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PROGRESS REPORT

A Center of Excellence for the Medical Application of Lasers (DOE Grant # DE-FG03-91ER61227)

DISCLAIMER

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Irvine, California

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BECKMAN LASER INSTITUTE AND MEDICAL CLINIC

Total Publications Resulting from DOE Grant # DE-FG03-91ER61227

TOTAL PUBLICATIONS (111)

ONCOLOGY (33)

1. New Sensitizers/Strategies (8)

1. Chapman J.A., Tadir Y., Tromberg B.J., Yu K., Manetta A., Sun C.H., Berns M.W. Effect of Administration Route and Estrogen Manipulation on Endometrial Uptake of Photofrin II. Am. J. Obstet. Gynecol. 168: 685-692, 1993.

2. Tadir Y., Tromberg B., Krasieva T., Steiner R., Chapman J., Berns M.W. Endometrial photosensitization: experimental models. In: Lasers in Gynecology. Ed. Donnez J. (in press).

3. Tadir Y., Tromberg B., Krasieva T., Berns M.W. Photodynamic therapy towards selective endometrial ablation. Proceedings S.P.I.E. 1879; 247-52, 1993.

4. Steiner R., Tromberg B., Weiss P., Krasieva T., Berns M.W., McCullough J., Tadir Y. Photodynamic destruction of rat endometrium using topically-administered Photofrin. Human Reproduction. (submitted)

5. Wyss P., Svaasand L., Tadir Y., Tromberg B.J., Berns M.W. Photomedicine of the endometrium: experimental concepts. Human Reproduction, (submitted)

6. Wyss P., Tadir Y., Tromberg B.J., Liaw L., Krasieva T., Steiner R., Villalon V.P., Berns M.W. Benzo Porphyrin Derivative (BPD): a potent photosensitizer for photodynamic destruction of the rabbit endometrium. Obstetrics and Gynecology, (submitted)

7. Wyss P., Tromberg B.J., Wyss M.T., Krasieva T., Liaw L., Schell M., Berns M.W., Tadir Y. Photodynamic destruction of endometrial tissue using topical 5-aminolevulinic acid (5-ALA) in rats and rabbits. Am. J. Obstet. Gynecol. (submitted)

8. Steiner R.A., Tadir Y., Tromberg B.J., Krasieva T., Ghazains T., Wyss P., Berns M.W. Photosensitization of the rat endometrium following 5-aminolevulinic acid (ALA) induced photodynamic therapy.

2. Non-Invasive Optical Fiber Diagnostic System Utilizing Photon Migration (25)

1. Tromberg B.J., Svaasand L.O., Tsay T-T., Haskell R.C., Berns M.W., "Optical Property Measurements in Turbid Media Using Frequency Domain Photon Migration", Proceedings of the SPIE, 1525, Berlin, 52-58 (1991).

2. Svaasand L.O., Tromberg B.J., "On the Properties of Optical Waves in Turbid Media", Proceedings of the SPIE, 1525, Berlin, 41-51 (1991).

3. Tromberg B.J., Svaasand L.O., Tsay T-T., Haskell R.C., Berns M.W., "Optical Property Measurements in the Multiple-scattering Regime Using Frequency-domain Photon Migration", Pacific Conference on Chemistry and Spectroscopy, Anaheim, October 7, 1991.

4. Tsay T-T., Tromberg B.J., Cho E., Vu K., Svaasand L.O., "Monitoring Photochemistry in Tumors Using Frequency Domain Photon Migration", Proceedings of the SPIE, Los Angeles, 213-218 (1991).

5. Svaasand L.O., Tromberg B.J., Tsay T-T., Haskell R.C., Berns M.W., "On the Properties of Photon Density Waves in Tissues", Advances in Biological Heat and Mass Transfer, ASME 1991, HTD-Vol. 189/BED-Vol. 18, 85-89, (1992).

6. Svaasand L.O., Tromberg B.J., Haskell R., Tsay T-T., Berns M.W. "Propagation of Photon Density Waves in Tissues", Lasers in Surgery and Medicine S4, 5, (1992).

7. Tromberg B.J., Svaasand L.O., Tsay T-T., Cho E., Vu K. "The Influence of Tissue Structure on the Phase Velocity of Photon Density Waves", Lasers in Surgery and Medicine S4, 5-6, (1992).

8. Tsay T-T., McAdams M.S., Tromberg B.J., Svaasand L.O., Haskell R.C., Ahdoot J. "Determination of Tissue Optical Properties Using Frequency Domain Photon Migration", Lasers in Surgery and Medicine S4, 6, (1992).

9. Tromberg B.J., Krasieva T., Shimizu S., Satorius A., and Forssen E.A., "Non-invasive laser-induced fluorescence imaging of daunorubicin in solid tumors in situ: comparison of conventional drug with tumor-targeting small unilamellar vesicles", Proc. Am. Assoc. Can. Res. 33, 2665 (1992).

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12. Svaasand L.O., Haskell R.C., Tromberg B.J., McAdams M. "Properties of Photon Density Waves at Boundaries", Proceedings of the International Society for Optical Engineering, 1888, B. Chance and R. Alfano editors, 214-226, (1993).

13. Tromberg B.J., Tsay T-T., Pham V., Svaasand L.O., "Frequency-Domain Spectroscopy in Turbid Media: Wavelength-Dependent Optical Properties", Lasers in Surgery and Medicine S5, 5, (1993).

14. Tromberg B.J., Svaasand L.O., Tsay T-T., Pham V., Haskell R.C., "Frequency-Domain Optical Spectroscopy in Turbid Media", Pacific Conference on Chemistry and Spectroscopy, Pasadena, October 21, 1993.

15. Madsen S.J., Tromberg B.J., Svaasand L.O., Haskell R.C. "Steady-state Versus Frequency-Domain Techniques for the Determination of Tissue Optical Properties", Optical Society of America Annual Meeting, October 5, 1993.

16. Tromberg B.J. "Non-Invasive Imaging of Tissue Optical Properties", Proceedings of the Chinese-American Workshop on Non-Invasive Medical Diagnostics, P.A. Lewin, editor, National Science Foundation, Wash., D.C. (1993).

17. Tromberg B.J., Madsen S.J., Wyss P., Svaasand L.O., Haskell R.C., Tadir Y. "Tissue characterization using frequency-domain photon migration", International Society for Photo-Optical Instrumentation Engineers (SPIE), Los Angeles, January 26, 1994.

18. Koenig K., Schneckenburger H., Walt H., Leeman T., Wyss-Desserich M. T., Ruck A., and Tromberg B.J. "Optical Methods for Tumor Treatment and Detection", Proceedings of the SPIE, Los Angeles, 2133, (in press).

19. Koenig K., Schneckenburger H., Hemmer J., Tromberg B.J. and Steiner R. *Advances in Laser and Light Spectroscopy to Diagnose Cancer and Other Diseases*, Proceedings of the SPIE, Los Angeles, 2135 (in press).

20. Peavy G.M., Krasieva T.B., Tromberg B.J., and Berns M.W., "Treatment Considerations for Photodynamic Therapy in the Cat", Proceedings of the SPIE, Los Angeles, 2128, (in press).

21, Madsen S.J., Tromberg B.J., Wyss P., Svaasand L.O., Haskell R.C. and Tadir Y., "The Optical Properties of Human Uterus at 630 nm", Proceedings of the Optical Society of America: Optical Imaging and Photon Migration", Orlando, March (1994).

22. Tromberg B.J., Svaasand L.O., Madsen S.J., Haskell R.C., Chapman C. "Frequency-Domain Photon Migration Spectroscopy In Turbid Media", Proceedings of the Optical Society of America: Optical Imaging and Photon Migration", Orlando, March (1994).

23. Haskell R.C., Svaasand L.O., Tsay T.-T., Feng T.- C., McAdams M.S., and Tromberg B.J. "Boundary Conditions for the Diffusion Equation in Radiative Transfer", JOSA-A, submitted.

24. Madsen S.J., Wyss P., Svaasand L.O., Haskell R.C., Tadir Y., Tromberg B.J. "Determination of the Optical Properties of Human Uterus Using Frequency-Domain Photon Migration and Steady-State Techniques", Physics in Medicine and Biology, submitted.

25. Wyss P., Svaasand L.O., Tadir Y., Berns M.W., and Tromberg B.J. "Photomedicine of the Endometrium: Experimental Concepts." Human Reproduction, submitted

PULMONARY/THORACIC SURGERY (14)

1. Thoracoscopic Laser Ablation of Emphysematous Pulmonary Bullae Using Laser Photo-Absorbing Dyes (14)

1. Boyajian J.G., Brenner M., Espinoza F.P., Milne E.C., Roeck W.W., Brown L., Chung E., Chen C., Wilson A.F., Kono T., Wakabayashi A., Berns M.W. Defocused carbon dioxide laser injury of the lung: radiographic and histopathologic findings in a rabbit model. Presented American Thoracic Society National Meeting, 1992.

2. Brenner M., Chung E., Boyajian J.G., Espinoza F.P., Brown L., Chung E., Chen C., Wilson A.F., Kono T., Wakabayashi A., Berns M.W. Interleukin 1B release during carbon dioxide laser injury of the lung in a rabbit model. Presented American Thoracic Society National Meeting, 1992.

3. Chung E., Brenner M., Boyajian J.G., Espinoza F.P., Brown L., Chung E., Chen C., Wilson A.F., Kono T., Wakabayashi A., Berns M.W. Systemic interleukin-1 beta in an acute CO_2 laser lung injury rabbit model. Presented at Southern California Pulmonary Research Conference., 1992.

4. Boyajian J.G., Brenner M., Espinoza F.P., Milne E.C., Roeck W.W., Brown L., Chung E., Chen C., Wilson A.F., Kono T., Wakabayashi A., Berns M.W. Defocused carbon dioxide laser injury of the lung: radiographic and histopathologic findings in a rabbit model. Presented at Southern California Pulmonary Research Conference., 1992.

5. Brenner M., Wakabayashi A., Kayaleh R.A., Milne E.N., Della Bella L., Osann K., Tadir Y., Berns M.W., Wilson A.F. Thoracoscopic laser ablation of emphysematous pulmonary bullae: Radiographic selection and treatment response. Presented Western Section AFCR 2/93.

6. Chung E., Brenner M., Schenkel T., Wang N., Boyajian J., Espinosa L., Wilson A.F., Osann K., Wakabayashi A., Tadir Y., Berns M.W. Effect of laser energy on Lung Injury in a Rabbit Model. Presented at Southern California Pulmonary Research Conference. 1993

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9. Brenner M., Waite T., Chung E., Schenkel T., Wang N., Boyajian J., Wilson L., Osann L., Wakabayashi A., Tadir Y., Berns M.W. A thoracoscopic small animal pulmonary laser exposure model. Accepted for presentation: Society for Optical and Quantum Electronics, 1993.

10. Shankel T., Brenner M., Waite T., Hamilton A., Wilson A.F., Tadir Y., and Berns M.W. An animal model for thoracoscopic laser ablation of emphysematous pulmonary bullae. Clinical Research 42(1):59A,1994.

11. Shankel T., Brenner M., Waite T., Hamilton A., Wilson A.F., Tadir Y., and Berns M.W. An animal model for thoracoscopic laser ablation of emphysematous pulmonary bullae. Submitted AFCR National Meetings Spring 1994.

12. Waite T., Brenner M., Shankel T., Hamilton A., Wilson A.F., Tadir Y., and Berns M.W. Thoracoscopic laser ablation of emphysematous pulmonary bullae in rabbits. Submitted Thoracoscopy and Panendoscopy International Meetings, Munich, GD 1994.

13. Brenner M., M.D., Milne E.N., Kayaleh R.A., Della Bella L., Osann K., Wilson A.F., Berns M.W. Thoracoscopic Laser Ablation of Pulmonary Bullae: Radiographic Selection and Physiologic Mechanisms of Treatment Response. In Press J Thoracic Cardiovasc Surg 7/93.

14. Brenner M., Shankel T., Waite T., et al. An animal model for thoracoscopic laser ablation of emphysematous pulmonary bullae. Submitted. 1994.

DERMATOLOGY/PLASTIC SURGERY (11)

1. Photochemotherapy of Psoriasis and Other Skin Diseases (2)

1. Weinstein G.D., McCullough J.L., Nelson J.S., Berns M.W., McCormick A.: Low Dose Photofrin II Photodynamic Therapy of Psoriasis, Journal of Investigative Dermatology 96:573, 1991.

2. Weinstein G..D., McCullough J.L., Nelson J.S., Berns M.W., McCormick A.: Low Dose Photofrin II Photodynamic Therapy of Psoriasis, Proceedings of the 5th International Psoriasis Symposium (1991).

2. Remote Infrared Sensing as a Method to Monitor and Improve Treatment of Vascular and Pigmented Lesions (9)

1. Nelson J.S., Jacques S.L., Wright W.H. Determination of thermal and physical properties of port wine stain lesions using pulsed photothermal radiometry. SPIE 1643:287-298, 1992.

2. Jacques S.L., Nelson J.S., Wright W.H., Milner T.E. Pulsed photothermal radiometry of port-wine-stain lesions. Appl. Optics. 32:2439-2446, 1993.

3. Milner T.E., Nelson J.S., Tran N., Katzir A., Svaasand L.O., Jacques S.L. Pulsed photothermal radiometry of port wine stains. SPIE 1881:34-42, 1993.

4. Milner T.E., Nelson J.S., Tran N., Katzir A., Svaasand L.O., Jacques S.L. Clinical use of pulsed photothermal radiometry. SPIE 1876:122-128, 1993.

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9. Nelson J.S., Milner T.E., Anvari B., Tanenbaum B.S., Kimel S., Svaasand L.O. Dynamic epidermal cooling during pulsed laser treatment of port wine stain - a new methodology with preliminary clinical evaluation. Arch. Dermatol. (in submission), 1994.

OBSTETRICS AND GYNECOLOGY (24)

1. Development of an Optical Force Trap for Diagnosis and Manipulation of Sperm (4)

1. Tadir Y., Neev J., Berns M.W. Laser Microsurgery and Manipulations of Single Cells. In: Practical manual of Gynecologic Endoscopy. Eds. C. Sutton, M. Diamond. Saunders Ltd. London, 1993. pp.379-386

2. Westphal L., El-Danasouri I.E., Shimizu S., Tadir Y., Berns M.W. Exposure of human sperm to the cumulus oophorus results in increased relative force as measured by a 760 nm laser optical tram. Human Reproduction. 8 (7) 1083-1086, 1993.

3. Araujo E., Tadir Y., Patrizio P., Ord T., Silber S., Berns M.W., Asch R. Relative force of human epididymal sperm correlated to the fertilizing capacity in vitro. Fertil Steril. (In press).

4. Zoentania, N.D., Araujo E., Berns M.W., Tadir Y., Schell M.W., Stone SC. Effect of freezing on the relative escape force of sperm as measured by laser optical trap. Fertil Steril. (submitted)

2. Laser Micromanipulations of Eggs and Embryos (12)

1. Tadir Y., Wright W.H., Vafa O., Liau L-H., Asch R., Berns M.W. Micromanipulation of Gametes Using Laser Microbeams. Human Reproduction. 6:1011-1016, 1991.

2. Neev Y., Tadir Y., Asch R., Ord T., Liaw L-H., Ho P.D., Berns M.W. Zona Drilling and Partial Zona Dissection using femto-, pico-, and nanosecond pulses: a comparative study. In: SPIE, 1650:61-69, 1992.

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7. Li L., Munne S., Licciardi F., Neev Y., Tadir Y., Berns M.W., Godke R., Cohen J. Microinjection of FITC-Dextran into mouse blastomeres to assess topical effects of zona penetration. Zygote (in press).

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9. Tadir Y., Neev J., Ho P., Berns M.W. Lasers for Gamete Micromanipulation: Basic concepts. J. Assist Reprod. Genetics. 10:121-125, 1993.

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3. The Development of PDT for the Treatment of Endometrial Disease (8)

1. Chapman J.A., Tadir Y., Tromberg B.J., Yu K., Manetta A., Sun C-H., Berns M.W. Effect of Administration Route and Estrogen Manipulation on Endometrial Uptake of Photofrin II. Am. J. Obstet. Gynecol. 168: 685-692, 1993.

