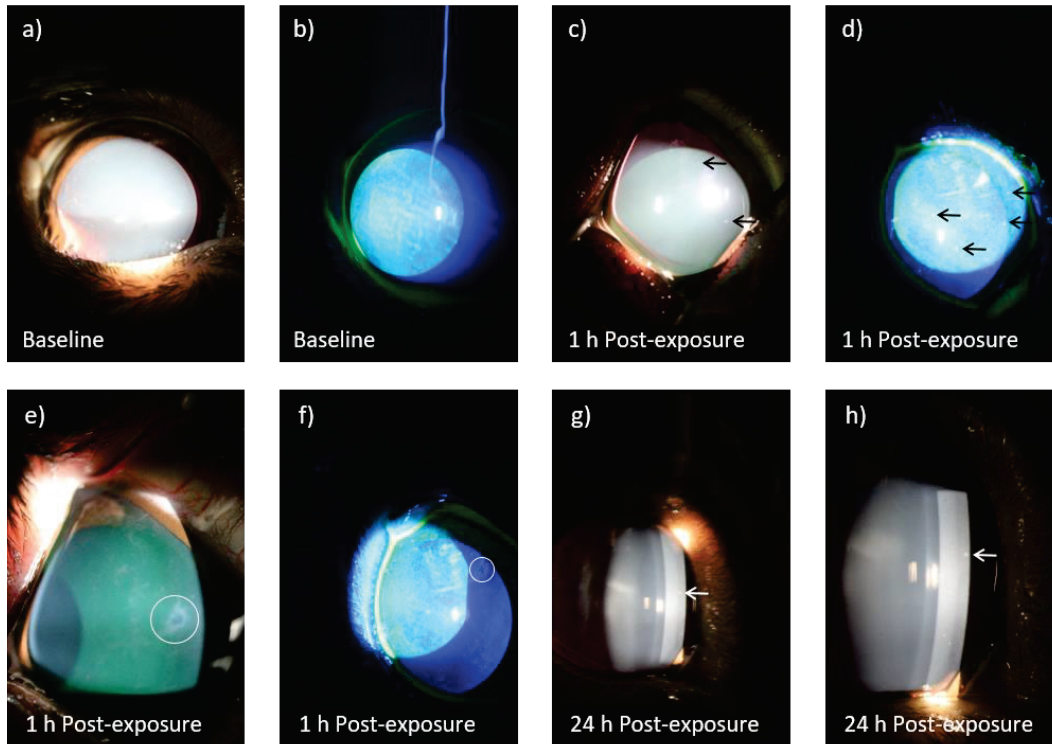


Figure 6. Slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-007 OD. Arrows indicate laser-induced lesions. Circles in panels e) and f) highlight a small abrasion unrelated to laser exposure. Panels b), d), and f) were taken after fluorescein was applied. One lesion identified in panel c) is also visible in panels d), g), and h). Panel h) is a magnified view of panel g).

Figure 7 is a composite image focused on a single lesion in a different subject, Ra21-006 OD. The arrow in panel a) points to the lesion identified in the OCT scans, visualized in the slit lamp after fluorescein was applied. In addition, a large abrasion in the central part of the cornea can be resolved (two bright approximately parallel horizontal lines). It was determined that this abrasion was not present during the baseline exam and was unrelated to the exposures. The abrasion may have been the result of the eyelids opening during transport and the cornea rubbing against the gauze or some other surface. The readers were able to identify 2 laser induced lesions. Panel b) is an optical image of the eye obtained with the OCT. The green box indicates the region which was examined in that particular scan with the OCT while the green arrow indicates the location of the B-scan shown in panel c). For this particular lesion the B-scan shows that there was damage to the epithelium that extends into the stroma creating a full-thickness lesion. This lesion corresponds to a delivered radiant exposure $\sim 30\%$ lower than the ED_{50} .

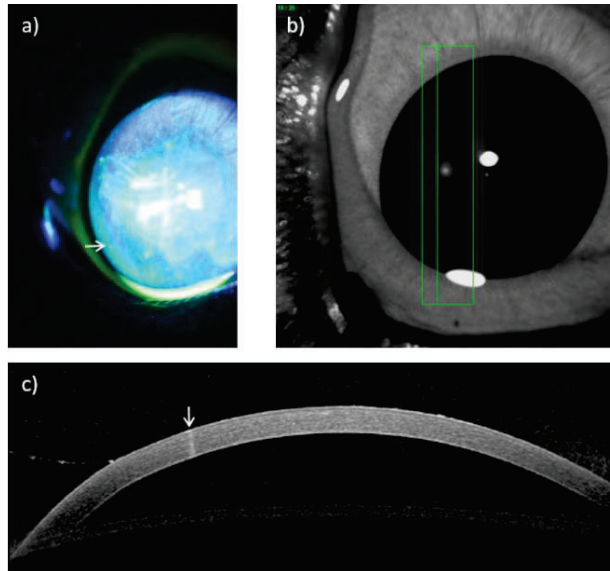


Figure 7. Subject Ra21-006 OD 0.7×ED₅₀. a) Slit lamp image showing the location of the lesion studied in the following panels as well as a large abrasion in the central region. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion.

Figure 8 is a composite image focused on a single lesion on subject Ra21-007 OD. For this lesion the B-scan appears to indicate that there was damage to the epithelium with possible minimal ablation that extends into the stroma as a full-thickness lesion. This lesion corresponds to a delivered peak radiant exposure of approximately 1.1 times the ED₅₀.

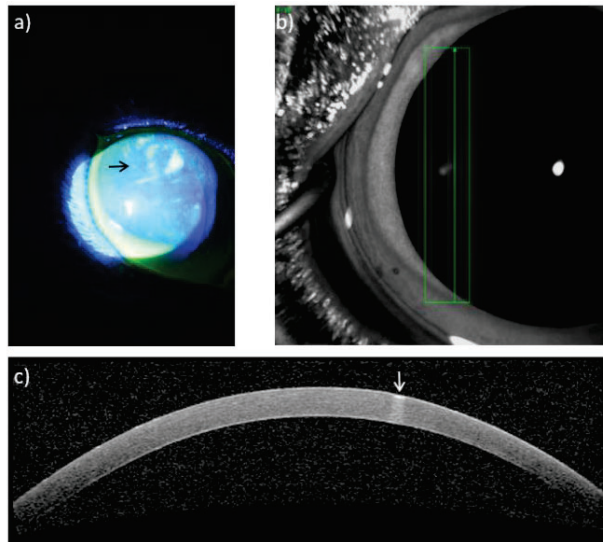


Figure 8. Subject Ra21-007 OD 1.1×ED₅₀. a) Slit lamp image showing the location of the lesion studied in the following panels. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion and possible damage to Descemet's membrane.

Figure 9 focuses on a different lesion in the same subject. For this lesion the B-scan appears to indicate that there was substantial damage to the epithelium, *i.e.* corneal ablation, and an injury

that extends into the stroma as a full-thickness lesion. This lesion corresponds to a delivered radiant exposure of 7.1 times the ED_{50} .

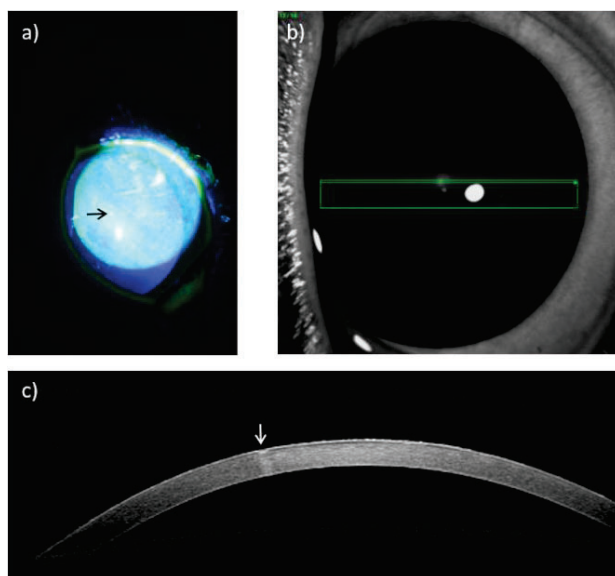


Figure 9. Subject Ra21-007 OD 7.1 $\times$ ED₅₀. a) Slit lamp image showing the location of the lesion studied in the following panels. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion and epithelial ablation.

Figure 10 shows slit lamp images extracted from the baseline, 1 h, and 24 h examinations of subject Ra21-003 OS. For this subject, the readers identified 3 laser induced lesions. From these images, there do not appear to be any differences between the lesions identified for this subject and those from other subjects. However, the OCT examination revealed that one of the lesions was a full-thickness corneal lesion with apparent disruption of the Descemet membrane, see Figure 11. Although this lesion corresponds to a delivered radiant exposure of 3.3 times the ED_{50} , the same behavior was observed for another lesion corresponding to a delivered radiant exposure of 2.2 times the ED_{50} (data not included). Unfortunately, no other lesions were found during the OCT examination of this subject. One additional observation was that the damage observed was not restricted to an individual B-scan, extending to over 6 frames in some cases. At this time this remains unexplained. Nevertheless, the data for this subject was included in the probit analysis given that the criteria for scoring the lesions is typically based solely on identifying any detectable alteration of the cornea using a slit lamp biomicroscope.

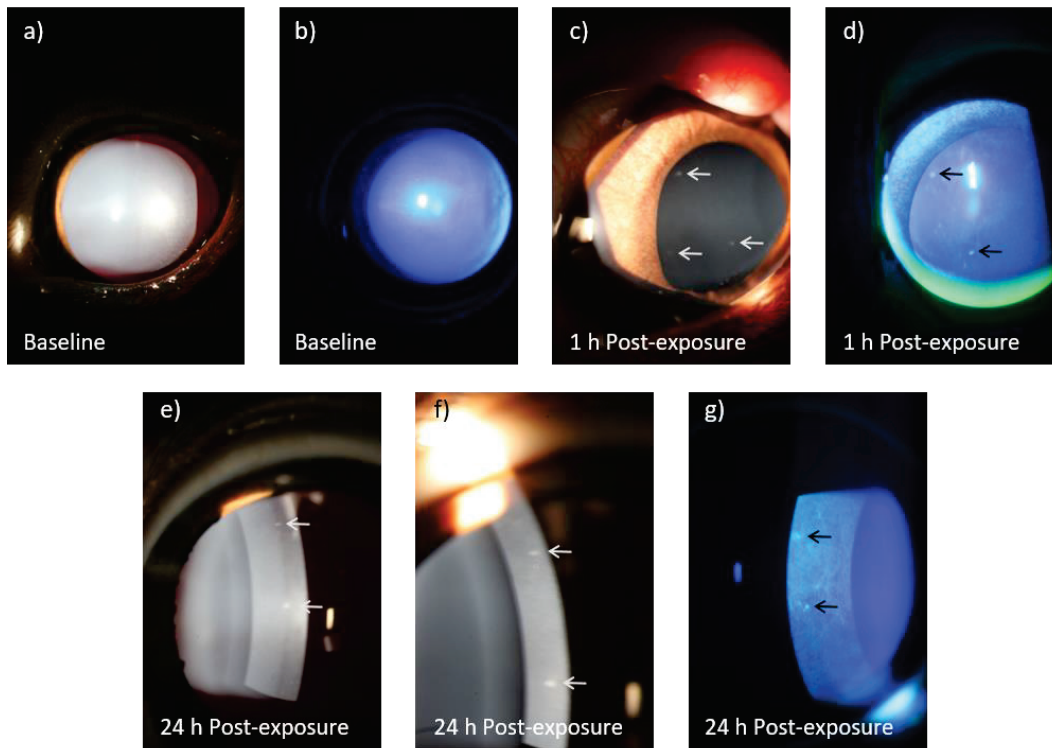


Figure 10. Slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-003 OS. Arrows indicate laser-induced lesions. Panels b), d), and g) were taken after fluorescein was applied. Panel f) is a magnified view of panel e). Two lesions identified in panel c) are also visible in panel d). Lesions identified in panel e) are also visible in panel g).

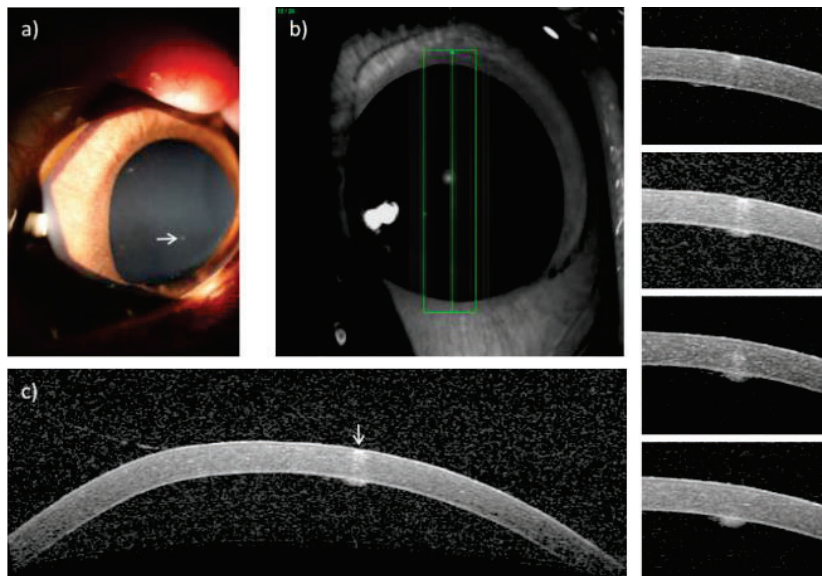


Figure 11. Subject Ra21-003 OS 3.3xED₅₀. a) Slit lamp image showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scans shown in the following panel. c) B-scan showing a full-thickness corneal lesion and apparent disruption of the Descemet membrane.

4.1.2. Probit analysis.

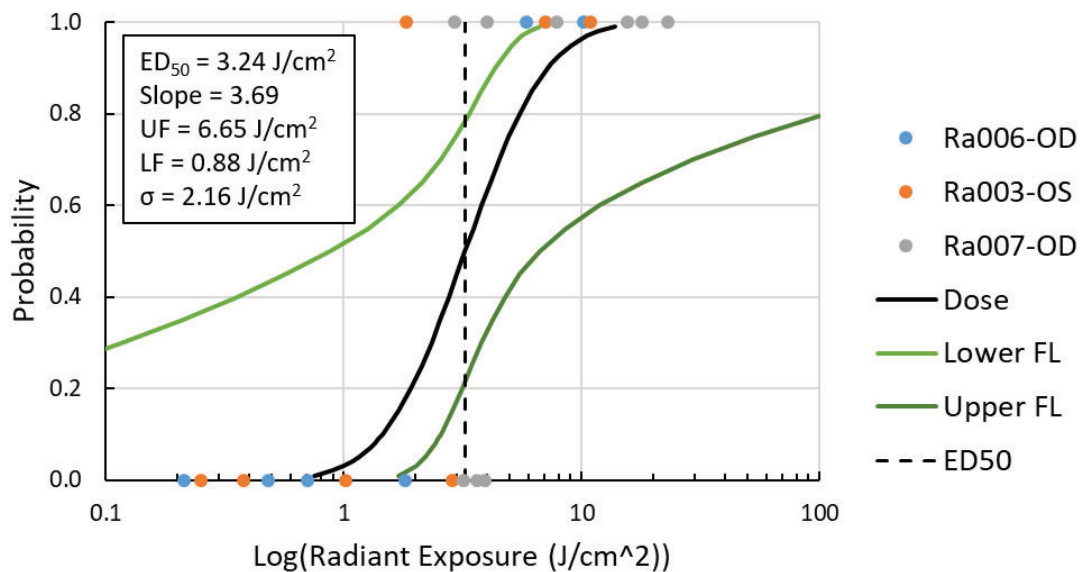


Figure 12. Probit analysis of exposures at 1318 nm for 3 subjects.

The graph showing the results of the probit analysis for the 1 h post-exposure examination as well as the 11 negative results and 11 positive ones is shown in Figure 12. The analysis was able to determine the peak ED_{50} threshold for damage, the lower and upper fiducial limits at 95% confidence, and the slope of the dose response curve at the peak ED_{50} value. The maximum uncertainty in the radiant exposure obtained for any exposure condition included in the analysis was 10.4% as shown in Appendix D.1 for each subject separately. Appendix D.1.6 includes the probit raw data file for the 1 h reads for exposures to 1318 nm.

4.2 Wavelength 2 – 1540 nm

Three subjects were exposed to 1540 nm and all three experiments showed consistent results.

4.2.1. Representative images.

Figure 13 shows slit lamp images extracted from the baseline, and 1 h examination of subject Ra21-010 OD. During the baseline examination this particular subject presented a small feature that resembled a laser-induced lesion. However, that feature was not visible when fluorescein was applied and did not hinder lesion identification at the 1 h post-exposure examination. In this particular subject, readers were able to distinguish all laser induced lesions. In the slit lamp images without fluorescein, the lesions appeared as a small white spot on the surface of the cornea, in the location of the laser exposure, panels d) and e). When fluorescein was added, the lesions appeared as bright white spots on a blue background, panels f) and g). When in focus, they appear to have clearly defined margins.

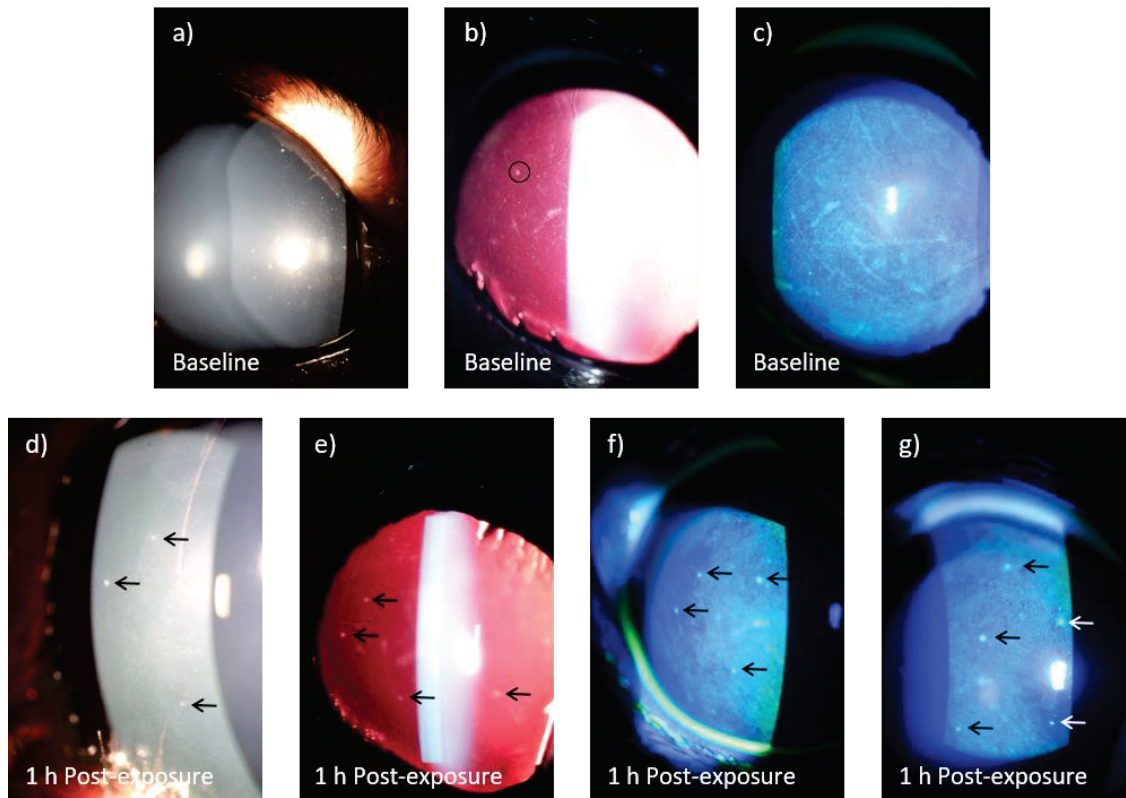


Figure 13. Representative slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-010 OD. Circle highlights a feature resembling a laser-induced lesion identified in the baseline examination. Arrows indicate laser-induced lesions. Panels c), f), and g) were taken after fluorescein was applied. The two lesions identified in panel d) are also visible in panels e) and f).

Figure 14 is a composite image focused on a single lesion in the same subject. The arrow in panel a) points to the identified in the OCT scans, visualized in the slit lamp after fluorescein was applied. Panel b) is an optical image of the eye obtained with the OCT. The green box indicates the region which was examined in that particular scan with the OCT while the green arrow indicates the location of the B-scan shown in panel c). For this particular lesion the B-scan appears to indicate that there was damage to the epithelium that extends into the stroma. However, the lesion is not a clear full-thickness lesion. This lesion corresponds to a delivered radiant exposure about 10% below the ED₅₀.

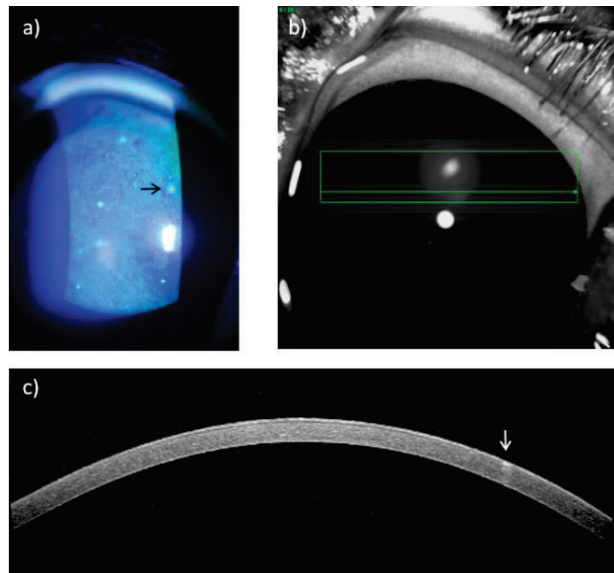


Figure 14. Subject Ra21-010 OD 0.9×ED₅₀. a) Slit lamp image showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing epithelial damage that possibly extends to Bowman's layer.

Figure 15 is focused on a different lesion in the same subject. For this lesion the B-scan appears to indicate that there was more substantial damage to the epithelium as represented by a more defined white region. The damage extends into the stroma down to the endothelium, *i.e.* full-thickness lesion. This lesion corresponds to a delivered radiant exposure ~20% above the ED₅₀.

