Rough draft

VI-A

EFFECTS OF WAVELENGTH, PULSE DURATION AND PULSE REPETITION FREQUENCY OF THE INTERACTIONS AND DESORPTION OF BRAIN TISSUE

M. AFFATIGATO,(1) B.A., R.J. MACIUNAS,(2) M.D., R.F. HAGLUND Jr.,(1) Ph.D., and R. CARVER,(2) B.S.

(1) Dept. of Physics and Astronomy
Vanderbilt University
Nashville, TN 37235

(2) Dept. of Neurosurgery
Vanderbilt University Medical Center
Nashville, TN 37235

ACKNOWLEDGEMENTS:

This research was supported in part by the Office of Naval Research under the auspices of the Vanderbilt Medical Free Electron Laser Project.

ADDRESS ALL CORRESPONDENCE TO:

Prof. Richard F. Haglund, Jr.
Department of Physics and Astronomy
Vanderbilt University
Nashville, Tn 37235 U.S.A.

PHONE: (615) 322 7964 FAX: (615) 343-7263

ABSTRACT

We report a phenomenological study of the effects of 532 and 308 nm laser light at nanosecond and picosecond pulse durations and at two different repetition rates on a rat-brain model. The quality and morphology of each ablation well and of its relationship with the laser parameters were made. Special attention was paid to unusual shapes of the cavity, such as "keyholes," and the effect of focussing on cavity shape was investigated.

INTRODUCTION: Neurosurgical applications of laser-tissue interactions have been directed to (1) singlet oxygen-mediated tumoricidal photodynamic therapy via excitation of various fluorescing compounds by fiber-delivered monochromatic light, and (2) precision tissue ablation, predominantly using the CO_2, Nd:YAG and KTP-532 lasers via microslade-directed intramicroscopic systems. While the successful application of photodynamic therapy techniques to central nervous system tissue requires fabrication of an efficient but relatively nontoxic agent with excitatory wavelengths suitable for maximal light penetration through brain parenchyma, precision ablation of central nervous system tissue requires the experimental identification of combinations of laser light wavelengths, pulse and power characteristics that are optimized for clinical use. This in turn requires a fundamental understanding of the processes leading to the laser irradiation-induced desorption of brain tissue.

In this paper we report observations of the effects of laser wavelength, pulse repetition frequency, pulse duration and fluence on the ablation of brain tissue. In this investigation, the influence of these parameters on the quality, morphology and depth of the ablation well was qualitatively described, and related to observed physical characteristics of rat brain. The consequences of shifting the focal spot deeper than the surface were considered along with other possible causes of the "keyhole effect," previously noted in the literature. We also noted the effect of the saline solution as a control for tissue hydration. The application of results from such an inquiry would allow the development of clinically optimal paradigms for laser wavelength,

pulse and related characteristics to guide appropriate selections for laser-induced ablation of neural tissue.

MATERIALS AND METHODS: Freshly excised, intact rat brains were prepared and placed in a normal saline solution (0.86 %). The brains were then taken to the laser site and irradiated in air, noting whether they were wet (saline solution on the surface), or dry (no liquid evident). An average of five spots per brain were exposed to the laser light, to facilitate histological analysis.. The brain was affixed to a microscope glass slide mounted on a translation stage. The laser beam was reflected from a mirror and passed vertically downward through a focussing lens to strike the surface of the brain, located near the focal plane of the lens.

The size of the laser spot was noted before the lens and at the surface of the brain, and used to estimate the fluence. The translation stage was used to move the brain and locate each new area of irradiation. Care was taken to allow for the curvature of the brain and the consequent shift in the placement of the focal spot above or below the surface. Lenses of different focal lengths and diameters were used, and the power levels of the laser were monitored several times during the experiment to insure the constancy of the experimental conditions.

