Tunable Organic Dye Laser Irradiation of Escherichia Coli.

Patrick Kenji Takahashi
Louisiana State University and Agricultural & Mechanical College

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Takahashi, Patrick Kenji, 1940-
Tunable Organic Dye Laser Irradiation of
Escherichia coli.

The Louisiana State University and Agricultural
and Mechanical College, Ph.D., 1971
Engineering, chemical

University Microfilms, A Xerox Company, Ann Arbor, Michigan
TUNABLE ORGANIC DYE LASER IRRADIATION OF
ESCHERICHIA COLI

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Chemical Engineering

by

Patrick Kenji Takahashi
B.S., Stanford University, 1962
M.S., Louisiana State University, 1969
August, 1971
PLEASE NOTE:

Some Pages have indistinct print. Filmed as received.

UNIVERSITY MICROFILMS
DEDICATION

To:

My wife Pearl, not only for first suggesting the use of a laser, but also for her understanding, encouragement, and physical assistance in bringing the project to this logical commencement.
ACKNOWLEDGMENT

The author is most deeply indebted to Dr. David Greenberg whose guidance and assistance were invaluable in initiating and developing the project to the present state. Sincere appreciation is given to Dr. George Dimopouloos who supervised the microbiological portion of the investigation. Grateful thanks also go to Dr. Louis Rusoff who, along with Drs. Greenberg and Dimopouloos, were instrumental in securing the finances to support the research.

The author also wishes to thank Dr. Bernard Weinstein and Mr. John Leigh of Milk Proteins, Incorporated. The investigation could not have started were it not for their personal interest and subsequent financial grant.

Sincere appreciation is extended to Mr. L. E. Veilleux for his research shop expertise, Mr. James Paulsen for troubleshooting various electronics problems, Dr. Philip A. Bryant for his statistical insight, Mrs. Eleanor S. Thibodeaux for her expeditious typing under somewhat trying conditions, and Mr. C. Richard Panico of the Xenon Corporation for his assistance during the laser construction phase of the project. Appreciation is tendered to Eastman Kodak Company, UV Products, HADRON, New Brunswick Scientific Company, and Goodall Rubber Company for supplying materials and supplies gratis.

Special thanks go to Mr. Harold Toups for materially aiding in the endeavor, and best wishes for continued success with the investigation.
PROLOGUE

It is of interest to present the evolution of this project for two reasons. First, by this means, the reader can more fully appreciate the long term implications instead of being overwhelmed by the minute fundamental details; hopefully, the "can't see the forest for the trees" syndrome will be precluded. Secondly, future graduate students who choose to start a dissertation at the very genesis can observe how this particular one was developed.

The dissertation started with several brainstorming sessions seeking topics that although untrammelled research-wise were showing signs of gaining prominence in the near future. In mid 1968, although pollution was still an accepted way of life and ecology hardly a household word, definite patterns of concern were being formed. All things considered, the general field of water pollution was selected as a start-off point for the project. The initial literature survey revealed that tertiary effluent treatment appeared somewhat open to pioneering investigation. With the above thought in mind, various tertiary treatments were selected and a second literature survey was undertaken. Since reverse osmosis was reduced to the object of searching for a super-membrane and considerable research was already being conducted in the use of nuclear radiation, very little was left of accepted methods of tertiary treatment. As earlier acknowledged, a suggestion of laser radiation was advanced as a possibility.

A third and more exhaustive literature survey was conducted on laser radiation. It was found that research on the microbiological effects of laser radiation were conductive to pioneering investigation.
Thus, a link was established between laser radiation and water pollution. A computerized literature search by the North Carolina Science and Technology Center served to fully cover the fields of laser sterilization of microorganisms and laser radiation in general.

Significantly enough, Kahn and Wiener, in their book *The Year 2000*, selected the use of lasers in two of the five most important areas of future technology. In addition to the general field of lasers was holography, a true-depth three-dimensional means of representation made possible only through the use of a laser. The other three areas were computers, nuclear power, and molecular biology.

Toward the end of the literature survey it became obvious that the nature of the project necessitated some means of financial support. Several sources were considered and private industry was selected as the area for solicitation. A method of saturation letter-writing was used to get in contact with as many corporations as possible that were even remotely concerned with sterilization of microorganisms. An example of a letter of this type is included in Appendix I.

Upwards of thirty-five companies oriented towards laser technology, biological processing and pharmaceuticals responded. Among the responses was a letter from a Pennsylvania-based research and development company. Liaison was subsequently made with Milk Proteins, Incorporated, a company interested in utilizing the laser for sterilization of dairy products. After several months of discussion, success was achieved with a unrestricted $10,000 grant for the purpose of supporting research on the microbiological effects of laser radiation. Several other

feelers indicated that industry in general was willing to finance research at the university level even though the initial investigation was to be devoted to fundamental studies and commercial application of the early work could not reach fruition until many years henceforth, if at all.

Laser technology in mid-1968 was at a stage where continuously tunable lasers were laboratory curiosities and an ultraviolet tunable laser was not yet a reality. Since the continuously tunable laser was still too new a concept to be for sale on the open market, the only available alternative was to build one.

Like any laboratory project devoted to developing a piece of hardware too new for the open market, progress was slow. In January of 1971, nearly a year after the decision to build a laser was made, success was achieved with the lasing of rhodamine 6G. After obtaining lasing action, efficiency and power upgrading attempts resulted in other unexpected problems. Only after acceptable and dependable lasing was achieved could serious thought be given to sterilization and catalysis of microorganisms.
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ABSTRACT

Very little knowledge is available on the microbiological effects of laser radiation. Firm theoretical evidence indicates that certain unique laser properties are ideal for the destruction of microorganisms. This investigation was conducted for the purpose of determining the feasibility of using a laser for the process of sterilization or catalysis.

The lack of data on the subject necessitated initial research at the most fundamental level. The use of tunable laser radiation for microbial sterilization was proposed at a time when the tunable laser was a brand new concept and was not yet available on the open market. Developmental work at synthesizing a tunable laser required an estimated $10,000. Success was achieved at obtaining a supporting grant and a liquid tunable organic dye flashlamp-pumped laser was designed and constructed. The fluorescent dyes rhodamine 6G, rhodamine B, and 7-diethylamino 4-methylcoumarin were lased and tuned. Ranges of wavelengths from 4410\(\text{nm}\) to 4750\(\text{nm}\) and 5567\(\text{nm}\) to 6330\(\text{nm}\) were obtained both in form of broadband and monochromatic outputs.

The most universal and widely studied organism, the *Escherichia coli*, was selected as the contaminant bacterium. In the experimental phase of the project, a twenty-four hour inoculant was irradiated as either a 0.2 or 0.5 microliter drop containing between 10,000 and 100,000 cells. The droplet was enclosed in an air-tight cavity to prevent drying. Various radiant flux densities at different narrowband and broadband wavelengths were used to determine the effect of total energy and wavelength. It was learned that proper alignment
of the laser beam was critical for inhibition of the organism. If the most intense portion of the beam profile missed the microbial droplet almost no effect was observed.

The efficiency of laser radiation comes very close to approximating the effect of conventional light in the visible. However, whereas conventional radiation requires irradiation for as long as four hours, an equivalent percentage kill with visible laser light requires an exposure time of less than one minute. In actuality, the time of exposure of the pulsed laser light is in terms of microseconds. The one minute figure includes the time between pulses, which for the experimental runs, amounted to several seconds. In short, laser light is far more effective than conventional light when the time factor is considered.

The use of exogenous photosensitizers in promoting photodynamic action significantly influenced the effect of the laser beam. It was found that toluidine blue at 5x10^{-6} M increased the susceptibility of E. coli to visible radiation between 5700Å and 6000Å. Acridine orange at 5x10^{-6} M seemed to aid bacterial growth in the 5700-6000Å and 4400-4600Å ranges when the laser beam was not focused and inhibit growth when the beam was made more intense by focusing.