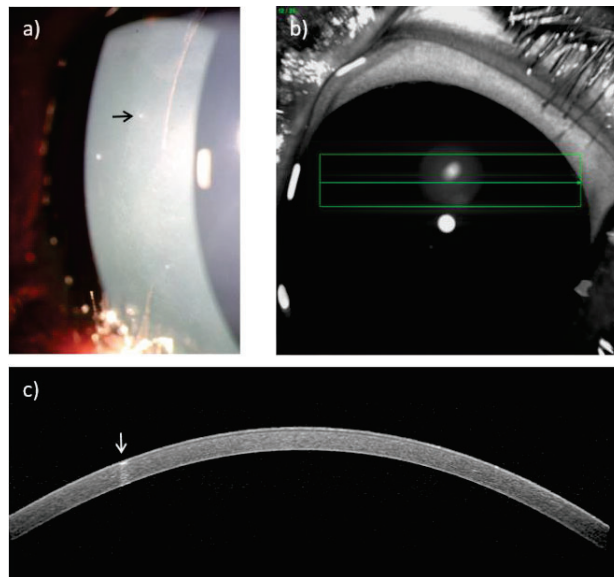


Figure 15. Subject Ra21-010 OD 1.2×ED₅₀. a) Slit lamp image showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion with damage to Bowman's layer.

Figure 16 is a composite image focused on a single lesion on subject Ra21-009 OD. For this lesion the B-scan appears to indicate that there was substantial damage to the epithelium with

possible minimal ablation and the injury extends into the stroma as a full-thickness lesion. This lesion corresponds to a delivered radiant exposure of 3.9 times the ED_{50} .

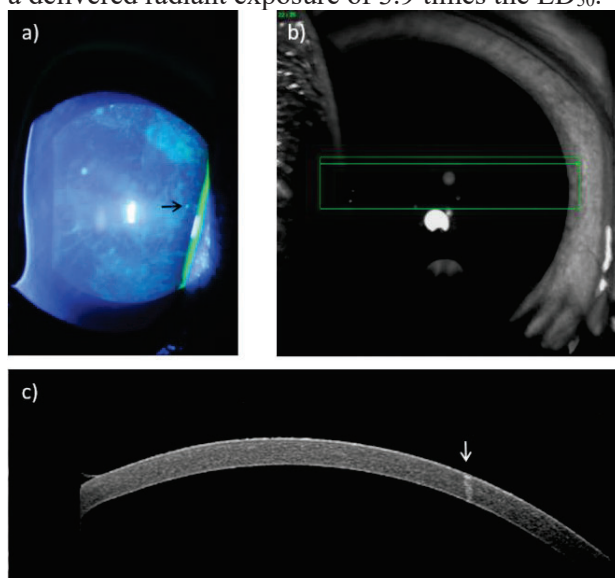


Figure 16. Subject Ra21-009 OD $3.9 \times ED_{50}$. a) Slit lamp image showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan shown in the following panel. c) B-scan showing a full-thickness corneal lesion with damage to the stroma below Bowman's layer.

4.2.2. Probit analysis.

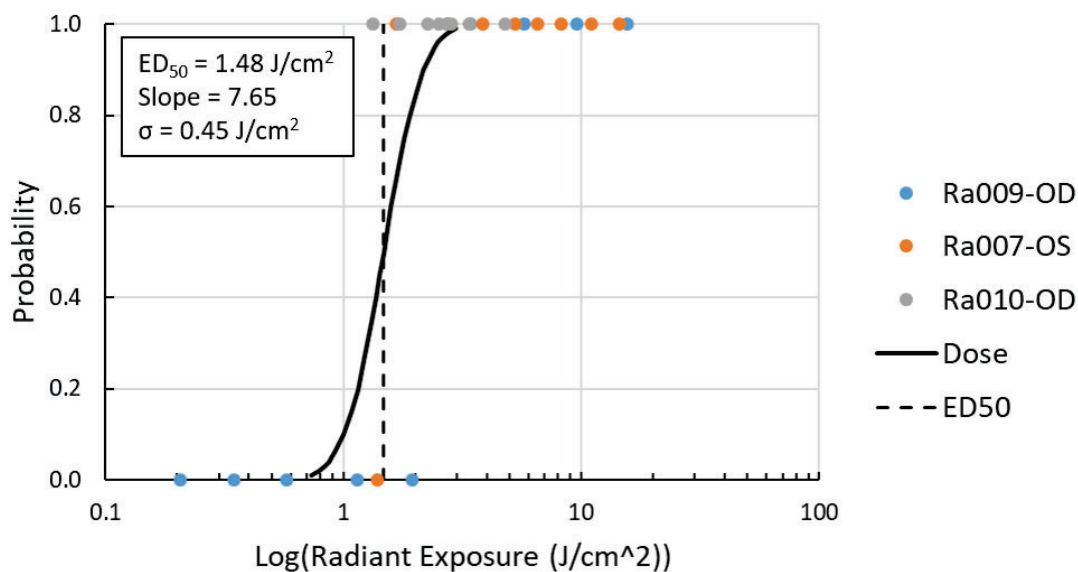


Figure 17. Probit analysis of exposures at 1540 nm.

The graph showing the results of the probit analysis for the 1 h post-exposure examination as well as the data points used is shown in Figure 17 where there were 6 negative results and 20 positive ones. Given the specific distribution of positive and negative results, the analysis was only able to determine the peak ED_{50} threshold for damage and the slope of the dose response

curve at the peak ED₅₀ value. The maximum uncertainty in the radiant exposure obtained for any exposure condition included in the analysis was 11.0% as shown in Appendix D.2 for each subject separately. Appendix D.2.4 includes the probit raw data file for the 1 h reads for exposures to 1540 nm.

4.3 Wavelength 3 – 2000 nm

Four subjects were exposed to 2000 nm and all experiments showed consistent results. However, we were not able to visualize with the OCT any of the lesions produced with delivered radiant exposures close to the ED₅₀ so we have not included any images under those conditions.

4.3.1. Representative images.

Figure 18 shows slit lamp images extracted from the baseline, 1 h, and 24 h examinations of subject Ra21-003 OD. In this particular subject, readers were able to distinguish six laser induced lesions. In the slit lamp images without fluorescein, the lesions appeared as a small white spot with clearly defined margins on the surface of the cornea, at the location of the laser exposure, panels c) and d). When fluorescein was added, the lesions appeared as bright white spots on a blue background, panel e). Lesions were no longer visible at the 24 h exam, panels f) and g).

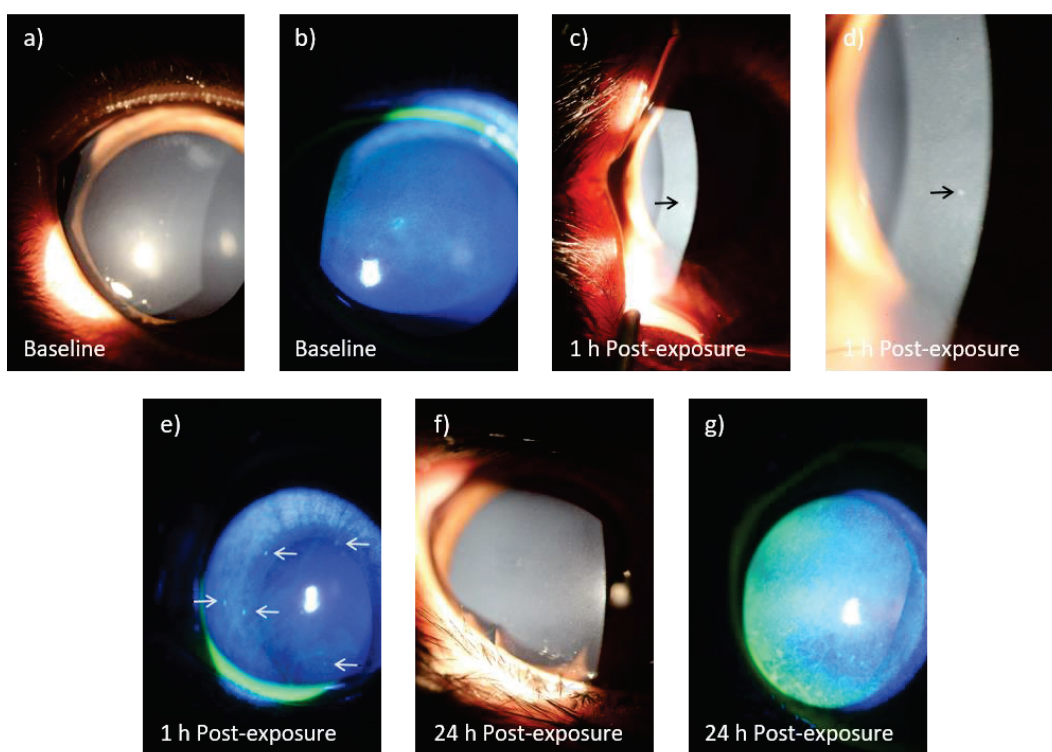


Figure 18. Representative slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-003 OD. Arrows indicate laser-induced lesions. Panels b), e), and g) were taken after fluorescein was applied. Panel d) corresponds to a magnified view of panel c).

Figure 19 is a composite image focused on a single lesion on subject Ra21-005 OS. The arrow in panel a) points to the lesion identified in the OCT scans, visualized in the slit lamp after fluorescein was applied. Panel b) is an optical image of the eye obtained with the OCT. The green box indicates the region which was examined in that particular scan with the OCT while the green arrow indicates the location of the B-scan shown in panel c). For this particular lesion the B-scan appears to indicate that the damage was mostly confined to the epithelium but may

extend slightly into the stroma. This lesion corresponds to a delivered radiant exposure about 20% above the ED₅₀.

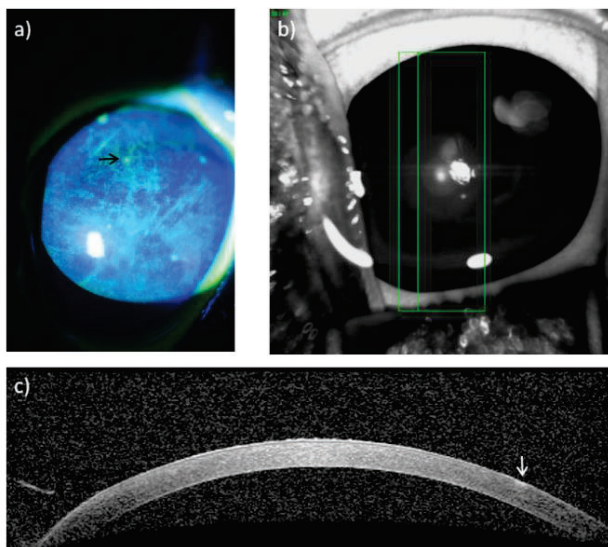


Figure 19. Subject Ra21-005 OS 1.2×ED₅₀. a) Slit lamp image with fluorescein showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan presented in the following panel. c) B-scan of the lesion showing damage to the epithelium.

Figure 20 is focused on a lesion in a different subject, Ra21-006 OS. The arrow in panel a) indicates the lesion that was also visualized with the OCT. For this lesion the B-scan appears to indicate that the damage is mostly superficial but penetrates slightly into the stroma. This lesion corresponds to a delivered radiant exposure about 2.3 times the ED₅₀.

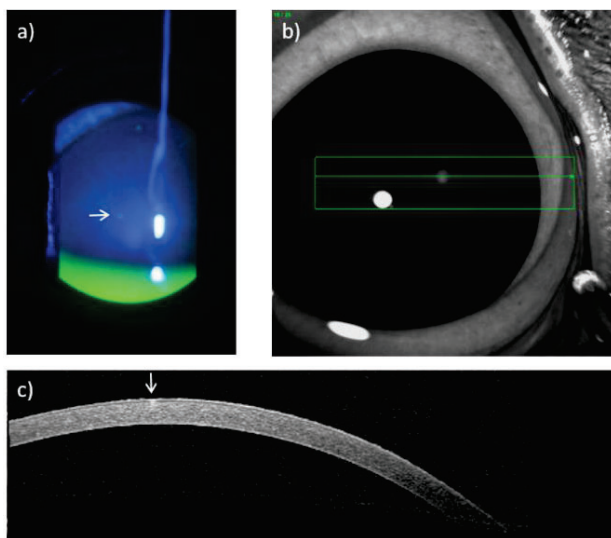


Figure 20. Subject Ra21-006 OS 2.3×ED₅₀. a) Slit lamp image with fluorescein showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan presented in the following panel. c) B-scan of the lesion showing epithelial ablation.

Figure 21 is focused on a different lesion in subject Ra21-006 OS. The arrow in panel a) indicates the lesion that was visualized with the OCT and shown in the other panels. For this lesion the B-scan appears to indicate epithelial ablation at the exposure location. However, the ablation does not appear to penetrate deeply into the stroma. This lesion corresponds to a delivered radiant exposure about 3 times the ED_{50} .

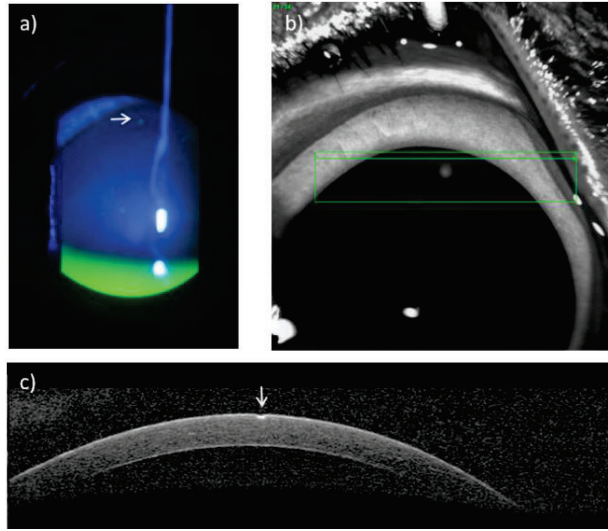


Figure 21. Subject Ra21-006 OS $3 \times ED_{50}$. a) Slit lamp image with fluorescein showing the location of the lesion studied in the following panels as indicated by the arrow. b) Optical image of the eye with green box indicating the region examined with the OCT and the green arrow indicating the location of the B-scan presented in the following panel. c) B-scan of the lesion showing epithelial ablation.

4.3.2. Probit analysis.

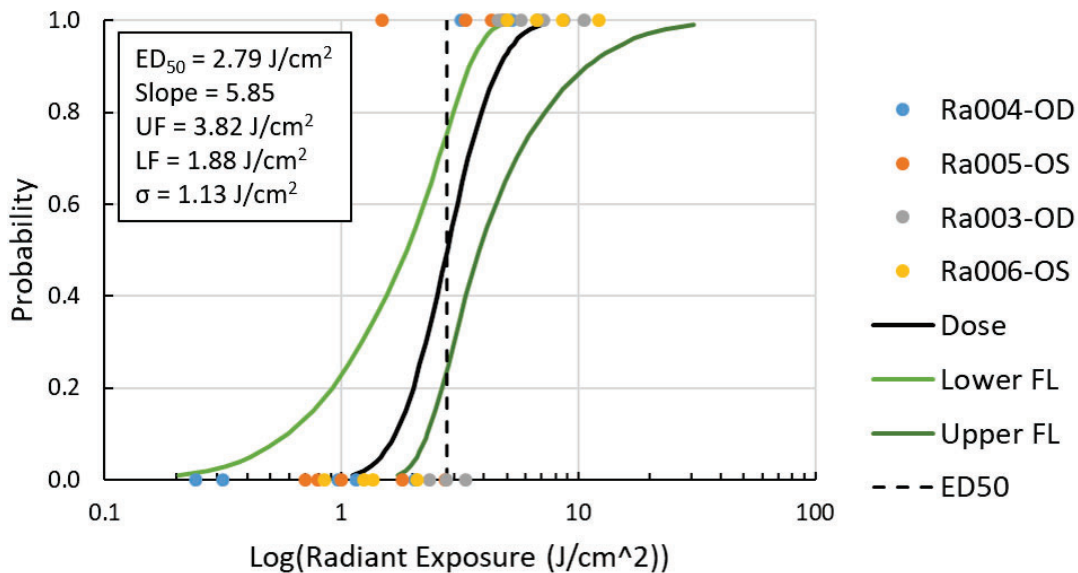


Figure 22. Probit analysis of exposures at 2000 nm.

The graph showing the results of the probit analysis for the 1 h post-exposure examination as well as the 17 negative results and 17 positive ones is shown in Figure 22. The analysis was able to determine the peak ED_{50} threshold for damage, the lower and upper fiducial limits at 95%

confidence, and the slope of the dose response curve at the ED₅₀ value. The maximum uncertainty in the radiant exposure obtained for any exposure condition included in the analysis was 10.5% as shown in Appendix D.3 for each subject separately. Appendix D.3.5 includes the probit raw data file for the 1 h reads for exposures to 2000 nm.

5.0 DISCUSSION

One of the goals of the bioeffects group at TRSL is to gather experimental data that will inform the laser safety community and enable them to evaluate if current MPE limits are sufficiently protective. In order to determine if the current ocular MPE limits are sufficiently protective, it is necessary to determine the median effective dose, or ED₅₀, that produces a minimum visible lesion (MVL) and compare to the current MPE limits. This particular study determined the corneal ED₅₀ upon exposure to a single, approximately 100 fs, pulse at three different NIR wavelengths; 1318 nm, 1540 nm, and 2000 nm. After exposures, the corneas were examined using a slit lamp microscope to identify what radiant exposure values produced a lesion on the cornea. In addition, the corneas were examined with an OCT in order to gain more information about the extent of the injuries. The fundus camera was used to evaluate if the retina was affected or not, however, there were no retinal lesions visible in any of the subjects during the fundus examination. Probit analysis was used to analyze the data collected at each wavelength and determine the peak ED₅₀ threshold for damage, the lower and upper fiducial limits at 95% confidence, and the slope of the dose response curve at the ED₅₀ value. From the dose response curve, the standard deviation for the median effective dose was calculated as $\sigma = (ED_{84} - ED_{16})/2$ where ED₈₄ and ED₁₆ represented the dose for 84% and 16% probability of damage, respectively. Note that the probit analysis was performed on the peak radiant exposure data, therefore we will refer to the resulting ED₅₀ as the *peak* ED₅₀.

Table 2 summarizes the number of subjects (N) and the number of negative (0 - no lesion) and positive (1 - lesion) scores that were used to perform the probit analysis as well as the parameters obtained from the analysis for each of the three wavelengths, *i.e.* the peak ED₅₀ threshold for damage to the cornea, the lower and upper fiducial limits at 95% confidence, the slope of the dose response curve at the peak ED₅₀ value, and the standard deviation. Note that for 1540 nm the overlap between the positive and negative scores was minimal therefore the probit analysis did not result in an upper and lower fiducial limit. The slope was larger than 2 in all cases.

Table 2. Parameters of the probit analysis for 1 h post-exposure damage thresholds

Wavelength (nm)	Number of subjects	N (0, 1)	Peak ED ₅₀ (J/cm ²)	σ (J/cm ²)	Lower Fiducial Limit (J/cm ²)	Upper Fiducial Limit (J/cm ²)	Probit Slope
1318	3	(11, 11)	3.24	2.16	0.88	6.65	3.69
1540	3	(6, 20)	1.48	0.45	NA	NA	7.65
2000	4	(17, 17)	2.79	1.13	1.88	3.82	5.85

Historically, the criteria used to consider the measurement of an ED₅₀ complete at a particular wavelength in the AFRL bioeffects group is to have a slope of the probit curve larger than 2 and an ED₅₀, with 95% confidence limits, within 50% of the ED₅₀. Upon examination of the data presented in Table 2 it is clear that although the data satisfied the criteria of having a slope of the probit curve larger than 2, the 95% confidence limits within 50% of the ED₅₀ were only satisfied at 2000 nm. The criteria listed above are satisfied by all 28 ocular studies to determine the corneal ED₅₀ found in the literature for UV and IR wavelengths. Therefore, there may be a need to perform more exposures in order to tighten the fiducial limits and increase the certainty with which we know the ED₅₀ at these wavelengths.

Section 5.1 will compare the results obtained for the ED₅₀ to the maximum permissible exposure (MPE) limit from current laser safety standards using two different approaches. In section 5.2, the characteristics of the injuries observed at the different wavelengths will be compared in light of the transmission characteristics of the cornea. Finally, in section 5.3 we will attempt to summarize some of lessons learned in the execution of this study from the perspective of performing *in vivo* cornea exposures and provide some concluding remarks in section 5.4.

5.1 Comparison of ED₅₀ Values to Current Laser Safety Standards

Two different approaches were used to evaluate if the current laser safety standards for ~100 fs single pulse exposures at 1318 nm, 1540 nm and 2000 nm provide adequate protection, see Table 3. First, the ED₅₀ obtained from the peak radiant exposure data, or peak ED₅₀, was compared to the MPE limits from the laser safety standards. For 1318 nm, the peak ED₅₀ was four orders of magnitude larger than the MPE indicating that current safety standards, using the peak radiant exposure, provide ample protection to a single femtosecond pulse at this wavelength. For 1540 nm, the peak ED₅₀ was about 15 times larger than the MPE indicating that current safety standards, using the peak radiant exposure, provide adequate protection to a single femtosecond pulse at this wavelength. For 2000 nm the peak ED₅₀ was almost 300 times the corresponding MPE indicating that current safety standards, using the peak radiant exposure, provide ample protection to a single femtosecond pulse at this wavelength.

Table 3. Comparison of the ED₅₀ to current MPE limits

Wavelength (nm)	Peak ED ₅₀ (J/cm ²)	Mean 1/e Focal Spot Diameter (μm)	Average ED ₅₀ (J/cm ²)	MPE (J/cm ²)	Peak ED ₅₀ / MPE	Average ED ₅₀ / MPE
1318	3.24	82 ± 15	0.0218	5.33×10 ⁻⁵	6.08×10 ⁺⁴	409
1540	1.48	97 ± 33	0.0139	0.1	14.8	0.139
2000	2.79	109 ± 11	0.0331	0.01	279.0	3.315

For the second approach, we utilized the mean NIR 1/e focal spot diameter to obtain the ED₅₀ expressed as the average radiant exposure over a 1 mm diameter limiting aperture, or average ED₅₀, see 3.4.5. The mean beam diameter (d_M) was the average of the focal spot 1/e diameters (d_x and d_y) on the different experimental days. The mean focal spot diameters were (82 ± 15 μm) at 1318 nm, (97 ± 33 μm) at 1540 nm, and (106 ± 11 μm) at 2000 nm where the uncertainties listed are the standard deviations obtained from the averages. The average ED₅₀ was compared to the MPE limit from the current laser safety standards for 100 fs pulse durations. From Table 3 it is clear that the average ED₅₀ for 1318 nm was ~400 times larger than the corresponding MPE limit therefore the current safety limit provides ample ocular protection. In contrast, the average corneal ED₅₀ value for 1540 nm was ~10 times smaller than the corresponding MPE value, indicating that the MPE limit does not provide adequate protection for this exposure conditions. For 2000 nm, the average ED₅₀ was ~3 times larger than the corresponding MPE value, indicating that the MPE limit provides moderate protection to a single femtosecond pulse exposure at this wavelength. It should be noted that the exposures here were for very small beam diameters, much smaller than the 1 mm limiting aperture - see Table 3.