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3. Tadir Y., Tromberg B., Krasieva T., Berns M.W. Photodynamic therapy towards selective endometrial ablation. Proceedings S.P.I.E. 1879; 247-52, 1993.

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7. Wyss P., Tromberg B.J., Wyss M.T., Krasieva T., Liaw L., Schell M., Berns M.W., Tadir Y. Photodynamic destruction of endometrial tissue using topical 5-aminolevulinic acid (5-ALA) in rats and rabbits. Am. J. Obstet. Gynecol. (submitted)

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OPHTHALMOLOGY (21)

A. New Ab-Interno and Contact Laser Surgeries for Glaucoma

I. Enhancement of Aqueous Outflow

a. Laser Trabecular Ablation (LTA) (5)

1. Hill R.A., Özler S.A., Profeta G.A., Baerveldt G., and Berns M.W. Laser trabecular ablation (LTA). Lasers Surg and Med. 11:431-346, 1991.

2. Hill R., Lesiecki M., Stern D., Hsia J. and Berns M. The effects of pulse width on laser trabecular Ablation. Lasers Surg Med. 13:440-446, 1993.

3. Hill R., Lesiecki M., Hsia J., Stern D., Brown L., Baerveldt G., and Berns M. Erbium:YAG laser trabecular ablation in primates. Manuscript under revision.

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1b. Abstracted by Lasers for sclerostomy Annual of Ophthalmic Laser Surgery, 1:82-90, 1992.

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3. Le M., Hill R., Yashiro H., Constan C., Stern D., Lesiecki M., Brown L. and Berns M. Ab-Interno Erbium(Er):YAG laser sclerostomy with iridectomy in dutch cross rabbits. Presented as an abstract and poster at the Annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 33(4):1266, 1992.

c. Ab-Externo Sclerostomy (3)

1. Dah M., Hill R.A., Ryan J., Lesiecki M., Liaw L-H., and Berns M.W. Ab-Externo erbium (Er):YAG laser sclerostomies with 200- 300- and 400-μm fiber optics in rabbits. Abstract and poster at the Annual meeting of the Association for Researchers in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 34(4): 737, 1993.

2. Erbium:YAG Sclerostomy in Humans-an Update. Beckman L.R., Baerveldt G., Beckman H., Hill R.A. and Simmons J.R. Abstract and paper at the Annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 34(4): 1071,1993.

3. Hill R.A., Invited Paper, IEEE Lasers and Electro-Optics Society 1992 Annual Meeting, November 16-19, Boston MA. New Applications for pulsed and CW lasers in the treatment of glaucoma; Conference Proceedings; 1992.

II. Decrease of Aqueous Formation

a. Destruction of the Ciliary Body by Photodynamic Therapy (PDT) (4)

1. Hill R.A., Esterowitz T., Ryan J., Yoshiro J., Shirk J., Kenney M., Shimuzu S., Liaw L-H., and Tromberg B.J. Photodynamic therapy of the ciliary body with silicon naphthalocyanine (SINc) in rabbits. Abstract and paper at the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 34(4): 1069,1993.

2. Esterowitz T., Hill R.A., Ryan J., Yashiro H., Kim J., Treadway A., Krasieva T., Nelson J.S., and Berns M.W. Photodynamic therapy (PDT) of the ciliary body with photofrin® II (PII) and chloroaluminum sulfonated phthalocyanine (CASPc) in dutch cross rabbits. Abstract and poster at the Annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 34(4):739, 1993.

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4. Hill R.A., Esterowitz T., Ryan J., Yashiro H., Kim J., Treadway A., Krasieva T., Nelson J.S., and Berns M.W. Photodynamic therapy (PDT) of the ciliary body with photofrin® II (PII) and chloroaluminum sulfonated phthalocyanine (CASPc) in dutch cross rabbits. Manuscript in preparation.

b. PDT Mediated Destruction of an Experimental Ocular Melanoma (1)

1. Hill R.A., Esterowitz T., Ryan J., Yoshiro J., Shirk J., Kenney M., Shimuzu S., Liaw L-H., and Tromberg B.J. Photodynamic therapy of experimental ocular melanoma with silicon naphthalocyanine (SINc) in rabbits. Abstract and paper at the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 35(4): 2120,1994.

B. Development of Excimer Laser Delivery Systems for the Treatment of Hyperopia and Astigmatism (2)

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2. "Expression of Stress-Inducible Heat Shock Protein 70 in Rabbit Corneal Cells after Excimer Laser Treatment" ARVO Proceedings, May 1994

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2. Stabholz A., Neev J., Liaw L-HL., Stabholz Ay., Khayat A., Torabinejad M.: Sealing of human dentinal tubules by XeCI-308nm excimer laser. Journal of Endodontics. In press, 1993.

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15. Wilder-Smith P., Arrastia A.M., Grill G., Liaw L-H., Berns M.W.: Effect of Nd:YAG laser radiation in conjunction with root planing on dentinal root surface: thermal and SEM studies. Submitted for publication

16. Wilder-Smith P., Grill G., Liaw L-H., Berns M.W.: Effect of Nd:YAG laser radiation and root planing on root surface: an SEM study. Accepted for presentation at ASLMS, Toronto, 1994

BECKMAN LASER INSTITUTE AND MEDICAL CLINIC

"A Center of Excellence for the Medical Application of Lasers"
(DOE Grant # DE-FG03-91ER61227)

PROGRESS REPORT

IVa. ONCOLOGY

1. New Sensitizers/Strategies

CO-PI's: Yona Tadir, M.D., Professor of Obstetrics and Gynecology, UCI; Philip DiSaia, M.D., Professor of Obstetrics and Gynecology, UCI; Roger Crumley, M.D., Professor and Chair of Otolaryngology, UCI

Progress

The BLIMC has been working collaboratively with Lawrence Livermore National Lab (LLNL) through a cooperative research and development agreement (CRADA) to develop a multiple-wavelength diode-based photodynamic therapy laser system. The proposed system would be a low-cost, compact, and high power (2-5 watt continuous wave) laser system emitting at 657-697 nm. All specifications have been provided to LLNL, and we anticipate having a prototype device to test in animals and cells by the initiation date of this renewal application. We anticipate applying this system to the treatment of human cancer patients under one of our FDA-approved protocols by the beginning of the second year of the renewal application. One of our corporate affiliates (Coherent, Inc., Palo Alto, CA) has indicated an interest in commercializing this system following "proof of principle" testing. This project is directly in line with the "technology transfer" goals of the DOE Medical Laser Center program.

In addition to the CRADA with LLNL, the BLIMC was recently awarded a $250,000 grant from the California Trade and Commerce Agency in support of this "defense conversion" activity.

<u>Cervical Intraepithelial Neoplasia (CIN)</u>

The objective of this clinical study is to determine the efficacy of topically applied Photofrin solution in the photodynamic therapy (PDT) of cervical intraepithelial neoplasias (CIN). This study is designed to gather information regarding the efficacy of using topical Photofrin + Azone conducted by laser light in this gynecological disease using fixed drug dose and application schedules, and a variable dose of activated red light delivered by an argon pumped dye laser system.

A 1% solution of Photofrin (DHE) in 4% Azone and isopropyl alcohol vehicle in a total volume of 1-3 cc's was applied to a cervical cap and inserted by the investigator or staff during the initial pelvic examination. This cap was removed 24 hours later and a variable dose of red light (630 nm) was delivered to the diseased area in the uterine cervix through a specially designed PDT speculum. 23 of the 24 patients were treated and follow up on these patients is in progress (including repeated colposcopy, Pap smears, guided biopsy [if indicated] and

photography at each visit). A progress report of phase I of the PDT study for CIN is detailed in table 1 (as of March 1994).

The topical application of a photosensitizer reduces the dose to the minimum needed with maximal drug concentration at the target area. It is expected that there will be a higher local concentration in the diseased area as compared to the surrounding normal tissue. None of the patients treated so far in this study experienced any local or systemic, early or late side effects following drug or light application. All patients experienced mild vaginal discharge (clear) in the first 2-5 days following PDT, but without any pain or other discomfort. All patients expressed their appreciation of this treatment modality in which no pain, local anesthetic or medication is needed during treatment or at any time thereafter.

Preliminary results suggests that the low light dose (40-60 J/cm^2) might not be sufficient to induce therapeutic effects. However, higher energy levels (>80 J/cm^2) appear to cure these patients. Completion of the current protocol requires one year of close follow up of all these patients.

Table 1. Progress report of phase I of PDT for CIN (as of March 1994):

	Patient Initial	Date of Treat.	Pre Op. Diag.	Treatment Laser Energy	3. Months Follow up: 1. PAP. smear 2. Biopsy	6. Months Follow up: 1. PAP. smear 2. Biopsy
1	AK	2/2/93	CIN I	40 J/cm2	1. CIN III 2. CIN III	Out of study: Conventional treat.
2	MC	3/11/93	CIN I	40 J/cm2	1. Normal 2. Normal	1. Normal 2. Normal
3	VW	3/11/93	CIN I-II	40 J/cm2	1. Few dysplastic cells 2. CIN I	1. Few dysplastic cells 2. Normal (+ condyloma)
4	AH	4/5/93	CIN I	40 J/cm2	1. Normal 2. Normal	1. CIN I 2. Normal
5	HT	4/15/93	CIN I	60 J/cm2	1. Normal 2. Normal	
6	TT	4/15/93	CIN I	60 J/cm2	1. Normal 2. Normal (+condyloma)	1. CIN I (+condyloma)
7	CD	6/3/93	CIN III	60 J/cm2	1. Few dysplastic cells 2. Normal	1. CIN II 2. CIN III
8	MS	6/30/93	CIN II	60 J/cm2	1. Atypical cells of Undetermined signif. 2. Normal (+condyloma)	1. CIN I 2. Normal
9	BF	7/8/93	CIN III	80 J/cm2	1. Normal 2. No need	1. Normal 2. Normal
10	NY	8/5/93	CIN II	80 J/cm2	1. Normal 2. CIN I (+ condyloma)	1. Normal 2. Normal
11	CH	9/21/93	CIN I	80 J/cm2		1. Normal 2. MILD focal dysplasia
12	SG	9/24/93	CIN II	80 J/cm2	1. Normal 2. Normal	
13	TN	9/24/93	CIN I	100 J/cm2	1. CIN I (+condyloma) 2. Normal	
14	DF	11/23/93	CIN I	100 J/cm2	2. Normal	
15	CS	12/3/93	CIN I-II	100 J/cm2		
16	JB	12/3/93	CIN I-II	100 J/cm2		
17	KR	12/3/93	CIN I	120 J/cm2		
18	AM	12/17/93	CIN I-II	120 J/cm2		
19	SM	1/6/94	CIN I	120 J/cm2		
20	BW	1/13/94	CIN I	120 J/cm2		
21	DG	1/27/94	CIN III	140 J/cm2		
22	WW	2/10/94	CIN I	140 J/cm2		
23	CH	2/17/94	CIN III	140 J/cm2		

Publications resulting from DOE grant (8):

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2. Tadir Y., Tromberg B., Krasieva T., Steiner R., Chapman J., Berns M.W. Endometrial photosensitization: experimental models. In: Lasers in Gynecology. Ed. Donnez J. (in press).

3. Tadir Y., Tromberg B., Krasieva T., Berns M.W. Photodynamic therapy towards selective endometrial ablation. Proceedings S.P.I.E. 1879; 247-52, 1993.

4. Steiner R., Tromberg B., Weiss P., Krasieva T., Berns M.W., McCullough J., Tadir Y. Photodynamic destruction of rat endometrium using topically-administered Photofrin. Human Reproduction. (submitted)

5. Wyss P., Svaasand L., Tadir Y., Tromberg B.J., Berns M.W. Photomedicine of the endometrium: experimental concepts. Human Reproduction, (submitted)

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7. Wyss P., Tromberg B.J., Wyss M.T., Krasieva T., Liaw L., Schell M., Berns M.W., Tadir Y. Photodynamic destruction of endometrial tissue using topical 5-aminolevulinic acid (5-ALA) in rats and rabbits. Am. J. Obstet. Gynecol. (submitted)

8. Steiner R.A., Tadir Y., Tromberg B.J., Krasieva T., Ghazains T., Wyss P., Berns M.W. Photosensitization of the rat endometrium following 5-aminolevulinic acid (ALA) induced photodynamic therapy.

2. Non-Invasive Optical Fiber Diagnostic System Utilizing Photon Migration
Co-PI's: Bruce J. Tromberg, Assistant Professor of Surgery and Biophysics/Physiology, UCI; Richard Haskell, Associate Professor of Physics, Harvey Mudd College, Claremont, CA; Lars O. Svaasand, Professor of Physical Electronics, Norwegian Institute of Technology, University of Trondheim, Norway

Progress

Our primary goal during the past three years has been to characterize the behavior of photon density waves in multiple scattering media using Frequency Domain Photon Migration (FDPM). The following list of accomplishments and publications summarizes our activities:

Specific Accomplishments

1) ***Develop and test analytical models for photon density wave behavior in infinite media and at an air/tissue boundary.*** We have conducted several studies which validate the accuracy of our analytical model for photon density wave behavior in infinite and semi-infinite media. Particular attention has been paid to partial internal reflection at the boundary (modified partial current model).

2) ***Develop reliable mathematical fitting routines for optical property calculations.*** We have developed algorithms for rapid fitting of experimental data which can be used on PC's and MAC's. This code is now running for infinite, semi-infinite, and slab geometries.

3) ***Use FDPM to measure optical properties of tissues and tissue phantoms.*** Several studies have been completed or are in progress which characterize the bulk optical properties of brain, sclera, uterus, and breast. Various structures in heterogeneous tissues have been characterized (e.g., white matter in brain, fibroid tumors in uterus) and optical properties correlated to conventional histology. In addition, FDPM measurements of model tumors have been recorded during photodynamic therapy.

4) ***Acquire quantitative absorption spectra in multiple scattering media.*** A multifrequency, multi-wavelength study of a phthalocyanine absorber in Intralipid has been completed.

5) ***Resolve fluorescence lifetimes from multiple scattering for fluorophores that are either homogeneously dispersed or spatially localized in scattering medium.*** Use this to discriminate between multiple fluorophores, evaluate microenvironments, and find buried objects in turbid media. Preliminary results indicate excellent agreement between data and fluorescence theory. Fluorescence lifetimes can be extracted from FDPM measurements in turbid media. A comprehensive study is now in progress.

6) ***Develop a low-volume, standard-sized cell with defined boundary conditions for evaluating optical properties of solutions, small tissue biopsies, and cell suspensions.*** We have completed preliminary studies characterizing the relationship between finite and infinite media measurements in low volume (10-100 mL) vessels. We are in the process of adapting boundary theory (using method of images) to derive analytical model which can be used to determine optical properties.

7) ***Conduct optical tomography experiments with buried absorber and attempt 3-d reconstructions.*** Preliminary results completed with buried absorber in breast tissue phantom using 50 frequencies (5-250 MHz) and over 276 independent views. Object was reconstructed using back-projection techniques.

8) ***Determine optimal method of modulating laser diode source(s) and coupling to avalanche photodiode detectors.*** We are currently testing the response of a prototype 800 nm laser diode-avalanche photodiode system which has a 1 GHz bandwidth.

9) ***Use diode sources and detectors to construct portable instrument for clinical monitoring.*** A prototype instrument is currently under design and construction.

Publications resulting from DOE grant (25):

1. Tromberg B.J., Svaasand L.O., Tsay T-T., Haskell R.C., Berns M.W., "Optical Property Measurements in Turbid Media Using Frequency Domain Photon Migration", Proceedings of the SPIE, 1525, Berlin, 52-58 (1991).

2. Svaasand L.O., Tromberg B.J., "On the Properties of Optical Waves in Turbid Media", Proceedings of the SPIE, 1525, Berlin, 41-51 (1991).

3. Tromberg B.J., Svaasand L.O., Tsay T-T., Haskell R.C., Berns M.W., "Optical Property Measurements in the Multiple-scattering Regime Using Frequency-domain Photon Migration", Pacific Conference on Chemistry and Spectroscopy, Anaheim, October 7, 1991.

4. Tsay T-T., Tromberg B.J., Cho E., Vu K., Svaasand L.O., "Monitoring Photochemistry in Tumors Using Frequency Domain Photon Migration", Proceedings of the SPIE, Los Angeles, 213-218 (1991).