After exposure, the rat brains were fixed in 10 % buffered neutral Formalin overnight. Graded alcohols and Xylene were allowed to infiltrate the tissue, and the whole brain was then embedded in paraffin. Sections were cut at 5 micron intervals and stained with Harris Hematoxylin and Eosin (HxE). The slices were then placed on microscope slides and microphotographs prepared, at two different magnifications (60 times, 26 times).to generate relative comparisons of ablation well depth and thermal damage zones.

The laser used varied in wavelength λ, power, pulse repetition frequency (PRF), and pulse durations. as shown in Table I.

Laser Type	λ (nm)	Pulse Duration	PRF (Hz)	P (W)	E (mJ)	I ($MW \cdot cm^{-2}$)
Nd:YAG, CW, mode-locked	532	100 ps	$76 \cdot 10^6$	1		0.03-0.08

Nd:YAG, Q-switched, mode-locked	532	35 ps	10		15	636
Nd:YAG, Q-switched	532	11 ns	10		400	46
Excimer (XeCl)	308	15 ns	10		230	1.7

RESULTS :

Figure 1 shows a comparison of the effects of two different wavelengths on ablation. The first dry sample (on the left), irradiated at 532 nm and magnified 60 times, shows less ablation and a greater zone of thermal damage. Yet the peak fluence of this irradiation was about 30 times that of the 308 nm exposure. Irradiation at 308 nm (on the right), also magnified 60 times, shows deeper ablation, and the thermal damage seems rather limited. The ultraviolet light is more attuned to the vibrational modes of the proteins and nucleic acids present in the components of gray matter; but this of course ignores the *in vivo* effect due to the presence of blood.

The heating of tissue by a continuous 1064-nm laser light has already been studied[1], and it has been shown that this wavelength of light heats the blood very quickly to 90 % of its maximum temperature within 3 s, and the thermal damage to the brain is still relatively severe. The thermal damage from 532-nm light follows the same pattern, although it is diminished.

Figure 2 illustrates the effects of high and low pulse repetition frequencies. The sample on the left was dry and irradiated with 532 nm light at 76 MHz and with picosecond pulses. Notice the greater thermal damage and immediate carbonization at the surface. The fast repetition rate does not allow for significant diffusion of the pulse energy. The sample on the right, dry and also exposed to 532 nm light with picosecond pulses but at 10 Hz shows an entirely different morphology. A keyhole effect now appears, and the surface damage is more contained. Both areas received the same approximate amount of total energy (≈ 35 J), and were photographed at 60 times magnification.

The dry sample on the left of Figure 3 was exposed to picosecond pulses of 532 nm light. The incision created ragged, deeper damage, with a relatively small zone of thermal damage. The same amount of total energy was deposited on each site, but the pulse energy is more

localized in time, and thus does not have sufficient time to diffuse. The sample on the right was irradiated with nanosecond pulses. Greater thermal damage and a shallower ablation well are apparent. The effect of pulse length becomes obvious if one considers the peak intensities involved: 636 MW/cm^2 for the picosecond pulses *vs.* 45.8 MW/cm^2 for the nanosecond ones.

The analysis of all the previous effects was done in the absence of blood flow. The *in vivo* case would of course require knowledge of the absorption bands due to oxyhemoglobin and other blood components, and of the interactions between light and liquid. Figure 4 shows the different absorption bands of heparinized rat blood (1:100 concentration in a saline solution): note the the absorbances at 308 nm and 532 nm are not too dissimilar. Also note the significant drop in absorbance near 290 nm, suggesting the utility of future study of the interactions of 248 nm excimer laser light with blood-irrigated brain tissue.

As a first order model, we considered the effect of the presence of the saline solution (0.86 % salt content). Although this solution contains none of the absorption bands of blood, it does provide a zeroth-order model of the light-liquid interaction. Figure 5 compares the ablation wells caused by 532 nm light (picosecond pulses) at 76 MHz on dry and wet samples. Dry samples had no solution apparent on the surface at the time of irradiation; the wet samples has a visible liquid layer on the surface. The exposed areas were on different hemispheres of the same rat brain.