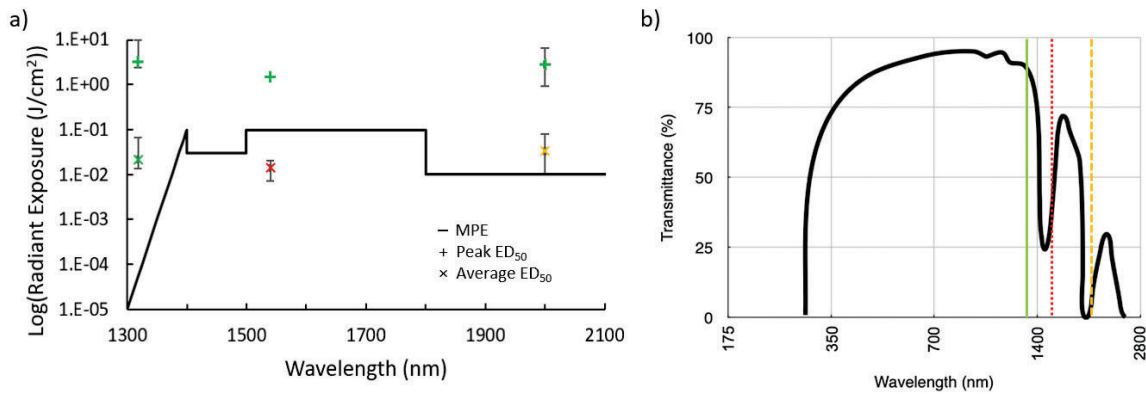


Figure 23. a) Comparison of MPE limits (black line), peak ED_{50} (crosses), and average ED_{50} values (exes). b) Cornea transmittance (black line) where vertical lines are located at 1318 nm (solid green line), 1540 nm (dotted red line), and 2000 nm (dashed yellow line).

The left panel in Figure 23 is a graphical representation of our results where the MPE limit is represented by the black line, the peak corneal ED_{50} values and the average corneal ED_{50} values obtained in this study are represented by crosses and exes respectively. The panel on the right in Figure 23 shows the corneal transmittance (black line) and the three wavelengths used in this study (vertical lines). From Figure 23a) and Table 3, it is evident that there is some ambiguity in how to evaluate the safety of a laser using the current ANSI standards. These results highlight the need for the ANSI standard to clarify how to assess small diameter beams and potentially review the MPE values for sub-nanosecond exposures at NIR wavelengths.

5.2 Comparison of Injury Characteristics Between Wavelengths

The 1 h post-exposure slit lamp examinations showed that for all three wavelengths, the laser induced lesions appeared as small white spots on the cornea with clearly defined edges. However, the OCT images revealed that there were some differences in the lesions as summarized in

Table 4. Note that these observations are based on a limited number of lesions/subjects/images therefore they are being considered as preliminary.

For 1318 nm, it appears that lesions visualized with the OCT were all full thickness, including subthreshold radiant exposures of $0.7 \times ED_{50}$. For a radiant exposure slightly above threshold, *i.e.* $1.1 \times ED_{50}$, the injury was clearly full thickness, there appeared to be damage to Bowman's layer, and the width of the affected region in the stroma increased relative to that at $0.7 \times ED_{50}$. For a radiant exposure of $7.1 \times ED_{50}$, there appeared to be ablation that penetrated slightly into the stroma. The width of the affected region in the stroma was comparable to that at $1.1 \times ED_{50}$. Out of four subjects that were exposed to 1318 nm, the OCT exam of only one subject showed an apparent disruption of the Descemet membrane in the two lesions that were visualized. It is unclear what caused that difference in behavior. Nevertheless, that subject was included in the probit analysis.

For 1540 nm, slightly subthreshold lesions from a radiant exposure of $0.9 \times ED_{50}$ produced epithelial damage, possibly affecting Bowman's layer as well. As the radiant exposure increased above threshold, *i.e.* for $1.2 \times ED_{50}$, the lesion became full thickness with clear damage to Bowman's layer. At $3.9 \times ED_{50}$, it was still a full thickness lesion with clear damage that extended slightly past Bowman's layer into the stroma. The width of the affected region in the stroma was comparable to that at $1.2 \times ED_{50}$.

The damage incurred at 2000 nm was significantly different than for the other wavelengths given that a full thickness lesion was never observed. At this particular wavelength, we were unable to visualize any subthreshold lesions with the OCT. For $1.2 \times \text{ED}_{50}$, there appeared to be minimal epithelial damage. When the radiant exposure increased to $2.3 \times \text{ED}_{50}$, there was clear epithelial damage that affected the Bowman's layer and appeared to extend slightly into the stroma. For a radiant exposure of $3 \times \text{ED}_{50}$, there appeared to be corneal ablation that affected the epithelial and Bowman's layers.

The difference in the lesion characteristics between 1318 nm, 1540 nm, and 2000 nm is expected to be correlated to the transmittance characteristics of the cornea at these wavelengths, see Figure 23b). Wavelengths between 1300 and 1400 nm lie within the transitional region, therefore which tissue is affected and in what manner depends on the wavelength and/or the duration of exposure. From our study, it appears that 1318 nm penetrates the cornea such that the damage involves the deeper corneal stroma. This results in a larger volume which absorbs the energy and a relatively high damage threshold. In addition, given that we observed damage involving the deeper corneal stroma, it appears that ablation is not the mechanism for damage at this wavelength. Wavelengths between 1500 and 1800 nm also penetrate the cornea, therefore the damage can involve deeper corneal stroma as seen in this study and reported in [22]. Wavelengths between 1800 and 2600 nm have moderate penetration into the cornea such that the damage is confined to the epithelium as seen in this study and documented in [16,21,37]. Given the moderate penetration of 2000 nm light into the cornea, it is expected that the damage threshold at 2000 nm would be smaller than at 1540 nm. However, our results do not support that conclusion. Instead, our study appears to indicate that the mechanism of damage is different for 1540 nm and 2000 nm where ablation is apparent in the latter but not in the former.

Table 4. Lesion characteristics based on the radiant exposure relative to the ED_{50}

Wavelength (nm)	Radiant Exposure (J/cm^2)	Observations
1318	$0.7 \times \text{ED}_{50}$	Full thickness lesion.
	$1.1 \times \text{ED}_{50}$	Full thickness lesion with damage to Bowman's layer. Increased width relative to $0.7 \times \text{ED}_{50}$.
	$7.1 \times \text{ED}_{50}$	Full thickness lesion with epithelial ablation. Approximately same width as for $1.1 \times \text{ED}_{50}$.
1540	$0.9 \times \text{ED}_{50}$	Epithelial damage, possibly affecting Bowman's layer.
	$1.2 \times \text{ED}_{50}$	Full thickness lesion with damage to Bowman's layer.
	$3.9 \times \text{ED}_{50}$	Full thickness lesion with damage extending past Bowman's layer into the stroma. Approximately same width as $1.2 \times \text{ED}_{50}$.
2000	$1.2 \times \text{ED}_{50}$	Minimal epithelial damage.
	$2.3 \times \text{ED}_{50}$	Epithelial damage, extending minimally into the stroma.
	$3.0 \times \text{ED}_{50}$	Apparent ablation confined to the epithelial and Bowman's layer.

The authors would like to make a few observations related to the determination of the ED_{50} at 1318 nm. 1) For one of the subjects, the lesions appeared deeper than an MVL given that the Descemet membrane was disrupted. 2) The absorption coefficient of water at 1318 nm is about 100 times lower than at 2000 nm, however, the ED_{50} values determined in this study were comparable for both wavelengths. Observation 1 supports the notion that the threshold value determined was actually higher than what was necessary to produce an MVL. Other studies have established that for pulse durations less than roughly 1 ms, the corneal ED_{50} in the 1000 nm to 5000 nm range follows the wavelength dependence of the absorption coefficient of water. Observation 2 above appears to contradict that notion. These observations indicate that there may be a need to validate the ED_{50} at 1318 nm by collecting more data.

5.3 Insights Gained

While performing this study, the authors noted that there were several actions which would facilitate the implementation of future studies similar in nature. Following is a list of the actions, some of which were implemented during the study while others are listed for reference purposes.

1. Make sure to irrigate the eye and blot the excess fluid at the limbus at least 10 s before exposure.
2. Always expose a set number of seconds after irrigation.
3. Make sure to keep the front facing camera's shutter open all the way while exposing in order to record the SC generated.
4. Keep track of the orientation of the eye while exposing.
5. Use the orientation information when doing the post-exposure eye exams.
6. Consider keeping the exposures closer to the center of the eye to avoid having inaccessible regions during post-exposure examinations.
7. Consider using a pen marker to locate the injuries.
8. Use petrolatum to lubricate the unexposed eye during anesthesia to prevent it from drying out in the event the eye opens up.
9. Setting up the ultrashort system for more than one wavelength per day is not feasible.
10. Improve the alignment of the digital camera on the slit lamp to ensure it is in focus and reflects what is visualized in the ocular.
11. If time permits, have the three readers perform the slit lamp examination. Otherwise, direct one of the readers to collect images from every accessible region of the cornea.
12. Readers may need to get together to reach a consensus on the location of the injuries.
13. Group lesions in sub-areas of the cornea with similar exposure energies. This will make finding and identifying lesions easier during slit lamp inspection.

5.4 Conclusions

This study investigated *in vivo* rabbit cornea damage from single femtosecond pulses at three NIR wavelengths. The peak ED₅₀ threshold for damage, the lower and upper fiducial limits at 95% confidence (LF and UF respectively), and the slope of the dose response curve at the ED₅₀ value were found using probit analysis for single femtosecond pulse exposures at 1318 nm, 1540 nm, and 2000 nm. It was determined that the (peak ED₅₀ ± σ) [LF, UF] for 1318 nm was (3.24 ± 2.16 J/cm²) [0.88 J/cm², 6.65 J/cm²], for 1540 nm it was (1.48 ± 0.45 J/cm²) where no fiducials were found, and for 2000 nm it was (2.79 ± 1.13 J/cm²) [1.88 J/cm², 3.82 J/cm²]. The values obtained for the ED₅₀ at these three wavelengths satisfy the criteria of having a slope of the probit curve larger than 2. However, the 95% confidence limits were only within 50% of the ED₅₀ at 2000 nm, therefore it may be necessary to perform more experiments in order to tighten the fiducial limits.

Nevertheless, the results obtained for the peak ED₅₀s were compared to the current MPE limits using two different approaches. In the first approach, the peak ED₅₀ was directly compared to the MPE as published in the ANSI Z136.1-2022 [68]. In this comparison, the MPE limits provide a safety margin of ~6000 times for corneal exposures at 1318 nm, ~15 times for corneal exposures at 1540 nm, and ~300 times for 2000 nm. Following the guidance from the ANSI standard to average the radiant exposure over a 1 mm limiting aperture for comparing to the MPE, we used the peak ED₅₀ values and the corresponding 1/e focal spot diameter to obtain the average ED₅₀ over a 1 mm diameter aperture. This resulted in average ED₅₀ values of 0.0218 J/cm² at 1318 nm, 0.0139 J/cm² at 1540 nm and 0.0331 J/cm² at 2000 nm. The average ED₅₀ values were directly compared to the current MPE limits. At 1318 nm, it was determined that the ANSI Z136.1-2022 MPE limit was ~400 times smaller than the average ED₅₀ therefore it is extremely protective of the cornea. However, the ANSI Z136.1-2022 MPE limit is not sufficiently

protective of the cornea for single pulse femtosecond exposures at 1540 nm given that the average ED₅₀ damage threshold was ~10 times smaller than the MPE limit. For 2000 nm, the average ED₅₀ damage threshold was ~3 times larger than the MPE limit, providing a relatively small safety margin for exposures. These results highlight the need for the ANSI standard to clarify how to assess small diameter beams and ultrashort pulses and to potentially review the MPE values for NIR sub-nanosecond exposures.

This study was also able to provide some preliminary observations concerning the appearance of the laser induced corneal alterations. The 1 h post-exposure slit lamp examinations showed that for all three wavelengths, the laser induced alterations, also referred to as lesions, appeared as small white spots on the cornea with clearly defined edges. However, the OCT examinations revealed that there were wavelength dependent differences in the depth of the corneal alterations. For 1318 nm, all the alterations visualized, including subthreshold radiant exposures, appeared to involve the full corneal thickness. For 1540 nm, slightly subthreshold lesions appeared to only produce epithelial damage, possibly affecting Bowman's layer as well. However, as the radiant exposure increased above threshold, the alterations involved deeper corneal layers, eventually affecting the full corneal thickness. In contrast, at 2000 nm, no subthreshold alterations were visualized with the OCT, and even for suprathreshold radiant exposures, the alterations remained confined to the epithelial layer. Overall, the results of this study contribute to the current understanding of breakdown dynamics in cornea as well as the knowledge base used for setting laser safety standards in the NIR range for ocular exposure to ultrashort pulses.

6.0 REFERENCES

1. W. Sibbett, A. A. Lagatsky, and C. T. A. Brown, "The development and application of femtosecond laser systems," *Opt. Express*, OE **20**, 6989–7001 (2012).
2. National Academies of Sciences, Engineering, and Medicine, *Opportunities in Intense Ultrafast Lasers: Reaching for the Brightest Light* (2017).
3. M. E. Fermann, A. Galvanauskas, and G. Sucha, "Ultrafast Lasers: Technology and Applications," <https://www.crcpress.com/Ultrafast-Lasers-Technology-and-Applications/Fermann-Galvanauskas-Sucha/p/book/9780367446963>.
4. S. Nolte, F. Schrepel, and F. Dausinger, eds., *Ultrashort Pulse Laser Technology: Laser Sources and Applications*, Springer Series in Optical Sciences (Springer International Publishing, 2016), Vol. 195.
5. K. Sugioka and Y. Cheng, "Ultrafast lasers—reliable tools for advanced materials processing," *Light: Science & Applications* **3**, e149–e149 (2014).
6. H. Lubatschowski, A. Heisterkamp, F. Will, A. Singh, J. Serbin, A. Ostendorf, O. Kermani, R. Heermann, H. Welling, and W. Ertmer, "Medical applications for ultrafast laser pulses," in (2003).
7. T. Udem, R. Holzwarth, and T. W. Hänsch, "Optical frequency metrology," *Nature* **416**, 233–237 (2002).
8. American National Standards Institute, *ANSI Z136.1-2022 American National Standard for Safe Use of Lasers* (Laser Institute of America, 2022).
9. International Commission on Non-Ionizing Radiation Protection, "ICNIRP Guidelines on Limits of Exposure to Laser Radiation of Wavelengths between 180 nm and 1,000 μm," *Health Phys* **105**, 271–295 (2013).
10. W. P. Roach, T. E. Johnson, and B. A. Rockwell, "Proposed maximum permissible exposure limits for ultrashort laser pulses," *Health Phys* **76**, 349–354 (1999).
11. V. Nuzzo, "Mechanisms of femtosecond laser-cornea interaction in keratoplasty," (2008).
12. D. J. Lund, P. R. Edsall, D. R. Fuller, and S. W. Hoxie, "Bioeffects of near-infrared lasers," *Journal of Laser Applications* **10**, 140–143 (1998).

13. J. A. Zuclich, D. J. Lund, B. E. Stuck, and P. R. Edsall, "Wavelength dependence of ocular damage thresholds in the near-IR to far-IR transition region (proposed revisions to MPEs)," *ILSC* **2005**, 58–66 (2005).
14. J. Navaratnam, T. P. Utheim, V. K. Rajasekhar, and A. Shahdadfar, "Substrates for Expansion of Corneal Endothelial Cells towards Bioengineering of Human Corneal Endothelium," *J Funct Biomater* **6**, 917–945 (2015).
15. G. Bozkir, M. Bozkir, H. Dogan, K. Aycan, and B. Güler, "Measurements of axial length and radius of corneal curvature in the rabbit eye," *Acta Med. Okayama* **51**, 9–11 (1997).
16. B. Chen, J. Oliver, S. Dutta, G. H. R. Iii, S. L. Thomsen, and A. J. Welch, "Corneal minimal visible lesion thresholds for 2.0 μm laser radiation," *J. Opt. Soc. Am. A, JOSAA* **24**, 3080–3088 (2007).
17. C. B. Barger, R. A. Farrell, W. R. Green, and R. L. McCally, "Corneal damage from exposure to IR radiation: rabbit endothelial damage thresholds," *Health Phys* **40**, 855–862 (1981).
18. C. B. Barger, O. J. Deters, R. A. Farrell, and R. L. McCally, "Epithelial damage in rabbit corneas exposed to CO₂ laser radiation," *Health Phys* **56**, 85–95 (1989).
19. T. F. Clarke, T. E. Johnson, M. B. Burton, B. Ketzenberger, and W. P. Roach, "Corneal injury threshold in rabbits for the 1540 nm infrared laser," *Aviat Space Environ Med* **73**, 787–790 (2002).
20. D. Courant, C. Chapel, C. Billy, N. Salès, J.-C. Pérot, and C. Pothier, "In vivo and in vitro evaluation of the corneal damage induced by 1573 nm laser radiation," *Journal of Laser Applications* **20**, 69–75 (2008).
21. R. L. McCally, R. A. Farrell, and C. B. Barger, "Cornea epithelial damage thresholds in rabbits exposed to Tm:YAG laser radiation at 2.02 μm ," *Lasers in Surgery and Medicine* **12**, 598–603 (1992).
22. R. L. McCally, J. Bonney-Ray, and C. B. Barger, "corneal epithelial injury thresholds for exposures to 1.54 microm radiation-dependence on beam diameter," *Health Phys* **87**, 615–624 (2004).
23. J. A. Zuclich, M. F. Blankenstein, S. J. Thomas, and R. F. Harrison, "Corneal damage induced by pulsed CO₂ laser radiation," *Health Phys* **47**, 829–835 (1984).
24. P. V. Algvere, P. A. Torstensson, and B. M. Tengroth, "Light transmittance of ocular media in living rabbit eyes," *Invest. Ophthalmol. Vis. Sci.* **34**, 349–354 (1993).
25. E. A. Boettner and J. R. Wolter, "Transmission of the Ocular Media," *Invest. Ophthalmol. Vis. Sci.* **1**, 776–783 (1962).
26. K. Schulmeister, D. H. Sliney, J. Mellerio, D. J. Lund, B. E. Stuck, and J. A. Zuclich, "Review of exposure limits and experimental data for corneal and lenticular damage from short pulsed UV and IR laser radiation," *Journal of Laser Applications* **20**, 98–105 (2008).
27. H. Chen, Z. Yang, J. Wang, P. Chen, and H. Qian, "A comparative study on ocular damage induced by 1319nm laser radiation: OCULAR DAMAGE INDUCED BY NEAR INFRARED LASERS," *Lasers Surg. Med.* **43**, 306–312 (2011).
28. Y. Guo, "Laser damage thresholds of ex-vivo pig and rabbit corneas at 2.5 and 2.7 μm with 8 nanosecond laser pulse duration," (2011).
29. J. Wang, L. Jiao, H. Chen, Z. Yang, and X. Hu, "Corneal thermal damage threshold dependence on the exposure duration for near-infrared laser radiation at 1319 nm," *J. Biomed. Opt.* **21**, 015011 (2016).
30. L. Jiao, J. Wang, X. Jing, H. Chen, and Z. Yang, "Ocular damage effects from 1338-nm pulsed laser radiation in a rabbit eye model," *Biomed. Opt. Express* **8**, 2745 (2017).
31. K. Schulmeister, M. Jean, D. Lund, and B. E. Stuck, "Comparison of corneal injury thresholds with laser safety limits," in *International Laser Safety Conference* (Laser Institute of America, 2019), p. 303.
32. G. M. Hale and M. R. Querry, "Optical Constants of Water in the 200-nm to 200- μm Wavelength Region," *Appl. Opt.* **12**, 555 (1973).

33. B. Ketzenberger, T. E. Johnson, Y. A. Van Gessel, S. P. Wild, and W. P. Roach, "Study of Corneal Lesions Induced by 1,318-nm Laser Radiation Pulses in Dutch Belted Rabbits (*Oryctolagus cuniculus*)," *Comparative Medicine* **52**, 513–517 (2002).
34. Y. D. Berezin, E. V. Boiko, V. V. Volkov, V. F. Danilichev, D. V. Ganin, A. F. Gatzu, N. N. Smirnov, V. V. Lazo, and A. M. Tkachuk, "Peculiarities of coagulation action of IR lasers (1-3 μm) radiation on cornea," in V. A. Serebryakov, ed. (1996), pp. 9–13.
35. B. E. Stuck, D. J. Lund, and E. S. Beatrice, "Ocular Effects of Holmium (2.06 μM) and Erbium (1.54 μM) Laser Radiation," *Health Physics* **40**, 835 (1981).
36. D. J. Lund, M. B. Landers, G. H. Bresnick, J. O. Powell, J. E. Chester, and C. Carver, "Ocular Hazards of the Q-Switched Erbium Laser," *Invest. Ophthalmol. Vis. Sci.* **9**, 463–470 (1970).
37. B. Chen, J. Oliver, S. Dutta, H. Rylander, S. Thomsen, and A. Welch, "Damage Threshold of In Vivo Rabbit Cornea by 2 micron Laser Irradiation," **9** (2007).
38. J. Zuclich, D. Gagliano, F. Cheney, B. Stuck, H. Zwick, P. Edsall, and D. Lund, *Ocular Effects of Penetrating Ir Laser Wavelengths* (Spie - Int Soc Optical Engineering, 1995), Vol. 2391, pp. 112–125.
39. J. A. Zuclich, S. T. Schuschereba, H. Zwick, F. Cheney, and B. E. Stuck, "Comparing laser-induced retinal damage from ir wavelengths to that from visible wavelengths," in *Laser-Inflicted Eye Injuries: Epidemiology, Prevention, and Treatment* (International Society for Optics and Photonics, 1996), Vol. 2674, pp. 66–79.
40. J. A. Zuclich, D. J. Lund, P. R. Edsall, B. E. Stuck, and G. T. Hengst, "High-power lasers in the 1.3- to 1.4- μm wavelength range: ocular effects and safety standard implications," in B. E. Stuck and M. Belkin, eds. (2001), p. 78.
41. J. A. Zuclich, D. J. Lund, B. E. Stuck, and P. R. Edsall, *Ocular Effects and Safety Standards Implications for High-Power Lasers in the 1.3–1.4 μm Wavelength Range.*, Air Force Technical Report AFRL-RH-FS-TR-2004-0187 (2004).
42. A. Vogel and V. Venugopalan, "Mechanisms of Pulsed Laser Ablation of Biological Tissues," *Chem. Rev.* **103**, 577–644 (2003).
43. J. M. Krauss, C. A. Puliafito, and R. F. Steinert, "Laser interactions with the cornea," *Surv Ophthalmol* **31**, 37–53 (1986).
44. J. Noack and A. Vogel, "Laser-induced plasma formation in water at nanosecond to femtosecond time scales: calculation of thresholds, absorption coefficients, and energy density," *IEEE Journal of Quantum Electronics* **35**, 1156–1167 (1999).
45. N. Linz, S. Freidank, X.-X. Liang, and A. Vogel, "Wavelength dependence of femtosecond laser-induced breakdown in water and implications for laser surgery," *Phys. Rev. B* **94**, 024113 (2016).
46. B. C. Stuart, M. D. Feit, S. Herman, A. M. Rubenchik, B. W. Shore, and M. D. Perry, "Nanosecond-to-femtosecond laser-induced breakdown in dielectrics," *Phys. Rev. B* **53**, 1749–1761 (1996).
47. A. Vogel, J. Noack, G. Hüttman, and G. Paltauf, "Mechanisms of femtosecond laser nanosurgery of cells and tissues," *Appl. Phys. B* **81**, 1015–1047 (2005).
48. C. Puliafito and R. Steinert, "Short-Pulsed Nd-Yag Laser Microsurgery of the Eye - Biophysical Considerations," *IEEE J. Quantum Electron.* **20**, 1442–1448 (1984).
49. A.-C. Tien, S. Backus, H. Kapteyn, M. Murnane, and G. Mourou, "Short-Pulse Laser Damage in Transparent Materials as a Function of Pulse Duration," *Phys. Rev. Lett.* **82**, 3883–3886 (1999).
50. A. Vogel, M. R. Capon, M. N. Asiyo-Vogel, and R. Birngruber, "Intraocular photodisruption with picosecond and nanosecond laser pulses: tissue effects in cornea, lens, and retina," *Invest. Ophthalmol. Vis. Sci.* **35**, 3032–3044 (1994).
51. A. Vogel, S. MS, K. MS, and R. Birngruber, "Mechanisms of Intraocular Photodisruption With Picosecond and Nanosecond Laser Pulses," *Lasers in Surgery and Medicine* **15**, 32–43 (1994).