Since radiation at 2650Å is from 10,000 to 50,000 times more efficient than visible light (4000Å-7000Å), it becomes immediately obvious that an ultraviolet laser would be an exceedingly powerful and eminently feasible device for sterilizing microorganisms. The investigation proved that although visible laser light can be used to sterilize biological systems, the development of a tunable ultraviolet laser holds greater promise for future commercial-scale operations.
CHAPTER I
INTRODUCTION

There has been very little research accomplished in the field of laser sterilization or catalysis of microbiological systems. Previous to the writing of this dissertation no work had been reported on the use of a tunable laser for these purposes. Although the ultimate goal of the project is the development of a full-scale chemical engineering process of sterilization or catalysis, the immensity of the work involved necessarily constrained the scope of this investigation to the very fundamental: feasibility study, grant acquisition, tunable laser construction, and programmed irradiation of very simple microbiological systems. Logical extensions aimed at the achievement of a pilot-plant and later commercial process will be undertaken by succeeding investigators. The object of this investigation was to obtain firm evidence supporting the practicability of using a laser for a process of sterilization or catalysis.

Contrary to studies on microbiological effects, a great amount of research has occurred on the general biological effects of laser radiation. Lasers have been used to kill insects, study the effects of in vivo and in vitro tissue cultures, and catalyze the growth of seeds. Furthermore, much progress has been made in the field of medicine, especially in ophthalmology and microcirculation, and to some degree, in an attempt at curing cancer.

A few research teams have reported on the use of lasers in microbiological inactivation. Klein, et al.\textsuperscript{1} irradiated several strains of bacteria photodynamically with a ruby laser as early as 1965.
The same type of research without the use of exogenous photosensitizers was conducted by a French research team. A comprehensive probe into the lethal photosensitization of microbes with light from a helium-neon laser was attempted by MacMillan, et al. Although the French group reported somewhat negatively as to the efficacy of laser radiation, the two studies performed in the United States were quite optimistic. The effects of laser radiation in the red ranged from death of the organisms to partial and no inhibition of the subculture. In all cases the presence of photosensitizers increased the sensitivity of the organism. The Klein group used methylene blue as the exogenous sensitizer and the MacMillan team used toluidine blue. Mention has not been made in the literature on any microbiological catalytic effect of laser radiation.

Stated in other words, the effectiveness of lasers emitting in the visible depends on the absorptive quality of the microorganism. Since most bacteria are rather transparent to visible light, either the addition of an exogenous photosensitizer, like a dye, or the use of an extremely high radiant flux density energy source is required when operating in the visible portion of the light spectrum. A pulsed tunable laser is ideal for visible applications because it combines both essentials. By being wavelength tunable, the laser can be tuned to the exact wavelength at which the photosensitizers absorb. Furthermore, the pulsing nature of the output means that very high radiant flux densities can be placed on the medium for a very short length of time. Harm has shown that a single exposure high dose rate is more effective than fractionated or continuous dosages of the same total energy at killing bacteria.
Ultraviolet radiation is considerably more efficient than visible light in inactivating bacteria. In fact, compared to the threshold of visible light at 4000Å, radiation at 2650Å is 10,000 times more efficient. The ideal laser for microbiological irradiation studies would be a tunable ultraviolet laser.

Although the very first laser was built only a little more than a decade ago, the first conceptual manifestation of stimulated emission goes back to the days of Niels Bohr when he suggested that atoms exist in distinct, separate energy levels and that when they go from one level to another they either emit or absorb these bundles of energy. The original mention of light amplification by stimulated emission of radiation was advanced by Albert Einstein in 1917. Einstein showed by analysis that electrons can be stimulated to jump up or down from one energy level to another by a photon of just the right amount, this amount depending on the frequency. He added that when a photon nudged an electron into dropping from one orbit to another, the electron would also emit another photon of the same frequency; and it would emit that photon in the same direction in such a manner that the emitted photon's energy would be added to the photon that stimulated the action.

However, the problems of developing a satisfactory quantum theory were of such magnitude that until the 1950's stimulated emission phenomenon was neglected by investigators in favor of the more tenable atomic and molecular spectroscopy. Interestingly enough, as early as 1946, a form of stimulated emission was observed, but not recognized. Inverted resonance occurring in nuclear induction experiments resulted in population inversion, one of the necessary conditions for laser
action.

By the early 1950's quantum electronics had reached such a state that three groups of physicists, working independently, proposed the use of stimulated emission for microwave amplification. This development was given the acronym MASER, for microwave amplification by the stimulated emission of radiation. A decade later Charles H. Townes of the United States, and Nikolai G. Basov and Alexander M. Prokhorov of the Soviet Union jointly received the 1964 Nobel Prize in Physics for this and their subsequent work in quantum electrodynamic theory.

Although Townes did succeed in constructing a workable maser at Columbia University, it was not until 1958, when Townes and Arthur L. Schawlow presented a paper on the feasibility of producing stimulated emission near the visible portion of the spectrum, that interest was renewed. Townes and Schawlow did most of the fundamental groundwork exploring the physical conditions necessary for laser action in both the solid and gaseous state. As precursive as Schawlow was, he nevertheless pointed out that a ruby laser would be difficult to produce because the R₁ ruby line terminated in the ground state.

As it turned out, in 1960, Theodore Maiman, Hughes Aircraft Company, used a brute-force technique on a ruby crystal to induce the first recognized stimulated emission. Later in the same year Ali Javan, Donald R. Herriott and William R. Bennett, Bell Telephone Laboratories, developed the helium-neon continuous gas laser. By 1962, various rare-earth and semiconductor lasers were in operation.

In August of 1962 fewer than 20 laser lines were known. Only two years later more than 1000 were known. However, the organic dye
laser was still not a reality. Fully two more years later, in 1966, Peter Sorokin and his co-workers finally reported success at producing stimulated emission from organic molecules. However, to achieve liquid laser action, a giant-pulse ruby laser was used to excite a dye solution of chloroaluminum phthalocyanine (CAP). Somehow, though, a "laser-pumped laser" did not seem entirely scrupulous. A year later a team of scientists at IBM led by Sorokin and John R. Lankard constructed and found success with a flashlamp excited organic dye laser.

The most interesting part about the discovery of the organic dye laser was that unlike the methodical perseverance characterizing the formulation and development of other types of lasers, the organic dye laser was stumbled upon by accident. Sorokin and Lankard were trying to observe the optical effect known as stimulated Raman emission from solutions of organic dye molecules in ethanol. As it happened, the emission obtained with a ruby laser was extremely diffuse at around 755Å. Since the band was diffuse, Raman emission was ruled out and the possibility of incipient laser action was suspected. This assumption was followed by incorporating the proper resonant cavity. The emergence of a powerful laser beam at a wavelength of 755Å along the axis of the secondary resonator serendipitously established Sorokin and Lankard as fathers of the organic dye laser. To this day they are pre-eminent in this field.

Only four years ago, then, was the basic tool necessary for the success of this project discovered. Yet to be realized in 1967 was tuning and frequency doubling into the ultraviolet. The background for these developments will be presented in succeeding sections.
It was the purpose of this investigation to conduct fundamental research on the use of a laser for sterilization or catalysis. It was obvious at the beginning of the project that a tunable laser was the ideal device to study these effects. Unfortunately, tunable laser technology had not progressed yet to the point that a product had reached the commercial market. As the only alternative available, a tunable organic dye laser was constructed by synthesizing components from diverse technical publications.

Specifically, the experimental phase of the study was designed to obtain data relating the efficiency of sterilization with laser output parameters. A preliminary mathematical model was developed in which energy flux, wavelength, and the physical system were utilized to predict the fractional survival of the irradiated organism.


CHAPTER II
THEORY

1. Laser Science

The presentation in this section will be brief and qualitative. The primary purpose will be to furnish the reader with the basics of laser science so that he can have an appreciation for the current state of laser affairs, a feel of how a laser works, and an awareness of how laser radiation differs from conventional light. For the peruser interested in the quantum electronics of stimulated emission, reference can be made to Appendix E. As an aid to definition, a glossary of laser science terms has also been included in Appendix B.

a. Fundamentals of Laser Science

As the laser, by definition, is a device wherein light is amplified by the stimulated emission of radiation, the process of obtaining laser light requires a light source exciting a very special type of material that has the property of absorbing the light in a particular way, and furthermore, in such a way that the emitted light magnifies itself. Thus, there are essentially two requirements for laser action: the particular way of absorption, called population inversion, and the special way of magnification, or stimulated emission amplification.