5. Svaasand L.O., Tromberg B.J., Tsay T-T.. Haskell R.C.,. Berns M.W., "On the Properties of Photon Density Waves in Tissues", Advances in Biological Heat and Mass Transfer, ASME 1991, HTD-Vol. 189/BED-Vol. 18, 85-89, (1992).

6. Svaasand L.O., Tromberg B.J., Haskell R., Tsay T-T., Berns M.W. "Propagation of Photon Density Waves in Tissues", Lasers in Surgery and Medicine S4, 5, (1992).

7. Tromberg B.J., Svaasand L.O., Tsay T-T., Cho E., Vu K. "The Influence of Tissue Structure on the Phase Velocity of Photon Density Waves", Lasers in Surgery and Medicine S4, 5-6, (1992).

8. Tsay T-T., McAdams M.S., Tromberg B.J., Svaasand L.O., Haskell R.C., Ahdoot J. "Determination of Tissue Optical Properties Using Frequency Domain Photon Migration", Lasers in Surgery and Medicine S4, 6, (1992).

9. Tromberg B.J., Krasieva T., Shimizu S., Satorius A., and Forssen E.A., "Non-invasive laser-induced fluorescence imaging of daunorubicin in solid tumors in situ: comparison of conventional drug with tumor-targeting small unilamellar vesicles", Proc. Am. Assoc. Can. Res. 33, 2665 (1992).

10. Tromberg B.J., Svaasand L.O., Tsay T-T., Haskell R.C., "Properties of Photon Density Waves in Multiple-Scattering Media", Applied Optics, 32, 607-616 (1993).

11. Svaasand L.O., Tromberg B.J., Haskell R.C., Tsay T-T.,. Berns M.W., "Tissue Characterization and Imaging Using Photon Density Waves", Optical Engineering, 32, 258-266 (1993).

12. Svaasand L.O., Haskell R.C., Tromberg B.J., McAdams M. "Properties of Photon Density Waves at Boundaries", Proceedings of the International Society for Optical Engineering, 1888, B. Chance and R. Alfano editors, 214-226, (1993).

13. Tromberg B.J., Tsay T-T., Pham V., Svaasand L.O., "Frequency-Domain Spectroscopy in Turbid Media: Wavelength-Dependent Optical Properties", Lasers in Surgery and Medicine S5, 5, (1993).

14. Tromberg B.J., Svaasand L.O., Tsay T-T., Pham V., Haskell R.C., "Frequency-Domain Optical Spectroscopy in Turbid Media", Pacific Conference on Chemistry and Spectroscopy, Pasadena, October 21, 1993.

15. Madsen S.J., Tromberg B.J., Svaasand L.O., Haskell R.C. "Steady-state Versus Frequency-Domain Techniques for the Determination of Tissue Optical Properties", Optical Society of America Annual Meeting, October 5, 1993.

16. Tromberg B.J. "Non-Invasive Imaging of Tissue Optical Properties", Proceedings of the Chinese-American Workshop on Non-Invasive Medical Diagnostics, P.A. Lewin, editor, National Science Foundation, Wash., D.C. (1993).

17. Tromberg B.J., Madsen S.J., Wyss P., Svaasand L.O., Haskell R.C., Tadir Y. "Tissue characterization using frequency-domain photon migration", International Society for Photo-Optical Instrumentation Engineers (SPIE), Los Angeles, January 26, 1994.

18. Koenig K., Schneckenburger H., Walt H., Leeman T., Wyss-Desserich M. T., Ruck A., and Tromberg B.J. "Optical Methods for Tumor Treatment and Detection", Proceedings of the SPIE, Los Angeles, 2133, (in press).

19. Koenig K., Schneckenburger H., Hemmer J., Tromberg B.J. and Steiner R. *Advances in Laser and Light Spectroscopy to Diagnose Cancer and Other Diseases*, Proceedings of the SPIE, Los Angeles, 2135 (in press).

20. Peavy G.M., Krasieva T.B., Tromberg B.J., and Berns M.W., "Treatment Considerations for Photodynamic Therapy in the Cat", Proceedings of the SPIE, Los Angeles, 2128, (in press).

21, Madsen S.J., Tromberg B.J., Wyss P., Svaasand L.O., Haskell R.C. and Tadir Y., "The Optical Properties of Human Uterus at 630 nm", Proceedings of the Optical Society of America: Optical Imaging and Photon Migration", Orlando, March (1994).

22. Tromberg B.J., Svaasand L.O., Madsen S.J., Haskell R.C., Chapman C. "Frequency-Domain Photon Migration Spectroscopy In Turbid Media", Proceedings of the Optical Society of America: Optical Imaging and Photon Migration", Orlando, March (1994).

23. Haskell R.C., Svaasand L.O., Tsay T.-T., Feng T.- C., McAdams M.S., and Tromberg B.J. "Boundary Conditions for the Diffusion Equation in Radiative Transfer", JOSA-A, submitted.

24. Madsen S.J., Wyss P., Svaasand L.O., Haskell R.C., Tadir Y., Tromberg B.J. "Determination of the Optical Properties of Human Uterus Using Frequency-Domain Photon Migration and Steady-State Techniques", Physics in Medicine and Biology, submitted.

25. Wyss P., Svaasand L.O., Tadir Y., Berns M.W., and Tromberg B.J. "Photomedicine of the Endometrium: Experimental Concepts." Human Reproduction, submitted

IVb. PULMONARY/THORACIC SURGERY

1. Thoracoscopic Laser Ablation of Emphysematous Pulmonary Bullae Using Laser Photo-Absorbing Dyes

Co-PI: Matthew Brenner, M.D., Assistant Professor of Medicine, UCI

Progress

Support from the Department of Energy Grant (DE-FG03-91ER61227) has enabled the Pulmonary Section to be extremely productive. This project has become the focus of Dr. Brenner's research. We have made a number of findings that will likely have considerable impact on the treatment of patients with bullous emphysema.

With funding from the DOE grant we have accomplished the following over the past 3 years:

1. There has been outstanding collaboration between basic laser research groups at the Beckman Laser Institute and Medical Clinic (BLIMC), and clinical biomedical researchers from University of California Irvine Medical Center (UCIMC) with direct application to the treatment of human diseases.

2. With regard to the animal projects:

   a. Nd:YAG laser pulmonary exposures were performed using continuous and pulsed modes in open thoracotomy experiments. There was excellent animal survival using a number of innovative techniques.

   b. Laser-induced parenchyma lung injury was seen and defined histopathologically. The diffuse nature and temporal evolution of injury were described.

   c. A series of experiments similar to the Nd:YAG studies was performed using CO_2 lasers. Differences between acute and long term effects of the CO_2 and Nd:YAG laser exposures were shown.

   d. Pulsed modes were found to cause identical injury to continuous mode laser settings in the ranges examined.

   e. Rapid infrared thermal imaging was performed and demonstrated rapid heating with long thermal relaxation times, explaining why pulsed modes produced similar injuries to continous mode injuries.

   f. Mediator studies showed considerable generalized mediator release following exposure to both CO_2 and Nd:YAG laser.

   g. Small animal thoracoscopy techniques were developed in the animal model. These will be of great value in current and future studies.

h. We developed and refined methods to reliably produce bullous emphysema in rabbits.

i. Methods were developed for thoracoscopic laser treatment of bullae in the rabbit model.

After analyzing the results of the above studies, we decided a radical new approach would be necessary in order to dramatically reduce the lung injury process. For this reason, we began a series of preliminary investigations to determine the feasibility of using photo-absorbing dyes thoracoscopically applied directly to the surface of pulmonary bullae to enhance effectiveness of laser treatment in the rabbit model. Our preliminary results suggest that this approach may significantly improve the selectivity of laser bullae ablation and change the approach to laser treatment of bullous emphysema in the future.

Studies performed over the past 3 years at BLIMC have already had a direct effect on the treatment of patients with bullous emphysema. More than 600 patients have now been treated with this procedure using thoracoscopic laser techniques originally developed at BLIMC. Lung injury data from the CO^2 laser studies have led to the use of Nd:YAG lasers for this procedure clinically, with some reduction in resultant injury. However, considerable morbidity and mortality remain, and indications for thoracoscopic laser ablation of emphysematous pulmonary bullae in humans remains controversial. Improvements in technique are needed. We have designed a series of studies to be conducted over the next three years that may alter the approach to this procedure.

Publications/Presentations resulting from DOE grant (14):

1. Boyajian J.G., Brenner M., Espinoza F.P., Milne E.C., Roeck W.W., Brown L., Chung E., Chen C., Wilson A.F., Kono T., Wakabayashi A., Berns M.W. Defocused carbon dioxide laser injury of the lung: radiographic and histopathologic findings in a rabbit model. Presented American Thoracic Society National Meeting, 1992.

2. Brenner M., Chung E., Boyajian J.G., Espinoza F.P., Brown L., Chung E., Chen C., Wilson A.F., Kono T., Wakabayashi A., Berns M.W. Interleukin 1B release during carbon dioxide laser injury of the lung in a rabbit model. Presented American Thoracic Society National Meeting, 1992.

3. Chung E., Brenner M., Boyajian J.G., Espinoza F.P., Brown L., Chung E., Chen C., Wilson A.F., Kono T., Wakabayashi A., Berns M.W. Systemic interleukin-1 beta in an acute CO^2 laser lung injury rabbit model. Presented at Southern California Pulmonary Research Conference., 1992.

4. Boyajian J.G., Brenner M., Espinoza F.P., Milne E.C., Roeck W.W., Brown L., Chung E., Chen C., Wilson A.F., Kono T., Wakabayashi A., Berns M.W. Defocused carbon dioxide laser injury of the lung: radiographic and histopathologic findings in a rabbit model. Presented at Southern California Pulmonary Research Conference., 1992.

5. Brenner M., Wakabayashi A., Kayaleh R.A., Milne E.N., Della Bella L., Osann K., Tadir Y., Berns M.W., Wilson A.F. Thoracoscopic laser ablation of emphysematous

pulmonary bullae: Radiographic selection and treatment response. Presented Western Section AFCR 2/93.

6. Chung E., Brenner M., Schenkel T., Wang N., Boyajian J., Espinosa L., Wilson A.F., Osann K., Wakabayashi A., Tadir Y., Berns M.W. Effect of laser energy on Lung Injury in a Rabbit Model. Presented at Southern California Pulmonary Research Conference. 1993

7. Brenner M., Wakabayashi A., Kayaleh R.A., Milne E.N., Della Bella L., Osann K., Tadir Y., Berns M.W., Wilson A.F. Laser ablation of pulmonary bullae: selection and treatment. AFCR National Meetings 1993. Clin Res 41(2):176A.

8. Brenner M., Chung E., Schenkel T., Wang N., Boyajian J., Espinosa L., Wilson A.F., Osann K., Wakabayashi A., Tadir Y., Berns M.W. Comparison of CO_2 and Nd:YAG Laser Lung Injury in a Rabbit Model. AFCR National Meetings 1993. Clin Res 41(2):175A

9. Brenner M., Waite T., Chung E., Schenkel T., Wang N., Boyajian J., Wilson L., Osann L., Wakabayashi A., Tadir Y., Berns M.W. A thoracoscopic small animal pulmonary laser exposure model. Accepted for presentation: Society for Optical and Quantum Electronics, 1993.

10. Shankel T., Brenner M., Waite T., Hamilton A., Wilson A.F., Tadir Y., and Berns M.W. An animal model for thoracoscopic laser ablation of emphysematous pulmonary bullae. Clinical Research 42(1):59A,1994.

11. Shankel T., Brenner M., Waite T., Hamilton A., Wilson A.F., Tadir Y., and Berns M.W. An animal model for thoracoscopic laser ablation of emphysematous pulmonary bullae. Submitted AFCR National Meetings Spring 1994.

12. Waite T., Brenner M., Shankel T., Hamilton A., Wilson A.F., Tadir Y., and Berns M.W. Thoracoscopic laser ablation of emphysematous pulmonary bullae in rabbits. Submitted Thoracoscopy and Panendoscopy International Meetings, Munich, GD 1994.

13. Brenner M., M.D., Milne E.N., Kayaleh R.A., Della Bella L., Osann K., Wilson A.F., Berns M.W. Thoracoscopic Laser Ablation of Pulmonary Bullae: Radiographic Selection and Physiologic Mechanisms of Treatment Response. In Press J Thoracic Cardiovasc Surg 7/93.

14. Brenner M., Shankel T., Waite T., et al. An animal model for thoracoscopic laser ablation of emphysematous pulmonary bullae. Submitted. 1994.

Invited Lectures

11/3/91 Advances in Pulmonary Medicine: Thoracoscopic Laser Treatment of Emphysematous Bullae. American College of Chest Physicians National Conference. Hyatt Hotel Conference Room, San Francisco, CA.

11/5/92 Thoracoscopic treatment of emphysematous pulmonary bullae. Long Beach Memorial Hospital Laser Thoracoscopy Course for Pulmonologists.

11/15/92 Thoracoscopy for bullous lung disease. Hoag Hospital Pulmonary Grand Rounds. Hoag Hospital Conference Center, Newport Beach, CA.

2/18/93 Palm Springs 1993: Southern California Pulmonary Research Conference. Limitations of Thoracoscopic Laser Ablation of Emphysematous Pulmonary Bullae. Palm Springs, CA.

Peer-Reviewed Position Paper

1. Brenner M. ALAC/ATS American Lung Association of California/American Thoracic Society of California Position Statement: Thoracoscopic Laser Ablation of Pulmonary Bullae. 1993.

Journal Articles Invited

1. Brenner M., Wakabayashi A. Thoracoscopic laser treatment of emphysematous bullae. Everyday Problems in Medicine 2(7). 1993.

2. Brenner M., Wakabayashi A. Thoracoscopic treatment of bullous emphysema. Byline, American Lung Association of Arizona. 1992.

IVd. DERMATOLOGY/PLASTIC SURGERY

1. Photochemotherapy of Psoriasis and Other Skin Diseases
Co-PI's: J. Stuart Nelson, M.D., Ph.D., Associate Professor of Dermatology and Surgery, UCI; Gerald D. Weinstein, M.D., Professor of Dermatology, UCI; Jerry L. McCullough, Ph.D., Professor of Dermatology, UCI

Progress

The objective of this dose-ranging pilot study was to obtain data relative to the clinical efficacy and safety of photodynamic therapy (PDT) of psoriasis using low dose systemic PHOTOFRIN™ II with various light sources. The variables included dose of Photofrin II, various light sources (UVA and visible), and time between drug administration and light treatment.

Patients received a single I.V. dose (0.5 - 1.0 mg/kg) Photofrin II. 48 hrs later, different sites of lesional and normal skin were treated with various dose/schedules of visible light: a single treatment with argon tuneable dye (ARD) laser 630 nm; or krypton (KRP) laser 405 nm; UVA: 9-20 treatments over 3-4 weeks. Treatment sites were evaluated weekly for 7 wks for lesion severity and normal skin photosensitivity.

Thirteen patients have thus far completed the study. The results to date have shown that the clinical responses were dose related. At a drug dose of 0.5 mg/kg, the low starting doses for

KRP (0.5 J/cm2 (J)); ARD (5 J); cumulative UVA (75 J) were clinically ineffective. Light doses were sequentially escalated in succeeding patients to maximize clinical response. 85-100% clearing was obtained in 2 out of 8 patients 3 weeks after a single treatment with 15 or 20 J ARD. The average maximum response obtained with KRP (1-30 J) was 25%; with UVA (cumulative 97-394 J) 20%. Escalating drug dose to 1.0 mg/kg in Patient 015 and treatment with 30-75 J/cm2 argon dye laser resulted in complete clearing of lesions that remained clear for 2 months. Maximum therapeutic effects with laser were obtained with light treatment 48 hr post Photofrin. At 24-48 hours post-Photofrin, selective fluorescence could be detected in psoriatic vs normal skin. There was only transient mild-moderate erythema in psoriatic and normal skin 24 hour post treatment. With patients using reasonable protection, there were no adverse experiences due to the prolonged cutaneous photosensitivity.

These preliminary results suggest that PDT with Photofrin and visible red light may be useful for the treatment of psoriasis. The most effective treatment obtained has been with 1 mg/kg Photofrin and red light (30-75 J/cm2) administered 48 hr post drug.