52. M. H. Niemz, "Threshold dependence of laser-induced optical breakdown on pulse duration," *Appl. Phys. Lett.* **66**, 1181–1183 (1995).
53. F. H. Loesel, M. H. Niemz, J. F. Bille, and T. Juhasz, "Laser-induced optical breakdown on hard and soft tissues and its dependence on the pulse duration: experiment and model," *IEEE Journal of Quantum Electronics* **32**, 1717–1722 (1996).
54. H. Sun, M. Han, M. H. Niemz, and J. F. Bille, "Femtosecond laser corneal ablation threshold: dependence on tissue depth and laser pulse width," *Lasers Surg Med* **39**, 654–658 (2007).
55. D. Giguere, G. Olivie, F. Vidal, S. Toetsch, G. Girard, T. Ozaki, J.-C. Kieffer, O. Nada, and I. Brunette, "Laser ablation threshold dependence on pulse duration for fused silica and corneal tissues: experiments and modeling," *J. Opt. Soc. Am. A-Opt. Image Sci. Vis.* **24**, 1562–1568 (2007).
56. G. Olivie, D. Giguere, F. Vidal, T. Ozaki, J.-C. Kieffer, O. Nada, and I. Brunette, "Wavelength dependence of femtosecond laser ablation threshold of corneal stroma," *Opt. Express* **16**, 4121–4129 (2008).
57. L. Hoffart, O. Utéza, N. Sanner, F. Matonti, M. Sentis, B. Ridings, and J. Conrath, "Nouvelle approche de détermination du seuil d'endommagement de tissus biologiques par laser femtoseconde: intérêt et application à la chirurgie de la cornée," [/data/revues/01815512/v33i9/S0181551210002743/](#) (2010).
58. L. Hoffart, P. Lassonde, F. Légaré, F. Vidal, N. Sanner, O. Utéza, M. Sentis, J.-C. Kieffer, and I. Brunette, "Surface ablation of corneal stroma with few-cycle laser pulses at 800 nm," *Opt. Express, OE* **19**, 230–240 (2011).
59. N. A. McPherson, T. E. Eurell, and T. E. Johnson, "Comparison of 1540-nm laser-induced injuries in ex vivo and in vitro rabbit corneal models," *J Biomed Opt* **12**, 064033 (2007).
60. G. W. Mikesell, "Corneal Temperatures—A Study of Normal and Laser-Injured Corneas in the Dutch Belted Rabbit," *Optometry and Vision Science* **55**, 108–115 (1978).
61. "Probit Analysis, 3rd ed. By D. J. Finney, Cambridge University Press, 32 E. 57th St., New York, Ny 10022, 1971. xv + 333 pp. 14.5 × 22 cm. Price \$18.50," *Journal of Pharmaceutical Sciences* **60**, 1432–1432 (1971).
62. Department of Defense (DoD), "Use of Animals in DoD Programs (DoD Instruction Number 3216.01).," (2017).
63. USDA, "Animal Welfare Regulations, 9 C.F.R. Subchapter A," (2013).
64. National Research Council (NRC), *Guide for the Care and Use of Laboratory Animals: Eighth Edition* (National Academies Press, 2011), p. 12910.
65. Army Regulation 40-33, "The Care and Use of Laboratory Animals in DOD Programs, AFMAN 40-401(1)," (n.d.).
66. C. P. Cain, G. D. Noojin, and L. Manning, *A Comparison Of Various Probit Methods For Analyzing Yes/No Data On A Log Scale*. (ANALYTIC SCIENCES CORP SAN ANTONIO TX, 1996).
67. B. Chen, J. Oliver, S. Dutta, G. H. R. Iii, S. L. T. M.d, and A. J. Welch, "Damage threshold of in-vivo rabbit cornea by 2-um laser irradiation," in *Optical Interactions with Tissue and Cells XVIII* (International Society for Optics and Photonics, 2007), Vol. 6435, p. 64350B.
68. American National Standards Institute, *ANSI Z136.1-2014 American National Standard for Safe Use of Lasers* (Laser Institute of America, 2014).

APPENDIX A – List of Instrumentation

The instruments used for this experiment are listed in Table 5. The table consists of 5 different blocks where block 1 is the laser, block 2 are the instruments used for laser characterization and alignment, block 3 are the components of the circuit to implement single shot capabilities, block 4 are the instruments used during exposures, and block 5 are used for the calibration procedures.

Disclaimer: Appearance of, or reference to, any commercial products or services does not constitute DoD endorsement of those products or services.

Table 5. List of instrumentation for laser exposures

Manufacturer	Description	Model	Purpose / Calibration date (if applicable)	Block
Light Conversion Technologies	Optical parametric amplifier	HE-TOPAS	Femtosecond laser system	1
Spectra Physics	Regenerative amplifier	Spitfire Ace SPTFPA-100F-1K-ACE		
Spectra Physics	HeNe laser	905	Alignment laser	2
Tektronix	Oscilloscope	MDO2054	Measure pulse duration	
Minioptic Technology, Inc	Autocorrelator	Delta	Measure pulse duration	
Ocean Optics	Spectrometer	NIR 512-2.5	Measure spectra	
Spiricon	Pyrocam	PY-111 h-C-A	Obtain beam profile	
Coherent	Pyroelectric detector	PM10	Laser's output power detector	
Moletron	Power meter	Powermax 500AD	Laser's output power meter	
Thorlabs	Photodiode	PDA10CF	100 Hz signal for delay generator	3
Stanford Research Systems	Delay generator	DG535	For single shot circuit	
Uniblitz	Shutter driver	T132	Mechanical shutter and driver	
Thorlabs	Photodiode	PDA20CS	Trigger for reference detector	
Tektronix	Oscilloscope	MDO3054	Visualize single shot pulse	
Teledyne	High speed camera	Genie Nano	Side-view camera	4
Imperx	High speed camera	IGV-B0610C-KC000	Front-view camera	
Coherent	Energy meter	LabMaxTOP	Reference meter	
Coherent	Energy sensor	J-10MB-LE	Reference detector	
Coherent	Energy meter	LabMaxTOP	Target meter	5
Coherent	Energy sensor	J-10MB-HE	Target detector	

The instruments used during the baseline, 1 h, and 24 h examinations are listed in Table 6.

Table 6. List of instrumentation used during eye exams

Manufacturer	Description	Model	Purpose
Topcon	Slit lamp microscope	SL-7E	Cornea examinations
Canon	Digital camera	EOS Rebel Ti3	Save images from slit lamp
Heidelberg Engineering	OCT	Spectralis HRA+OCT	Cornea examinations
Topcon	Retinal camera	TDC-50DX	Fundus examinations
Nikon	Digital camera	Teleplus PRO 300	Save images from retinal camera

The serial number and calibration date of the relevant instruments are listed in Table 7.

Table 7. Instrument make, model, serial number, and calibration date

Manufacturer	Description	Model	Serial number	Calibration date	Notes
Tektronix	Oscilloscope	MDO2054	B020542	2021/12/10	
Coherent	Sample meter	LabMaxTOP	0021C20R	2021/06/03	
Coherent	Sample meter	LabMaxTOP	0024I09R	2022/04/29	Replaced meter above on 2021/05/17
Coherent	Sample detector	J-10MB-HE	0226H18R	2021/08/14	
Coherent	Reference meter	LabMaxTOP	0728B20R	2021/06/02	
Coherent	Reference detector	J-10MB-LE	0699G18R	2021/08/11	
Coherent	Pre-shutter meter	LabMaxTOP	0024I09R	2022/04/29	Removed from setup 2021/05/10
Coherent	Pre-shutter detector	J-10MB-LE	0362K17R	2021/11/02	Removed from setup 2021/05/10
Mollectron	Laser output meter	PowerMax500AD	0356L03	2021/10/06	
Coherent	Laser output detector	PM10	0379H04	2022/07/09	

APPENDIX B – Laser Beam Profiles

The sensitivity of the laser system required a daily beam characterization consisting of measuring the spectra, beam profile, collimated beam diameter, focal spot diameter, and pulse width. In addition, an energy calibration was performed daily.

On the day of the experiment, the laser was tuned to the desired wavelength. The collimated beam was characterized by collecting laser beam profiles immediately after the waveplate from which the $D4\sigma$ output beam diameter was obtained. The spectral content of the beam was measured at the target location using a spectrometer. The beam diameter at the target location was measured using a knife edge technique. The pulse duration was measured using an autocorrelator at least once during the experiments for each wavelength.

Table 8 lists the peak wavelength obtained from the measured spectra, the $D4\sigma$ beam diameters obtained from the pyrocam software, and the pulse durations measured using the autocorrelator for the four days we performed exposures at 1318 nm. The uncertainty associated with the peak wavelength was 6.6 nm.

Table 8. Beam parameters for exposures at 1318 nm

Date	Subject Number	Eye	Nominal Wavelength (nm)	Measured Wavelength (nm)	Beam Diameter in X (mm)	Beam Diameter in Y (mm)	Pulse duration (fs)
05/12/2021	Ra21-006	OD	1318	1321.5	6.7	7.8	90
05/17/2021	Ra21-003	OS	1318	1321.5	7.4	8.1	94
05/24/2021	Ra21-007	OD	1318	1314.8	7.9	8.2	94
06/14/2021	Ra21-010	OS	1318	NA	8.9	9.1	97

Figure 24 shows the beam profiles of the beam before the focusing lens, on the four days we performed exposures at 1318 nm. Note that there were high intensity regions, or hot spots, in the beam profile. The location of the hot spots changed from day to day. However, the ratio of the major axis to the minor axis of the beams remained within 16% of each other while the $D4\sigma$ beam diameters varied by as much as ~25% between the different days of exposure. It should be noted that on 06/14/2021, panel d), the hot spots in the beam rendered were somewhat asymmetric, with an approximately crescent-like distribution towards the right hand side. However, the $D4\sigma$ beam diameters did not reflect that distribution as they were within 2% from each other. Note that the intensity distribution in panel d) is similar to the one in panel a) for a smaller beam.

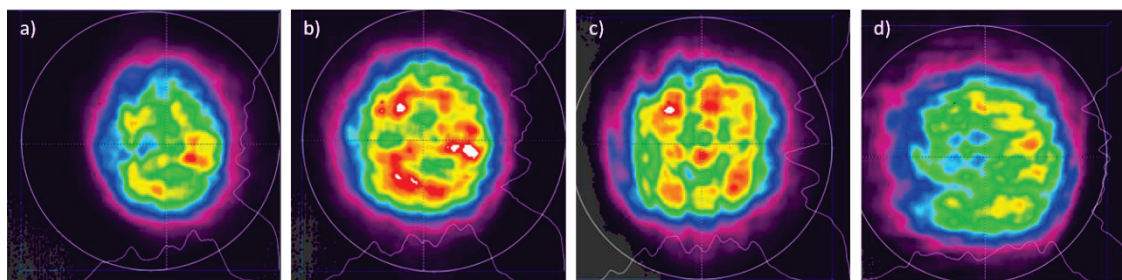


Figure 24. Beam profiles at 1318 nm for exposures performed on a) 05/12/2021, b) 05/17/2021, c) 05/24/2021, and d) 06/14/2021.

Table 9 lists the peak wavelength obtained from the measured spectra, the $D4\sigma$ beam diameters obtained from the pyrocam software, and the pulse durations measured using the autocorrelator

for the three days we performed exposures at 1540 nm. The uncertainty associated with the peak wavelength was 6.6 nm.

Table 9. Beam parameters for exposures at 1540 nm

Date	Subject Number	Eye	Nominal Wavelength (nm)	Measured Wavelength (nm)	Beam Diameter in X (mm)	Beam Diameter in Y (mm)	Pulse duration (fs)
05/26/2021	Ra21-009	OD	1540	1542.9	7.4	8.0	136
06/01/2021	Ra21-007	OS	1540	1522.8	7.8	8.6	136
06/21/2021	Ra21-010	OD	1540	1536.2	6.2	6.1	136

Figure 25. Beam profiles at 1540 nm for exposures performed on a) 05/26/2021, b) 06/01/2021, and c) 06/21/2021. Figure 25 shows the beam profiles of the beam before the focusing lens on the three days we performed exposures at 1540 nm. At this wavelength, the location of the hot spots was somewhat similar from day to day. The ratio of the major axis to the minor axis of the beam remained within 10% of each other although the beam diameters varied by as much as ~20% between the different days of exposure.

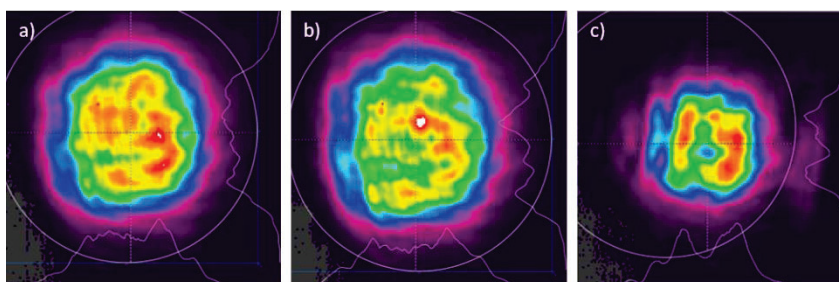


Figure 25. Beam profiles at 1540 nm for exposures performed on a) 05/26/2021, b) 06/01/2021, and c) 06/21/2021.

Table 10 lists the peak wavelength obtained from the measured spectra, the D4 σ beam diameters obtained from the pyrocam software, and the pulse durations measured using the autocorrelator for the four days we performed exposures at 2000 nm. The uncertainty associated with the peak wavelength was 6.6 nm.

Table 10. Beam parameters for exposures at 2000 nm

Date	Subject Number	Eye	Nominal Wavelength (nm)	Measured Wavelength (nm)	Beam Diameter in X (mm)	Beam Diameter in Y (mm)	Pulse duration (fs)
05/04/2021	Ra21-004	OD	2000	1992.4	6.6	6.9	241
05/06/2021	Ra21-005	OS	2000	2005.7	6.8	7.0	241
05/10/2021	Ra21-003	OD	2000	1999.1	7.5	7.3	241
06/03/2021	Ra21-006	OS	2000	1999.1	8.8	9.2	241

Figure 26 shows the beam profiles of the beam directed at the target on the four days we performed exposures at 2000 nm. The beam profiles have an asymmetric distribution of the intensity with a distinct hot spot which remained in the upper half of the beam. The ratios of the major axis to the minor axis of the beam remained within 5% of each other while the beam diameters varied by as much as ~25% between the different days of exposure.

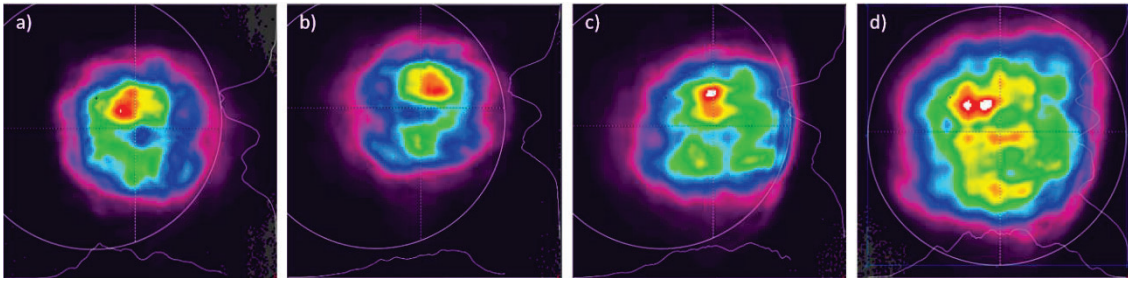


Figure 26. Beam profiles at 2000 nm for exposures performed on a) 05/04/2021, b) 05/06/2021, c) 05/10/2021, and d) 06/03/2021.

APPENDIX C – Subject Summary and Exposure Characteristics

On the day of the exposures, the knife edge technique was used to obtain the beam diameter at the focal point. This measurement was regularly performed in one direction, labeled as x . However, during the 1 h post-exposure examination on day 06/14/2021, it was noted that the lesions were elongated vertically. This observation prompted us to perform the knife edge measurement in the perpendicular direction (y) as well. The focal spot size diameter in the y -direction was calculated as $d_{y,1/e^2} = d_{x,1/e^2}/r$ where $r = D_x/D_y$ and D_x and D_y were the collimated beam diameters in both directions. The focal spot diameters obtained in this way correspond to the $1/e^2$ beam diameters. However, the calculation of the area to obtain the radiant exposure for each exposure used the $1/e$ focal spot size diameter in both x and y , labeled as d_x and d_y and obtained from the knife edge measurements by dividing by $\sqrt{2}$. Table 11 summarizes the subject and eye that was exposed at each wavelength, the wavelength that it was exposed to, the measured (x) and calculated (y) $1/e$ focal spot diameters, pulse duration, and if eyes were collected for histology or not and the time point at which this happened.

Table 11. Summary of subjects and exposure parameters

Date	Subject Number	Eye	Wavelength (nm)	1/e Focal Spot Diameter in X (μm)	1/e Focal Spot Diameter in Y (μm)	Pulse duration (fs)	Histology (1 h / 24 h)
05/12/2021	Ra21-006	OD	1318	86	101	90 ± 10	--
05/17/2021	Ra21-003	OS	1318	87	95	94 ± 10	24 h
05/24/2021	Ra21-007	OD	1318	62	64	94 ± 10	--
06/14/2021	Ra21-010	OS	1318	See D.1.4	See D.1.4	97 ± 11	--
05/26/2021	Ra21-009	OD	1540	74	79	136 ± 15	24 h
06/01/2021	Ra21-007	OS	1540	71	78	136 ± 15	24 h
06/21/2021	Ra21-010	OD	1540	117	161	136 ± 15	1 h
05/04/2021	Ra21-004	OD	2000	109	114	241 ± 27	24 h
05/06/2021	Ra21-005	OS	2000	120	123	241 ± 27	24 h
05/10/2021	Ra21-003	OD	2000	96	94	241 ± 27	--
06/03/2021	Ra21-006	OS	2000	93	98	241 ± 27	1 h

The focal spot diameters in X varied as much as 29%, 39%, and 22% from day to day for 1318 nm, 1540 nm, and 2000 nm respectively. Note that the mean $1/e$ focal spot diameters in X were $(82 \pm 15 \mu\text{m})$ at 1318 nm, $(97 \pm 33 \mu\text{m})$ at 1540 nm, and $(106 \pm 11 \mu\text{m})$ at 2000 nm where the uncertainties listed are the standard deviations obtained from the averages. For more details on the issues with determining the focal spot diameters on 06/14/2021, please see section D.1.4.

APPENDIX D – Cornea Exposures

D.1 Wavelength 1 – 1318 nm

D.1.1. Subject Ra21-006 OD.

D.1.1.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp with and without fluorescein and the fundus camera.

D.1.1.2. Post-exposure examination and evaluation.

Readers were provided with a hand drawn map of exposure locations and a power point slide of the pre-shot images (Figure 27) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

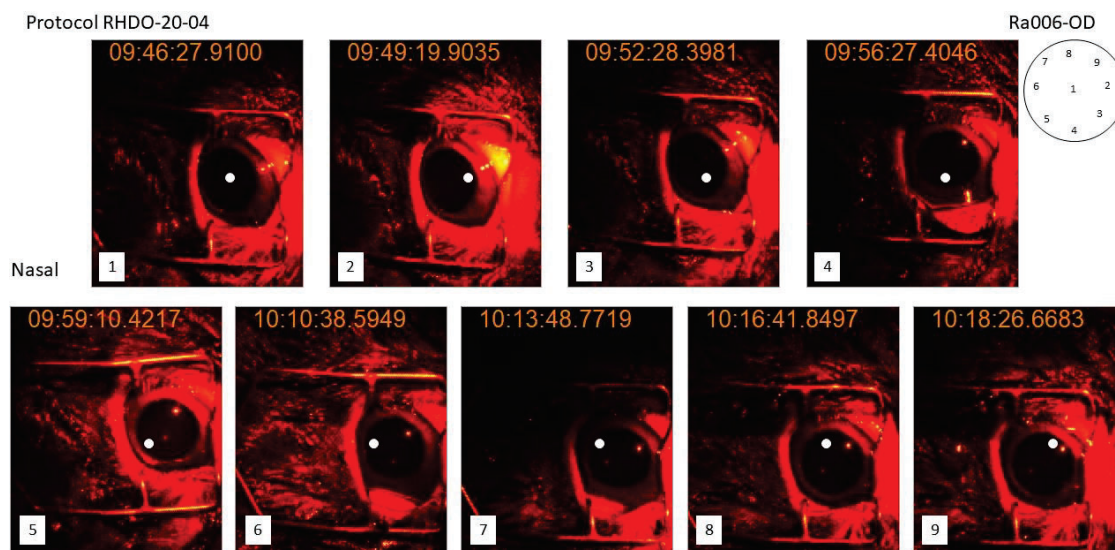


Figure 27. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

From the images above it appears that the subject's eye was not looking directly into the laser beam for several exposures and it moved significantly from exposure to exposure. This exemplifies some of the uncertainties associated with locating the injuries and assigning them to a specific radiant exposure as describe in section 3.4.2.1 above. Representative images of the lesions are presented in section 4.1.1 above.