Population inversion describes a condition where more atoms or molecules of a lasing medium are found in the excited state than in the ground state. This condition occurs when the proper type of light
is absorbed by the right type of material. Given a material, the numbers of atoms in different energy levels are exponentially related. In Figure 1-A, the ordinate E represents the energy levels and the abscissa N the total number of atoms in each energy level. For laser action to occur, a metastable condition called population inversion must be present. Figure 1-B is the electronic conformation after the lasing material has absorbed photons of radiant light and population inversion has occurred. Note should be made of the fact that there are now more atoms in the first excited level than the ground state.

For a ruby laser a flashlamp is used to pump the chromium atoms from the ground state to the second excited state. Although the formula for ruby is Al₂O₃, contained within the ruby crystal are molecules of chromium which are responsible for the laser process. In Figure 2, this excitation is represented by the transition from G to 2. After a buildup of atoms in level 2 spontaneous decay transfers the excited atoms into level 1. A return to the ground state is characterized by both spontaneous and stimulated emission of red light. The stimulated emission portion is the potential source of laser action. The spontaneous emission is wasted as heat.

If one considers the normal situation, light that passes through a substance diminishes in intensity. Stimulated emission, as such, works in just the opposite manner. Using the standard attenuation equation, we have:

\[ I = I_o \exp\left[\frac{(N_2 - N_1)\delta L}{\lambda}\right] \]

where
\[ I = \text{transmitted light intensity} \]
\[ I_o = \text{incident light intensity} \]
FIGURE 1. Energy Level Diagram Before and After Excitation
(M. Guidry, EE 161 classroom notes, 1969)

A. ENERGY LEVEL DIAGRAM BEFORE EXCITATION

B. ENERGY LEVEL DIAGRAM AFTER EXCITATION
FIGURE 2.—Energy Level Diagram of Chromium Ions in Ruby (Lengyel, 1966.)

Excitation

Spontaneous decay

Spontaneous and stimulated emission of light at 6943 Å
\[ N_2 = \text{number of atoms in the higher excited state} \]
\[ N_1 = \text{number of atoms in the lower excited or ground state} \]
\[ \delta = \text{absorption coefficient} \]
\[ L = \text{traverse length of the light beam.} \]

Normally, population inversion is not present and the emerging intensity is smaller than the incident intensity. That is, \( N_1 > N_2 \).

In population inversion, \( N_2 > N_1 \), and the light beam grows exponentially as it passes through the sample.

The second requirement is an amplification process to build-up intensity. In other words, a feedback system with a high \( G \) is required. The gain, \( G \), is defined as

\[ G = \frac{\text{maximum energy stored}}{\text{energy lost}} \]

For laser cavities, that is, the volume inside of which lasing is amplified, mirrors are used on both ends for amplification. Figure 9 in Chapter III is a schematic of a laser cavity. The mirror on one side transmits a certain percentage of light as laser radiation. Only the radiations along the optical axis are emitted as laser radiation because only these are amplified. Radiation in other directions are reflected back and forth within the laser reflector.

The primary source of energy can be a xenon lamp, as for the ruby laser, or electric currents (AC, DC, or RF), as for gas and semiconductor lasers. The efficiency of conversion is only 1% for the ruby laser but as high as 70% for the gallium arsenide semiconductor laser, where efficiency is defined as the ratio of output to input energy. In comparison, the conventional ultraviolet lamp
has about a 20% efficiency.

In summary, practically any material, given sufficient energy and conditions for amplification, can lase. From the first two-level ammonia maser to several hundred now well defined materials capable of inducing laser action, the list will no doubt continue to grow.

b. Unique Laser Parameters

Six properties that can distinguish laser light from conventional light sources are intense radiant flux density, tunable monochromaticity, wavelength selectivity, Gaussian mode structure, coherence, and beam collimation. All these parameters are of utmost significance to the future course of this project.

Radiant flux is defined as the rate at which radiant energy passes through a surface. It is measured in units of power, that is, watts or joules per second. The actual intensity of radiation imparted by a laser is the radiant flux density. It is convenient to measure the radiant flux density in units of watts per square centimeter. Appendix D delves into the details of units used in laser science and radiation microbiology.

When comparing conventional radiation with laser light, a popular method is to relate radiant flux densities at the wavelength of emission. The radiant flux density of an elaborate ruby laser can easily reach tens of thousands of megawatts per square centimeter. All this radiation is in the spectral interval about 0.1 Å units wide. The exact wavelength of emission of a ruby laser is 6943 ± 0.05 Å. Conventional black body radiation whose peak emission is at 6943 Å has a temperature that can be calculated by Wein's displacement law:
\[ \lambda_m T = 2.898 \times 10^7 \text{°K} \]

where \( T \) is the temperature at wavelength of emission \( \lambda_m \). Since \( \lambda_m \) is taken to be the emission wavelength of a ruby laser,

\[ T = \frac{2.898 \times 10^7}{6943} \]

and

\[ T = 4174 \text{°K}. \]

The total power flux is calculated using the Stephen-Boltzmann Law:

\[ W = \int_0^\infty W(\lambda,T) \, d\lambda = \sigma T^4 \]

\[ W = 5.679 \times 10^{-12} \frac{\text{watt}}{\text{cm}^2 \cdot \text{°K}} \times (4174 \text{°K})^4 \]

\[ W = 1730 \text{ watts/cm}^2 \]

However, using the radiation formula for wavelength distribution, only the following power flux falls in the 0.1\( \lambda \) peak around 6943\( \lambda \)

\[ W(\lambda,T) \, d\lambda = \frac{C_1 \lambda^{-5} \Delta \lambda}{\exp(C_2/\lambda T) - 1} \]

where

\[ C_1 = \frac{2\pi hc^2}{\lambda} = 3.74 \times 10^{20} \text{ watts/cm}^2 (\lambda)^4 \]

\[ C_2 = \frac{hc}{k} = 1.438 \times 10^8 \text{ A} \cdot \text{°K} \]

where \( h \) is Planck's constant, \( k \) is Boltzmann's constant, and \( c \) is the velocity of light. Substituting the appropriate numbers, the total power flux is determined to be equal to 0.0164 watts/cm\(^2\). Dividing 10\(^10\) watts/cm\(^2\), the radiant flux density of a ruby laser, by 0.0164 watts/cm\(^2\), the radiant flux density of a conventional source of radiation, the resulting ratio is an almost inconceivable figure approaching one trillion. Comparison with an organic dye laser yields
an equally imposing ratio of approximately 100 million. It is valid to restrict the emission to a narrow bandwidth because future selective sterilization studies will exploit this particular property of the tunable laser.

Laser radiation is the only continuously tunable and yet monochromatic source that has a high radiant flux density. All conventional sources are either essentially polychromatic and therefore complicate a photodynamic mathematical model beyond reasonable utility or tunable only in discrete or low radiant flux density steps. With frequency doubling a non-linear optical process wherein the wavelength is halved, it will be possible to continuously tune a laser from the far ultraviolet through the visible and into the infrared. Furthermore, the laser bandwidth can be as narrow as 0.01Å.

Laser mode structure refers to the pattern of radiant flux density across the cross-section of the beam. The purest form of mode structure results in a Gaussian intensity distribution and is termed TEM$_{00}$ mode. TEM$_{00}$ mode will be seen to be the most ideal for frequency doubling visible light into ultraviolet light.

Coherence can best be explained mathematically. There are two types of coherence, spatial and temporal. One is dependent on the other. Coherence refers to the correlation between phase of monochromatic radiation evaluated at two different points. Electromagnetic radiation is represented by the following equation:

\[ E = A \sin(2\pi ft - k_x x) \]

where

- \( A \) = amplitude
- \( f \) = frequency of radiation
\[ t = \text{time} \]

\[ K_f = \text{spatial frequency, or phase, where } \frac{1}{K_f} \text{ is the period of the spatial frequency, that is, the number of waves in a distance } 2\pi \text{ unit length} \]

\[ x = \text{space distance along optical axis, where } x = 0 \text{ at point of beam emanation.} \]

Laser radiation is coherent, conventional radiation is not. That is, laser radiation has the ability to keep \( f \) and \( K_f \) constant for significant periods and distances. The coherent path length for laser radiation is on the order of \( 10^7 \) meters and coherent time is 0.2 seconds. Conventional monochromatic sources have path lengths of about 0.7 meters and times of \( 10^{-9} \) seconds. This ability of laser light to keep coherence is the principle behind holography and one of the reasons why frequency doubling is enhanced.