There are two practical limitations of this therapy for the treatment of psoriasis. One is the limited area of skin that can be treated using laser. Future studies will be directed at investigating non-laser visible light sources for PDT of more extensive disease. The other disadvantage is that patients must take precautions for several weeks post treatment to limit exposure to sunlight and high-intensity artificial light. The use of other photosensitizers, (i.e., benzoporphyrin derivative (BPD)), that have limited photosensitivy will be more useful for PDT. In addition, the prolonged photosensitivity that occurs with Photofrin II limits the repeated dosing for additional PDT treatments. Since psoriasis may respond to more frequent treatments with low dose light, the use of a photosensitizer which does not cause prolonged photosensitization of the normal skin would be desirable.

Publications resulting from DOE grant (2):

1. Weinstein G.D., McCullough J.L., Nelson J.S., Berns M.W., McCormick A.: Low Dose Photofrin II Photodynamic Therapy of Psoriasis, Journal of Investigative Dermatology 96:573, 1991.

2. Weinstein G.D., McCullough J.L., Nelson J.S., Berns M.W., McCormick A.: Low Dose Photofrin II Photodynamic Therapy of Psoriasis, Proceedings of the 5th International Psoriasis Symposium (1991).

2. Remote Infrared Sensing as a Method to Monitor and Improve Treatment of Vascular and Pigmented Lesions

Co-PI's: J. Stuart Nelson, M.D., Ph.D., Associate Professor of Dermatology and Surgery, UCI; Thomas E. Milner, Ph.D., Assistant Professor of Surgery (Pending), UCI

Progress

I. Summary of Work Completed:

I.A *In-vitro* PWS model: We have developed an *in-vitro* model using collagen films consisting of variable amounts of absorber to simulate the multi-layered composite port wine

stain (PWS) skin (Figure 1). This PWS model makes use of thin (125-250 μm) type I collagen films and an organic optical absorbing dye. The collagen films (Collatec, Plainview, NJ) are prepared to various thicknesses and optical absorption to simulate various PWS. Each PWS tissue model is prepared by staining the collagen films with Brilliant Blue® [triphenylmethane dye (Aldrich Chemical Co., Milwaukee WI)] which absorbs optimally in water at a wavelength of 585 nm. The variation of the dye absorbance with concentration is calibrated spectrophotometrically. When collagen films are placed in a dye solution with a known concentration and volume, the quantity of blue dye that binds to the collagen fibers is computed by measuring the optical absorption of the free dye remaining in solution. With this technique, collagen layers corresponding to epidermis, dermis and PWS are prepared.

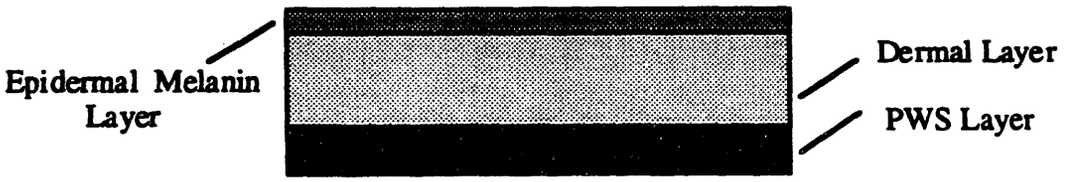

Figure 1: Illustration of an *in-vitro* collagen film model PWS.

The pulsed photothermal radiometry (PPTR) signals from various *in-vitro* PWS models have been measured and characterized in our laboratory. The effect of epidermal absorption was investigated by increasing the optical absorption of the epidermal collagen layer while keeping the depth and concentration of the PWS layer constant. The PPTR signals corresponding to three simulations involving epidermal layers with different dye concentrations are shown in figure 2.

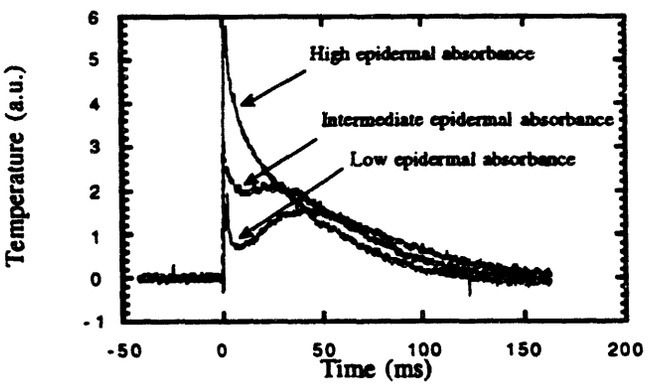

Figure 2: Effect of different epidermal absorbance on PPTR signal

I.B Clinical PPTR instrument: For clinical PWS measurements our laboratory-based PPTR system will require modification. Site-to-site calibration of the tissue-detector geometry is

difficult to control when using a liquid-N_2-cooled detector. A thermo-electric cooled detector is a possible solution to this problem but substantially adds to the total system cost. Because of its flexibility and maneuverability an infrared collection fiber has greater skin site-to-site accessibility. Additionally, an infrared fiber has engineering, economic and clinical advantages over a lens collecting system. Despite these benefits, an alternate signal processing scheme is needed to compensate for the substantial loss in the signal-to-noise ratio.

Modulation of the infrared signal combined with homodyne detection offers a solution to the signal detection problem. We have constructed a hand piece which houses infrared and visible collection fibers (Figure 3). Diffusely reflected laser light scattered from the skin is collected by a GRIN® lens, coupled to a visible fiber and detected with a silicon photo diode. The PPTR signal is collected by the infrared fiber (Ag-Halide), modulated with a mechanical chopper, and focused into a liquid-N_2-cooled HgCdTe detector. The signal is demodulated with a lock-in amplifier, digitized with an A/D converter and stored in a computer for further analysis.

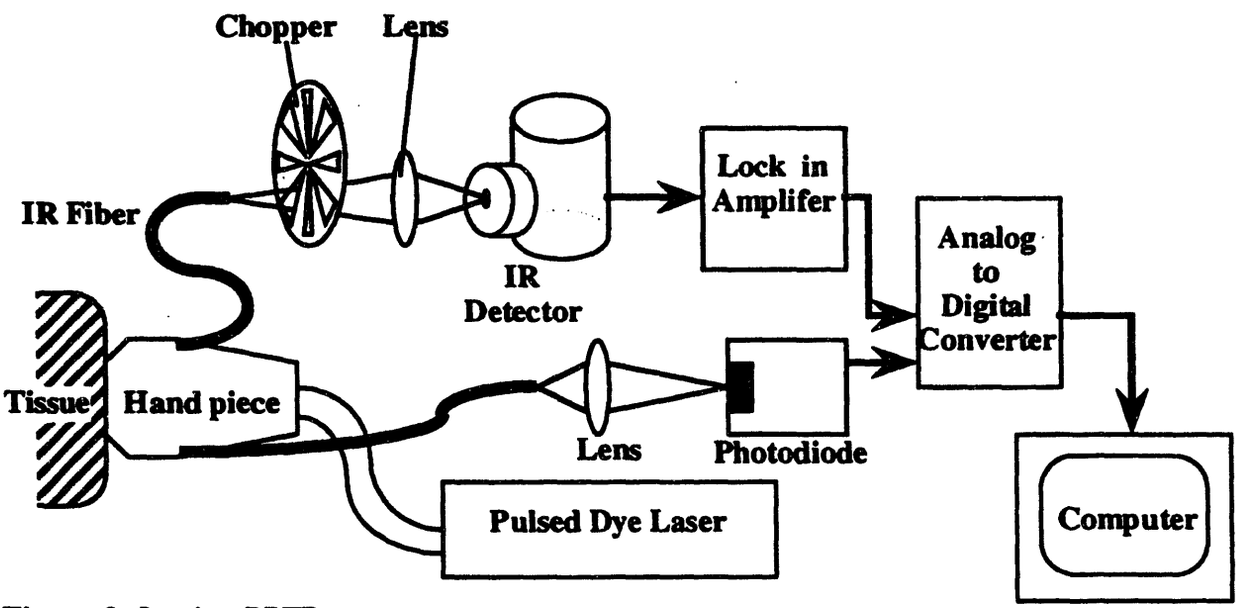

Figure 3: In-vivo PPTR system.

We have carried out initial clinical tests of the fiber-based PPTR instrument diagrammed in Figure 3. With a sub-therapeutic light dose of 4 (Jcm^{-2}) from a flash lamp pumped pulsed dye laser (λ_p=585 nm, t_p=0.45 ms), the PPTR signals from a patient's normal and PWS skin were measured. The PPTR signal exiting an infrared fiber was modulated at 3 kHz with a mechanical chopper and the time constant of the lock-in amplifier was set at 10 ms. The PPTR signals corresponding to normal and PWS skin are illustrated in Figure 4a and 4b, respectively.

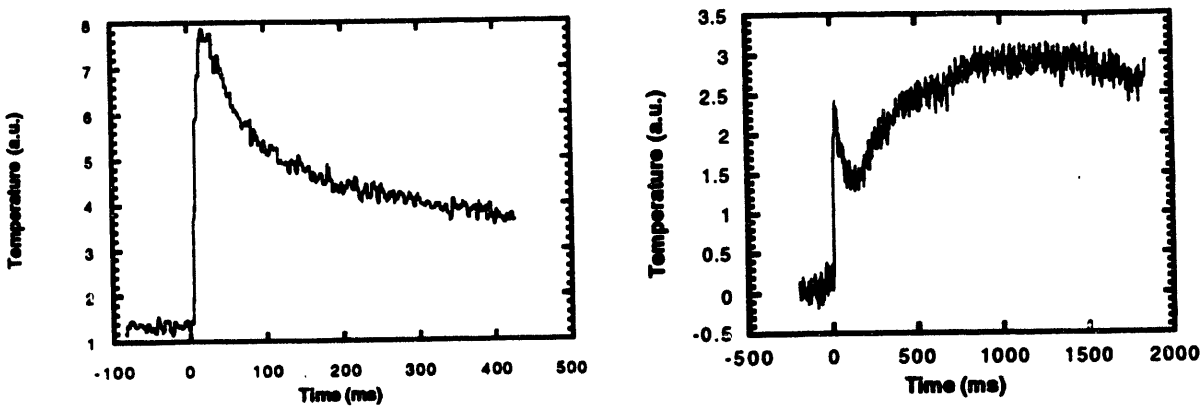

Figure 4 Patient PPTR measurements of (a) normal and (b) PWS skin.

The PPTR signal from normal skin shows an initial epidermal "T-jump" followed by characteristic monotonic cooling. The PPTR response of PWS tissue shows a relatively smaller epidermal "T-jump" with a large amplitude delayed thermal wave which peaks at ~1 s corresponding to a PWS depth of ~750 μm. The smaller initial epidermal "T-jump" in PWS skin is partially due to the decrease in the back scattered component resulting from PWS absorbance.

Ic. PPTR Inversion Problem: In practice, use of PPTR requires estimation of the initial temperature distribution ($\Delta T(\zeta,0)$) from a measured PPTR signal (S(t)). Knowledge of the initial temperature distribution reveals the depth and concentration of subsurface chromophores. The measured PPTR signal is related to the initial temperature distribution (Eq. 1) through a Fredholm integral equation of the first kind.

$$S(t) = (C_d\mu/2)\int_{\zeta=0}^{\zeta=\infty} \Delta T(\zeta,0)e^{-\zeta^2/4\pi}[\mathrm{erfcx}(u_-) + \mathrm{erfcx}(u_+)]d\zeta \qquad (1)$$

Determination of the initial temperature distribution ($\Delta T(\zeta,0)$) from the measured PPTR signal (S(t)) requires solution of the inverse problem defined in Eq. 1. Numerical computation of the singular values for the integral equation reveals the *severely* ill-posed nature of the PPTR inversion problem. Ill-posed inverse problems are difficult to solve because solution estimates are often susceptible to signal noise (e.g., PPTR signal noise).

In collaboration with Dennis M. Goodman, Ph.D., at Lawrence Livermore National Laboratory, we have developed a constrained conjugate gradient (CCG) numerical algorithm that solves the PPTR inversion problem. By requiring estimates of the initial temperature distribution to be non-negative, solutions are found by minimizing a regularized norm (Eq. 2).

$$f(\Delta T_j,\lambda) = \min\left\{\left\|K_{i,j}\Delta T_j - S_i\right\|^2 + \lambda\left\|\Delta T_j\right\|^2\right\} \qquad (2)$$

The regularization parameter (λ) determines the degree of smoothening of a solution estimate ($\Delta T(\zeta,t=0)$); large (small) regularization parameters increase (decrease) the degree of smoothening.

Using the non-negative CCG algorithm, solution estimates for the initial temperature distribution have been computed for a number of cases that include (1) simulated PPTR signals; (2) a subsurface absorbing collagen film in a PWS tissue phantom; and (3) blood vessels in PWS skin. We illustrate one such solution estimate (Figure 5b) for the initial temperature distribution ($\Delta T(\zeta,t=0)$) corresponding to a light subsurface absorbing collagen film positioned 225 μm below the surface.

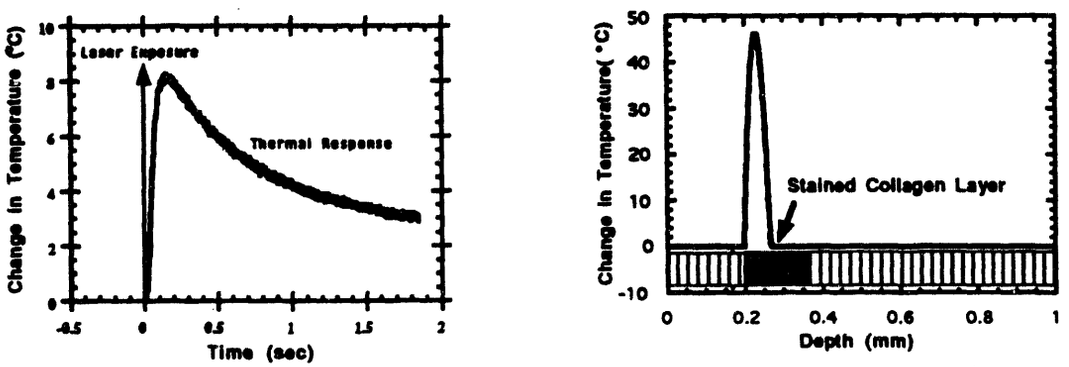

Figure 5: (A) PPTR signal for buried absorbing collagen film; (B) computed initial temperature distribution

II. Planned Studies:

II.A Pulsed Photothermal Tomography: Pulsed photothermal tomography (PPT) uses a fast infrared camera to detect temperature rises in a substrate, induced by pulsed radiation. In practice, PPTR and PTT are similar in that both record the time evolution of infrared emission from a test object in response to pulsed laser radiation. The two methodologies differ in that PTT makes use of a fast infrared camera to record infrared emission *images* of the irradiated test site. A potentially useful application of PPT, is tomographic imaging of individual blood vessels that comprise PWS birthmarks. Following pulsed laser irradiation of PWS skin, an increase in infrared emission will occur due to light absorption by hemoglobin contained within the blood vessels. The spatial distribution of the initial infrared emission, shortly after the laser pulse is delivered, can provide a measure of the PWS blood vessel diameters (Figure 6). Analysis of the time evolution of the surface temperature provides a means to determine the depth of laser-heated PWS blood vessels.

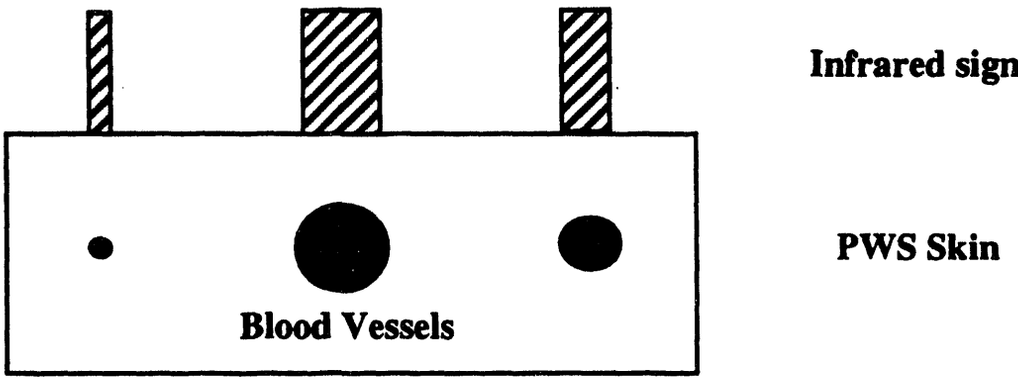

Figure 6: Initial infrared emission measurement of PWS skin.