During the 1 h post-exposure examination it was noted that there was a large abrasion in the central part of the cornea which appears almost like two horizontal lines and one approximately vertical line (see Figure 28). It was determined that this abrasion was not present during the baseline exam and was unrelated to the exposures.

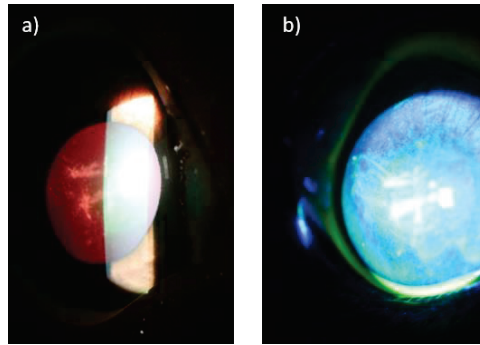


Figure 28. Slit lamp images a) without and b) with fluorescein showing a large abrasion in the central region of the cornea.

D.1.1.3. Data.

The readers were able to identify 2 laser induced lesions and there were 3 disqualified exposures that were excluded from the probit analysis (shaded rows in Table 12).

Table 12. Exposure parameters and 1 h post-exposure scoring: Ra21-006 OD at 1318 nm

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm \mu(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm \mu(H)$ (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	9.93	70.7	701.91	28.87	10.20	0.73	1	1	1	1
2	0.88	16.57	14.63	1.25	0.21	0.02	0	0	0	0
3	9.93	5.05	50.14	2.07	0.73	0.05	1	1	1	1
4	9.93	3.33	33.06	1.37	0.48	0.03	0	0	0	0
5	9.93	16.55	164.31	6.76	2.39	0.17	1	1	1	1
6	0.88	2.85	2.52	0.22	0.04	0.00	1	0	0	0
7	9.93	40.6	403.08	16.58	5.86	0.42	0	1	1	1
8	9.93	4.88	48.45	2.00	0.70	0.05	0	0	0	0
9	9.93	12.61	125.19	5.15	1.82	0.13	0	0	0	0

Table 13. 24 h post-exposure scoring for Ra21-006 OD at 1318 nm

Location	24 h Post-exposure Scoring			
	Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	1	1	1	1
2	0	0	1	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	1	1	1	1
7	1	1	1	1
8	0	0	1	0
9	0	0	0	0

D.1.2. Subject Ra21-003 OS.

D.1.2.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp with and without fluorescein and the fundus camera.

D.1.2.2. Post-exposure examination and evaluation.

Readers were provided with a power point slide of the pre-shot images (Figure 29) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

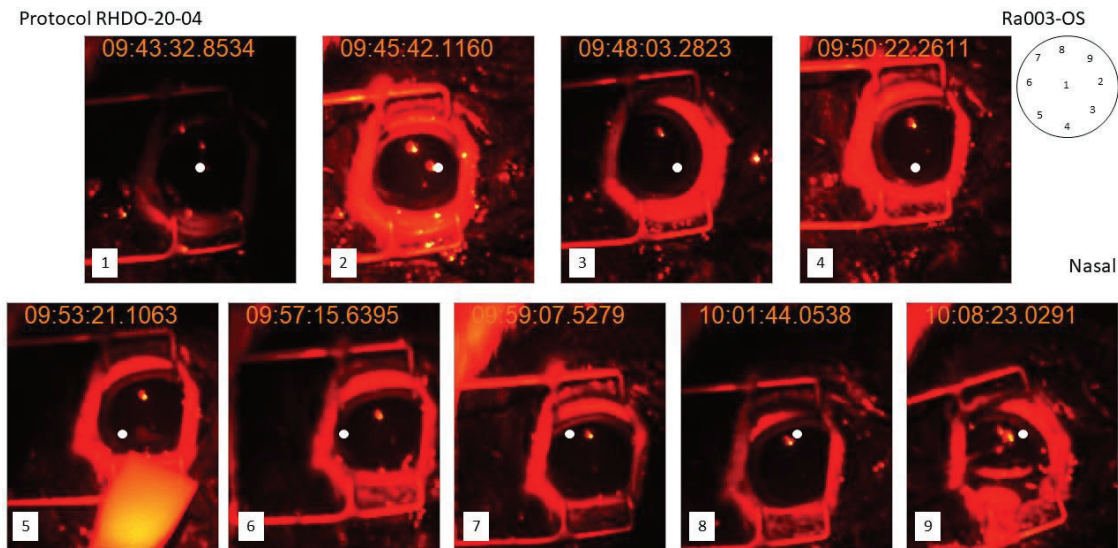


Figure 29. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

Upon examining the video recorded during exposures, it was noted that there was a blueish light visible in one of the frames, see Figure 30. This light is the result of a supercontinuum (SC) being generated as the femtosecond pulses travel from air into the cornea. The nucleus of the comet-like structure is assumed to be the location at which the NIR beam impinges on the cornea, generating the SC. The comet-like appearance may be caused by the camera being positioned above the eye, viewing it at an angle of approximately 14° , and the cornea being transparent to visible wavelengths. Therefore, the camera is seeing a diverging SC traveling into the eye. More details on SC generation can be found in 0. Visualizing the SC generated provides us with an intrinsic check on the location of the NIR laser beam, *i.e.* when comparing the location of the nucleus of the comet-like shape to the location of the alignment laser (white circle) we can see that the alignment laser is positioned within 1 to 2 mm of the NIR beam.

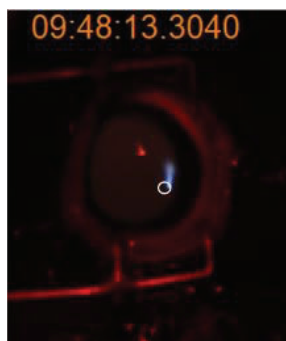


Figure 30. Image showing the SC light generated during exposure 3 as captured by the front-viewing camera. The open white circle corresponds to the location of the alignment laser. The blueish comet shape is the SC generated on the surface of the cornea as viewed from the vantage point of the front-viewing camera.

Figure 31 shows the images captured by the front viewing camera of the SC generated during 7 of the 9 exposures. It is unclear why the front-viewing camera did not capture the SC generated in two of the exposures but given that the pulse duration is so short, it could be an issue with timing. When the radiant exposure is low, another explanation could be that no SC is generated because the incoming energy is below the threshold for SC generation, or the camera is not sensitive enough to detect it. One interesting observation is that, to some extent, the intensity of the SC generated correlated with the magnitude of the radiant exposure, see Table 14.

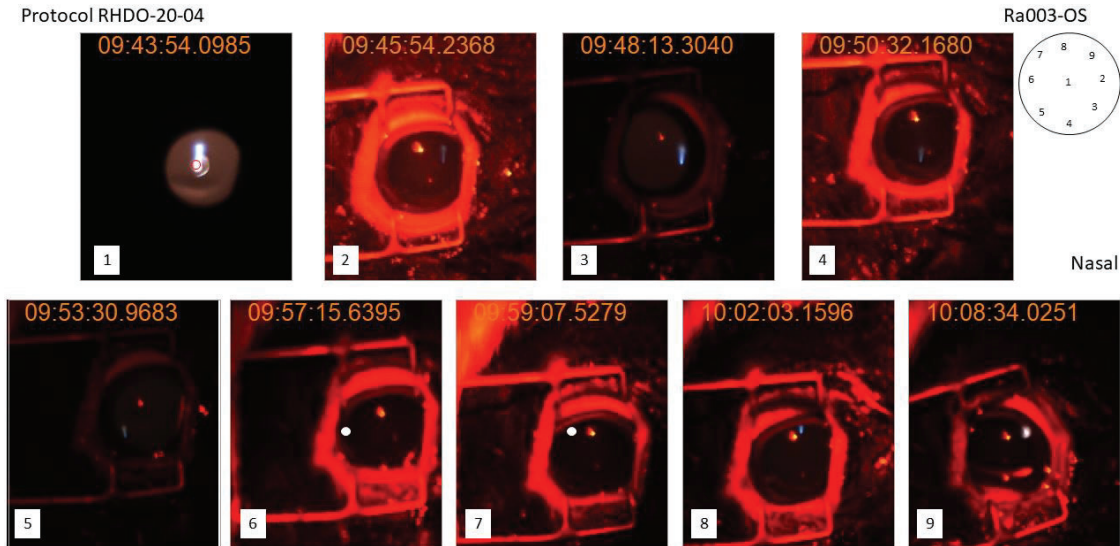


Figure 31. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. Note that in exposures 1, 3, and 5 the eye was not adequately illuminated with the red LED light during the exposure. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure therefore that image is the corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

Representative images of the lesions in this subject are presented in section 4.1.1 above.

D.1.2.3. Data.

The readers were able to identify 3 laser induced lesions and there were 2 disqualified exposures that were excluded from the probit analysis (shaded rows in Table 14).

Table 14. Exposure parameters and 1 h post-exposure scoring: Ra21-003 OS at 1318 nm

Location	Energy and Radiant exposure Determination						1 h Post-exposure Scoring			
Exposure Number	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse ± u(Q) (μJ)		Peak Radiant Exposure per Pulse ± u(H) (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	10.2	68.6	700.27	27.75	10.862	0.610	1	1	0	1
2	0.9	17.7	16.20	0.68	0.251	0.015	0	0	0	0
3	10.2	11.6	118.31	4.69	1.835	0.103	1	1	1	1
4	0.9	20.0	18.34	0.77	0.284	0.016	0	0	0	0
5	10.2	3.3	33.69	1.35	0.523	0.029	0	1	1	1
6	0.9	26.6	24.39	1.02	0.378	0.022	0	0	0	0
7	10.2	44.6	455.28	18.04	7.062	0.396	1	1	1	1
8	10.2	6.4	65.43	2.60	1.015	0.057	0	0	0	0
9	10.2	18.1	184.87	7.33	2.867	0.161	0	0	0	0

Table 15. Exposure energy and 24 h post-exposure scoring for Ra21-003 OS at 1318 nm

Location	24 h Post-exposure Scoring			
	Reader 1 1 (0/1)	Reader 2 2 (0/1)	Reader 3 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	1	1	1	1
2	0	0	0	0
3	1	1	0	1
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	1	1	1	1
8	0	0	0	0
9	0	0	0	0

D.1.3. Subject Ra21-007 OD.

D.1.3.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp, with and without fluorescein, and the fundus camera.

D.1.3.2. Post-exposure examination and evaluation.

Readers were provided with a power point slide of the pre-shot images (Figure 32) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

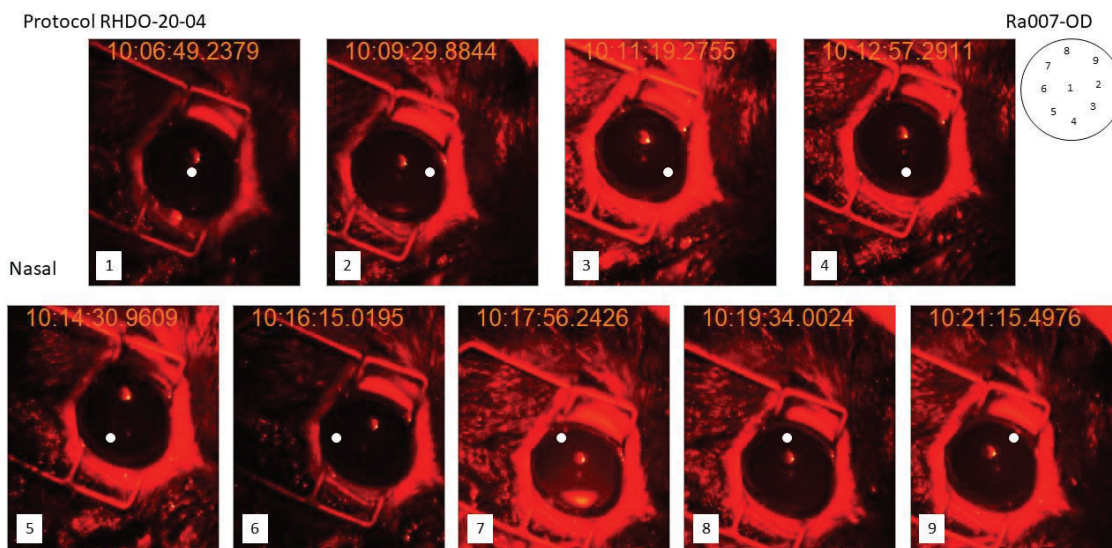


Figure 32. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

During the 1 h post exposure examination it was determined that this subject had a small abrasion on the upper right hand side of the cornea (see Figure 33). The abrasion was unrelated to the laser exposures.

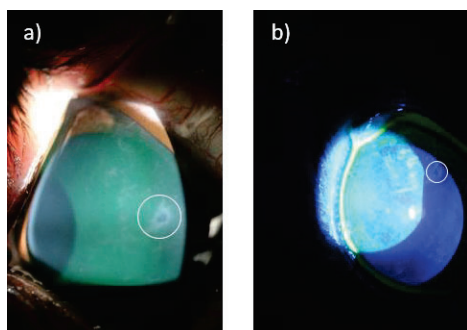


Figure 33. Slit lamp images a) without and b) with fluorescein showing a small abrasion in the upper right hand side of the cornea (white circle).

Figure 34 shows the SC generated in all 9 exposures. The intensity of the SC generated correlated with the magnitude of the radiant exposure, see Table 16.

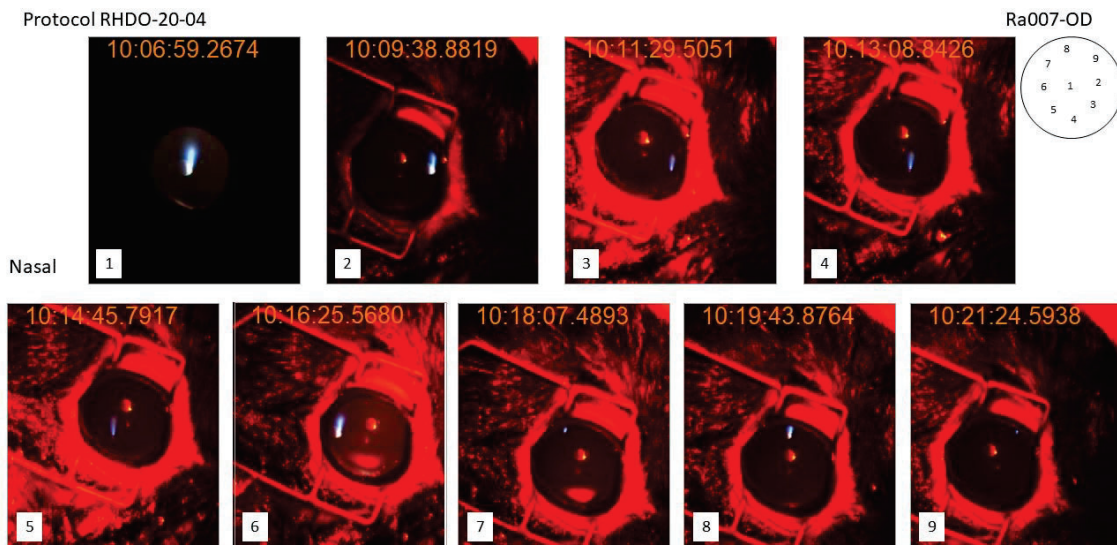


Figure 34. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. Note that in exposure 1 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

Representative images of the lesions in this subject are presented in section 4.1.1 above.

D.1.3.3. Data.

The readers were able to identify 6 laser induced lesions.

Table 16. Exposure parameters and 1 h post-exposure scoring: Ra21-007 OD at 1318 nm

Location Exposure Number	Energy and Radiant exposure Determination						1 h Post-exposure Scoring			
	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm \mu(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm \mu(H)$ (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	10.3	68.9	711.53	28.46	23.09	1.89	1	1	1	1
2	10.3	46.4	479.17	19.16	15.55	1.27	1	1	1	1
3	10.3	11.9	122.79	4.91	3.98	0.33	1	1	1	1
4	10.3	11.7	121.24	4.85	3.93	0.32	1	0	0	0
5	10.3	8.7	89.95	3.60	2.92	0.24	1	1	1	1
6	10.3	53.5	552.49	22.10	17.93	1.47	1	0	1	1
7	10.3	10.9	112.25	4.49	3.64	0.30	0	0	0	0
8	10.3	23.3	241.03	9.64	7.82	0.64	1	1	1	1

9	10.3	9.5	98.52	3.95	3.20	0.26	0	0	1	0
---	------	-----	-------	------	------	------	---	---	---	---

Table 17. 24 h post-exposure scoring for Ra21-007 OD at 1318 nm

Exposure Number	24 h Post-exposure Scoring			
	Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	1	1	1	1
2	1	0	1	1
3	1	0	1	1
4	1	0	0	0
5	1	0	0	0
6	1	0	1	1
7	0	0	1	0
8	1	0	1	1
9	0	0	0	0

D.1.4. Subject Ra21-010 OS.

D.1.4.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp, with and without fluorescein, and the fundus camera.

D.1.4.2. Post-exposure examination and evaluation.

Readers were provided with a power point slide of the pre-shot images (Figure 35) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

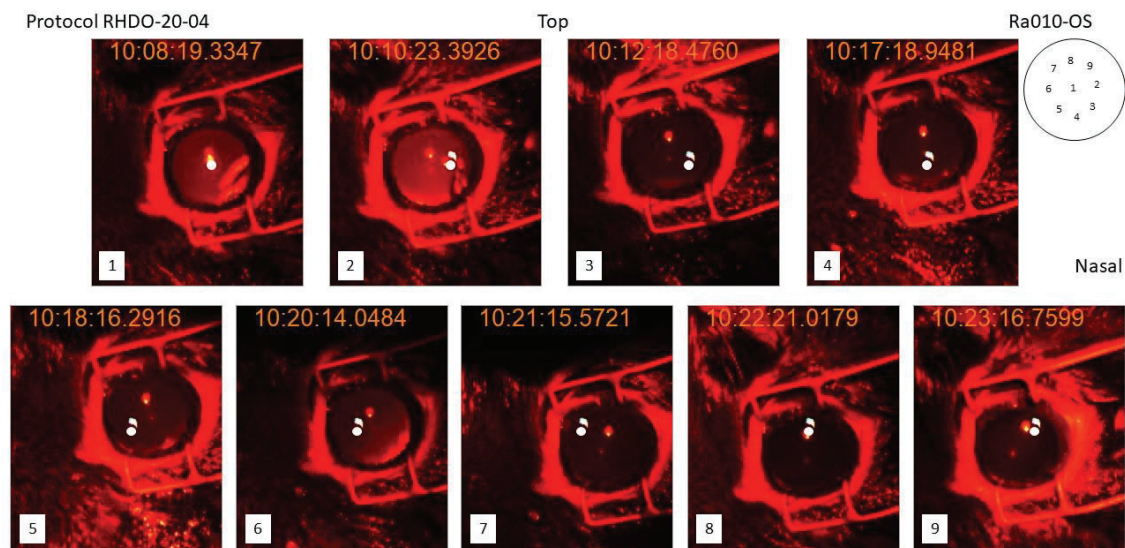


Figure 35. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations. During the first exposure, some pixels in the front-view camera got damage, appearing as a white crescent above and to the right of the solid dot representing the alignment laser.

It should be noted that during the first exposure, there was an unexplained incident which resulted in permanent damage of some of the pixels in the front-viewing camera. The incident manifested

itself in a broad streak-like area, accompanied by a large circular area of saturated pixels (white areas) as shown in panel 1 of Figure 36. Subsequent images showed a crescent-like area of damaged pixels above and to the right of the exposure location. The SC generated was captured in 7 of the 9 exposures and its intensity correlated with the magnitude of the radiant exposure, see Table 19 below.

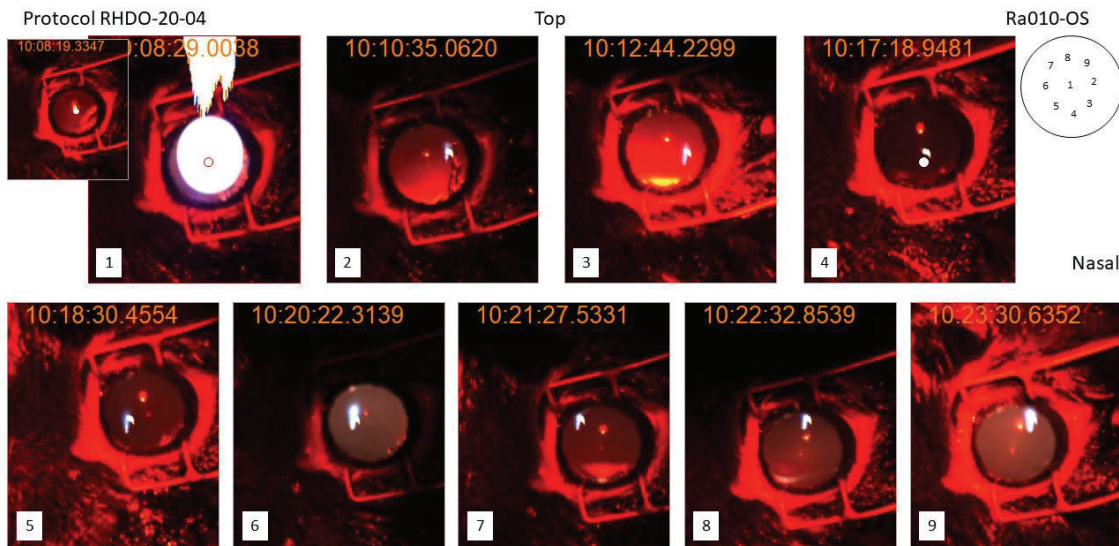


Figure 36. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. The image for exposure 1 has a large number of pixels saturated, presumably due to the high energy incoming NIR beam. The corresponding pre-shot image is shown as an inset on the upper left for reference. Pixels that got damaged during the first exposure form a white crescent shape above and to the right of the exposure location. Note that in exposures 2 and 6 the eye was not adequately illuminated with the red LED light during the exposure. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure therefore that image is the corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

During the 1 h post-exposure examination, it was noted that the lesions appeared to be elongated vertically, see Figure 37, indicating that the beam had an elliptical focal spot. However, the $D_{4\sigma}$ beam diameters obtained from the pyrocam software were within 2% of each other, indicating an approximately circular beam. In trying to find an explanation for this discrepancy, an attempt was made to perform the knife edge measurement in the y -direction. Unfortunately, the laser had already been turned off and the measurement had to wait until the following day. However, on the day following the exposure, the focal spot diameter in the x -direction was 25% smaller than on the day of exposure, introducing an uncertainty into what the actual focal spot shape and size was during the exposures. In an attempt to resolve this question, during the data analysis phase, the slit lamp images were used to estimate the focal spot size of the minor (D_{x_SL}) and major (D_{y_SL}) diameters. Mimicking the procedure used to determine the focal spot diameter in the y -direction used for the other subjects, the ratio $r_{SL} = D_{x_SL}/D_{y_SL}$ was used to find the focal spot diameter in the y -direction (d_{y_SL}) from the knife edge measurement of the focal spot diameter in the x -direction from the day of exposure as $d_{y_SL} = d_x/r_{SL}$. Table 18 below, shows the results of these different measurements and calculations.