The wet sample shows a ragged incision, characteristic of a "boiling away" of material. The dry sample, albeit smoother, shows a greater degree of carbonization. Thermal damage seems to be limited in both areas.

The "keyhole" effect appeared when the brain was irradiated with 532 nm light picosecond pulses at 10 Hz. Note that this exposure has the highest peak power used, about 636 MW/cm^2. This strange shape seemed to be related to the depth of the placement of the focal spot, appearing only when the spot was underneath the surface, but within the penetration depth of gray matter ($\approx$ 0.6 mm). This suggests that the keyhole effect is simply a focussing problem, with the following explanation: the first (conical) part of the well is due to the continued convergence within the

brain; the second part, a spherical cavity, occurs when the reduced area causes the power to pass a threshold value, on the order of 1 GW. This causes an explosion of ablation centered around the focal point, due perhaps to thermal lensing.

DISCUSSION:

The presence of a liquid is also important in another respect. Figure 6 is a photograph of a side slice of an ablation cavity. Being near the edge of the well, it is completely covered by tissue. Thus, the morphology of the cavity is more like a sub-surface "bubble", with a small centrally located hole through which the laser light penetrates. Hillenkamp has suggested that the bubble is caused by the vaporization of liquid within the tissue, and the cover remains because the liquid present is able to evaporate from the surface, thus dissipating the energy. For the 76 MHz, 532 nm picosecond laser irradiation, an audible "pop" was heard after 4 or 5 seconds of exposure. It is assumed that this is the sound of the bubble bursting due to the superheated vapor inside: the heated vapor cannot leave through the central hole faster than the rate of energy being deposited. The following simple calculation seems to support the hypothesis.

The penetration depth[2] of 532 nm light in gray matter is ≈ 0.6 mm. If we assume a cylindrical volume, with the upper and lower circular areas equal to the area of the focal spot, and the height of the cylinder to be the penetration depth, we then have:

$$\Delta Q = m\, c_p\, \Delta T + m\, L$$

The mass is given by the volume and the density of water, so m= 1.2 mg, and Δ T= 78 C. If we assume the mass of the tissue to be 80 % water, then m= 0.965 mg. Thus , Δ Q = 2.26 J. We now use the average power of the laser, 1.25 W, after noting that the absorbance of gray matter is 42 % at 532 nm. So the time it takes to boil the water contained in the cylindrical volume is approximately 4.3 s, well in agreement with the observed results for brain tissue with no water on the surface. In the case of the collimated (unfocussed) beam, the radius was approximately 2.2 times the focussed beam waist, so m= 5.8 mg, and Δ Q = 10.86 J, leading to an evaporation time of 21 s. The actual time, as determined by the audible "pop", was 25-26 sec. The differences might be attributed to the heating of the vapor to the point of tissue explosion. Sa'ar[3] *et al.* have shown that cavitation facilitates the transmission of laser light in liquids, but in this case the liquid is trapped within the tissue, allowing for localized dessication of the material and explosive liberation of the heated steam, and this was observed in 1064-nm light incident on brain tissue.[1] The clinical importance of understanding such explosive ablation processes is obvious and well documented.[4]

The quick rise in the depth of penetration for the focussed beam occurs around 5 s, due to the explosion of the vapor bubble and the consequent rapid increase in yield. For the unfocussed, collimated beam, the rate of removal also increases dramatically after approximately 20 s, again due to the vapor bubble explosion. It is also worthy of note that the depth of penetration for the focussed beam is expected to saturate after some time, due to the divergence of the beam waist, which leads to a decrease in the fluence and in the removal of new tissue. This is not observed, however, even after 2 min of exposure. The collimated beam diverges only slightly, and it could penetrate the entire brain if the irradiation lasted long enough. This might suggest the use of lenses with short focal lengths, which might lead to lesser damage in the tissue underneath the irradiated area due to their greater divergence with respect to depth and consequent decrease in the fluence of the beam after it passes its focal point.

