ULTRASHORT LASER PULSE EFFECTS ON OCULAR TISSUE: HISTOPATHOLOGIC ANALYSIS AND SURGICAL TECHNIQUES

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Toth et al. identified the acute pathologic retinal events which determined the extent of tissue damage from minimal laser energy and suprathreshold energy. She identified the acute pathology of primate maculas with minimal visible retinal lesions generated by 90 femtosecond and 5 picosecond pulses of 580 nm laser energy. Dr. Toth's study included light microscopic evaluation of macular damage and electron microscopic ultrastructural analysis of melanosome ruptures within the retinal pigment epithelium. The pathology was reported at AFSOR with a CDROM summary of lesion data provided to Armstrong Laboratory for reference. Dr. Toth scored the area and extent of tissue damage and compiled a list for Dr. Rockwell and Dr. Clarence Cain at Armstrong Laboratory, Brooks, AFB for analysis and comparison to the ED50 MVL data. A paper summarizing this work has been published as the lead article in October, 1997 in Investigative Ophthalmology and Visual Science (IOVS). Dr. Toth's Laboratory group gathered significant tissue data on ultrashort pulses of other laser wavelengths. They collaborated with Armstrong Laboratory (Dr. Cain et al.) in analyzing near-infrared ultrashort laser pulse injury MVL data which was published at Society of Photo-Optical Instrumentation Engineers (SPIE), 1997 (Cair, Toth, et al.).
Dr Toth also consulted with the Armstrong laboratory group on numerous related publications. She interpreted all macular fluorescein angiograms for the laser safety publications. Toth worked diligently at publishing the results of her research in the ophthalmic and engineering literature.
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Principal Investigator:

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Reporting Period:

15 April 1995 - 14 April 1998
II. OBJECTIVES:

Unchanged from Statement of Work

III. STATUS OF EFFORTS:

Toth et al. identified the acute pathologic retinal events which determined the extent of tissue damage from minimal laser energy and suprathreshold energy. She identified the acute pathology of primate maculas with minimal visible retinal lesions generated by 90 femtosecond and 5 picosecond pulses of 580 nm laser energy. Dr. Toth's study included light microscopic evaluation of macular damage and electron microscopic ultrastructural analysis of melanosome ruptures within the retinal pigment epithelium.

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IV. ACCOMPLISHMENTS / NEW FINDINGS:

Dr. Toth's histologic information regarding the effect of visible ultrashort laser pulses on the retina was reported to the military, scientific and medical laser and safety community. This new data included the first reports of the narrow columnar damage from ultrashort laser pulses, and supported the bench and theoretical work of Armstrong Laboratory. This histopathology report also demonstrated intraretinal, not choroidal, hemorrhages in relatively low energy lesions from fs and ps pulses. This validated our initial clinical observations and clarified theoretical predictions from the Armstrong Laboratory. With this histopathology, Toth demonstrated that the mechanism of injury is clearly quite different than that of longer pulse widths. With the Armstrong group, Toth has shown that laser induced breakdown is a probable cause for lesions above threshold at 90 fs. Toth et al also demonstrated that ruptured melanosomes are not the cause of the MVL damage at 90 fs, though fractured and striated melanosomes are a component of higher energy suprathreshold lesions. This information was important to the interpretation of Dr. Glickman's studies of oxidative damage from ruptured melanosomes.

In collaboration with international scientists and the Armstrong laser group, Toth demonstrated the in vivo difference in evolution of a thermal laser lesion such as
from a cw argon laser versus evolution of a focal inner retinal lesion from an ultrashort laser pulse. Utilizing optical coherence tomography, she followed lesion evolution in vivo over the first seconds after laser delivery. In Archives of Ophthalmology, she published a report of this timecourse of development of edema, distortion of tissue and bleeding after laser delivery.

The histopathologic data was the first available for these ultrashort laser pulsewidths and has been essential to the military and civilian laser safety community as they determine safe standards for the use of ultrashort lasers. This data was previously requested by numerous military and civilian researchers, at national and international meetings and has been referenced at numerous laser meetings over the past several years. This data has also been of interest to the medical community as physicians considered the use of novel laser pulse structures for clinical applications in patient care. For the first time, clinicians knew which areas of the retina would be affected by low or high energy visible ultrashort laser pulses. As Toth demonstrated, focal retinal pigment epithelial damage can be created by these laser pulses without significantly affecting overlying neurosensory retina. Thus she proposed that ultrashort pulses might be a useful method for the future treatment of drusen associated with age-related macular degeneration.