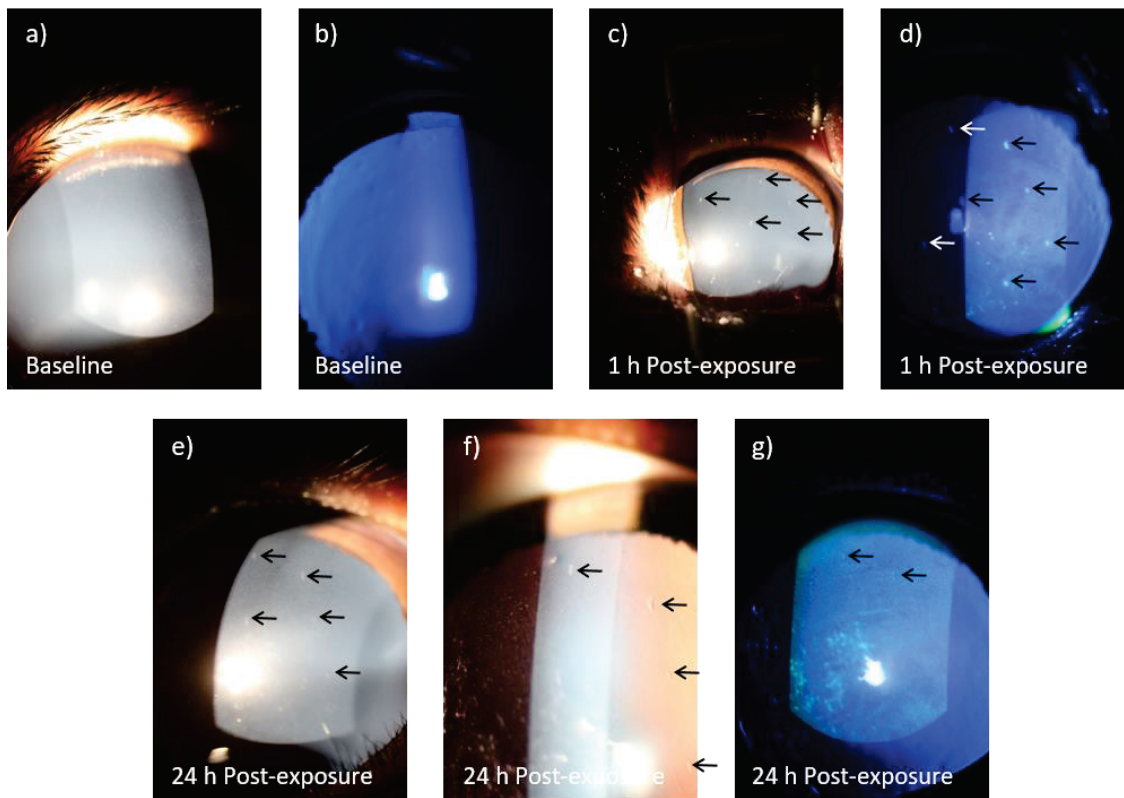


Figure 37. Slit lamp images of the baseline, 1 h post-exposure, and 24 h post-exposure examinations of subject Ra21-010 OS. Arrows indicate laser-induced lesions. Panels b), d), and g) were taken after fluorescein was applied. Panel f) is a magnified view of panel e). Several of the lesions identified in panel c) are also visible in the other panels. The elongated appearance of the lesions is more apparent in panels d), e), and f).

Table 18 summarizes the 3 different methods used to estimate the focal spot size diameters for the exposures of subject Ra21-010 OS and the measurements that they rendered. The column labeled Ref. denoted a reference number used to identify that method and the corresponding measurements.

Table 18. Focal spot size diameter calculations

1/e Measured Focal Spot Diameter in X (μm)	Calculation	1/e Focal Spot Diameter in Y (μm)	Notes	Ref.
143	$d_y = d_x/r$	145	d_x measured on day of exposure, r obtained from collimated beam diameters	1
143	$d_{y,SL} = d_x/r_{SL}$	357	d_x measured on day of exposure, r_{SL} obtained from slit lamp images	2
108	NA	252	d_x and d_y measured on the day following exposures	3

D.1.4.3. Data.

For this subject, the readers were able to identify all 9 laser induced lesions where they all appeared to be vertically elongated. Method 1 in Table 18 was not used to calculate the radiant exposures given that it indicates an approximately circular spot that does not reflect the shape of

the lesions observed. Table 19 shows the results of the radiant exposure when utilizing Method 2 in Table 18 to calculate the area of the focal spot.

Table 19. Exposure parameters (Method 2) and 1 h post-exposure scoring: Ra21-010 OS

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm u(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm u(H)$ (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	8.87	78.7	698.31	29.87	1.74	0.09	1	1	1	1
2	8.87	26.1	231.59	9.91	0.58	0.03	1	1	1	1
3	8.87	36.1	320.32	13.70	0.80	0.04	1	1	1	1
4	8.87	52.1	462.28	19.78	1.15	0.06	1	1	1	1
5	8.87	31	275.06	11.77	0.69	0.04	1	1	1	1
6	8.87	74.3	659.26	28.20	1.64	0.08	1	1	1	1
7	8.87	41.1	364.68	15.60	0.91	0.05	1	1	1	1
8	8.87	64.7	574.08	24.56	1.43	0.07	1	1	1	1
9	--	--	861.33	34.00	2.15	0.10	1	1	1	1

*Maximum energy was determined after the exposure by placing a detector in place of the sample and firing a single shot.

Table 20 shows the results of the radiant exposure when utilizing Method 3 in Table 18 to calculate the area of the focal spot. Note that the radiant exposures obtained using Method 3 are approximately twice as large as those obtained using Method 2.

Table 20. Exposure parameters (Method 3) and 1 h post-exposure scoring: Ra21-010 OS

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm u(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm u(H)$ (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	8.87	78.7	698.31	29.87	3.28	0.18	1	1	1	1
2	8.87	26.1	231.59	9.91	1.09	0.06	1	1	1	1
3	8.87	36.1	320.32	13.70	1.50	0.08	1	1	1	1
4	8.87	52.1	462.28	19.78	2.17	0.12	1	1	1	1
5	8.87	31	275.06	11.77	1.29	0.07	1	1	1	1
6	8.87	74.3	659.26	28.20	3.09	0.17	1	1	1	1
7	8.87	41.1	364.68	15.60	1.71	0.10	1	1	1	1
8	8.87	64.7	574.08	24.56	2.69	0.15	1	1	1	1
9	--	--	861.33	33.64	4.04	0.21	1	1	1	1

Table 21. 24 h post-exposure scoring for Ra21-010 OS at 1318 nm

Location	24 h Post-exposure Scoring			
	Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	1	1	1	1
2	1	1	1	1
3	1	1	1	1
4	1	1	1	1
5	1	1	1	1
6	1	1	1	1
7	1	1	0	1
8	1	1	1	1
9	1	1	1	1

D.1.5. Comparison of various probit analyses.

Probit analysis was performed on the aggregated data from subjects Ra21-006 OD, Ra21-003 OS, Ra21-007 OD, and Ra21-010 OS using both Methods 2 (Table 19) and 3 (Table 20) to obtain the radiant exposures.

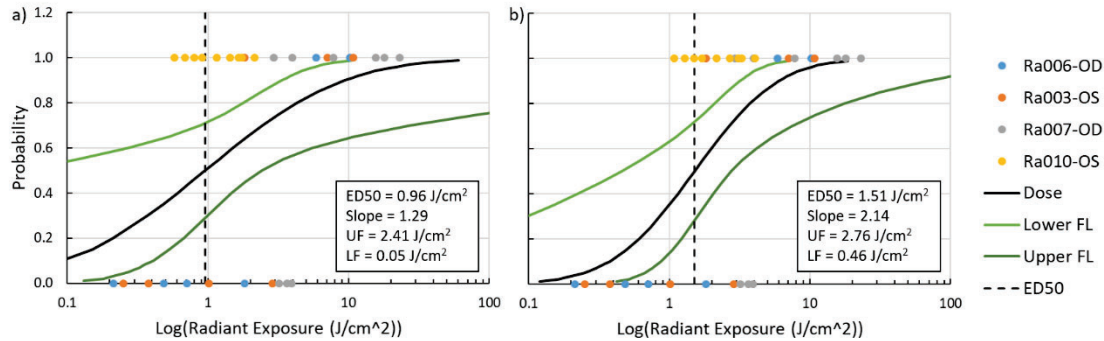


Figure 38. Probit analysis for 4 subjects exposed to 1318 nm. The area used to calculate the peak radiant exposure for Ra010-OS utilized a) the knife edge measurement for d_x from the day of the exposures and the ratio obtained from the slit lamp images of the injuries for d_y (Method 2) and b) the knife edge measurements for d_x and d_y from the day following the exposures (Method 3).

Figure 38 shows the probit analysis for the 1 h post-exposure examination for all 4 subjects as well as the data points used, where the data in panel a) was obtained using Method 2 and that in panel b) using Method 3. Table 22 summarizes the results obtained for the probit analyses conducted with four subjects as well as the one presented in section 4.1.2 with only 3 subjects.

Table 22. Summary of the probit analyses conducted

Date of Exposure	Subject Included in Analysis	Focal Spot Diameter in X (μm)	Focal Spot Diameter in Y (μm)	N (0, 1)	1 h ED ₅₀ Peak Radiant Exposure per Pulse (J/cm ²)	Lower Fiducial (J/cm ²)	Upper Fiducial (J/cm ²)	Probit Slope
05/12/2021	Ra21-006-OD	87	101	(11, 20)	0.96	0.05	2.41	1.29
05/17/2021	Ra21-003-OS	87	95					
05/24/2021	Ra21-007-OD	62	64					
06/14/2021	Ra21-010-OS	143	357					
05/12/2021	Ra21-006-OD	87	101	(11, 20)	1.51	0.46	2.76	2.14
05/17/2021	Ra21-003-OS	87	95					
05/24/2021	Ra21-007-OD	62	64					
06/14/2021	Ra21-010-OS	108	252					
05/12/2021	Ra21-006-OD	87	101	(11, 11)	3.24	0.88	6.65	3.69
05/17/2021	Ra21-003-OS	87	95					
05/24/2021	Ra21-007-OD	62	64					

Note that although there is an overlap of ~85% between the ranges spanned by the lower and upper fiducials obtained using Methods 2 and 3, the ED₅₀ increased by ~60% between them. This large difference caused us to eliminate subject Ra21-010 OS from the final probit analysis used to determine the ED₅₀ for femtosecond pulses at 1318 nm. When doing so, the ED₅₀ increased by more than 2 times relative to the one obtained from 4 subjects.

D.1.6. Cumulative probit analysis.

Table 23. Three subject raw probit data for exposures at 1318 nm

Thu Apr 14 10:29:02 2022							
Input File							
ONES = 11 ZEROES = 11 TOTAL = 22							
Percent confidence = 0.95							
ED50 = 3.24 Upper FL = 6.65 Lower FL = 0.877							
Intercept = -1.88 Slope = 3.69							
Pearson's Chi-Sq = 11.8105 Probability of Chi-Sq = 0.9224							
h = 1.00 g = 0.74 t = 1.96							
Log XBAR = 0.555 Log YBAR = 5.16							
SYY = 16.972 SXY = 1.399 SXX = 0.379 S0 = 6.427							
Iterations = 23 DF = 21							
Prob	Dose	Lower FL	Upper FL	Prob	Dose	Lower FL	Upper FL
0.01	0.759	4.03e-005	1.70	0.55	3.51	1.26	8.55
0.02	0.899	0.000138	1.88	0.60	3.80	1.69	11.9
0.03	1.00	0.000300	2.01	0.65	4.12	2.13	17.9
0.04	1.09	0.000537	2.11	0.70	4.50	2.56	29.5
0.05	1.16	0.000864	2.20	0.75	4.94	2.97	52.9
0.06	1.23	0.00129	2.28	0.80	5.48	3.37	105.
0.07	1.29	0.00184	2.36	0.85	6.19	3.81	241.
0.08	1.35	0.00253	2.43	0.90	7.21	4.33	701.
0.09	1.40	0.00336	2.49	0.91	7.48	4.46	909.
0.10	1.46	0.00438	2.56	0.92	7.79	4.60	1.21e+003
0.15	1.70	0.0130	2.86	0.93	8.14	4.75	1.65e+003
0.20	1.92	0.0305	3.15	0.94	8.55	4.92	2.34e+003

0.25	2.13	0.0628	3.45	0.95	9.05	5.11	3.50e+003	
0.30	2.34	0.119	3.79	0.96	9.67	5.34	5.61e+003	
0.35	2.55	0.211	4.21	0.97	10.5	5.64	1.00e+004	
0.40	2.77	0.357	4.75	0.98	11.7	6.04	2.18e+004	
0.45	3.00	0.574	5.50	0.99	13.8	6.71	7.42e+004	
0.50	3.24	0.877	6.65					
Dose	Tries Hits		Dose	Tries Hits		Dose	Tries Hits	
0.210	1	0	2.87	1	0	7.82	1	1
0.250	1	0	2.92	1	1	10.2	1	1
0.380	1	0	3.20	1	0	10.9	1	1
0.480	1	0	3.64	1	0	15.5	1	1
0.700	1	0	3.93	1	0	17.9	1	1
1.01	1	0	3.98	1	1	23.1	1	1
1.82	1	0	5.86	1	1			
1.84	1	1	7.06	1	1			
Totals					22	11		
*****Original Data*****								
0.21	0	0.25	0	0.38	0	0.48	0	0.70 0
1.01	0	1.82	0	1.84	1	2.87	0	2.92 1
3.2	0	3.64	0	3.93	0	3.98	1	5.86 1
7.06	1	7.82	1	10.20	1	10.86	1	15.55 1
17.93	1	23.09	1					

D.2 Wavelength 2 – 1540 nm

D.2.1. Subject Ra21-009 OD.

D.2.1.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp, with and without fluorescein, and the fundus camera.

D.2.1.2. Post-exposure examinations.

Readers were provided with a power point slide of the pre-shot images (Figure 39) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

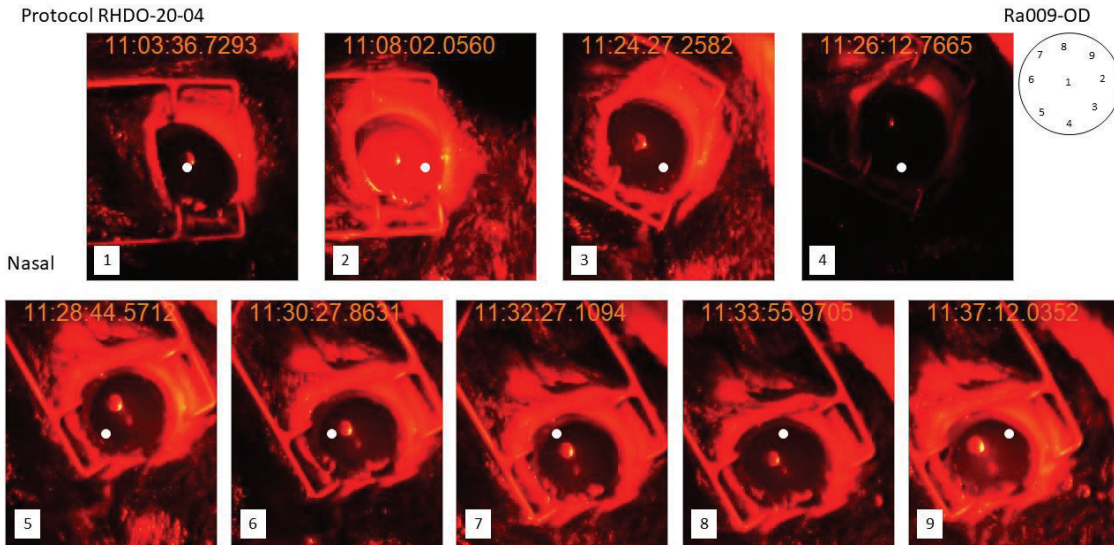


Figure 39. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Note that in exposure 4 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

From the images above it appears that the subject's eye was not looking directly into the laser beam for several exposures and it moved between exposures 1 and 3. This exemplifies the uncertainties associated with locating the injuries and assigning them to a specific radiant exposure as describe in section 3.4.2.1 above.

Figure 40 shows the SC generated in 5 exposures. The intensity of the SC generated correlated with the magnitude of the radiant exposure, see Table 24.

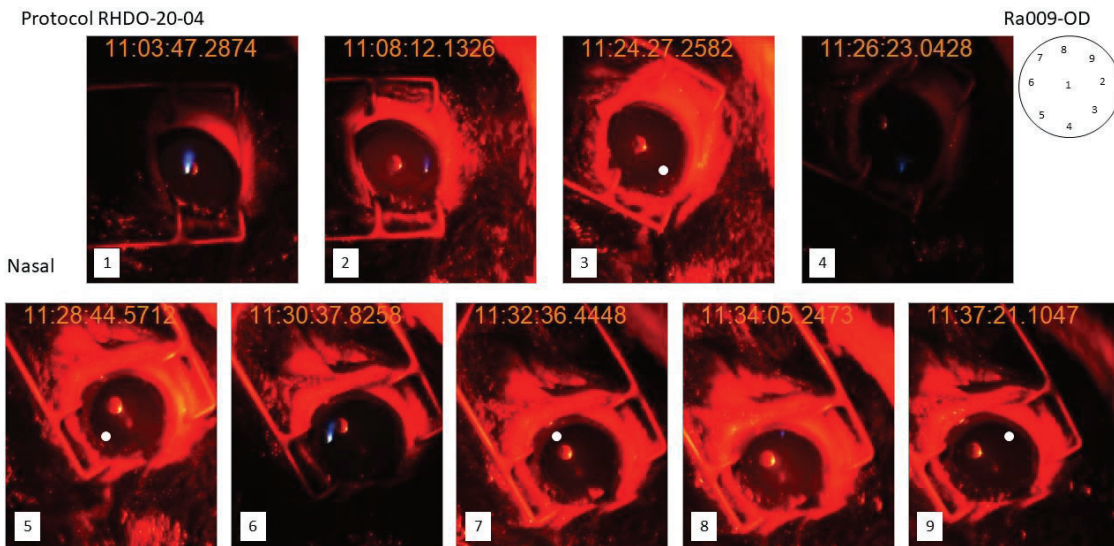


Figure 40. Images showing the SC light generated during the exposure as captured by the front-viewing camera. Note that in exposures 1, 4, and 6 the eye was not adequately illuminated with the red LED light during the exposure. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

A representative composite image of one of the lesions is presented in section 4.2.1 above.

D.2.1.3. Data.

The readers were able to identify 4 laser induced lesions.

Table 24. Exposure parameters and 1 h post-exposure scoring: Ra21-009 OD at 1540 nm

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
Exposure Number	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm u(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm u(H)$ (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	9.30	76.7	713.54	32.86	15.56	1.26	1	1	1	1
2	9.30	28.3	263.27	12.12	5.74	0.47	1	1	1	1
3	2.26	4.16	9.40	0.47	0.20	0.02	0	0	0	0
4	9.30	9.58	89.12	4.11	1.94	0.16	1	0	0	0
5	2.26	7.04	15.90	0.80	0.35	0.03	0	0	0	0
6	9.30	47.1	438.17	20.18	9.55	0.78	1	1	1	1
7	9.30	5.62	52.28	2.41	1.14	0.09	0	0	0	0
8	9.30	16.87	156.94	7.23	3.42	0.28	1	1	1	1
9	2.26	11.7	26.43	1.33	0.58	0.05	0	0	0	0

Table 25. 24 h post-exposure scoring for Ra21-009 OD at 1540 nm

Location	24 h Post-exposure Scoring			
Exposure Number	Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	1	1	1	1
2	1	1	1	1
3	0	1	0	0
4	0	0	0	0
5	0	0	0	0
6	1	1	1	1
7	0	1	0	0
8	0	1	0	0
9	0	0	0	0

D.2.2. Subject Ra21-007 OS.

D.2.2.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp, with and without fluorescein, and the fundus camera.

D.2.2.2. Post-exposure examinations.

Readers were provided with a power point slide of the pre-shot images (Figure 41) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

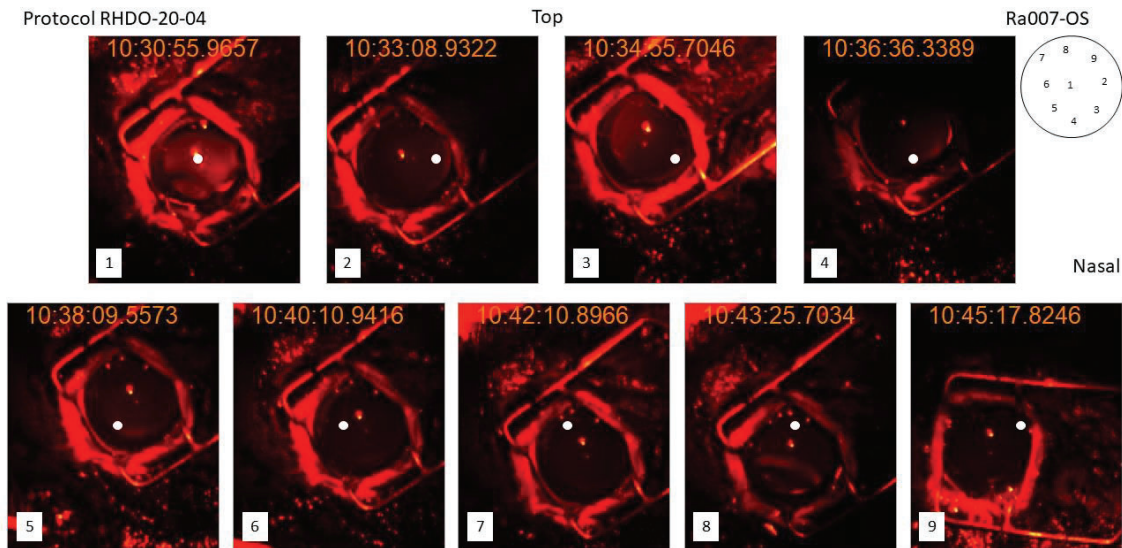


Figure 41. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Note that in exposure 4 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

Figure 42 shows the SC generated in 8 exposures. The intensity of the SC generated correlated with the magnitude of the radiant exposure, see Table 26.