Finally, a laser beam does not diverge as much as conventional light because coherent radiation has a lower tendency to diverge. For example, take the case of transmitting a beam of light from the Earth to the Moon and back. One would not succeed with a gigantic conventional flashlight as the beam would diverge rapidly and lose intensity along a constant cross-section. Conversely, specially built lasers can be made to diverge only 1/40,000 of a degree of an arc. It is understandable how transmission over long distances is possible with relatively little loss of power. This last property of the laser will be useful when a counter- or co-current dynamic experimental model is utilized.
c. Types of Lasers

There are essentially four types of lasers. They are:

a) Solid or ionic: ruby, Neodymium-YAG in glass, parametric oscillator

b) Semiconductor: gallium arsenide, gallium phosphide

c) Gas: helium-neon, carbon dioxide, argon, nitrogen

d) Liquid: rare earth, organic dye

The ruby laser was described in some detail in the previous section. The neodymium-YAG laser is normally used as part of the parametric oscillator system. They will both be discussed in a later section on tunable lasers. Semiconductor lasers, although highly efficient, have not proved yet to be of commercial interest because of their low output powers. Furthermore these lasers emit mostly in the infrared.

Gas lasers are rapidly achieving prominence due to their ease of portability, reliability and high powers. The carbon dioxide laser especially has been an intriguing product as the military has for some time now kept its high-powered development confidential. The helium-neon laser is of significance to this project as one is utilized to align the optical components. Argon and nitrogen lasers are characterized by very short pulse risetimes and are gaining prominence as dye exciters in laser-pumped lasers.

The organic dye laser is of primal importance to this project. The next section will present a theoretical discussion on the organic dye laser.

d. Tunable Lasers

There are two categories of tunable lasers. The first category
includes only discretely tunable lasers. That is, these lasers can be wavelength tuned only in discrete steps. A typical discretely tunable laser is a neodymium:YAG laser which can emit more than a dozen different finite wavelengths. This type of laser is analogous to a pushbutton car radio or the VHF channels on a TV set. The other type of tunable laser is a continuously tunable laser. That is, the laser can be tuned to any wavelength. In relation to the analogy of the discretely tunable laser, a continuously tunable laser is like a dial type radio or the UHF section of a TV set.

Three types of continuously tunable lasers are common. They are the parametric oscillator system, the semiconductor laser, and the organic dye laser. There are other types of tunable lasers. For example, Tiffany has reported on the dynamics of selective molecular excitation by laser photocatalysis of bromine reactions. Although he referred to his ruby laser as tunable, the tunability extended only from 694.3 nm to 693.4 nm by cooling with liquid nitrogen. However, it is obvious that in the practical sense this is not acceptable as the tunability range is far too narrow. All the following continuously tunable lasers have ranges of several thousands of angstroms.

1) Parametric Oscillator Laser

In a theoretical sense, the parametric oscillator laser is the ideal tunable laser for this project. However, there are several overriding reasons why one was not used for this investigation:

a) The state of the art is at least one year behind dye laser technology.

b) The cost is prohibitive. When a tunable ultraviolet parametric oscillator system reaches the market, the cost
will be in the area of $50,000.

c) Construction of one is beyond the means of a dissertation in chemical engineering.

It is nevertheless of interest to present a short theory on how a system of this type works. The idea of the parametric oscillator arose through the study of a much contemplated, but never before realized field of physics known as non-linear optics. This is the study of the behaviour of transparent materials subjected to light of such intensity as to change some of the parameters of the material. The following section on second harmonic generation is another type of non-linear reaction. Significantly, these extremely high optical fields can only be obtained from lasers.

A parametric oscillator consists of a crystal, like lithium niobate, which converts a powerful laser beam, as from a neodymium:YAG laser, at one wavelength into another coherent beam of light at another wavelength. In actual practice, the system is far more complicated than as presented.

The only commercial system, marketed by a California firm,* uses a krypton arc lamp to energize a neodymium:YAG rod. Mirrors and a double prism assembly working in conjunction with an acousto-optic Q-switch of brewster-angle fused quartz with a lithium niobate transducer provides "burst" mode operation giving high average output power without high gain and resultant loss of mode control. To tune the laser, the mirror is rotated allowing for oscillation at each of the 13 available wavelengths. This system typically operates at -40°C to depopulate an unwanted lower energy state. Cooling also

* Chromatix, Inc., Mountain View, California
narrow the linewidth and improves efficiency and stability. A look at Figure 3 indicates that the above is only the energizing source. We have yet to even approach the principle of parametric oscillation.

Further along in Figure 3, a lithium iodate crystal is then utilized to frequency double the neodymium:YAG laser output. Whereas the neodymium:YAG output was in the infrared, the output after frequency doubling is exactly at double the frequency or half the wavelength.

Neglecting a discussion of indicated required optics, the doubled output finally reaches the area of parametric oscillation, the lithium niobate crystal in an oven. Tuning is accomplished by varying the crystal’s index of refraction through temperature control. The oscillator simultaneously generates two frequencies, whose sum is equal to the pumping frequency. A tuning range from $3700\lambda$ to $5400\lambda$ is possible using the above system. Tunable ultraviolet outputs are also attainable through the use of a temperature controlled ADP crystal. Eighty milliwatts was obtained by the Chromatix group at $2650\lambda$.

2) Semiconductor Diode Laser

The semiconductor laser is pumped in a different manner from conventional lasers as an electric current is used to inject electrons from low-lying levels of the conduction band to the uppermost levels of the valance band; the frequency corresponds to the band gap energy. The actual lasing conditions, though, follow the normal population inversion/amplification course. Gallium arsenide lasers can be tuned by changing the temperature of the material. The temperature can be altered by modulating the current density. Shorter wavelengths are
FIGURE 3.—Parametric Oscillator System
(Wallace, 1970.)

Rotating concave mirror

Double prism

Nd:YAG rod pumped by krypton arc lamp

Acousto-optic Q switch of Brewster angle fused quartz with a lithium-niobate transducer

Laser output in the infrared

Lithium-niobate frequency doubler

Appropriate filter

Laser frequency doubled output in the visible

Matching lens

Concave mirror

Lithium niobate parametric oscillator in oven

Concave mirror

Laser output tunable in the visible
obtained by increasing the current density.

The lead selenide diode laser has been shown to be eminently tunable by using hydrostatic pressure to alter the band gap. By increasing the pressure up to 14,000 atmospheres, the laser, immersed in a liquid helium bath, was tuned from 75,000Å to 220,000Å.

Progress in the development of visible and ultraviolet tunable semiconductor diode lasers has been very slow. Present technology has not come up with a sufficiently powerful tunable semiconductor diode laser capable of sterilizing bacteria.

3) Tunable Organic Dye Laser

Two types of organic dye lasers are prominent. One type is energized by another laser, usually Nd:YAG, nitrogen, argon, or ruby. The other type is pumped by a fast pulse flashlamp. In general, the output wavelengths of the laser-pumped laser are shorter than that of the flashlamp type. With respect to efficiency, starting with the original energy input, they are about equal. Only the flashlamp-pumped laser will be discussed because it is the type utilized in this investigation. To begin the discussion a short history of the tunable organic dye laser is warranted.

The first published hint of using organic dyes as the active lasing media was proposed by Rautian and Sobel'mann and Brock in 1961. Stockman collaborated on the first real attempt at using a flashlamp to pump the dye perylene. However, the first truly successful effort to produce stimulated emission from organic molecules was reported in 1966 by Sorokin and co-workers. They used a giant pulse ruby laser to excite a solution of the dye CAP.
Similar results were obtained later by many others using cyanine dyes. After discovery of the laser-pumped laser, Sorokin and Lankard, in 1967, built and had success with flashlamp pumped excitation of several xanthene dyes.

Early studies noted the shift in wavelength with dye concentration. Here was a simple, though highly broadband means of obtaining tunability. Farmer further obtained tunability by merely altering the physical length of the dye cell. The most elegant means of tuning the dye laser, and the technique used in this investigation, was demonstrated by Soffer and McFarland in 1967. They replaced the totally reflecting mirror of a laser-pumped rhodamine 6G laser with a diffraction grating. The grating was mounted in the Littrow arrangement and was adjusted to the angle for which the first-order reflection of the desired wavelength was reflected back upon itself along the axis of the optical cavity. Other means of tuning include mixing of dyes, Q-switching, temperature change, pH change, and uses of a Fabry-Perot interferometer, prism, and an echelle-etalon.