The technical summary of the work is comprehensively covered in the publications listed below. These data will, therefore, not be repeated in this summary.
V. PERSONNEL SUPPORTED:

<table>
<thead>
<tr>
<th>Personnel</th>
<th>Job Title</th>
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<tbody>
<tr>
<td>Cynthia A. Toth, M.D.</td>
<td>Principal Investigator</td>
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<tr>
<td>Diane Hatchell, Ph.D.</td>
<td>Co-Investigator</td>
</tr>
<tr>
<td>Alan D. Proia, M.D., Ph.D.</td>
<td>Pathologist</td>
</tr>
<tr>
<td>Ewa Worniallo</td>
<td>Histology Laboratory Analyst</td>
</tr>
<tr>
<td>Katrina Winter</td>
<td>Laboratory Research Analyst</td>
</tr>
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</table>

Collaborators:

| Dr. Benjamin A. Rockwell       | Armstrong Laboratory, Brooks AFB, TX         |
| Lt. Daniel X. Hammer           | Armstrong Laboratory, Brooks AFB, TX         |
| Maj. Cheryl D. DiCarlo         | USUHS, Bethesda, MD                          |
| Capt. William P. Roach        | AFOSR, Bolling AFB, DC                       |
| Dr. Clarence P. Cain          | TASC, San Antonio, TX                        |
| Gary D. Noojin                 | TASC, San Antonio, TX                        |
| David J. Stolarski            | TASC, San Antonio, TX                        |

Other Academic Collaborators:

| James G. Fujimoto, Ph.D.       | Massachusetts Institute of Technology       |
| Reginald Birngruber, Ph.D.     | Medizinisches Laserzentrum Lubeck            |
| Stephen A. Boppart, MSEE       | Massachusetts Institute of Technology       |
| Michael R. Hee Ph.D.           | Massachusetts Institute of Technology       |
VI. PUBLICATIONS:

Refereed Journals:


Non-Refereed Journals:


Published Abstracts:


### VII. Transitions:

1997 Basic Research Technology Transitions: F46920-95-1-0226

<table>
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<tr>
<th>Sub Area</th>
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<tr>
<td>X</td>
<td>Ultrashort laser bioeffects</td>
<td>Kozumbo</td>
<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>Transmission electron micrographs of ultrashort effects on the RPE demonstrate striate and fractured melanomas</td>
<td>Data supported the concept of microbubble effects as well as LIB.</td>
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<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>Comprehensive histopathology documenting the minimal level of pathologic response to ultrashort laser pulses in IOLS</td>
<td>Comprehensive data to be used in establishing national laser safety standards, i.e., the maximum permissible exposure (MPE) limits of ultrashort laser pulses</td>
</tr>
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<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>Histopathology data demonstrating choroidal damage from the first infrared laser retinal injuries</td>
<td>Data demonstrate a different retinal effect at short pulse widths with wavelength dependence</td>
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<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>Dr. Charles Lin Wollman Labs 617-724-3957</td>
<td>Light micrographs of ultrashort prime lesions show lifting and vacuolization which supports his microbubble theory</td>
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<td>Dr. Charles Lin Wollman Labs 617-724-3957</td>
<td>Melanosome response to femtosecond laser pulses includes ruptured and striated melanomas in moderate lesions</td>
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<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>Dr. Clarence P. Cain The Analytic ScienceCorp San Antonio, TX (210) 536-4794</td>
<td>Fluorescein angiography data on laser lesions of primate retina</td>
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<td>X</td>
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<td>Kozumbo</td>
<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>Bobbie Geurther, PhD Duke FEL facility 919-660-2674</td>
<td>Overview of pulsed laser effects on ocular tissue</td>
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<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>Dr. Steve Jacques UT Health Science Center 512-648-7874</td>
<td>Melanosome response to femtosecond laser pulses: striated melanomas are also present in moderate lesions</td>
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<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>VayTek, Fairfield, Iowa 515-472-2227</td>
<td>Three dimensional imaging software applied to transmission electron micrographs</td>
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<td>X</td>
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<td>Kozumbo</td>
<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>ANSI subcommittee for laser safety standards update William P. Roach</td>
<td>Laser injury data for ultrashort laser pulses to the eye with interpretation of effect on risk of injury with vision loss</td>
</tr>
<tr>
<td>X</td>
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<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>International Ophthalmic community</td>
<td>Clinopathologic correlation of laser retinal lesions and optical coherence tomography</td>
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<td>X</td>
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<td>Cynthia A. Toth, M.D., Duke University Eye Center (919) 684-5631</td>
<td>Humphrey Instruments San Leandro CA 510-895-9110</td>
<td>Information regarding applications of optical coherence tomography of evolving laser and retinal lesions.</td>
</tr>
</tbody>
</table>
VIII. NEW DISCOVERIES, INVENTIONS, OR PATENT DISCLOSURES:

- Our published comprehensive studies provide much basic data, for the ANSI "Standard for the safe use of lasers" revision to include ultrashort laser pulses. This data will thus also be used for military laser standards. This data will similarly be used by the European community when the "IEC International Standard for the Safety of Laser Products" is revised to include ultrashort laser pulses.