The long term objective of our proposed research is to apply the principles of pulsed photothermal tomography (PPT) for improved diagnostics and treatments in laser surgical procedures that require photothermal effects. Because most laser surgical procedures in practical use today require a photothermal mechanism, we believe our research plan can realize substantial improvement in existing treatment methodologies. We propose basic research on two levels to realize our goals: (1) development of a tomographic computer algorithm that will reduce a time series of infrared emission images to a spatial temperature distribution arising from absorption of laser light; (2) experimental investigation of in-vitro model phantoms that simulate the optical and thermal properties of human skin. Although our studies will be directed toward improvement of PWS laser diagnostics and treatments, we believe that the techniques described herein can be successfully applied to a diverse number of other laser surgical procedures.

II.A.1 Tomographic Algorithm Development: Results obtained for inversion of the one dimensional PPTR problem (Eq. 1) using the non-negative CCG algorithm provide a sound basis for application to the PTT inversion problem. The goal of our research is to determine the initial three-dimensional temperature distribution in skin, $T(\xi,\eta,\zeta,t=0)$, immediately following exposure to a sub-therapeutic laser pulse, given only the recorded time sequence of PPT infrared emission frames. Knowledge of the initial temperature distribution in the PWS, will allow determination of blood vessel sizes and depths and result in improved clinical laser dosimetry. An infrared emission frame, R(x,y,t), measured in units of temperature and recorded at time t is related to the initial three-dimensional temperature distribution in the PWS by a convolution integral with the PPT point spread function, $g(x-\xi,y-\eta,-\zeta,t)$,

$$R(x,y,t) = \iiint T(\xi,\eta,\zeta,t=0)\cdot g(x-\xi,y-\eta,-\zeta,t)\, d\xi\, d\eta\, d\zeta \qquad (3)$$

The PPT point spread function, g(x,y,z,t), is defined (Eq. 2),

$$g(r,t) = \frac{\mu}{(2\sigma_s^2 + 4\chi t)2\pi} e^{-\frac{r^2}{4\chi t+2\sigma_s^2}} e^{-\frac{\zeta_o^2}{4\chi t}} \left(e^{u_+^2}\mathrm{erfc}(u_+) + e^{u_-^2}\mathrm{erfc}(u_-)\right) \qquad (4)$$

where,

$$u_\pm = \mu\sqrt{\chi t} \pm \frac{\zeta_o}{2\sqrt{\chi t}} \qquad (5)$$

χ is the thermal diffusivity, and erfc() is the complementary error function. Determination of the PPT point spread function allows solution of the forward problem by computation of the convolution integral (Eq. 3) relating the measured infrared emission frames, R(x,y,t), to the initial temperature distribution, $T(\xi,\eta,\zeta,t=0)$, immediately following laser exposure. Alternatively, Eq. (3) and (4) define an inverse problem: given a time series of measured infrared emission frames, R(x,y,t), determine the initial temperature distribution, $T(\xi,\eta,\zeta,t=0)$,in the skin.

Examination of Eqs. 3 and 4 reveals that the three-dimensional inverse problem consists of two separable problems representative of heat diffusion in two lower dimensional orthogonal subspaces. The PPTR inverse problem (Eq. 1) corresponds to one-dimensional diffusion of heat perpendicular to the skin surface and can be adequately solved using the non-negative CCG algorithm. Computation of the singular values for the lateral inverse problem indicate it is *less* ill-posed than the one-dimensional PPTR problem and, therefore, can be solved with non-negative CCG algorithm with appropriate regularization. Solution of the two-dimensional lateral diffusion inverse problem will allow reconstruction of the initial temperature distribution, $T(\xi,\eta,\zeta,t=0)$, that defines the spatial location and initial temperature of subsurface laser-heated PWS blood vessels.

We propose a series of numerical experiments that will allow iterative development, testing, and optimization of the three-dimensional tomographic reconstruction algorithm. First, we will determine the optimum degree of regularization (λ) for the lateral inverse problem. Because the lateral problem becomes more ill-posed at later times, the optimum degree of regularization is dependent upon time (i.e., $\lambda_o = \lambda(t)$). We propose to use an L-Curve analysis (Hansen, 1994) to determine the optimum degree of lateral regularization corresponding to time (t_i) at which the i-th frame is recorded. Knowledge of the regularization parameters for both the lateral and PPTR inverse problems, will allow computation of an initial temperature distribution solution estimate, $T_s(\xi,\eta,\zeta,t=0)$, from a given sequence of simulated infrared emission frames, $R_s(x,y,t_i)$. Computed solution estimates will be compared with the known initial temperature distribution used to generate the simulated infrared emission frames. Numerical experiments that utilize simulated infrared emission frames allow testing of the three-dimensional tomographic reconstruction algorithm.over a wide parameter space of possible PWS lesions. Once the three-dimensional tomographic reconstruction algorithm is properly regularized and tested, the source code will be optimized and recompiled for fast execution on a risc-based computer system.

II.A.2 Experimental Investigation: We propose a series of experiments to develop and test our understanding of PTT for improved laser dosimetry required for the treatment of PWS. Our goal is to determine the three dimensional spatial temperature distribution ($T(\xi,\eta,\zeta,t=0)$) which the subsurface PWS blood vessels reach immediately after laser irradiation. Measurement of this temperature distribution in response to a diagnostic light dose will allow determination of the appropriate laser dose and pulse width necessary to produce adequate core vessel temperatures and consequential irreversible damage. Experimental studies are proposed at two levels:

(1) in-vitro collagen film model
(2) chicken comb animal model

II.A.2.a In-vitro Collagen Film Model PWS: We propose to test the validity of our three-dimensional reconstruction tomographic algorithm with in-vitro PWS phantoms using collagen strips and films consisting of variable amounts of absorber to simulate multilayered composite PWS skin. Studies will be conducted in two phases:

(1) experimental tests over a wide PWS parameter space
(2) determination of PWS model parameters

Given an in-vitro model PWS system, we will compute the infrared emission intensity in response to pulsed laser irradiation. Model PWS skin phantoms will be selected that simulate different kinds of PWS encountered clinically. In particular, we will prepare each PWS model phantom with known: (1) epidermal absorbance; (2) PWS vessel depth; (3) PWS vessel size; and (4) PWS vessel absorbance.

The tomographic reconstruction algorithm will compute the model parameters of each in-vitro phantom (i.e., epidermal absorbance, PWS vessel depth, and PWS vessel size) from a measurement of infrared emission frames. The computed model parameters of each phantom will be compared with known prepared values. Proceeding in this manner any limitations of the proposed tomographic reconstruction algorithm will be identified.

II.A.2.b Chicken Comb Animal Model Studies: To develop PTT for application in the clinical management of PWS patients, studies must be conducted on animal models. The highly vascularized chicken comb model has been developed and extensively studied in our laboratory because its histoanatomy is analogous to that found in PWS.

On a predefined site representative of the entire comb, a PTT measurement will be made in response to a diagnostic laser dose (D_d) to determine the initial three-dimensional spatial temperature distribution, $T(\xi,\eta,\zeta,t=0)$. The temperature generated in individual comb blood vessels immediately following exposure to a diagnostic laser pulse will be used to compute a therapeutic light dose (D_t) and laser pulse width (t_p) necessary for photothermolysis of the largest fraction of subsurface vessels.

With optimum therapeutic dose (D_t) and pulse width (t_p) determined, immediately adjacent to the test site, each comb will have a 3 x 3 matrix of nine test sites selected for laser treatment over a dose range $D_t \pm 1$ (Jcm^{-2}) and pulse widths $t_p \pm 20\%$. The animals will then be observed for several months to determine the optimum clinical response (i.e. significant blanching). The data will be correlated with histopathological measurements of the size and depth of individual comb blood vessels. Specifically, damage to the overlying epidermis as well as the extent of blood vessel damage will be documented. The experimental procedure will be conducted with a number of combs so that statistically significant conclusions may be drawn. The proposed experiments will: (1) allow practical "hands on" experience with our PTT instrumentation in a preclinical model; and (2) test the ability of the proposed three-dimensional tomographic algorithm to predict the therapeutic light dose, (D_t), and laser pulse width (t_p) necessary to produce blood vessel destruction, as assessed objectively by comb blanching and histopathology.

Publications resulting from DOE grant (9):

1. Nelson J.S., Jacques S.L., Wright W.H. Determination of thermal and physical properties of port wine stain lesions using pulsed photothermal radiometry. SPIE 1643:287-298, 1992.

2. Jacques S.L., Nelson J.S., Wright W.H., Milner T.E. Pulsed photothermal radiometry of port-wine-stain lesions. Appl. Optics. 32:2439-2446, 1993.

3. Milner T.E., Nelson J.S., Tran N., Katzir A., Svaasand L.O., Jacques S.L. Pulsed photothermal radiometry of port wine stains. SPIE 1881:34-42, 1993.

4. Milner T.E., Nelson J.S., Tran N., Katzir A., Svaasand L.O., Jacques S.L. Clinical use of pulsed photothermal radiometry. SPIE 1876:122-128, 1993.

5. Kimel S., Svaasand L.O., Milner T.E., Schell M.J., Hammer-Wilson M., Nelson J.S., Berns M.W. Laser photothermolysis of single blood vessels in the chick chorioallantoic membrane (CAM). SPIE 2077:216-227, 1993.

6. Milner T.E., Norvang L.T., Svaasand L.O., Tran N., Tanenbaum B.S., Nelson J.S. Photothermal tomography of subcutaneous chromophores. SPIE 2077:228-236, 1993.

7. Kimel S., Svaasand L.O., Hammer-Wilson M., Schell M.J., Milner T.E., Nelson J.S., Berns M.W. Differential vascular response to laser photothermolysis. J. Invest. Dermatol. (in press), 1993.

8. Milner T.E., Hall R.L., Svaasand L.O., Jacques S.L., Nelson J.S. Infrared filter for laser therapy of port wine stain. Optics Letters (in submission), 1994.

9. Nelson J.S., Milner T.E., Anvari B., Tanenbaum B.S., Kimel S., Svaasand L.O. Dynamic epidermal cooling during pulsed laser treatment of port wine stain - a new methodology with preliminary clinical evaluation. Arch. Dermatol. (in submission), 1994.

IVf. OBSTETRICS AND GYNECOLOGY

1. Development of an Optical Force Trap for Diagnosis and Manipulation of Sperm
Co-PI's: Yona Tadir, M.D., Professor, of Obstetrics and Gynecology, UCI: Gregory Sonek, Ph.D., Associate Professor of Electrical and Computer Engineering, UCI; Ricardo H. Asch, M.D., Professor of Obstetrics and Gynecology, UCI

Progress

Several collaborative studies were conducted with the infertility specialists at Stanford University and with the team at the Center for Reproductive Health at UCI Medical Center.

In our initial studies, an Nd:YAG (1.06 μm) laser beam was directed into a conventional microscope and focused onto the viewing plane by the objective lens. The laser beam power

at which human sperm were released from the trap was measured and correlated to the sperm's linear velocity prior to trapping. The mean trapping laser power readings for slow, medium and fast motile sperm were 57, 73, and 84 mW respectively. The analysis of measurements over the total population demonstrated that zig-zag motile sperm had significantly higher mean laser power readings when compared to straight motile sperm with similar mean linear velocities.

Cryopreservation of human sperm is a standard laboratory procedure in many fertility centers. Several methods of freezing and thawing are being applied and various cryoprotectants are being used in order to preserve sperm motility and minimize damage to the fertilizing potential. Micromanipulation of human spermatozoa with laser generated optical trap has been used to assess the relative force generated by each single spermatozoon (Dantas 1994, in submission). This novel technology was applied to assess the relative escape force of human ejaculated sperm in fresh and frozen-thawed samples in the same subject to determine any possible adverse effects of the freezing process on this sperm parameter. The relative escape force generated by fresh and frozen-thawed ejaculated sperm was analyzed in an 800 nm (Ti: Sapphire) laser generated optical trap system. Normal sperm samples based on WHO criteria from ten (10) individual subjects were included. Each sample was split into two aliquots for fresh and frozen-thawed analysis, 2 to 10 days later, after a slow freezing process. Sperm samples were prepared in washed, resuspended pellets using HEPES buffered HTF media. A single beam gradient optical trap equipped with 100X magnification (Neofluar) was used to analyze the relative escape force in an average of 100 sperm for each aliquot. In total, 2130 morphologically normal spermatozoa were trapped at room temperature: 1160 sperm from fresh samples and 970 from the frozen-thawed samples. The overall mean relative escape force of the ejaculated sperm in fresh samples was 78.00 ± 43.03 mW and 76.28 ± 48.65 mW, in frozen-thawed samples (P.0.05). However, wide individual variations were noted: there was a significant decrease in 5 subjects and an increase in the other 5 (significant for 3) in the capacity of the sperm to be released from the optical trap. Since no consistent pattern was found, there may be other unknown or unpredictable sperm parameters that may be influenced by freezing/thawing. As measured by the laser optical trap, freezing of normal sperm does not appear to affect the relative escape force of ejaculated sperm and it was suggested that any detrimental effect of freezing on sperm function may be related to other sperm parameters.

It is known that spermatozoa change their movement characteristics in response to different environmental conditions and the fertilizing potential is improved following capacitation. To investigate the relative force of spermatozoa exhibiting different motility patterns and compare it to the force following exposure of sperm to the cumulus mass prior to IVF, a 760 nm laser optical trap was used (Westphal 1993). Spermatozoa were trapped at 300 mW, and laser power was reduced until spermatozoa could escape. Three motility patterns were studied: linear, hyper activated, and cumulus-related. Mean escape power for spermatozoa displaying linear motility was 59.5 ± 43 mW, for hyperactivated motility 122.3 ± 67 mW ($P < 0.0001$) and for cumulus-related motility 200.6 ± 44.2 mW ($P < 0.0001$). In this study, we showed that human spermatozoa generated more relative force upon exposure to the cumulus mass. The combination of small-amplitude lateral head displacement and higher relative force may produce a drilling effect which is synergistic with the enzymatic digestion of the cumulus matrix during the fertilization process.

One of the major problems in male infertility is congenital blockage of the vas deference (sperm duct). Males with this condition may have normal testicular function but the ejaculate contain glandular secretions without any sperm and as such they are infertile. It is known that sperm has to pass through the epidydimis in order to complete maturation. Very little is known about the motility patterns and fertilizing potential of human epidydimal sperm, since the only way that one can test this semen is following surgical aspiration and this was not available prior to the new IVF-MESA treatment. The center for reproductive health at UCI was the first in the world to combine microscopic epidydimal sperm aspiration (MESA) with IVF. Having access to this unique specimens we designed a study oriented towards three main goals: 1) to assess the relative escape force of human epididymal sperm using a laser generated optical trap, 2) to compare it with that of human ejaculated sperm; 3) to evaluate if a relative sperm force-threshold could ultimately serve as discriminatory factor for its fertilizing capacity in vitro (Araujo 1994, in submission).

Evaluation of the relative threshold escape force generated by epididymal and ejaculated sperm in an 800 nm (Ti: Saph) laser generated optical trap system and fertilization of human eggs in vitro. A total of 2720 sperm from twenty eight samples were randomly analyzed. Fifteen were ejaculated samples (1650 sperm) obtained from normal men and thirteen were epididymal samples (1070 sperm) aspirated microsurgically from patients with obstructive azoospermia. All samples were diluted in HEPES buffered human tubal fluid supplemented with 0.5% human serum albumin and washed once. The aliquots used for the optical trap were from the same specimens used for IVF. A single beam gradient optical trap equipped with the 100 X Neofluar objective was used to analyze an average of 100 sperm per patient.