The mathematics of organic dye lasers are similar to that of other types of lasers. Much of the discussion located in part two of Appendix E was extracted from Snavely's treatment of the organic dye laser and Keller's work on organic dye laser quenching. In particular, the dye disodium fluorescein salt was utilized to illustrate quantitative use of the important equations with the hope that insight could be obtained into determining why lasing could not be achieved.

An energy level diagram of an organic dye laser is shown in
Figure 4. In the drawing, the heavy horizontal lines represent vibrational states and the lighter lines represent the rotational fine structure. There are three singlet states, electronic ground state $S_0$ and the first and second excited singlet states $S_1$ and $S_2$, and two triplet states $T_1$ and $T_2$. Other higher energy levels are present but are inconsequential to the discussion.

A short pulse flashlamp pumps the molecules of the dye solution from the ground singlet state to higher vibrational-rotational levels of the $S_1$ state—as represented by the transition $A\rightarrow b$. Nonradiative molecular energy decay occurs from $b\rightarrow B$. Both stimulated and spontaneous emission occurs in the $B\rightarrow a$ step. The laser process terminates by the nonradiative decay $a\rightarrow A$.

For lasing action to occur the concentration of molecules in the $S_1$ state must reach the critical inversion value. Whether this value is reached or not depends on the losses inherent in the process. One of these losses is spontaneous decay from $B\rightarrow a$. This is known as fluorescence. Spontaneous fluorescence can override stimulated emission when the dye molecule cannot store enough molecules at the $B$ level. Another very serious loss is the nonradiative relaxation from $B$ to a lower lying triplet state $T_1$, indicated by the dashed line in Figure 4. This process called intersystem crossing is detrimental for it acts as a trap for the excited molecules and depletes the supply of molecules available for the lasing process. The decay process $T_1\rightarrow S_0$ may be nonradiative or radiative, the latter popularly known as phosphorescence.

In order to minimize the detrimental effects of the molecular triplet state it is necessary to reach laser threshold before a
FIGURE 4.—Energy Level Diagram of an Organic Dye Laser. (Snavely, 1969.)
A significant number of molecules have accumulated in the triplet state. This requires either an excitation source with a very quick risetime in the order of one microsecond for most dyes and as short as 100 nanoseconds for others, or an additive such as cyclooctatetraene which acts on the molecular level to quench the triplet state.

Two other losses are triplet-triplet, $T_1-T_2$, and singlet-singlet, $S_1-S_2$, transition. The large optical absorption losses associated with these processes can be strong enough to prevent the laser from operating.

The hardware aspects of the tunable organic dye laser will be covered in a later section. In the meantime, it is only important to state that the dye laser works when a flashlamp emits radiation into a quartz dye-cell containing a dye solution; the dye molecules absorb the light and themselves emit light in such a way that nearly perfectly aligned mirrors bounce the dye-emitted light between the mirrors and amplify the beam within the resonating cavity. A useful laser output is achieved because one mirror is partially transparent and transmits a portion as the laser beam.

e. Second Harmonic Generation

Second harmonic generation (SHG), or its synonym, frequency doubling, is a physical process that takes incident light at one wavelength and converts a portion of the light into a second harmonic of the incident frequency, i.e., doubling the frequency or halving the wavelength. The extremely high germicidal efficiencies of ultraviolet light emitting in the area of 2650Å, combined with the present nonexistence of dye molecules that lase below 3400Å, make SHG an attractive alternative means of obtaining tunable ultraviolet
light. A short discussion of SHG will be presented here for edification purposes.

The principle of SHG was suspected by physicists for some time, and attempts were previously made using carbon-arc and high-pressure discharge lamps to observe non-linear effects. However, for the effects to be realized a great deal of energy had to be concentrated in a narrow band of wavelengths. Until the advent of the laser this has not been possible.

In 1961, a research group headed by Peter A. Franken focused onto a quartz crystal the 6943 Å beam of a ruby laser. Of the light striking the crystal one part in $10^8$ was converted to second harmonic light at 3471.5 Å. Since then progress has been impressive as efficiencies of 40% and greater have been obtained with potassium dihydrogen phosphate (KDP) crystals.

A quantum electronic treatment of SHG can be found in the last part of Appendix E. SHG can be described in the following manner. When normal light waves pass through a transparent crystal the weakly bound valence electrons redistribute themselves in step with the alternating electric field of the light. As long as the light wave electric field is small compared to the cohesive electric fields within the crystal the polarization current faithfully follows the electric field of the light wave. The situation is quite different when very intense light with an electric field comparable to the cohesive local electric fields in the crystal is beamed through the crystal. At $10^6$ volts/cm, there is a massive redistribution of the electrons and the resulting polarization is no longer proportional to the optical electric field. The resultant distorted polarization
gives rise to light containing an overtone, or second harmonic, of the fundamental frequency.

In addition to high intensity, SHG is dependent upon several other unique laser parameters. Fine monochromaticity, that is, a bandwidth of 1Å or less, is up to ten times more effective than broadband power. Furthermore, shorter wavelengths are more efficient by an inverse square relationship. In addition, high directionality is necessary for polarization in the correct optical axis; a laser beam is highly collimated, conventional light sources are not. Finally, the pattern of a beam is important. TEM$_{\infty}$ mode, where the beam profile is Gaussian, is most effective, as the highest concentration of power is located at the middle of the cross-section. A later discussion on mode structure will more fully describe TEM$_{1j}$ terminology.

2. Radiation Microbiology

Coincidentally, radiation microbiology also begins with Einstein. The classical reference in this field, Radiation Biology, by Alexander Hollaender, begins with Einstein's photochemical equivalence law: a photon can induce a photochemical reaction only by being absorbed and, on being absorbed, will activate one and only one molecule. This section will start with a short discussion on the various means of sterilization available and progress to a qualitative analysis of sterilization by irradiation. The Escherichia coli will be used to illustrate most of the radiative effects. A brief treatment of the mathematics involved will serve as background material for a later more detailed presentation on biological mathematical models.
a. Sterilization Technology

Sterilization in a biochemical process can be carried out in a variety of ways:

a) Application of heat; such as in an autoclave.
b) Mechanical removal; such as by ultra filtration.
c) Treatment with chemical agents; as in disinfection.
d) Irradiation with ultraviolet radiation from conventional lamps, high energy Roentgen rays, high energy cathode rays, ultrashort electrical impulses, high frequency sound waves, and laser radiation.

At present continuous sterilization with steam is the most universally used method. However, the disadvantages of steam are manifold. The initial cost of equipment is quite high. Thermal effects on the medium can be serious. Time lag can be a problem. Finally, there is relatively little flexibility - steam kills all living organisms on exposure at 250°F for 10 minutes.²⁴ There is reason to believe that laser radiation can selectively kill certain bacteria and leave other enzymes, bacteria, and the product itself unharmed.²⁵ Differing biochemical structures exhibited by microorganisms suggest that each type of organism has its own characteristic energy absorption signature. It is anticipated that selective sterilization of a contaminant along with the concomitant unrestrained growth of beneficial bacteria can be realized by taking advantage of such absorption patterns.

Furthermore, advantage can be taken of the non-thermal nature of laser radiation. Numerous industrial processes use steam or heat only because it has been the most practical. Although heat serves its purpose by eliminating contaminant growth, high temperatures also tend to degrade the product.
b. Microorganism Inactivation by Radiation

All irradiation methods of sterilization obey the same law of kinetics. The mechanism of kill, almost without exception, follows first order kinetics or the logarithmic law or the general exponential attenuation law. Although this discussion will treat laser radiation in particular, it should be remembered that in general, any form of electromagnetic radiation will be applicable. Laser radiation is different from other types of radiation only in its extreme intensity, monochromaticity, and wavelength selectivity; mathematically, laser radiation is still electromagnetic radiation.

Death itself is a confusing term, as although the normal definition alludes to the cessation of the life supporting processes or the inability to utilize materials and energy, microbiological death is something else again. The literature reports that a bacterium is considered dead if it or its progeny is unable to reproduce. The reason why "death-life" is extended to the next generation is that the inhibiting agent might have acted as the bacterium was undergoing the process of cell division. For the experimental analysis, a readable colony consisted of hundreds of millions of cells. As such, this fine definition of microbiological death does not complicate the study.