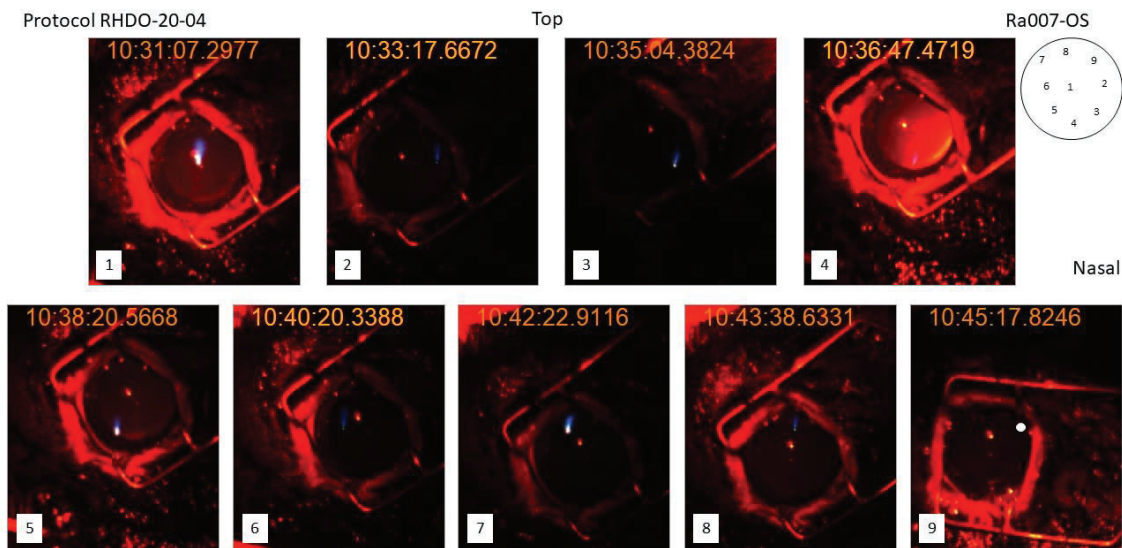


Figure 42. Images showing the SC light generated during the exposure as captured by the front-viewing camera. In exposures 2, 3, 5, 6, and 7 the eye was not adequately illuminated with the red LED light during the exposure. In exposures 2, 4, and 6 the SC light generated is extremely weak so the brightness and contrast of the image was adjusted. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure so that image is the corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

Figure 43 contains representative slit lamp images collected during the 1 h examination showing some of the lesions identified for this subject.

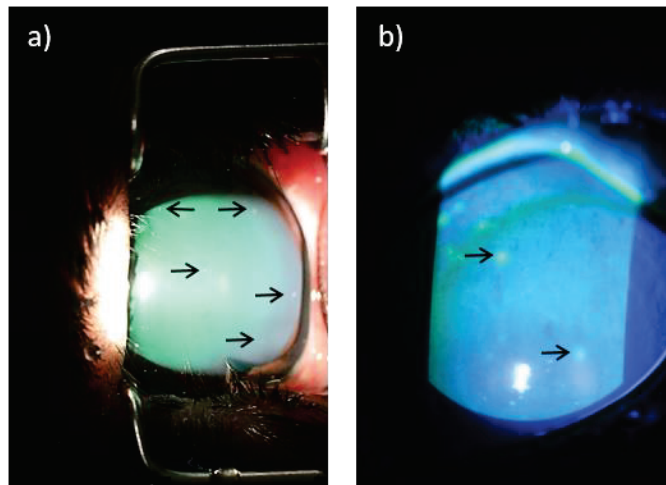


Figure 43. Representative slit lamp images a) without and b) with fluorescein of subject Ra21-007 OS. Arrows indicate identified laser induced lesions.

D.2.2.3. Data.

The readers were able to identify 8 laser induced lesions.

Table 26. Exposure parameters and 1 h post-exposure scoring: Ra21-007 OS at 1540 nm

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
Exposure Number	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm u(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm u(H)$ (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	8.67	73.5	637.47	25.98	14.51	1.58	1	1	1	1
2	8.67	8.48	73.55	3.00	1.67	0.18	1	1	1	1
3	8.67	26.6	230.70	9.40	5.25	0.57	1	1	1	1
4	8.67	13.9	120.55	4.92	2.74	0.30	1	1	1	1
5	8.67	41.6	360.80	14.70	8.21	0.89	1	1	1	1
6	8.67	7.05	61.14	2.50	1.39	0.15	0	0	0	0
7	8.67	55.7	483.09	19.69	10.99	1.20	1	1	1	1
8	8.67	19.5	169.12	6.89	3.85	0.42	1	1	0	1
9	8.67	33.3	288.81	11.77	6.57	0.72	0	1	1	1

Table 27. Exposure energy and 24 h post-exposure scoring for Ra21-007 OS at 1540 nm

Location	24 h Post -exposure Scoring			
Exposure Number	Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	1	1	1	1
6	0	0	0	0
7	1	1	1	1
8	0	0	0	0
9	0	0	0	0

D.2.3. Subject Ra21-010 OD.

D.2.3.1. Pre-exposure examination.

The pre-exposure examination appeared to show that this subject presented a small feature that resembled a laser-induced lesion in the upper left hand side of the eye, Figure 44. Slit lamp

examinations showing an apparent feature in the cornea of subject Ra21-010 OD during the baseline examination. Feature is highlighted by a black circle in panel b) but is not visible when fluorescein was applied, panel c).Figure 44 panel d). However, that feature was not visible when fluorescein was applied panel c).

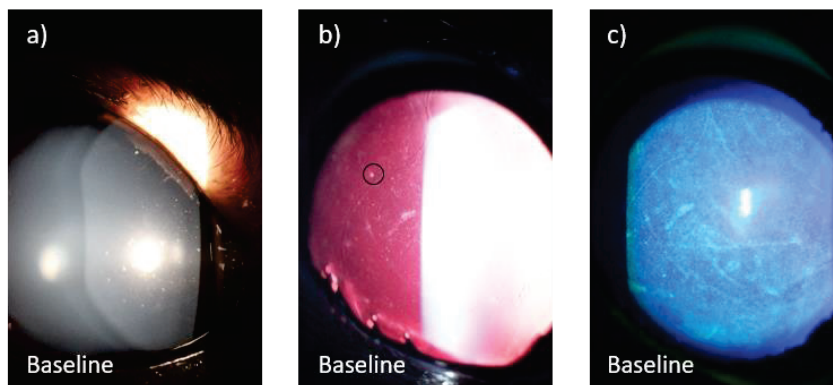


Figure 44. Slit lamp examinations showing an apparent feature in the cornea of subject Ra21-010 OD during the baseline examination. Feature is highlighted by a black circle in panel b) but is not visible when fluorescein was applied, panel c).

D.2.3.2. Post-exposure examinations.

Readers were provided with a power point slide of the pre-shot images (Figure 45) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

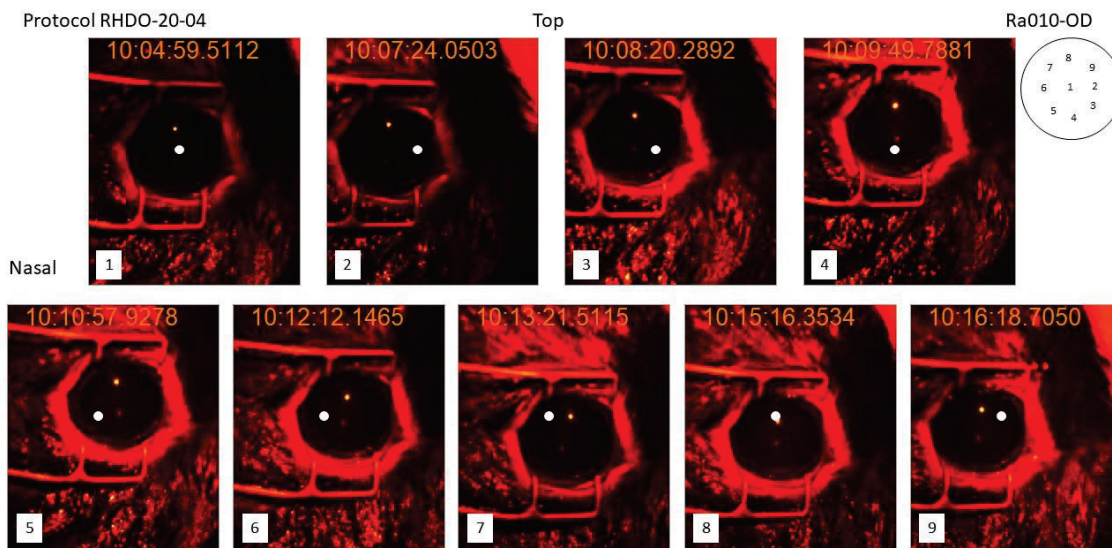


Figure 45. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

Figure 46 shows the SC generated in 8 exposures. The intensity of the SC generated correlated with the magnitude of the radiant exposure, see Table 28.

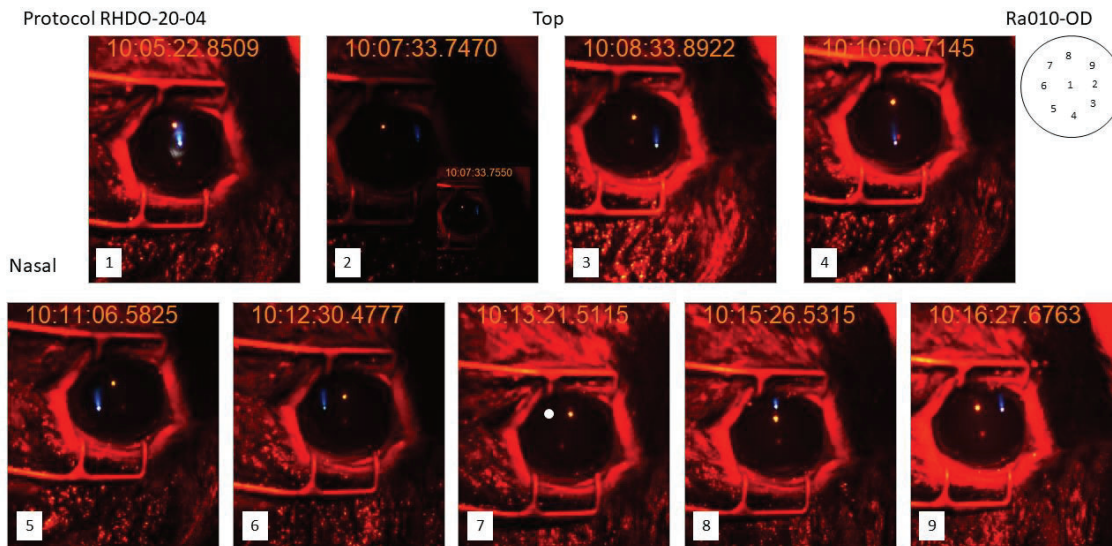


Figure 46. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. Note that in exposure 1 the eye was not adequately illuminated with the red LED light during the exposure. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure therefore that image is the corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

Representative images of some of the lesions in this subject are presented in section 4.2.1 above.

D.2.3.3. Data.

Readers were able to distinguish all laser induced lesions and there was one disqualified exposure. The eye was collected after the 1 h exposure for histological purposes.

Table 28. Exposure parameters and 1 h post-exposure scoring: Ra21-010 OD at 1540 nm

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
Exposure Number	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse ± u(Q) (μJ)		Peak Radiant Exposure per Pulse ± u(H) (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	9.81	72	706.32	27.84	4.78	0.23	1	1	1	1
2	8.44	23.4	197.50	9.04	1.34	0.07	1	1	1	1
3	9.81	41	402.21	15.85	2.72	0.13	1	1	1	1
4	9.81	34	333.54	13.15	2.25	0.11	1	1	1	1
5	9.81	51.2	502.27	19.80	3.40	0.16	1	0	1	1
6	9.81	26	255.06	10.05	1.72	0.08	1	1	1	1
7	9.81	2.6	25.51	1.02	0.17	0.01	0	1	0	0
8	9.81	38.1	373.76	14.73	2.53	0.12	1	1	1	1
9	9.81	42.9	420.85	16.59	2.85	0.14	1	1	1	1

D.2.4. Cumulative probit analysis.

Table 29. Raw probit data for exposures at 1540 nm.

Thu Apr 14 13:01:25 2022

Input File

ONES = 20 ZEROES = 6 TOTAL = 26

Percent confidence = 0.95

ED50 = 1.48 Upper FL = 2.21 Lower FL = 31.6

Intercept = -1.29 Slope = 7.65

Pearson's Chi-Sq = 8.2906 Probability of Chi-Sq = 0.9988

h = 1.00 g = 1.08 t = 1.96

Log XBAR = 0.225 Log YBAR = 5.43

SYX = 11.847 SXY = 0.465 SXX = 0.061 S0 = 4.266

Iterations = 36 DF = 25

Prob	Dose	Lower FL	Upper FL	Prob	Dose	Lower FL	Upper FL
0.01	0.732	2.33e+009	1.17	0.55	1.53	9.60	2.84
0.02	0.795	2.87e+008	1.23	0.60	1.59	-1.#J	-1.#J
0.03	0.837	-	-	0.65	1.66	-	-
0.04	0.871	-	-	0.70	1.73	-	-
0.05	0.899	-	-	0.75	1.81	-	-
0.06	0.924	-	-	0.80	1.90	-	-
0.07	0.946	-	-	0.85	2.02	-	-
0.08	0.966	-	-	0.90	2.17	-	-
0.09	0.985	-	-	0.91	2.21	-	-
0.10	1.00	-	-	0.92	2.25	-	-
0.15	1.08	-	-	0.93	2.30	-	-
0.20	1.15	-	-	0.94	2.36	-	-
0.25	1.20	-	-	0.95	2.42	-	-
0.30	1.26	-	-	0.96	2.50	-	-

0.35	1.31	-	-	0.97	2.60	-	-
0.40	1.37	-	-	0.98	2.74	-	-
0.45	1.42	-	-	0.99	2.97	-	-
0.50	1.48	-	-				
Dose	Tries	Hits	Dose	Tries	Hits	Dose	Tries Hits
0.200	1	0	2.25	1	1	5.25	1 1
0.350	1	0	2.53	1	1	5.74	1 1
0.580	1	0	2.72	1	1	6.57	1 1
1.14	1	0	2.74	1	1	8.21	1 1
1.34	1	1	2.85	1	1	9.55	1 1
1.39	1	0	3.40	1	1	11.0	1 1
1.67	1	1	3.42	1	1	14.5	1 1
1.72	1	1	3.85	1	1	15.6	1 1
1.94	1	0	4.78	1	1		
Totals				26 20			
*****Original Data*****							
0.2	0	0.35	0	0.58	0	1.14	0 1.34 1
1.39	0	1.67	1	1.72	1	1.94	0 2.25 1
2.53	1	2.72	1	2.74	1	2.85	1 3.40 1
3.42	1	3.85	1	4.78	1	5.25	1 5.74 1
6.57	1	8.21	1	9.55	1	10.99	1 14.51 1
15.56	1						

D.3 Wavelength 3 – 2000 nm

D.3.1. Subject Ra21-004 OD.

This was the first experimental subject to undergo exposures, therefore, some of the procedures and techniques were not optimized. In particular, no pre-shot images were recorded as the front view camera was misaligned and the researchers were not systematic at waiting a set amount of time between irrigating the eye and performing the laser exposure. Lighting in the rooms was optimized as well as the procedure for positioning the subject among others.

D.3.1.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp, with and without fluorescein, and the fundus camera.

D.3.1.2. Post-exposure examinations.

Readers were provided with a hand drawn map of exposure locations to aid in finding the lesions when available, see Figure 47.

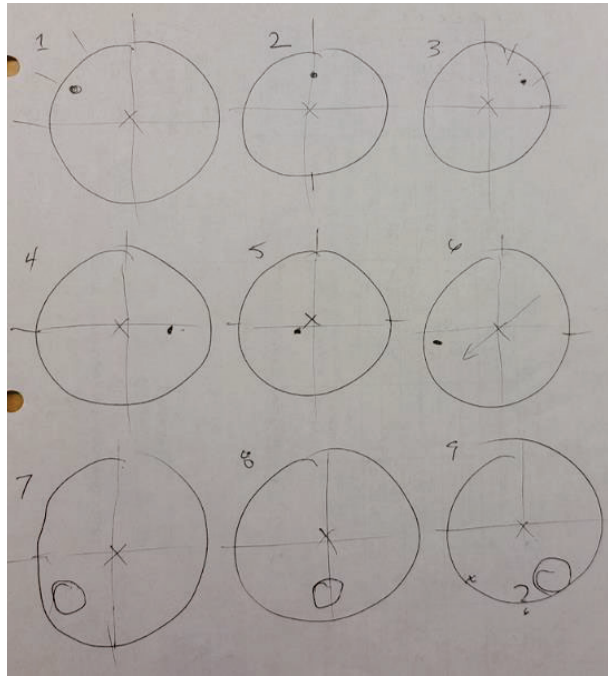


Figure 47. Hand drawn map showing the exposure locations for subject Ra21-004 OS.

Figure 48 shows two slit lamp images taken with fluorescein which show lesions.

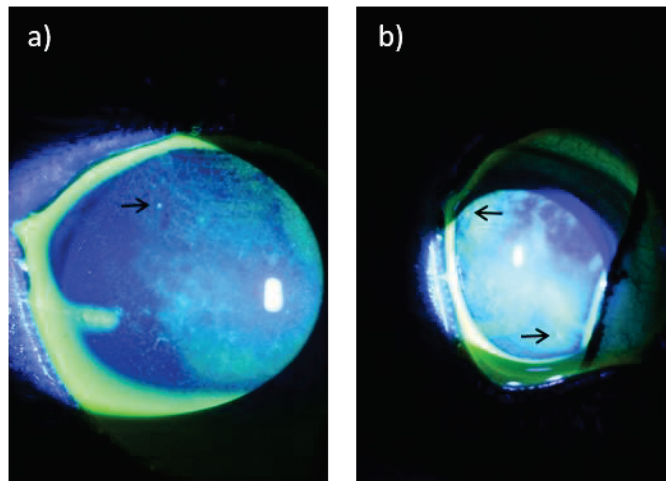


Figure 48. Slit lamp images with fluorescein of subject Ra21-004 OD 1 h post-exposure. Arrows indicate laser-induced lesions. One lesion identified in a) is also visible in b).

D.3.1.3. Data.

Readers were able to distinguish 3 laser induced lesions and there was 1 disqualified exposure.

Table 30. Exposure parameters and 1 h post-exposure scoring: Ra21-004 OD at 2000 nm

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
Exposure Number	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm u(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm u(H)$ (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	85.51	7.63	652.42	25.86	6.72	0.69	1	1	1	1
2	8.02	2.92	23.41	1.81	0.24	0.03	0	0	0	0
3	85.51	2.31	197.52	8.00	2.04	0.21	0	1	0	0
4	85.51	1.092	93.37	4.07	0.96	0.10	1	0	0	0
5	85.51	1.312	112.19	4.75	1.16	0.12	0	1	0	0
6	8.02	6.12	49.07	1.98	0.51	0.05	0	0	0	0
7	85.51	3.63	310.39	12.39	3.20	0.33	1	1	1	1
8	8.02	3.83	30.71	1.24	0.32	0.03	0	0	0	0
9	85.51	5.97	510.48	20.26	5.26	0.54	1	1	1	1

Table 31. 24 h post-exposure scoring for Ra21-004 OD at 2000 nm

Location	24 h Post-exposure Scoring			
Exposure Number	Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	0	--	0	
2	0	0	0	0
3	0	1	1	1
4	0	0	0	0
5	0	1	1	1
6	0	0	0	0
7	0	--	0	
8	0	0	0	0
9	1	1	1	1

D.3.2. Subject Ra21-005 OS.

D.3.2.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp, with and without fluorescein, and the fundus camera

D.3.2.2. Post-exposure examinations.

Readers were provided with a power point slide of the pre-shot images (Figure 49) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

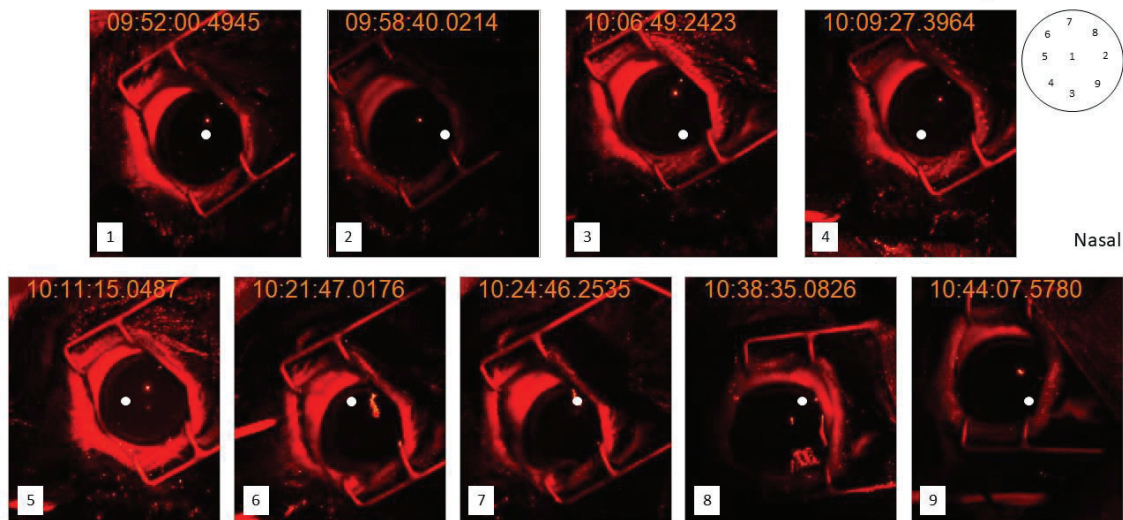


Figure 49. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Note that in exposure 1, 2, 8, and 9 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

From the images above it appears that the subject's eye was not looking directly into the laser beam for several exposures and it moved significantly between exposures 7 and 8. This exemplifies the uncertainties associated with locating the injuries and assigning them to a specific radiant exposure as described in section 3.4.2.1 above.

The settings of the front-view camera prevented us from capturing the SC generated during the exposures.

A representative image of one of the lesions in this subject is presented in section 4.3.1 above.

D.3.2.3. Data.

Readers were able to identify 4 laser induced lesions.