CONCLUSIONS:

First order observations of the effects of wavelength, pulse length and pulse repetition frequency were reported. Ultraviolet light (308 nm), attuned to the vibrational modes of the gray matter components (such as proteins and the bases of the nucleic acids), showed better ablation profiles, causing deeper etch wells and lesser thermal damage than the 532 nm light. Picosecond pulses (35 ps) created deep, ragged ablation wells, whereas nanosecond pulses (11 ns) made smoother but shallower impressions and caused greater thermal damage. The optimal length ought to be between these two extremes, perhaps around 1 ns. A weaker dependence on thee pulse repetition frequency was observed, in which light at 76 MHz inflicted more surface damage, but did not etch any deeper than light at 10 Hz. The "keyhole" effect was also studied, and an explanation was suggested according to which the morphology is due to the continued convergence of high peak-power laser light within the penetration depth of gray matter. Other factors were also considered in the creation of the ablation well, such as the saline solution and the influence due to the absence of blood flow.

ACKNOWLEDGEMENT

Thank go to Ms. Deborah Hefner for her extraordinary work in the preparation of the slices, and to Dr. Glenn Edwards for the use of the Quantel nano-picosecond laser.

FIGURE CAPTIONS

Figure 1: Comparison of the effect of irradiation with 532 nm light (on the left), and 308 nm ultraviolet light (on the right) on the ablation well morphology.

Figure 2: Comparison of two different pulse repetition frequencies on the desorption of gray matter. The sample on the left was shot at 76 MHz, while the one on the right at 10 Hz (the wavelength was kept constant at 532 nm).

Figure 3: Picosecond-duration pulses of 532 nm light were used to irradiate the sample on the left, whereas nanosecond pulses were used on the sample on the right.

Figure 4: Absorption bands of heparinized rat blood, in a 1:100 saline solution.

Figure 5: Effect of the presence of water on the tissue. The sample on the left was wet and irradiated with 532 nm light at 76 MHz; the one on the right v as dry.

Figure 6: Sub-surface bubble in gray matter, near the edge of the ablation well.

Figure 7: Penetration depth of 532 nm light in gray matter, approx. 0.6 mm.

Figure 8: Illustration of the keyhole effect, created with 532 nm, 10 Hz, light with nanosecond-length pulses.

---

[1] Wharen, R.E. et al. "The Nd:YAG lser in neurosurgery; Part 1". J. Neurosurg. 60, March 1984, pgs. 531-539.

[2] Eggert, H.R. and Blazek, V., Neurosurgery, Vol. 21, No. 4, 1987.

[3] Sa'ar, A. et al. "Transmission of pulsed laser beams through "opaque" liquids by a cavitation effect". Appl. Phys. Lett. 50 (22), 1 June 1987, pgs. 1556-1558.

[4] Jain, K.K. "Complications of the Use of the Nd:YAG Laser in Neurosurgery", Neurosurgery, Vol. 16, No. 6, June 1985, pgs. 759-762.