When a laser beam is incident on an organism, the biological matter may be modified by a number of physical and chemical processes. These effects include photochemical reactions; evaporation; irradiation by locally generated x-rays, ultraviolet rays, high-energy electrons and ions; acoustic waves; and thermo-chemical reactions. In the
visible, especially with lasers having wavelengths longer than 6000Å, the primary damaging mechanism appears to be thermo-chemical reactions, specifically denaturization of proteins. In the case of ultraviolet radiation, cell death is primarily caused by destruction of the nucleic acids DNA and RNA.

If one compares the action spectra of any live organism with that of DNA, remarkable note can be made of the similarity. Both absorption curves peak at around 2650Å and dramatically fall at longer wavelengths. Since bactericidal efficiency is highest at 2650Å, it is understandable why cell inactivation in the ultraviolet is attributed to absorption and subsequent disruption of the nucleic acids.

1) Anatomy of the *Escherichia coli*

Very early in the project several organisms were considered as candidates for the biological system. Although it was decided that the primary organism should have some industrial significance, it was felt that at the earliest stage of irradiation it was more important to use a simple one organism system in transparent media. Furthermore, the microorganism should be one which is commonly studied so that convenient comparison could be made of the available conventional light irradiated data with the to be obtained laser irradiated data. The universal nature of *Escherichia coli*, both environmentally and research-wise, made this organism a logical choice for the initial irradiation studies.

The following describes the *E. coli*:
Taxonomy

Order ..................... Eubacteriales
Family ................. Enterobacteriaceae
Tribe ................. Escherichieae
Genus ................. Escherichia
Species ................. coli

Cellular Characteristics

Morphology .................. Rods, ranging in size from long to coccoid
Motility .................. Motile and nonmotile forms
Staining .................. Gram negative
Occurrence .................. Singly, in pairs, or in short chains
Size .................. 0.5 by 1.0 to 3.0 microns
Habitat .................. Intestinal tract of human beings

Growth Factors

Oxygen .................. Aerobic, facultatively anaerobic

Temperature

Optimum .................. 30 to 37°C
Range .................. 10 to 45°C

Thermal death time ........ Usually 30 minutes at 60°C

Color Characteristics ........ Some strains produce a yellow pigment

Pathogenicity ........ Usually nonpathogenic but can cause infection of the genitourinary tract; known to cause chronic respiratory disease in chickens

Like in photography, the bactericidal effect of radiation results
from an exposure, i.e., intensity multiplied by time. However, it has recently been observed by Harm\textsuperscript{27} that exposure of \textit{E. coli} at low average dose rates of ultraviolet radiation produced either by fractionated doses or by continuous irradiation at a very low dose rate results in much increased survival compared to an equivalent total energy exposure at a single high dose rate. The significance of this statement is that reciprocity of time and intensity does not hold under certain conditions of irradiation. This phenomenon is of great import to this project as the laser used in the experiments imparted exceedingly high dose rates in a very short length of time. In short, the kill effectiveness of a pulsed laser should be greater than that of a continuous laser or a continuous mercury lamp, all other things being equal.

The basic factors in exposure are the incident power, the time of exposure, and the irradiated area. It is most convenient to use microwatts per square centimeter when referring to contact irradiance and watts per square centimeter when talking about radiant flux density of the laser output. Appendix D provides a complete discussion on units used in radiation microbiology.

Several curves are available illustrating the effect of light on microorganisms. A very useful curve relating the effectiveness of the wavelength of radiation relative to 2650\AA, the optimal germicidal wavelength, is given in Figure 5.\textsuperscript{28,29} Since the tunable laser will emit at various wavelengths in the visible, convenient comparison can be made with the optimal kill wavelength. Figure 6 is an adaptation of several curves from Hollaender,\textsuperscript{28} and when used with Figure 5, direct comparison can be made with the results of the
FIGURE 5.—Effectiveness of Ultraviolet and Visible Radiation on *E. coli* Relative to the Optimal Bactericidal Wavelength at 2650Å. (Luckiesh, 1946.)
FIGURE 6.—Sterilization of Typical Microorganisms Showing Reciprocity of Time and Intensity for Various Exposures. (Hollaender, 1955)

Radiant Flux Density

milliwatts per cm$^2$
of 2537Å radiation

$10^{-6}$ $10^{-4}$ $10^{-2}$ $1$ $10^2$

Exposure Time, seconds

$10^2$ $10^4$ $10^6$ $10^8$

99% killing of resistant fungi
99% killing of E. coli in water
99% killing of E. coli (dry)
70% killing of E. coli (dry)
Different organisms have different susceptibilities to radiation. Table 1 reveals that E. coli is about average in sensitivity to radiation. Energy flux is measured in joules/cm² at 2537Å and is the energy flux necessary to inhibit colony formation in 90% of the organism. If there is any real inconsistency about the table, it is the disparity in energy flux densities required to inhibit the same organism. For example, factors of two differences can be found within Staph. aureus and Staph. albus runs. The variations can be attributed to differences in experimental conditions. The more remarkable outcome is that the energy flux required for 90% inhibition all fall within an order of magnitude from each other. This fact suggests that all living microorganisms are essentially inhibited by the same process at 2537Å. As indicated for Bacillus subtilis, spores are approximately twice as difficult to inhibit as the vegetative organism. Although not included in the table, the resistances of viruses and bacteriophages are comparable to bacteria while molds and yeasts are from 100 to 1000 times more resistive.

Thus, a sterilization study of E. coli should give a good indication of how other bacteria will react to tunable laser light. As such, it is propitious to concentrate efforts on one bacterial organism.

2) Sterilization Kinetics

It has been earlier stated that cell death by radiation is first order. The following discussion examines why this fact is true.

Destruction of complex molecules in any sterilization process may
Table 1

Susceptibility of Typical Microorganisms to Total Energy and Relative to *Escherichia coli*. (Hollaender, 1955)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Energy Flux*</th>
<th>Relative to <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>300x10^{-5}</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Shigella paradysenteriae</em></td>
<td>168x10^{-5}</td>
<td>0.56</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>218x10^{-5}</td>
<td>0.717</td>
</tr>
<tr>
<td>b</td>
<td>260x10^{-5}</td>
<td>0.867</td>
</tr>
<tr>
<td>c</td>
<td>495x10^{-5}</td>
<td>1.650</td>
</tr>
<tr>
<td><em>Staphylococcus albus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>184x10^{-5}</td>
<td>0.613</td>
</tr>
<tr>
<td>b</td>
<td>330x10^{-5}</td>
<td>1.100</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> (mixed)</td>
<td>710x10^{-5}</td>
<td>2.700</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> (spores)</td>
<td>1200x10^{-5}</td>
<td>4.000</td>
</tr>
</tbody>
</table>

* Energy flux was measured in joules per square centimeter at 2537Å.
occur in one of two main ways:

a) Direct activation of the molecule by some external energy source, followed by an internal breakdown without the intervention of other molecules. A reaction of this type is unimolecular. Chemical reaction kinetics show that this type of reaction is also first order.

b) Reaction between a complex molecule in the organism and an external agent. Since the external sterilizing agent is usually present in large excess, the reaction is typically pseudo-first order.

In other words, even if the sterilizing agent is radiation as in the first case or a disinfectant as in the second, the actual sterilization mechanism approximates the first order. Therefore, the change in the number of organisms with time can be related as follows:

\[- \frac{dx}{dt} = k'x\]  

(2-1)

where \(X\) = population of organism  
\(t\) = time since inoculation  
\(k'\) = an inhibition minus growth constant which depends on several microbiological and environmental conditions.

The more important parameters upon which \(k'\) depends include the type of organism; the type of medium, that is, liquid, gaseous, nutrient media, opacity of media; intensity of radiation; wavelength of radiation; environmental conditions such as \(pH\), temperature, humidity; organism growth cycle; and the form of inhibiting agent. If the initial population is taken as \(X_0\), solution of equation (2-1) gives

\[X = X_0e^{-k't}\]  

(2-2)
c. Microorganism Photoactivation

The type of photoactivation reported in the literature normally applies to photoreactivation, a photoprocess where visible radiation in the 420Å to 5400Å range is used to resuscitate organisms that were exposed to and thereby inactivated by ultraviolet radiation. This ability of visible light to cause a photoreversal has been traced to an enzyme that combines specifically with thymine dimers. On exposure to visible light, the dimers, which were initially formed when the organism was irradiated with ultraviolet light (which effectively block reproduction), are cleaved, thus restoring the original thymine residues.