Table 32. Exposure parameters and 1 h post-exposure scoring: Ra21-005 OS at 2000 nm

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm u(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm u(H)$ (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	78.80	6.33	498.78	20.23	4.28	0.21	1	1	1	1
2	78.80	1.174	92.51	4.06	0.79	0.04	0	0	0	0
3	78.80	1.473	116.07	4.95	1.00	0.05	0	1	0	0
4	78.80	2.68	211.17	8.68	1.81	0.09	1	0	0	0
5	78.80	4.05	319.12	13.00	2.74	0.13	0	0	0	0
6	78.80	4.95	390.04	15.85	3.35	0.16	1	1	1	1
7	78.80	7.13	561.82	22.77	4.82	0.23	0	1	1	1
8	78.80	1.042	82.11	3.67	0.70	0.04	0	0	1	0
9	78.80	2.19	172.56	7.15	1.48	0.07	1	1	1	1

Table 33. 24 h post-exposure scoring for Ra21-005 OS at 2000 nm

Location	24 h Post-exposure Scoring			
	Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	0		1	0
2	0		0	0
3	0		0	0
4	0		0	0
5	0		0	0
6	1		0	0
7	0		0	0
8	0		0	0
9	0		1	0

D.3.3. Subject Ra21-003 OD.

D.3.3.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp, with and without fluorescein, and the fundus camera

D.3.3.2. Post-exposure examinations.

Readers were provided with a power point slide of the pre-shot images (Figure 50) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

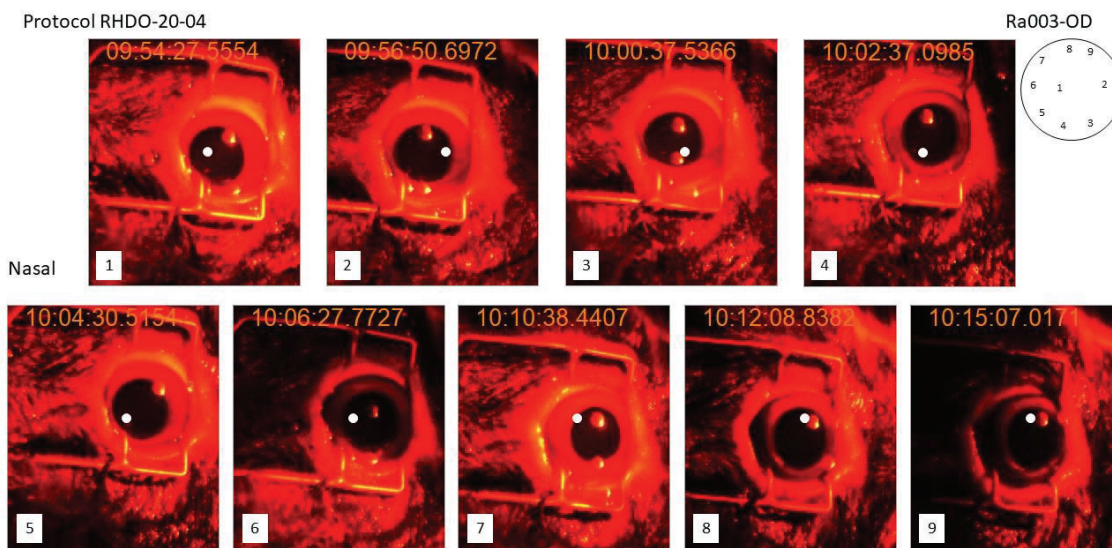


Figure 50. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Note that in exposure 6 and 9 the eye was not adequately illuminated with the red LED light during the exposure. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

From the images above it is clear that the imaging area of the front view camera was smaller than in previous exposures, the orientation of the eye varied significantly from exposure to exposure. This exemplifies the uncertainties associated with locating the injuries and assigning them to a specific radiant exposure as described in section 3.4.2.1 above. In particular, exposure 7 appeared to be very close to the edge of the cornea, which could potentially make it difficult to visualize during examinations.

The settings of the front-view camera prevented us from capturing the SC generated during the exposures.

Representative images of some of the lesions in this subject are presented in section 4.2.1 above.

D.3.3.3. Data.

Readers were able to identify 6 laser induced lesions.

Table 34. Exposure parameters and 1 h post-exposure scoring: Ra21-003 OD at 2000 nm

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
	Exposure Number	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm \mu(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm \mu(H)$ (J/cm^2)	Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	1	68.78	10.87	747.67	30.15	10.53 0.66	1	1	1	1
2	2	68.78	2.43	167.14	6.87	2.35 0.15	0	0	0	0
3	3	68.78	5.04	346.67	14.03	4.88 0.31	1	1	0	1
4	4	68.78	3.44	236.61	9.63	3.33 0.21	0	0	1	0
5	5	68.78	2.87	197.41	8.07	2.78 0.18	0	0	1	0
6	6	68.78	8.97	616.98	24.89	8.69 0.55	1	1	1	1
7	7	68.78	4.72	324.66	13.15	4.57 0.29	1	1	1	1
8	8	68.78	5.93	407.88	16.49	5.74 0.36	1	1	1	1
9	9	68.78	7.36	506.24	20.44	7.13 0.45	1	1	1	1

Table 35. 24 h post-exposure scoring for Ra21-003 OD at 2000 nm

Location	24 h Post-exposure Scoring			
Exposure Number	Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0

D.3.4. Subject Ra21-006 OS.

D.3.4.1. Pre-exposure examination.

The pre-exposure examinations did not show anything unusual or noteworthy on the cornea or retina as visualized with the slit lamp, with and without fluorescein, and the fundus camera

D.3.4.2. Post-exposure examinations.

Readers were provided with a power point slide of the pre-shot images (Figure 51) with a white dot marking the location of the alignment laser to aid in finding the lesions when available.

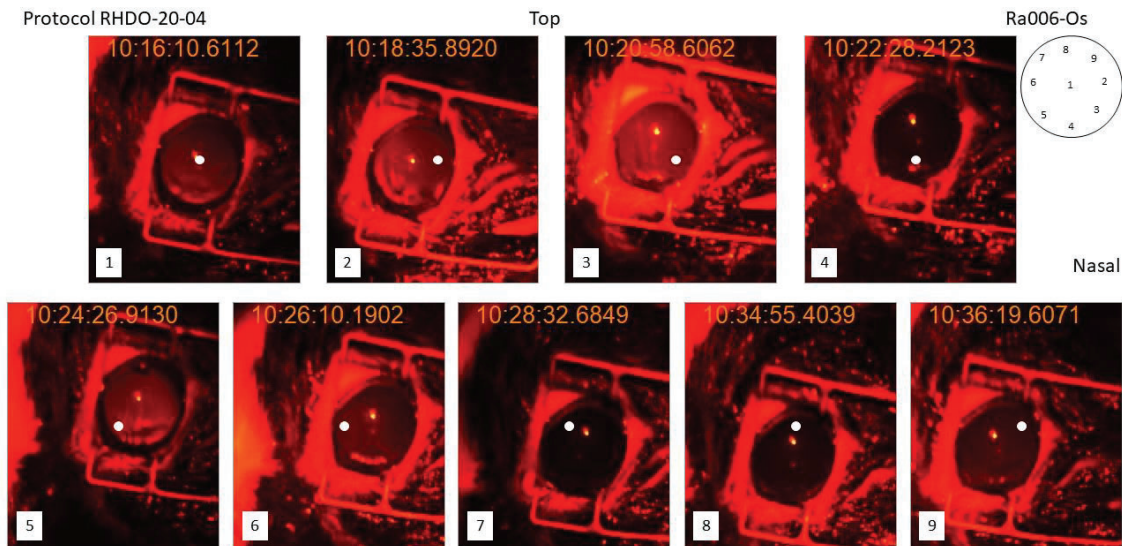


Figure 51. Pre-shot images showing exposure locations as indicated by the position of the alignment laser (white solid dot). Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

Figure 52 shows the SC generated in 5 exposures. At 2000 nm, the SC generated as captured by the camera appears to be white and does not have such a long tail as for 1318 nm and 1540 nm. The latter may be due to the higher absorption coefficient of the cornea at this wavelength when compared to the others. In addition, it is much harder to visualize it, however, the intensity of the SC generated correlated with the magnitude of the radiant exposure, see Table 36.

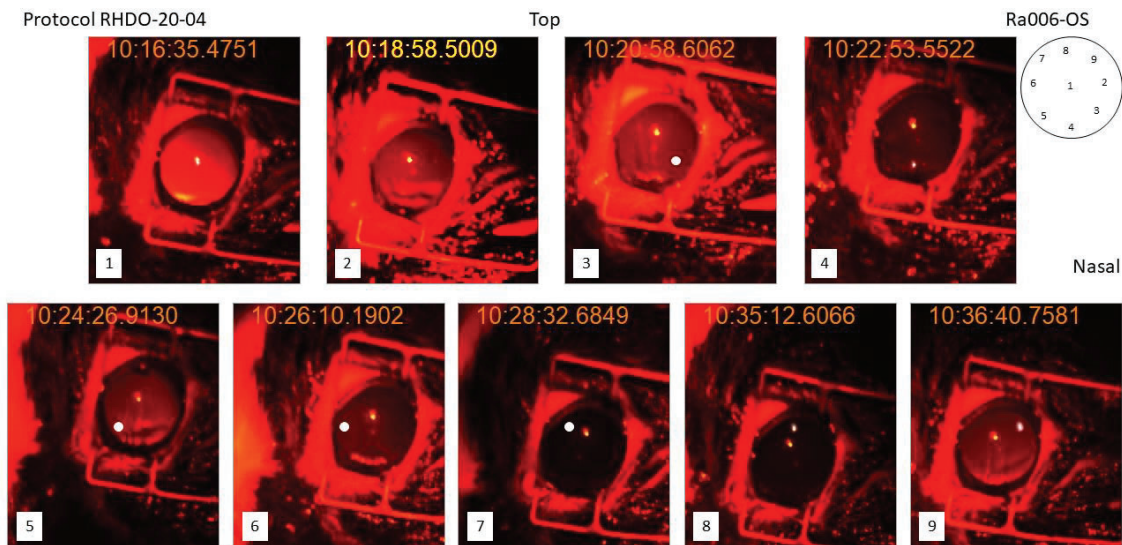


Figure 52. Images showing the SC light generated during the exposure as captured by the front-viewing camera for all exposure locations. Given that the SC light captured in the image corresponding to exposure 2 was extremely weak, the brightness and contrast was adjusted to aid in locating it. Images that contain a solid white dot indicate no SC was captured by the camera during the exposure therefore that image is the corresponding pre-shot image. Insert in the upper left hand corner is a schematic diagram of the intended exposure locations.

Representative images of some of the lesions in this subject are presented in section 4.2.1 above.

D.3.4.3. Data.

Readers were able to distinguish 4 induced lesions and there was one disqualified exposure. The eye was collected after the 1 h exposure for histological purposes.

Table 36. Exposure parameters and 1 h post-exposure scoring: Ra21-006 OS at 2000 nm

Location	Energy and Radiant Exposure Determination						1 h Post-exposure Scoring			
Exposure Number	Sample to Reference Ratio	Reference Energy per Pulse (μJ)	Delivered Energy per Pulse $\pm u(Q)$ (μJ)		Peak Radiant Exposure per Pulse $\pm u(H)$ (J/cm^2)		Reader 1 (0/1)	Reader 2 (0/1)	Reader 3 (0/1)	Observed Damage 2 out of 3 (0/1)
1	21.98	21.61	475.03	19.43	6.64	0.35		1	1	1
2	21.98	5.35	117.60	4.83	1.64	0.09		1	1	1
3	7.18	8.44	60.62	2.44	0.85	0.04		0	0	0
4	21.98	16.28	357.87	14.64	5.00	0.27		1	1	1
5	7.18	12.33	88.57	3.57	1.24	0.07		0	0	0
6	7.18	20.65	148.33	5.97	2.07	0.11		0	0	0
7	7.18	13.59	97.62	3.93	1.36	0.07		1	0	0
8	21.98	28.01	615.72	25.18	8.61	0.46		1	1	1
9	--	--	870.82*	35.29	12.17	0.65		1	1	1

*Maximum energy was determined after the exposure by placing a detector in place of the sample, firing a single shot several times and calculating the average over the measured values.

D.3.5. Cumulative probit analysis

Table 37. Raw probit data for exposures at 2000 nm.

Thu Apr 14 13:56:40 2022

Input File

ONES = 17 ZEROES = 17 TOTAL = 34

Percent confidence = 0.95

ED50 = 2.79 Upper FL = 3.82 Lower FL = 1.88

Intercept = -2.61 Slope = 5.85

Pearson's Chi-Sq = 24.7679 Probability of Chi-Sq = 0.8153

h = 1.00 g = 0.39 t = 1.96

Log XBAR = 0.474 Log YBAR = 5.16

SYY = 34.595 SXY = 1.679 SXX = 0.287 S0 = 8.118

Iterations = 35 DF = 33

Prob	Dose	Lower FL	Upper FL	Prob	Dose	Lower FL	Upper FL
0.01	1.12	0.206	1.73	0.55	2.94	2.05	4.11

0.02	1.25	0.272	1.86	0.60	3.09	2.23	4.47
0.03	1.33	0.325	1.95	0.65	3.25	2.40	4.92
0.04	1.40	0.370	2.02	0.70	3.43	2.58	5.48
0.05	1.46	0.412	2.08	0.75	3.64	2.77	6.20
0.06	1.52	0.452	2.13	0.80	3.89	2.97	7.18
0.07	1.56	0.489	2.18	0.85	4.20	3.19	8.58
0.08	1.61	0.525	2.23	0.90	4.63	3.47	10.8
0.09	1.65	0.560	2.27	0.91	4.74	3.54	11.5
0.10	1.69	0.594	2.31	0.92	4.86	3.61	12.2
0.15	1.86	0.756	2.49	0.93	4.99	3.69	13.1
0.20	2.01	0.912	2.66	0.94	5.15	3.78	14.2
0.25	2.14	1.07	2.82	0.95	5.34	3.89	15.5
0.30	2.27	1.22	2.98	0.96	5.56	4.01	17.2
0.35	2.40	1.38	3.15	0.97	5.86	4.16	19.6
0.40	2.53	1.55	3.35	0.98	6.27	4.37	23.3
0.45	2.66	1.71	3.56	0.99	6.98	4.71	30.8
0.50	2.79	1.88	3.82				
Dose	Tries	Hits	Dose	Tries	Hits	Dose	Tries Hits
0.240	1	0	1.81	1	0	4.82	1 1
0.320	1	0	2.04	1	0	4.88	1 1
0.700	1	0	2.07	1	0	5.00	1 1
0.790	1	0	2.35	1	0	5.26	1 1
0.850	1	0	2.74	1	0	5.74	1 1
0.960	1	0	2.78	1	0	6.64	1 1
1.00	1	0	3.20	1	1	6.72	1 1
1.16	1	0	3.33	1	0	7.13	1 1
1.24	1	0	3.35	1	1	8.61	1 1
1.36	1	0	4.28	1	1	8.69	1 1
1.48	1	1	4.57	1	1	10.5	1 1

Totals				34 17					
*****Original Data*****									
0.24	0	0.32	0	0.70	0	0.79	0	0.85	0
0.96	0	1.00	0	1.16	0	1.24	0	1.36	0
1.48	1	1.64	1.81	0	2.04	0	2.07	0	
2.35	0	2.74	0	2.78	0	3.20	1	3.33	0
3.35	1	4.28	1	4.57	1	4.82	1	4.88	1
5	1	5.26	1	5.74	1	6.64	1	6.72	1
7.13	1	8.61	1	8.69	1	10.53	1	12.17	1

APPENDIX E – Evaluation of the Supercontinuum Generated

In order to confirm that the blueish (white) light that we saw in the images during laser exposures of the cornea was SC generated by the ultrashort pulses, we focused the NIR beam onto a regular microscope glass slide using the exact same setup as that used for the cornea exposures. The idea of this experiment was that the ultrashort pulse would interact with the glass slide in a similar manner as the cornea. The glass slide was 1 mm thick and was placed at the exposure plane. An integrating sphere connected to a spectrometer was located behind the glass slide in order to collect the SC light generated, see Figure 53. The spectra of the incoming NIR beam was measured and the SC spectra was measured with both a visible and a NIR spectrometer. At high energy, the NIR beam underwent nonlinear interactions with the optical components in our system, generating 3rd harmonic. We documented the presence of the third harmonic by removing the glass slide and measuring the spectra of the incoming beam. The spectra of the low energy NIR beam without the glass slide in place was considered as the baseline. The baseline was subtracted from all visible spectra. The alignment laser was turned off during the measurements and the room lights were set exactly the same as during the cornea exposure experiments.

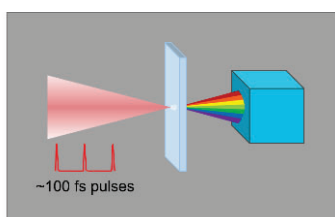


Figure 53. Optical setup used to measure the spectra of the SC generated by a 1 mm thick glass slide.

In the following sections, we will present the spectra of the incoming NIR beam, the visible and NIR spectra of the SC, and the visible spectra of the 3rd harmonic. In addition, we present a photograph of the SC generated at each of the 3 wavelengths used as viewed on a white paper. The maximum intensity of the incoming NIR light as well as that of the 3rd harmonic have been scaled for presentation purposes. However, the incoming light was set to a pulse energy of 90 J when we measured the visible spectra of the SC generated. The incoming pulse energy used for the photographs of the SC was not recorded and they are included for illustration purposes only.

E.1 Wavelength 1 – 1318 nm

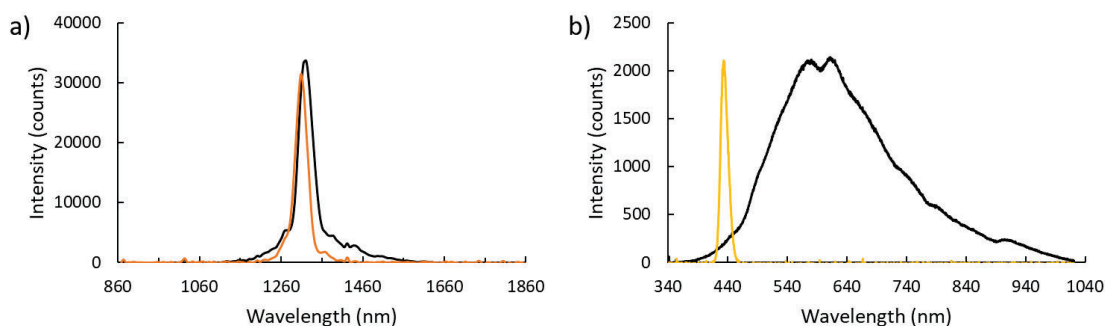


Figure 54. a) Spectra of incoming NIR laser beam (orange) and SC spectra (black). b) Spectra of the SC generated with an incoming energy of 90 μ J at 1318 nm (black) and the 3rd harmonic of 1318 nm (yellow).

The NIR spectra of the generated SC is slightly red-shifted and broadened relative to the incoming NIR light. The visible spectra of the SC contains wavelengths between 340 nm and 1040 nm, resulting in a bandwidth of approximately 700 nm. The peak is located around 600 nm.



Figure 55. Photograph of the SC generated by 1318 nm on a white piece of paper.

E.2 Wavelength 2 – 1540 nm

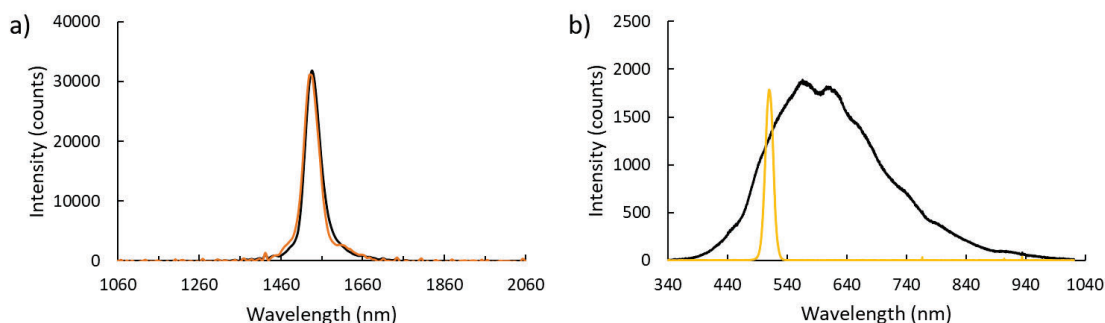


Figure 56. a) Spectra of incoming NIR laser beam (orange) and SC spectra (black). b) Spectra of the SC generated with an incoming energy of 90 μ J at 1540 nm (black) and the 3rd harmonic of 1540 nm (yellow).

The NIR spectra of the generated SC is basically unmodified relative to the incoming NIR light. The visible spectra of the SC contains wavelengths between 340 nm and 940 nm, resulting in a bandwidth of approximately 600 nm. The peak is located at 572 nm.

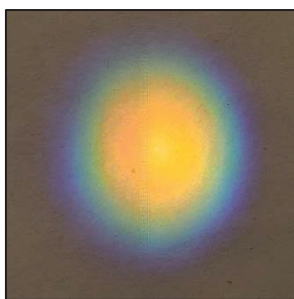


Figure 57. Photograph of the SC generated by 1540 nm on a white piece of paper.

E.3 Wavelength 3 – 2000 nm

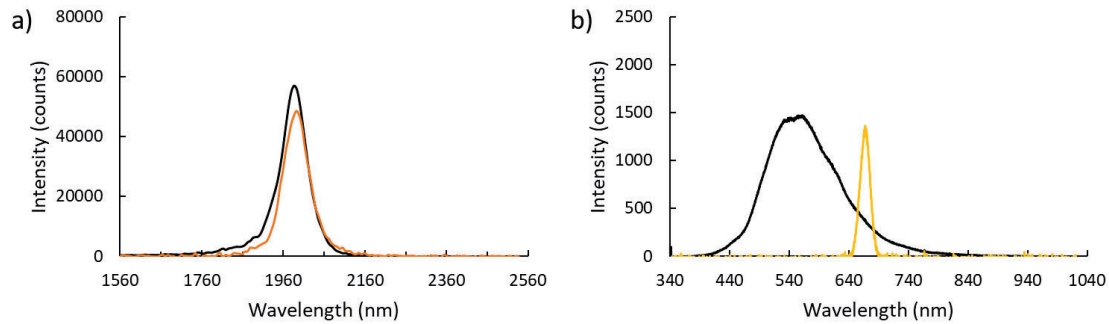


Figure 58. a) Spectra of incoming NIR laser beam (orange) and SC spectra (black). b) Spectra of the SC generated with an incoming energy of 90 μJ at 2000 nm (black) and the 3rd harmonic of 2000 nm (yellow).

The NIR spectra of the generated SC is slightly broadened relative to the incoming NIR light. The visible spectra of the SC contains wavelengths between 440 nm and 740 nm, resulting in a bandwidth of approximately 300 nm. The peak is located at 564 nm.

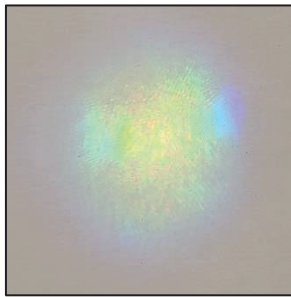


Figure 59. Photograph of the SC generated by 2000 nm on a white piece of paper.