The mean relative escape force of the epididymal sperm was 32.4 mW (+/- 0.98 SEM), significantly lower than the normal ejaculated sperm, 85.1 mW (+/- 1.2 SEM) [$p<0.0001$]. By correlating epididymal sperm relative force with fertilization in vitro, it was found that no fertilization occurred if a sample had < 13% of the sperm population with a relative force of 30 mW or higher (0 out of 3 patients). In contrast, epididymal samples containing > 13 % of the sperm population with a relative force of 30 mW or higher, fertilization of at least one oocyte was observed in 8 out of 10 patients (80%). In the ejaculated sperm, 15 out of 15 patients (100%) had at least one embryo, and considering all of them together, 1526 out of 1655 (92%) sperm had >30 mW of relative force.

It was concluded that:

1) The average relative threshold escape force of the epididymal sperm was found to be 60% weaker than that of ejaculated sperm.

2) An arbitrary cut-off point of 30 mW determined that 92% of the ejaculated sperm and only 28% of the epididymal sperm further fertilized human oocytes in vitro ($p<10^{-6}$)

3) It was demonstrated that the non-contact laser optical trap is a sensitive tool that can evaluate new physiologic parameters and possibly predict fertilization.

Publications resulting from DOE grant (4):

1. Tadir Y., Neev J., Berns M.W. Laser Microsurgery and Manipulations of Single Cells. In: Practical manual of Gynecologic Endoscopy. Eds. C. Sutton, M. Diamond. Saunders Ltd. London, 1993. pp.379-386

2. Westphal L., El-Danasouri I.E., Shimizu S., Tadir Y., Berns M.W. Exposure of human sperm to the cumulus oophorus results in increased relative force as measured by a 760 nm laser optical tram. Human Reproduction. 8 (7) 1083-1086, 1993.

3. Araujo E., Tadir Y., Patrizio P., Ord T., Silber S., Berns M.W., Asch R. Relative force of human epididymal sperm correlated to the fertilizing capacity in vitro. Fertil Steril. (In press).

4. Zoentania, N.D., Araujo E., Berns M.W., Tadir Y., Schell M.W., Stone SC. Effect of freezing on the relative escape force of sperm as measured by laser optical trap. Fertil Steril. (submitted)

2. Laser Micromanipulations of Eggs and Embryos

Co-PI's: Yona Tadir, M.D., Professor of Obstetrics and Gynecology, UCI; Joseph Neev, Ph.D., Assistant Professor of Surgery, UCI; Mitchel Schiewe, Ph.D., Assistant Professor of Obstetrics and Gynecology, UCI.

Progress

Since we described the potential use of the laser for gametes micromanipulation (MM) in 1989, and especially during the last 3 years, we have focused on questions related to laser zona pellucida (ZP) interaction and defining laser parameters that influence effects and survival of eggs and embryos.

Studies were undertaken in order to identify appropriate laser wavelengths for cutting the zona pellucida of oocytes and embryos.

364 unfertilized oocytes served as an experimental model. Oocytes were micromanipulated with two 308 nm XeCl lasers (Neev 1992a). Images and effects were video recorded and analyzed by computerized image processing and SEM. The XeCl excimer laser, delivered in a non-contact mode through the viewing microscope objective, was found to be an efficient, convenient, and highly accurate mode of oocyte micromanipulation. Laser pulse parameters such as energy, repetition rate, duration, and microscope optical properties were characterized as to their effect on the dimensions and quality of the drilled site. Ablation holes smaller than 1μm were obtained in a reproducible fashion without causing any apparent damage to neighboring areas. This non-contact mode allows for simultaneous viewing and cutting provided proper laser parameters are chosen. Pulse energy and the beam focal plane position were shown to be the most critical parameters in determining the ablated spot diameters.

Effects on further embryonic development were evaluated following use of the same laser (Neev 1992b). Zonae of 8 to 16 cell mouse embryos were either lased (n=189), zona drilled

with acidified Tyrode's solution (n=183), or left zona intact (n=188). Blastocyst formation (99-100%) was similar in the three groups. Hatching occurred earlier in the lased embryos compared to those of the control groups. These embryos hatched through the laser ablated area. Significantly more embryos were hatching on days 4 and 7 in the conventionally drilled group when compared to the laser treated group. However, implantation rates of morphologically normal laser ablated embryos were not impaired when compared to the control embryos.

Li et al. (in press) studied topical effects of lasing on mouse blastomeres using a XeCl 308 nm. Effects were determined by microinjection of a vital fluorescence dye (fluoresein isothiocyanate [FITC] dextran) into the cell immediately adjacent to the site of zona photoablation. This dye is only passed onto daughter blastomeres and therefore allows study of specific cell lines. Embryonic growth was assessed following cell separation at the morula and blastocyst stage. Four-cell embryos treated with this laser had significantly fewer cells 72 hours after zona photoablation than control embryos. This information may suggest that the 308 nm excimer laser had some detrimental effect on precompacted mouse embryos; however, a different choice of laser parameters with the same wavelength could correct this problem.

El-Danasouri et al. (1993) used the 308 nm non-contact pulsed laser directed through a 100X quartz objective to perform LZD before insemination. The laser energy at the objective focal point was 0.4 μJ/pulse with a spot diameter of 1 μm. The microscope stage was moved until the zona approached the laser tangentially and a photoablation slit was made. Zonae-opened oocytes were inseminated with low sperm concentration (2×10^4 sperm/ml) and compared to two control groups consisting of zona intact oocytes inseminated with either similarly low sperm concentration or normal sperm concentration (2×10^6 sperm/ml). Laser manipulated oocytes resulted in more than a 6-fold increase in fertilization rate over the low sperm IVF, non-manipulated oocytes (65% vs. 10.4%), and 94.9% of the zygotes developed to the 2-cell stage. However, blastocyst formation in the laser manipulated eggs was significantly lower than that of the control group inseminated with normal sperm concentration (68.5% vs. 90.2%, $p<0.01$). The authors concluded that the 308 nm laser has potential as a simple non-contact drill for improving fertilization with low sperm count. However, further characterization of laser parameters is needed to improve embryo growth prior to human IVF. In another study, we further investigated the possible effect of superoxide anion on the fertilization and cleavage rate of a similar animal models (El-Danasouri 1993). The idea was based on the principle that this compound is known to reduce intra- or extra-cellular free radicals, particularly singlet oxygen, that may be generated by laser irradiation. Results showed no effect on blastocyst formation.

Although the structure and chemical properties of the ZP have been investigated, there are still many questions with respect to "zona hardness" and its relevance to fertilization and implantation of in vitro cultured oocytes and embryos.

The objectives of these collaborative studies were to characterize possible hardening of the zona pellucida, evaluate the effect of culture duration, patient age, and effect of chemicals to induce zona hardening (Tadir 1993 a). A specific aim was to develop a new laser-based method that would permit accurate determination of ZP hardness (ZPH) during in vitro development. The ZP evaluated in these studies were obtained, as available, from the discarded unfertilized eggs (n=376) and abnormal (polyspermic) or poor-quality embryos

(n=52). In addition, fresh and cryopreserved mouse embryos (FVB-N) were also analyzed. ZPH was determined by two different methods: 1) enzymatic and 2) non-contact laser micro ablation. Upon recovery, eggs and embryos were cultured in 1 ml of HTF medium supplemented with 6% plasminate in a 5% CO_2, humidified air incubator (37°C). The ZP were randomly designated for enzyme treatment (a-chymotrypsin) after 0, 24, 48, 72, 96, 120 and 144 hours of in vitro culture. The eggs were cleansed free of cellular debris, washed in PBS and pipetted into 3 mg/ml of chymotrypsin solution. The dispersion of the ZP glycoproteins was assessed at 200x using differential interference contrast light microscopy and the duration of time for complete digestion recorded. The embryos manipulated with the laser were further cultured following initial hardness measurements under the same culture conditions. Laser ZPH measurements were performed with a XeCl (308 nm) excimer laser directed through quartz microscope optics. Calibration of the laser micromanipulator to define baseline levels was performed at 60 spots of human ZP. Normal ZPH was defined according to time of laser exposure at constant output. Enzyme digestion duration increased ($P<0.05$) in the first 24 hours in vitro, but did not significantly change over an additional 4 days in culture. Zona hardening of fertilized eggs using the enzymatic method also revealed a longer ($P<0.01$) digestion time (32.2 ± 1.8 vs. 25.8 ± 0.6 min.). Although, there were significant patient to patient variations, age was not correlated to enzyme digestion duration. Based on the enzymatic ZP digestion measurements, it is apparent that spontaneous zona hardening does occur within 24 hours of in vitro culture, similar to levels achieved post-fertilization. The data do not support, however, the concept that additional, abnormal hardening of the ZP occurs during extended culturing.

The laser ZPH measurements of non-fertilized human oocytes revealed that ethanol at various concentration (9.5; 24; 47; and 95%) gradually increased zona hardness. In turn, it appears that the non-contact laser micromanipulator can be a valuable tool which permits accurate ZPH measurements of live oocytes and embryos, in conjunction with continuous in vitro culture.

Several review articles on laser micromanipulations were published in the last two years. In these articles, we described basic concepts of laser-gamete interaction (Tadir 1993 b) and discussed advantages and disadvantages as compared to other techniques (Tadir 1994).

Most studies suggested in our previous DOE proposal (1991) were completed as planned, and the concept of the laser as a potential tool for gamete micromanipulations was adopted and tested by other groups as well. The proliferation of conventional micromanipulation procedures created an interesting situation in which competing technologies are being developed and simultaneously tested. Background information that is available today on advantages and disadvantages of the laser microbeam for gamete manipulations and specific needs as defined in the IVF laboratory in the last two years calls for phase II studies in which the optimal parameters as previously defined will be tested on biological models and will be compared to non-laser MM techniques.

Publications resulting from DOE grant (12):

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2. Neev Y., Tadir Y., Asch R., Ord T., Liaw L-H., Ho P.D., Berns M.W. Zona Drilling and Partial Zona Dissection using femto-, pico-, and nanosecond pulses: a comparative study. In: SPIE, 1650:61-69, 1992.

3. Tadir Y., Neev Y., Berns M.W. Laser micromanipulation of Oocytes and sperm. Lancet (Letter) 339: 1424, 1992.

4. Tadir Y., Neev J., Berns M.W. Laser in Assisted Reproduction and Genetics. J. Assisted Reproduc. and Genetics. 9: 303-305, 1992

5. Neev J., Tadir Y., Ho P., Asch R.H., Ord T. and Berns M.W. Microscope-delivered UV laser zona dissection: principles and practices. J. Assist. Reprod. and Genetics. 9:513-523, 1992.

6. Neev J., Gonzales A., Licciardi F., Alikani M., Tadir Y., Berns M.W., Cohen J.A. contact-free microscope delivered laser ablation system for assisted hatching of the mouse embryo without the use of a micromanipulator. Human Reprod 8;939-944, 1993.

7. Li L., Munne S., Licciardi F., Neev Y., Tadir Y., Berns M.W., Godke R., Cohen J. Microinjection of FITC-Dextran into mouse blastomeres to assess topical effects of zona penetration. Zygote (in press).

8. Tadir Y., Neev Y., Tromberg B.J., Berns M.W. Laser technology in reproductive medicine. In: Reproductive endocrinology, surgery and technology. Eds. Adashi, Rock, Rosenwaks. Raven Press. NY, NY. (in press).

9. Tadir Y., Neev J., Ho P., Berns M.W. Lasers for Gamete Micromanipulation: Basic concepts. J. Assist Reprod. Genetics. 10:121-125, 1993.

10. Tadir Y. Microsurgical fertilization techniques in IVF: past, present and future developments. Proceedings. VIII World Congress of In Vitro Fertilization and Assisted Reproductive Technologies. Serono Symposia Publication, Raven Press. (In Press).

11. Tadir Y., Neev Y., Berns M.W. Lasers in micromanipulation of Pre-implantation Embryos and gametes. Seminars in Reproductive Endocrinology. (In press).

12. Tadir Y. Microsurgical Fertilization: world survey 1993. J. Assisted Repr. Genetics, Jan. 1994

3. The Development of PDT for the Treatment of Endometrial Disease
Co-PI's: Yona Tadir, M.D., Professor of Obstetrics and Gynecology, UCI; Bruce Tromberg, Ph.D., Assistant Professor of Surgery and Biophysics/Physiology, UCI; Alberto Manetta, M.D., Associate Professor of Obstetrics and Gynecology, UCI

Progress

Researchers at the BLIMC have studied photochemical effects at many levels: sub cellular, cellular (Roberts WG, and Berns MW. 1989), animal (Nelson JS, and Berns MW. 1987, Tromberg, BJ et al. 1990), and human (Wile AG, et al. 1984, Rettenmaier MA. et al. 1984, Nelson JS. 1990). In order to better understand the determinants of selective uptake and retention of various photosensitizers in uterine tissue, we have systematically investigated this topic in rat models (Chapman et al. 1993, Steiner et al, in submission [a]). The three main questions addressed in this work were: a) what is the preferred mode of drug application, b) what is the influence of estradiol on the selectivity and duration of drug uptake, and c) reproductive performance of rat uteri following PDT as a marker for endometrial destruction.

Since Photofrin is currently the most commonly used photosensitizer, this compound was the main drug employed in these studies. Initially, we evaluated the relative merits of intravenous (IV), intraperitoneal (IP), and intrauterine (IU) administration methods in medically or surgically castrated rats. Extraction of Photofrin from uterine tissue was conducted according to a modified porphyrin fecal extraction technique (Rossi E and Curnow D 1986). Frozen sections were analyzed by fluorescence microscopy.

Our preliminary studies combined with the limited information available in the literature encouraged further investigation of this approach. Although the I.U. application of drug appeared promising, drawbacks to these studies included the relatively high Photofrin concentration in the columnar epithelium and the absence of data correlating our drug distribution and studies with photodynamic efficacy. For that reason, in the "reproductive performance" study we have added to the Photofrin a penetration-enhancing agent - Azone (1-dodecylazacycloheptane-2-one), (Whitby Research, Inc. Richmond VA). It was selected based on our previous studies demonstrating effective per cutaneous penetration of HPD in normal skin (McCullough J, et al. 1988).

Table 1. Summary of our preliminary studies.

Author	Model	Photosensitizer / Application mode	Dose (mg/kg)	Laser -W.L.	Aim
Chapman et al (1993)*Ph. I*	**Rat-** *Endometrium*	**DHE:** - IV - IP - IU -	7 - 7 - 0.7	/	*Pharmacokinetics,: Fluorescence study of various applications*
		Topical application			
Chapman et al (1993)*Ph. II*	**Rat-** *Endometrium*	**DHE** - IU	0.7	/	*Pharmacokinetics,: -Fluorescence study -Influence of E2*
Steiner et al. (In submission-a)	**Rat-** *Endometrium*	**DHE;** - IU **DHE + Azone** - IU	} 0.7	/	*Pharmacokinetics, Fluorescence study*
Steiner et al. (In submission-a)	**Rat-** *Endometrium*	**DHE;** - IU **DHE + Azone** - IU	} 0.7	630nm	*Histology following PDT*
Steiner et al. (In submission-a)	**Rat-** *Endometrium*	**DHE;** - IU **DHE+Azone** - IU	} 0.7	630nm	*Reproductive performance*
Steiner et al. (In submission-b)	**Rat-** *Endometrium*	**ALA** - IU	58	630nm	*- Pharmacokinetics, Fluorescence - Histology following PDT -Reproductive performance*

In order to improve the accuracy of our measurements, we have constructed a sensitive, high dynamic range fluorescence system which substantially improves drug detectability and spatial resolution in each uterine layer. The system consists of a Zeiss Axiovert 10 inverted microscope which can be configured to visualize phase contrast and fluorescent images of tissue frozen sections. All images are recorded using a thermoelectrically cooled, slow-scan CCD camera interfaced to a computer. Camera resolution is determined over 2.2 x 10^5 pixels with 16-bits per pixel dynamic range. Due to the exceptional sensitivity of the system, typical exposure times are about 1 sec for most frozen section fluorescent images.

Once the sensitivity of our system was established, a study was designed (Steiner et al, in submission [a]) to compare the distribution and photodynamic efficacy of two forms of topically-administered Photofrin in the uterus: (1) 0.7 mg/kg aqueous Photofrin and (2) 0.7 mg/kg Photofrin + 4% Azone, a penetration-enhancing agent (Steiner et al, in submission).