Another type of photoactivation advanced by Dr. Youn Han deals with the acceleration of cell reproduction. Given a system where cells are dividing, an external agent, such as light, can increase the normal reproduction rate by physically enhancing cell division.

A third type of photoactivation depends upon the medium on which the organism is cultured. According to Marshall, the choice of medium probably has the greatest effect on total count. All of the viable organisms that could normally grow don't. The percentage of live organisms that do grow remain relatively constant within the same medium but varies considerably between media. Although McConkey Agar, the medium used to culture the E. coli organism utilized in these irradiation studies, is acceptable for all coliforms, the agar itself is better suited for the Shigella and Salmonella strains. There is sufficient evidence to believe that with photoactivation more than the normal numbers of colonies can be induced to grow. If light was
the agent behind this catalytic effect, experienced would be a third type of photoactivation.

Yet another kind of photoactivation is related to type three. Bacteria, like E. coli, form chains. E. coli group themselves in very small clusters, especially in pairs. If the photo-beam is of sufficient intensity, these clusters can be split. The resultant plate count will be higher as each cluster will form only one colony whereas a split cluster will form as many colonies as there are individual bacteria.

In summary, therefore, photoactivation can result from either physical cleavage, growth acceleration, or activation. In any case, the outcome is more individual bacterial colonies on plate count.

3. Theoretical Irradiation Models

The mathematical models presented in this section will be highly simplified and largely qualitative. An extension of this dissertation to be conducted by a succeeding graduate student will involve a generalized hybrid or digital solution of the total biological model. The mathematical treatment will start with the differential equations describing the transient behavior of a continuous microbiological reactor. The experimental batch model utilized in this investigation will be derived from the dynamic model.

If the bacterial population of the biological system is measured before and after irradiation, the fraction of bacteria surviving the radiation, \( \frac{X}{X_0} \), can be determined. This fraction is largely dependent on three factors: 1) the total energy flux and wavelength of laser
radiation, 2) the survival characteristics of the organism, and 3) the physical system. The object of this section will be to develop the relationship among these three factors.

To simplify the development, as many factors as possible will be kept constant. The organism utilized will be *E. coli* at 24 hours after inoculation; the medium, nutrient broth; the environmental conditions such as pH, temperature, and humidity, constant; and the general experimental conditions, standardized. The proportionality constant, $k'$ from equation (2-2) will be converted to a parametric constant that will vary with the total energy flux and wavelength of the laser beam.

The absorption spectra of the biological organism is a most useful curve to have on hand when relating survival, radiant flux density, and wavelength. These curves are not available for most types of bacterial cells. Fortunately, *E. coli* is a widely studied microbe and adequate curves are available. Figures 5 and 6 are examples of these curves.

Living cells show high absorption in the far ultraviolet region because they all contain considerable amounts of protein and nucleic acid. The ability of a living cell to absorb in the near ultraviolet and visible region primarily depends on the internal presence of dye molecules. These dye molecules, called endogenous photosensitizers if naturally occurring, and exogenous photosensitizers if artificially added, significantly aid in inhibiting cell growth through visible light radiation. A separate section in Appendix F provides a theoretical treatment on the kinetics of such photodynamic type of action.

The *E. coli* contains between 60-90% water, which can be disregarded
as an absorbing substance. The next most common constituent is protein, which accounts for more than 50% of the dry weight. Nucleic acids make up 10-20% of the dry weight, and carbohydrates and lipids less than 10% each.

An action spectra for *E. coli* is shown in Figure 5. Between 2400-3000Å, the primary absorbers are the nucleic acids and protein. The nucleic acids are far more important in cell death because alteration of but one gene or two in the genetic material can adversely affect reproduction. In contrast, loss of a few molecules of protein would rarely have drastic consequences. Pinpointing the exact area of damage, recent studies have indicated that thymine in the DNA molecule is the most important target.

Of immediate interest to the project is the effect of near ultraviolet and visible radiation. Figure 7 is a curve adapted from the data of Luckie\textsuperscript{35} showing the effect of visible radiation relative to radiation at 2650Å. Radiation of 3500-4900Å has been shown by Bruce \textsuperscript{36} to cause leakage of ions, a phenomenon that does not occur too readily in the far ultraviolet. As mentioned earlier, chromophores can be found that absorb visible radiation. Exogenous photosensitizers can be tried and wavelength matched with the tunable laser for maximum absorption, and therefore, maximum bactericidal efficiency.

In general, the experimental work concerned itself with the establishment of precedents on the effectiveness of pulsed tunable laser radiation. The laser wavelength and energy flux were measured with an appropriate instrument and the bacterial survival ratio was determined through standard microbiological means.

Once the effective intensity required to inhibit a set percentage
FIGURE 7.—Effectiveness of Visible Radiation on E. coli Relative to Ultraviolet Radiation at 2650Å. (Adapted from Luckiesh, 1946)
of organisms at a selected wavelength is determined, comparison can be made with conventional radiation. The models will enable calculation of this required intensity, or alternately, given the energy flux and wavelength, predict the population of the organism after irradiation.

a. Dynamic Model

The purpose of the math models will be to predict the concentration of viable organisms knowing radiation conditions and time-growth characteristics of the organism. The manipulative or independent variables are the radiation parameters (wavelength, radiant flux density, and time of exposure), and the time-growth variables (growth characteristics, environmental characteristics, point on the time-concentrations curve at which organism was exposed to radiation, and the susceptibility of the organism to radiation).

Several simplifications are necessary and experimentally feasible. The initial concentration of the organism, \( X_0 \), will be kept constant. Since \( \text{E. coli} \) is the only microorganism in the medium, known and constant will be the doubling time, yield constant, half-life, sensitivity, and mutation rates. For the above variables to be kept constant, the following substrate or environmental conditions must also be kept constant:

a) concentration of limiting substrate

b) incubation time

c) pH

d) temperature

e) pressure
The differential equations describing a continuous stirred tank microbiological reactor are as follows:

\[
\frac{dX}{dt} = k'X = DX - \mu X \tag{2-3}
\]

\[
\frac{dS}{dt} = DS_0 - DS - \frac{\mu X}{Y} \tag{2-4}
\]

where

- \(X\) = cell mass concentration, gm/liter
- \(S\) = residual concentration of substrate nutrient, gm/liter
- \(D\) = dilution rate, or volumetric flow rate divided by the reactor volume, hr\(^{-1}\)
- \(\mu\) = growth rate, hr\(^{-1}\)
- \(Y\) = yield coefficient, gram cells produced per gm nutrient consumed

The growth rate, \(\mu\), has been empirically expressed by Monod\(^{37}\) as a function of the substrate nutrient \(S\).

\[
\mu = \hat{\mu} \left( \frac{S}{K_s + S} \right) \tag{2-5}
\]

where

- \(\hat{\mu}\) = maximum growth rate in the absence of inhibition, hr\(^{-1}\)
- \(K_s\) = saturation constant, numerically equals the lowest concentration of the substrate at which the specific growth rate is equal to one-half the maximum specific growth rate in the absence of inhibition, gm/liter.

b. Static Model

For the static or "batch" reactor the dilution rate, \(D\), is zero, and the descriptive equations simplify to
Andrews developed a model incorporating an inhibitory substrate, and used the following relationship for $\mu$, the growth rate.

\[
\frac{d\mu}{dt} = \sqrt{\mu} \left( \frac{S}{K_S + S} \right) x
\] 

(2-6)

\[
\frac{dS}{dt} = -\frac{\mu}{Y} \left( \frac{S}{K_S + S} \right)
\]

(2-7)

Andrews developed a model incorporating an inhibitory substrate, and used the following relationship for $\mu$, the growth rate.

\[
\mu = \frac{\sqrt{\mu}}{K_S + \frac{HS}{K_i}}
\]

(2-8)

where

$K_i$ = inhibition constant, numerically equal to the highest substrate concentration at which the specific growth rate is equal to one-half the maximum specific growth rate in the absence of inhibition, gm/liter

$HS$ = un-ionized substrate concentration, a pH effect, gm/liter.