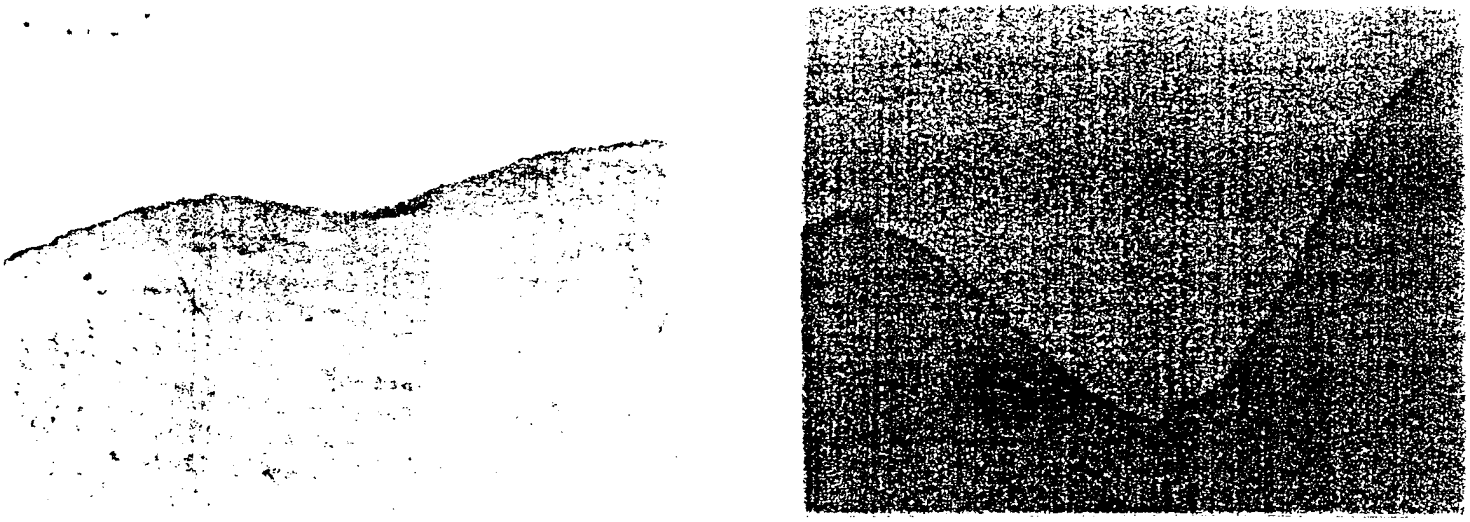


Figure 1. Compari[illegible] (left) vs. [illegible] nm light (right).

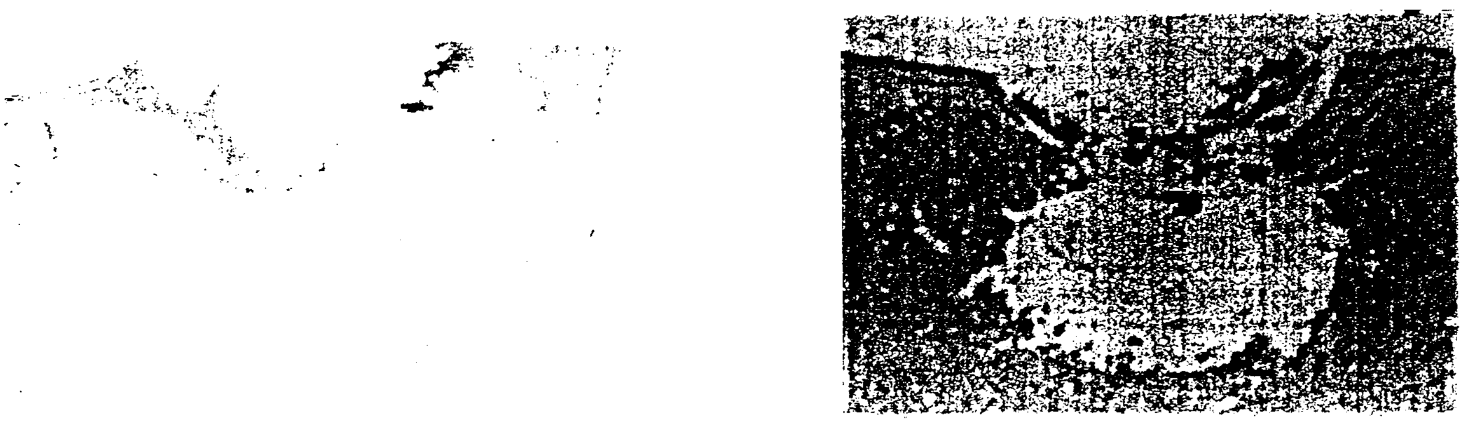
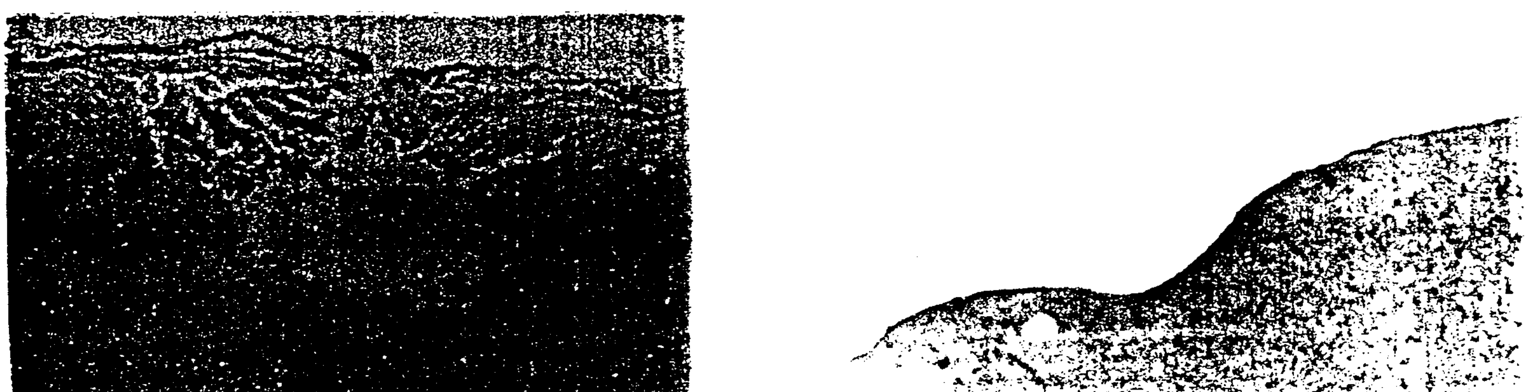