Photofrin and Photofrin/Azone were topically applied to 124 experimental animals. Fluorescence microscopy was employed to determine drug localization in uterine frozen sections at various times after drug administration. Optimal pharmacokinetic parameters determined from fluorescence studies were used to estimate photodynamic dose. Uterine structure and reproductive performance were evaluated following illumination with 80 J/cm^2 of 630 nm light.

A rapid increase in drug uptake was observed for all layers (mainly the endometrium) within the first 3 hours of topical application for both forms of Photofrin. Drug uptake increased more gradually for up to 72 hours. However peak levels in the endometrial glands were reached earlier with Photofrin/Azone (24 hr. compared to 72 hr. for Photofrin alone). Functionality studies demonstrated a significant reduction in the number of implantations per rat for treated uterine horns compared to control horns. The mean implantation rate decreased systematically by increasing the interval between Photofrin administration and light application. At 72 hours, 0.88 (± 0.52) gestational sacs per rat were observed with Photofrin therapy (vs. 8.1 ± 1.12; $p = 0.01$ in the untreated side), indicating nearly complete loss of reproductive capability. In contrast, similar results were achieved after only 3 hours with Photofrin/Azone treatment (0.38 ± 0.26 sacs per rat vs. 7.5 ± 1.07 in the untreated side; $p = 0.01$).

Pharmacokinetic studies indicated that topically-administered Photofrin/Azone is diffusely distributed throughout the endometrium within 3 hours of application. In contrast, the columnar epithelium appears to act as a partial barrier to Azone-free Photofrin diffusion. Uniform endometrial drug distribution is not achieved in the later case for several days, perhaps due to additional systemic contribution of residual Photofrin. Post-irradiation structural studies confirm pharmacokinetic findings that Photofrin's destructive effect can be enhanced either by extending the drug incubation period from 3 hours to 72 hours or by adding Azone, a penetration enhancing compound. Reproductive performance studies further support pharmacokinetic findings that the penetration-enhancing Azone can accelerate the effect of Photofrin-based endometrial destruction.

Following completion of the Photofrin studies and in view of limitations with drug penetration, the impact of PDT on the endometrium following topical application of 5-Aminolevulinic acid (ALA) was studied in a similar model (Steiner et al, in submission [b]). ALA is a precursor of protoporphyrin IX (Pp IX) in the biosynthetic pathway of heme and occurs in all aerobic cells. The slowest process in heme-synthesis is the Pp IX to heme conversion. Therefore, administration of exogenous ALA induces the accumulation of Pp IX, a potent photosensitizer (Kennedy 1992). The use of ALA for photodynamic therapy can also provide selectivity, since the capacity to synthesize Pp IX varies between cells.

Eighty-seven (87) female rats were divided into three groups for three complimentary studies: A) determination of the uptake, distribution and conversion to Pp IX in the uterine layers by fluorescence microscopy; B) morphological changes, and C) reproductive performance following PDT. Digital fluorescence microscopy revealed peak values at 3-6 hours, with significant higher concentration in the endometrial glands. Return to pre-injection levels is visible 12-24 hours later.

Histological studies revealed PDT induced endometrial destruction with marked atrophy at 7-10 weeks after treatment. The reproductive performance study demonstrated a significant implantation failure in the treated uterine horns as compared to various controls. The number of implantation sacs in the treated area was 0.4 ± 0.3 as compared to 8.9 ± 1.0 in the untreated control side ($p < 0.01$). Control animals (light without drug and drug without light) exhibited no significant difference in the number of implantations (8.2 ± 1.0 vs. 6,8 ± 0.9 and 8.3 ± 0.7 vs. 7.2 ± 0.6).

It was concluded that ALA is a promising photosensitizer for endometrial targeting. Selective uptake by endometrial glands resulting from local conversion to Pp IX may be used as a new tool in studying various aspects of endometrial physiology such as implantation and regeneration mechanisms. This concept may also be developed to a new contraception and as well as a treatment modality for dysfunctional uterine bleeding.

Publications resulting from DOE grant (8):

1. Chapman J.A., Tadir Y., Tromberg B.J., Yu K., Manetta A., Sun C-H., Berns M.W. Effect of Administration Route and Estrogen Manipulation on Endometrial Uptake of Photofrin II. Am. J. Obstet. Gynecol. 168: 685-692, 1993.

2. Tadir Y., Tromberg B., Krasieva T., Steiner R., Chapman J., Berns M.W. Endometrial photosensitization: experimental models. In: Lasers in Gynecology. Ed. Donnez J. (in press).

3. Tadir Y., Tromberg B., Krasieva T., Berns M.W. Photodynamic therapy towards selective endometrial ablation. Proceedings S.P.I.E. 1879; 247-52, 1993.

4. Steiner R., Tromberg B., Weiss P., Krasieva T., Berns M.W., McCullough J., Tadir Y. Photodynamic destruction of rat endometrium using topically-administered Photofrin. Human Reproduction. (submitted)

5. Wyss P., Svaasand L., Tadir Y., Tromberg B.J., Berns M.W. Photomedicine of the endometrium: experimental concepts. Human Reproduction, (submitted)

6. Wyss P., Tadir Y., Tromberg B.J., Liaw L., Krasieva T., Steiner R., Villalon V.P., Berns, M.W. Benzo Porphyrin Derivative (BPD): a potent photosensitizer for photodynamic destruction of the rabbit endometrium. Obstetrics and Gynecology, (submitted)

7. Wyss P., Tromberg B.J., Wyss M.T., Krasieva T., Liaw L., Schell M., Berns M.W., Tadir Y. Photodynamic destruction of endometrial tissue using topical 5-aminolevulinic acid (5-ALA) in rats and rabbits. Am. J. Obstet. Gynecol. (submitted)

8. Steiner R.A., Tadir Y., Tromberg B.J., Krasieva T., Ghazains T., Wyss P., Berns M.W. Photosensitization of the rat endometrium following 5-aminolevulinic acid (ALA) induced photodynamic therapy.

IVg. OPHTHALMOLOGY

1. New Ab-Interno and Contact Laser Surgeries for Glaucoma

Co-PI: Richard A. Hill, M.D., Assistant Professor of Ophthalmology, UCI

Progress

Summary
The initial proposal to DOE contained two approaches to the treatment of glaucoma: I. Ab-Interno techniques focused on the enhancement of aqueous outflow as a mechanism to lower intra ocular pressure; II. Contact laser investigations were focused on decreasing the formation of aqueous by destroying some of the tissue producing it. Pertinent abstracts, publications, clinical trials, and courses resulting from DOE research are listed following the progress report on each section.

I. Enhancement of Aqueous Outflow

1. Laser Trabecular Ablation (LTA)

This is an area of large productivity. During the past three years, we investigated all available pulsed infrared lasers for the ablation of trabecular meshwork. Although 2.94 μm produced minimal thermal damage, it did pose some difficulties in designing an efficient delivery system. We are currently using a compound fiber which works well in clinical situations. To further minimize thermal damage, it was necessary to find a pulse width (around 100 μs) which did not let peak power exceed the structural limits of the trabecular meshwork for single pulse ablation (4 mJ). In our *in-vivo* testing, we discovered a "hypertensive phase" at one week post-operatively in both humans and non-human primates. It is believed that this is secondary to repair of the endothelial lining of the aqueous collector channels. Efforts are now being directed at decreasing further blast and thermal damage.

A micro transducer was developed in conjunction with Innerspace. This device utilized a 25 gauge stand off needle to record intraocular pressures. The post-surgical increase in outflow was determined after LTA by adding balanced salt solution with a micro syringe pump at a known rate through the irrigating laser probe until the pre-surgical intraocular pressure was again reached. Trajectory analysis was not needed as a new steady state could be reached in minutes. The measured increases in out flow were very similar, limiting the clinical usefulness of this calculation as a predictive value for clinical success. We have developed a successful relationship with Candela Laser Company and this has led to SBIR funding for further clinical development. It is expected that LTA will go to a multi-center clinical trial in late 1994.

Publications resulting from DOE grant (5)

1. Hill R.A., Özler S.A., Profeta G.A., Baerveldt G., and Berns M.W. Laser trabecular ablation (LTA). Lasers Surg and Med. 11:431-346, 1991.

2. Hill R., Lesiecki M., Stern D., Hsia J. and Berns M. The effects of pulse width on laser trabecular Ablation. Lasers Surg Med. 13:440-446, 1993.

3. Hill R., Lesiecki M., Hsia J., Stern D., Brown L., Baerveldt G., and Berns M. Erbium:YAG laser trabecular ablation in primates. Manuscript under revision.

4. Hill R., Lesiecki M., Hsia J., Stern D., Brown L., Baerveldt G., and Berns M. Erbium:YAG laser trabecular ablation in primates. Presented as an abstract and poster at the Annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 33(4):1018, 1992.

5. Lesiecki M., Hill R., Stern D., Hsia J. and Berns M. The effects of pulse width on laser trabecular ablation. Presented as an abstract and poster at the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 33(4):12661992.

2. Ab-Interno Sclerostomy with Iridectomy

The early results of these studies placed glaucoma surgeons creating sclerostomies with lasers into two main groups. One group believed that large thermal effects were not undesirable (<2.1 μm). It was felt by this group that carbonization and thermally altered collagen was not efficiently removed by macrophages. The other group (2.94 μm) believed that it was necessary to minimize thermal effects in an attempt to limit the inflammatory response. It was felt that this response lead to sub-conjunctival scarring and to failure of sclerostomies. Ab-interno approaches have the additional theoretical advantage of not violating conjunctiva. Our group was the first to describe an Ab-Interno sclerostomy with laser iridectomy, utilizing end firing and side firing probes. However, most ophthalmologists use an Ab-Externo approach to surgery and the first large human trials utilized this approach. Currently Premier Laser Company is investigating the Ab-Interno approach with iridectomy formation from a side firing probe.

Publications resulting from DOE grant (6):

1. Özler S.A., Hill R.A., Andrews J.J., Baerveldt G., Heuer D.K., and Berns M.W. Infrared laser sclerostomies. Presented as an abstract and poster at the Annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. 31 (Suppl): 1675, 1990.

1a. Abstracted by Current Science; Iwach; Current Opinion in Ophthalmology Vol 4; Number 2.

1b. Abstracted by Lasers for sclerostomy Annual of Ophthalmic Laser Surgery, 1:82-90, 1992.

2. Hill R.A., Özler S.A., Baerveldt G., Viscardi J.J., Keates R.H., Lee M., Harrington J.A. and Berns M.W. Ab-Interno Neodymium:YAG versus Erbium:YAG sclerostomies in a rabbit model. Presented as an abstract and poster at the Annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. 32 (Suppl) 744,1991.

2a. Abstracted by Current Science; Iwach; Current Opinion in Ophthalmology Vol 4; Number 2.

3. Le M., Hill R., Yashiro H., Constan C., Stern D., Lesiecki M., Brown L. and Berns M. Ab-Interno Erbium(Er):YAG laser sclerostomy with iridectomy in dutch cross rabbits. Presented as an abstract and poster at the Annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 33(4):1266, 1992.

3. Ab-Externo Sclerostomy

This area of investigation is a spin-off of the original investigation of infrared laser sclerostomies. The majority of ophthalmic surgeons utilize an Ab-Externo approach. It followed that the Ab-Externo approach was the first to reach clinical trials in large numbers. The current status of these lasers (in humans) is that when energies or fibers are optimized to produce larger sclerostomies (≥ 300 μm) and used with anti-metabolites such as 5-FU, there is no clinical difference between lasers producing large and small thermal effects. Published rates of complications and energies used are higher for lasers producing larger thermal effects. Although surgical success rates are improving, both types of lasers are slightly less successful than traditional non-laser methods such as trabeculectomy.

Publications resulting from DOE grant (3):

1. Dah M., Hill R.A., Ryan J., Lesiecki M., Liaw L-H., and Berns M.W. Ab-Externo erbium (Er):YAG laser sclerostomies with 200- 300- and 400-μm fiber optics in rabbits. Abstract and poster at the Annual meeting of the Association for Researchers in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 34(4): 737, 1993.

2. Erbium:YAG Sclerostomy in Humans-an Update. Beckman L.R., Baerveldt G., Beckman H., Hill R.A. and Simmons J.R. Abstract and paper at the Annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 34(4): 1071,1993.

3. Hill R.A., Invited Paper, IEEE Lasers and Electro-Optics Society 1992 Annual Meeting, November 16-19, Boston MA. New Applications for pulsed and CW lasers in the treatment of glaucoma; Conference Proceedings; 1992.

II. Decrease of Aqueous Formation

1. Destruction of the Ciliary Body by Photodynamic Therapy (PDT)

Previous attempts at the use of PDT for destruction of the ciliary body may not have worked well because of the minimal transmission of visible light through sclera. We have used contact fiber optics to increase efficiency of transscleral transmission of visible laser light. We were the first to report the PDT mediated selective destruction of the ciliary based on a selective retention of photochemicals by the ciliary body. We have found some thermal effects and the possibility of retinal phototoxicity also exists for shorter wavelengths (Photofrin® II (PII) 633 nm). These effects are lessened and transmission efficiency increased with a shift into the deep red (silicon naphthalocyanine SINc; 770 nm). Our current effort is focused on photochemicals that absorb in the deep red region and the creation of

derivatives that are in the very near infrared. It is our hope to create a selective injury to the ciliary body with minimal chance of retinal or dermal phototoxicity and good transscleral transmission characteristics.

Publications resulting from DOE grant (4):

1. Hill R.A., Esterowitz T., Ryan J., Yoshiro J., Shirk J., Kenney M., Shimuzu S., Liaw L-H., and Tromberg B.J. Photodynamic therapy of the ciliary body with silicon naphthalocyanine (SINc) in rabbits. Abstract and paper at the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 34(4): 1069,1993.

2. Esterowitz T., Hill R.A., Ryan J., Yashiro H., Kim J., Treadway A., Krasieva T., Nelson J.S., and Berns M.W. Photodynamic therapy (PDT) of the ciliary body with photofrin® II (PII) and chloroaluminum sulfonated phthalocyanine (CASPc) in dutch cross rabbits. Abstract and poster at the Annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 34(4):739, 1993.

3. Hill R.A., Esterowitz T., Ryan J., Yoshiro H., Shirk J., Kenney M., Shimuzu S., Liaw L-H. and Tromberg B.J. Photodynamic therapy of the ciliary body with silicon naphthalocyanine (SINc) in rabbits. Manuscript in preparation.

4. Hill R.A., Esterowitz T., Ryan J., Yashiro H., Kim J., Treadway A., Krasieva T., Nelson J.S., and Berns M.W. Photodynamic therapy (PDT) of the ciliary body with photofrin® II (PII) and chloroaluminum sulfonated phthalocyanine (CASPc) in dutch cross rabbits. Manuscript in preparation.

2. PDT Mediated Destruction of an Experimental Ocular Melanoma

This work is an extension of our work in PDT mediated destruction of the ciliary body. There has been some earlier work utilizing photosensitizers (Rose Bengal, and chloroaluminum sulfonated phthalocyanine (CASPc; BLIMC)) that absorb at shorter wavelengths. These studies were limited by phototoxicity, exudative retinal detachments at higher light doses and limited tissue penetration at shorter wave lengths. In our pilot studies, we have implanted Greene Hamster Melanoma Tumor in the subchoroidal space of a pigmented Dutch Cross rabbit model. To increase tissue penetration and decrease the possibility of retinal phototoxicity and exudative retinal detachment, we are currently working in the deep red (770nm) with silicon naphthalocyanine (SINc.). Fluorescence localization studies showed a largely perivascular localization of (SINc) at 24 hours post injection. PDT therapy (24 hours post injection) is achieved with the application of 625 μw CW (40-80 $MW/^{cm2}$) for 2-10 minutes. Early results have showed tumor regression without exudative retinal detachment formation. We are encouraged and an ACS seed grant was awarded to supplement these pilot studies.

Publications resulting from DOE grant (1):

1. Hill R.A., Esterowitz T., Ryan J., Yoshiro J., Shirk J., Kenney M., Shimuzu S., Liaw L-H., and Tromberg B.J. Photodynamic therapy of experimental ocular melanoma with

silicon naphthalocyanine (SINc) in rabbits. Abstract and paper at the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO). Invest Ophthalmol Vis Sci. (Suppl) 35(4): 2120,1994.