Figure 2. Compari[illegible]



[illegible] Compari[illegible]

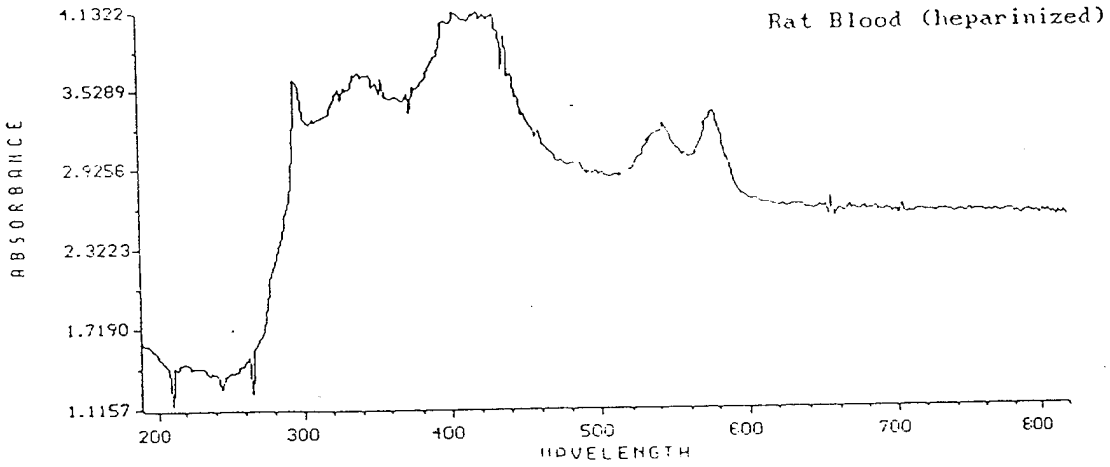


Figure 4. The absorption of heparinized rat blood.

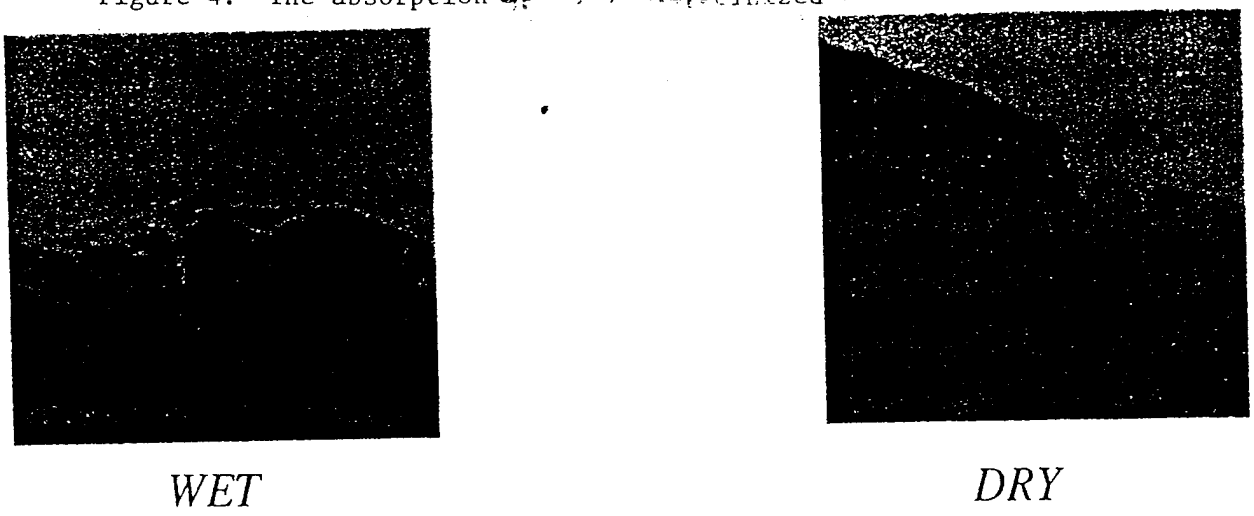


Figure 5. Comparison of ablation wells from wet and dry sites

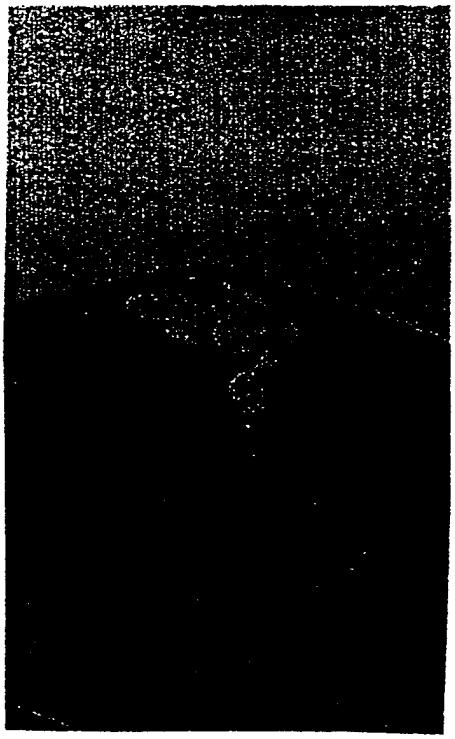


Figure 6. Sub-surface vapor bubble.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (*Leave Blank*)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	See Title Page	1 February 1987 to 31 January 1991

4. TITLE AND SUBTITLE

Use Title on Reprint

5. FUNDING NUMBERS

N00014-87-C-0146
0R0A444C-
43051
43051S4

6. AUTHOR(S)

See Individual Articles

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Vanderbilt University
Nashville TN 37332
(615-322-2786)

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5660

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

Each Paper Summarized on first page. Journal articles submitted as contract reports. All work performed under Government contract.

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (*Maximum 200 words*)

See first page of Article

14. SUBJECT TERMS

Free Electron Lasers Medicine Biology
Biomedical Instrumentation
Energy Cells

15. NUMBER OF PAGES

00

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std 239-18
298-102