2. Development of Excimer Laser (193 nm) Delivery Systems for the Treatment of Hyperopia and Astigmatism
Co-PI: Richard Keates, M.D., Professor and Chair of Ophthalmology, UCI

Progress

We have replaced our Tauton Excimer laser with a Chiron Technolas laser. The Chiron Technolas laser is a second-generation excimer laser which incorporates beam wobble for a smoother ablation profile. We have begun to treat patients from between -1.50 and -10.00 diopters of myopia. After the new laser was received, an initial plastic ablation study was completed which involved the photoablation of PMMA material.

Preclinical studies were completed on 12 rabbits. One group of 4 was sacrificed immediately and one group was sacrificed after 30 days. Light microscopy and electron microscopy was performed on these animals. Analysis of wound-closure rates, corneal thickness and topographical data was completed prior to the initiation of clinical trials with the laser.

We completed a hyperopia trial on 10 rabbit eyes using the new laser for the treatment of hyperopia. Results indicate that 8 of the 10 rabbits had their hyperopia substantially shifted with 7 of these 8 rabbits becoming myopic at 7 days. A paper entitled, "Excimer Laser Keratectomy for Hyperopia" which discusses the results of this study is in the final preparation stages and will be submitted for consideration within 30 days.

Two abstracts have been submitted and accepted for the Association for Research in Vision and Ophthalmology (ARVO) meeting in May, 1994. One is entitled, "The Keracor 116 Excimer Laser: A Second Generation ArF 193nm Laser for the Correction of Myopic Refractive Errors." This paper presents clinical data obtained from 236 eyes. The other abstract entitled "Expression of Stress-Inducible Heat Shock Protein 70 in Rabbit Corneal Cells after Excimer Laser Treatment" presents data on stress-inducible heat shock protein in rabbit cornea following excimer laser treatment of corneal cells.

We have also completed a study on unity cuts with the new Chiron Technolas laser. This involves doing a myopic correction followed by a hyperopic correction of equal power which in theory should produce no refractive change. Four animals were treated with unity cuts and we are awaiting the histology results.

A project dealing with a multiphase approach to the laser has also been completed. In this study, one treatment was given removing half the myopia and in 30 days another treatment was given to complete the correction. Two animals were treated with the new Chiron Technolas laser and at this time histology results are being reviewed.

Dr. Quishi Ren, a Ph.D. physicist has joined our faculty. Dr. Ren has extensive background in solid state lasers and will be working with scanning algorithms on the laser and tieing topography to the actual ablations.

Publications resulting from DOE grant (2):

1. "The Keracor 116 Excimer Laser: A Second Generation ArF 193nm Laser for the Correction of Myopic Refractive Errors" ARVO Proceedings, May 1994

2. "Expression of Stress-Inducible Heat Shock Protein 70 in Rabbit Corneal Cells after Excimer Laser Treatment" ARVO Proceedings, May 1994

IVh. DENTISTRY (Dental Applications of Lasers)

Co-PI's: Petra Wilder-Smith D.D.S., Ph.D., Assistant Professor of Surgery (Pending), UCI; Joseph Neev, Ph.D., Assistant Professor of Surgery, UCI; Joel White, D.D.S., Department of Restorative Dentistry; UC San Francisco

Progress

Since we discussed the potential applications of lasers in dentistry in 1991, several studies were undertaken and completed with DOE support:

1. *Stabholz A., Neev J., Liaw L-HL., Stabholz Ay., Khayat A., Torabinejad M.: The effect of ArF-193nm excimer laser on human dentinal tubules: Journal of Oral Surgery, Oral Medicine and Oral Pathology, Vol. 75 1:90-94, 1993.*

We studied the effect of the ArF excimer laser on human dentinal tubules. The effect was characterized using SEM methods. The ArF excimer laser was applied for 5 seconds on three quadrants with fluences that ranged from 0.2 J/cm^2 to 15 J/cm^2 and PRR of 25Hz. The untreated quadrant served as control. The effect of the ArF irradiation varied. Laser fluences of 0.2, 0.5, and 1.0 J/cm^2 had no effect. Melting and resolidification of dentinal smear layer was observed under the scanning electron microscope with a fluence of 5J/cm^2. Fluence of 15J/cm^2 caused significant removal of peritubular dentin.

2. *Stabholz A., Neev J., Liaw L-HL., Stabholz Ay., Khayat A., Torabinejad M.: Sealing of human dentinal tubules by XeCl-308nm excimer laser. Journal of Endodontics. In press, 1993.*

In a subsequent study, the effect of XeCl excimer laser on sealing of human dentinal tubules was investigated. Fifteen 3mm thick slices were cut at the cementoenamel junction from extracted human teeth by an electric saw. Diamond bur was used to remove the cementum layer and expose the dentinal tubules. Each slice was divided into four quadrants. Three were irradiated for 4 seconds by the laser with fluences ranging from 0.5 to 7.0 J/cm^2, and PRR of 25 Hz. The forth quadrant served as control. The specimens were mounted on a stub sputter coated by gold and examine by SEM. Untreated surfaces showed numerous exposed dentinal tubules. In contrast, all specimens lased at fluences of up to 1 J/cm^2 showed the presence of melted dentine which closed the dentin tubules at. At fluences of 4 J/cm^2 and higher, rupture of molten materials and exposure of dentin tubules were noted. The results indicate that application of XeCl excimer at specific fluences can cause melting of dentin and closure of exposed dentin tubules.

3. *Stabholz A., Kettering J., Neev J., Torabinejad M.: Effects of the XeCl excimer laser on Streptococcus mutans. Journal of Endodontics, 19:232-235, 1993.*

The effect of XeCl laser irradiation on the growth of *Streptococcus mutans* in liquid media and on agar plates was also studied. Bacterial suspensions of *S. mutans* were placed in 96 wells of well culture plates. The contents of 72 wells (three experimental groups of 24 wells each) were lased for different time duration (2,4, and 8 s). The remaining 24 wells were left unlased to be used as controls. Samples were withdrawn from all wells and examined for surviving bacteria. In addition, blood agar plates were inoculated with *S. mutans* and were lased with different energy densities (fluences). Zones of bacterial inhibition were measured. Analysis of variance test was used to determine statistical differences.

The bactericidal effect of the laser applications was directly related to the amount of radiation time. Laser irradiation for 4 and 8 resulted in bactericidal effect that was statistically significant compared with no treatment or to 2-s exposure. The effect of different energy levels was studied by irradiating inoculated blood agar plates. The zones of inhibition produced by higher energy levels (0.5 J/cm^2, 0.7 J/cm^2, and 1.0 J/cm^2) were larger in comparison to the lowest fluence used (0.1 J/cm^2). Application of the laser to the surface of the agar plates produced and indentation with a surrounding halo. The indentations and the zones of inhibition were more pronounced as the fluences increased. Based on our results it appears that the XeCl 308-nm excimer laser can kill *S. mutans*. This effect should be tested on other bacteria commonly present in infected root canals.

4. *J. Neev, A. Stabholz, L. L. Liaw, M. Torabinejad, J.T. Fujishige, P.H. Ho, M.W.Berns, "Scanning Electron Microscopy and Thermal characteristics of Dentin ablated by a short-pulse XeCl Laser", Lasers in Surgery and Medicine Vol 13, No 3:353-361, 1993.*

In an additional study, we have correlated IR thermography measurements with microstructural changes due to irradiation of XeCl lasers. We demonstrated that this excimer laser which is often characterized as a "cold ablation" laser, can result in significant thermal modification in the dentin surfaces. Changes include the formation of melted dentin grains which uniformly cover the surface and the exposed dentin tubules. Maximum temperatures of the ablated surfaces, however, remained relatively low at most laser parameters used. Also, the immediate neighborhood of the root canal was essentially undisturbed at most laser parameters. Our studies suggest that with the appropriate choice of parameters XeCl lasers can be effective in producing surface structures which may prove useful in enhancing bond strength or other applications in dentistry, without exposing tooth pulp to significant temperature elevation.

5. *White JM, Neev J, Goodis H, and Berns MW, Surface temperature and penetration depth of Nd:YAG laser on Enamel and Dentin. SPIE, Vol 1643 Laser Surgery: 423-436, 1992.*

Using IR-thermography we investigated thermal effects of clinical pulsed Nd:YAG laser energy on enamel and dentin. 150 μm pulses were delivered through 320 μm fiber for exposure times of 1,10, and 30 seconds. Laser parameters varied from 0-.3 to 3.0 W and from 10 to 30 Hz. Other treatment conditions included applications of hot coffee, carbide bur in a dental air cooled turbine drill and soldering iron. IR thermograph was used to measure maximum surface temperature and thermal penetration depth into enamel and dentin. Surface temperature was found to range 34 to 110^{0}C on enamel, and 62 to 392 on dentin. As power

and time were increased both maximum surface temperature and thermal penetration distance increased. The greatest length of thermal effect on the surface and (11.0 cm) and thermal penetration depth (4.7mm) were caused by the air-cooled turbine drill on dentin, and while the temperatures created with the laser were higher then the drill, the diameter of the hot spot on the surface, and the thermal penetration depth in the pulpal direction were significantly less than those of the dental drill. The pulse Nd:YAG laser with 320 μm fiber optic system was thus determined to be safe for application to enamel and dentin without detrimental thermal or pulpal effects.

6. *Harold E. Goodis, Joel M. White, and Joseph Neev "Thermal Measurement of Root Surface Temperatures During Application of Intracanal Laser Energy, In Vitro" SPIE, Vol. 1880, Lasers in Orthopedic, Dental, and Veterinary Medicine II. 226 - 234, 1993.*

In this study, the use of laser energy to clean, shape and sterilize a root canal system space was investigated. Since such a procedure involves the generation of heat due to the thermal effect of the laser on the organic tissue contents and dentin walls of that space a thermographic study was beneficial. Indeed, if heat generation is above physiologic levels, irreparable damage may occur to the periodontal ligament and surrounding bone. Our study measured temperature rise on the outer root surfaces of extracted teeth during intracanal laser exposure. 30 single rooted, recently extracted teeth free of caries and restorations, were accessed, pulps extirpated and divided into three groups. Each root canal system was treated with a 1.06 μm pulsed Nd:YAG laser with quartz contact probes under the following laser parameters: 1 - 100 μm probe, 1W, 10Hz; Group 2 - 200 μm probe, 2W, 20Hz; Group 3 - 320 μm probe, 3W, 30Hz, with an exposure time of 10 sec/canal space. Temperatures were recorded for all surfaces (mesial distal, buccal, lingual, apical) with infrared thermography utilizing a detector response time of 1usec, sensitivity range (infrared) of 8 to 12um and a scan rate of 30 frames/sec.

7. *Joseph Neev, Harold E. Goodis, and Joel M. White "Thermal Characteristics during Nd:YAG and Carbon dioxide laser application on enamel and dentin" SPIE, 1994 in press.*

To determine the effects of highly absorbed IR radiation on enamel and dentin we have also considered the effects of CO_2 laser (both pulsed and CW mode), and tested its effects using published parameters value. Thermal effects were then further investigated using SEM and light microscopy. We found that while IR thermography indicated smaller thermal effects (TPD, DHS and RTA) compared to the dental drill, heat accumulation in outer layer resulted in excessive charring of large neighboring area. Optimal operating parameters were therefore not identified.

8. *Effects of lasers on enamel caries resistance, caries progression, microhardness and microstructure*

Studies performed at BLIMC on effects of different laser wavelengths and parameters on dental caries resistance included investigations using the ArF and the XeCL excimer lasers in the presence or absence of adjunct fluoride applications. Studies were performed on freshly extracted teeth, which showed no clinical or radiographic signs of caries. These specimens were completely varnished with acid-resistant shellac, leaving uncovered a window of 3mm x 3mm. Initial microhardness profiles were determined for the window area on the surface and

at 25 μ intervals to a depth of 200 μ. Samples were then randomly allocated to one of four groups:

A. Control, no treatment
B. Fluoride only (APF, 1.23%)
C. Fluoride, then irradiation
D. Irradiation, then Fluoride

After microhardness re-measurement, these samples were subjected to standard artificial decay induction. Then microhardness, light microscope and SEM determinations were undertaken to document decay-resistance, decay progress, as well as microhardness changes at the tooth surface and again at 25 μ intervals to a depth of 200 μ. Microstructural changes were also observed. Preliminary results showed excellent decay-enhancement using the XeCL laser followed directly by topical fluoride application. These samples are currently undergoing atomic force microscopy investigations to determine precise structural changes: first results indicate a development of micro-voids within the outermost enamel structures after lasing, possibly due to preferential removal of softer, more decay-susceptible organic matter. These microvoids appear to be eliminated by subsequent fluoride application, perhaps by the deposition of more mineralized, more decay-resistant fluoridated hydroxyapatite structures.

Publications resulting from DOE grant (5):

1. Wilder-Smith P., Phan T., Liaw L-H., Berns M.W. Effects of XeCL Excimer lasers and fluoride application on artificial caries-like lesions. Presented at SPIE, 1994, and accepted for publication in this society's Journal.

2. Wilder-Smith P., Arrastia A.M., Neev J., Liaw L-H., Berns M.W. Caries Inhibition by ArF, XeCL lasers and fluoride application. J. Dent. Res. 1993, 72: 1994

3. Wilder-Smith P., Arrastia A.M., Neev J., Liaw L.H., Berns M.W. Caries Inhibition by ArF, XeCL lasers and fluoride application. Presented at SPIE, Los Angeles, 1994

4. Wilder-Smith P., Phan T., Berns M.W. Effect of XeCl excimer laser irradiation and fluoride application on artificial caries-like enamel lesions. Accepted for presentation at ASLMS, Toronto, 1994

5. DiRubio L.A., Tangyunyoung P., Warren O.L., Houston J.E., Michalske T.A., Wilder-Smith, P. Nano-Scale Mechanics and Morphology of Laser Ablated Tooth Enamel, accepted for presentation at American Vacuum Society, 1994

9. *Effects of lasers on dentin caries resistance, caries progression, microhardness and microstructure*

Methods as above, but investigations were performed on healthy dentin instead of enamel. In these early studies it has proved more difficult to define optimal parameters for achieving the desired effects without inducing concomitant structural defects in the dentin as well. Thus we are still focusing our efforts on identifying appropriate parameters to consistently and

reliably achieve increased microhardness and decay resistance without excessive detrimental microstructural effects.

Publications resulting from DOE grant (1):

1. Wilder-Smith P., Desai T.J., Berns M.W. Effect of XeCl excimer laser irradiation and fluoride application on artificial caries-like lesion formation in dentin. Accepted for presentation at ASLMS, Toronto, 1994

10. *Effect of Nd:YAG laser radiation and root planing on root surface*

After scaling and root-planing procedures are performed, a residual surface smear layer containing cytotoxic and inflammatory mediators as well as bacteria and bacterial products which adversely affect periodontal health remain on the tooth root surface. Chemicals currently utilized for removal of this residue are associated with many adverse effects. In first studies at BLIMC, the effects of pulsed Nd:YAG irradiation on extracted teeth were investigated. The teeth used were freshly extracted and identified as stemming from healthy or periodontally diseased oral milieus. Both groups of teeth were subdivided into subentities defined by treatment:

A. Control
B. Irradiation only using a range of parameters
C. Root planing + irradiation
D. Root planing only

Intra-canal and surface thermography were performed during laser irradiation, SEM was carried out on all samples. Using a 300µ fiber, 5W, pulse durations and intervals of up to 0.1s and energy densities of approximately 700J/^{cm2}, smear layer and bacterial deposits were effectively removed without microstructural damage to surrounding structures. Early bacteriological studies have documented effective elimination of the 5 most common bacterial contaminants of the root surface using the parameters described above.

Publications resulting from DOE grant (3):

1. Wilder-Smith P., Grill G., Liaw L-H., Berns M.W.: Effect of Nd:YAG laser radiation at various parameters alone and in conjunction with root planing on root surface: an SEM study. Submitted for publication

2. Wilder-Smith P., Arrastia A.M., Grill G., Liaw L-H., Berns M.W.: Effect of Nd:YAG laser radiation in conjunction with root planing on dentinal root surface: thermal and SEM studies. Submitted for publication

3. Wilder-Smith P., Grill G., Liaw L-H., Berns M.W.: Effect of Nd:YAG laser radiation and root planing on root surface: an SEM study. Accepted for presentation at ASLMS, Toronto, 1994

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