Since the experimental conditions kept pH constant and assumed no inhibitory substrate, the descriptive equations revert back to equations (2-6) and (2-7). The inhibitory agent, laser radiation, is quite unlike a substrate type of suppressor. The effect is virtually instantaneous and the result is assumed to be a step-wise reduction in viable organisms. Furthermore, since the immediate study did not limit the substrate, the simplifying result is the equation which defines the microbiological batch reactor.

\[
\frac{dX}{dt} = \mu X
\]

(2-9)

When a laser beam is used to irradiate the microbial reactor, the growth rate, $\mu$, is reduced by the effect of laser radiation Thus
equation 2-9 is modified as:

\[
\frac{dX}{dt} = (\mu - \mu_L)X
\]  

(2-10)

and

\[
\frac{dX}{dt} = -\mu \left( \frac{\mu_L}{\mu} - 1 \right)X = -\mu L_K X
\]  

(2-11)

where

\[
\begin{align*}
\mu_L & = \text{laser radiation kill rate, hr}^{-1} \\
L_K & = \text{laser radiation kill coefficient} = \left( \frac{\mu_L}{\mu} - 1 \right)
\end{align*}
\]

Equation (2-11) can be solved to yield

\[
\frac{X}{X_0} = e^{-\mu L_K t}
\]  

(2-12)

The above equation can be used to determine the fraction survival of the organism at any point in time \( t \) after inoculation of the sample, knowing \( \mu \) and either \( \mu_L \) or \( L_K \), where \( \mu = \mu_L \left( \frac{S}{K_s + S} \right) \) when \( S \) is the concentration of the nutrient media at the time of exposure. \( \mu_L \) and \( L_K \) will depend on the wavelength, radiant flux density, and total exposure time of the laser radiation.

The microbiological system will be exposed to laser radiation at the same point in time after the sample is inoculated. Therefore, \( t \) will be a constant. Since only a relative effect of wavelength and intensity is desired, equation (2-12) can be used to predict the survival ratio of the organism,

\[
\frac{X}{X_0} = e^{-k_1 \psi(\lambda, E_L)}
\]  

(2-13)

where

\[
\begin{align*}
k_1 & = \text{a constant dependent on microbiological parameters} \\
\psi(\lambda, E_L) & = \text{the energy flux dependent on the laser irradiation parameters wavelength and energy flux (equal to } E_L \text{ at the wavelength of emission)}
\end{align*}
\]
\[
\lambda = \text{wavelength of radiation}
\]
\[
E_L = \text{total energy flux, joules/cm}^2
\]

The object will now be to develop a math model based on the experimental system to obtain an equation of the form expressed in (2-13). Figure 8 contains two diagrams depicting the experimental systems used. Part A is with focused beam irradiation and B is with unfocused beam irradiation.

The approach to this static model will be based on the assumption that the droplet and beam will form a lumped parameter system. The discussion will bear out this premise.

Figure 6 can be transformed into equation form. The result is

\[
\frac{X}{X_0} = e^{k_c I_c L}
\]

where \( k_c = 0.398 \), microbiological kill factor for conventional light sources
\( I_c \) = radiant flux density, mw/cm²
\( t_L \) = radiation exposure time, seconds

Since equation (2-14) is specifically for radiation at 2537Å, a wavelength correction factor is required to enable usage at any wavelength.

\[
\frac{X}{X_0} = e^{-0.398 I_c t_L W(\lambda)}
\]

where
\( I_c t_L \) = exposure of conventional light at any \( \lambda \), mj/cm²
\( W(\lambda) \) = relative effect of wavelength

For E. coli, \( W(\lambda) \) can be represented by Figure 5. If only visible radiation is used, Figure 7 is an accurate representation of \( W(\lambda) \).

The product \( I_c t_L \) is equal to the total energy placed on the drop.
FIGURE 8. --Microbiological Irradiation Systems.

A. FOCUSED BEAM IRRADIATION

B. UNFOCUSED BEAM IRRADIATION
If a powermeter is used to measure $I_c t_L$, measurement would be in joules/cm$^2$. In the absence of accurate powermeter readings, $I_c t_L$ can be approximated by relating the pertinent irradiation variables.

To obtain the relative power output from differing lasing systems, a comparative equation will be developed. The starting points for this equation are the general energy equation, $E = \frac{1}{2} CV^2$, the laser input, and the laser parameter values most used in the experimental runs. These values are: 1) kilovolt setting on voltage supply, 10KV, 2) bore diameter of flashlamp, 5mm, 3) bore diameter of dye cell, 3mm, 4) reflectance of front end mirror, 0.5, 5) dye used, rhodamine 6G, 6) concentration of dye, $3-4 \times 10^{-4}$ M, 7) reflectance of mirror placed behind the droplet, 1.0, and 8) the laser efficiency at the above conditions.

The energy flux emitted by a laser is equal to the input energy multiplied by an efficiency factor and divided by the cross-sectional beam area.

$$E'_L = \frac{1}{2} CV^2 \phi_L P_u \left( \frac{4}{\pi d_d^2} \right)$$

where

- $E'_L =$ total energy flux emitted, joules/cm$^2$
- $C =$ capacitance of micropulser, farads
- $V =$ voltage across flashlamp, volts
- $\phi_L =$ laser efficiency of energy conversion
- $P_u =$ number of pulses
- $d_d =$ bore diameter of dye cell, cm.

In expanding equation (2-16), the voltage ratio, $\left( \frac{V}{10^4} \right)$, should be squared because output energy flux varies as the square of the
input voltage. The flashlamp ratio, \(\frac{d_f}{d_0}\), should be taken to the minus two power because the output energy flux increases with the square of decreasing flashlamp bore diameter. The exponent for the dye cell ratio, \(\frac{d}{d_0}\), will be equal to \(m\). The type and concentration of the dye used to promote lasing action also should be included in the equation as some dyes were easier to lase than others. The values for these factors, \(D_y\), for the type of dye, and \(C_D\), for the concentration, will be given in a later discussion. The three mirrors used in the irradiation system also should be incorporated as they directly determined the amount of energy contacting the microbial droplet (See Figure 8). The mirror factor behind the droplet is actually a ratio, \(\frac{M_g}{1}\). As \(M_g\), the mirror reflectance is increased, more light should be reflected back on the drop. The back end optics ratio, \(\frac{M_e}{d}\), depends on the number of effective reflections on mirror \(M_e\). For the present, the exponent will be \(n\). The front end optics ratio, \(\frac{1-M_e}{d}\), should also be a linear relationship because the light output is directly proportional to the percent transmission of the mirror. Focused light should have an effect on the energy flux as the beam is made more concentrated with convergence. At this time, the focused light factor will be taken as \(f_o\).

Inclusion of the appropriate comparative ratios expands equation (2-16) to

\[
E_L = \frac{kCV^2\phi P}{\pi d_d^2} \left(\frac{4}{\pi d_d^2}\right)^2 \left(\frac{d_f}{d_0}\right)^m \left(\frac{d}{d_0}\right) (C_D) (M_R) \left(\frac{1-M_e}{d}\right) f_o \tag{2-17}
\]

where \(E_L\) = total energy flux emitted by laser, joules/cm\(^2\)

\(V_K\) = power supply voltage, kilovolts

\(d_f\) = bore diameter of flashlamp, cm.
\[ D_Y = \text{dye used (see following discussion)} \]
\[ C_D = \text{concentration-threshold factor dependent on concentration of dye (see following discussion)} \]
\[ M_R = \text{reflectance of mirror behind the drop} \]
\[ M_B = \text{factor depending on optics used at back end of cavity; numerically equal to mirror reflectance} \]

- 100% reflecting mirror = 1.0
- Diffraction grating = 0.8 (PTR Data sheet \textsuperscript{39})
- 90\% reflecting mirror = 0.9

\[ M_F = \text{factor depending on optics used at front end of cavity; numerically equal to mirror reflectance.} \]

\( n \) and \( m \) are parameters to be determined.

The dyecell ratio was not squared because although a larger dyecell absorbs more radiation as a square of the diameter, larger diameters require passage of pumping light through more dye solution, thus reducing the lasing effect. The two conditions counteract each other, and the overall effect is approximately a linear increase in output energy with increasing dyecell bore diameter. As a result, a reasonable value for \( m \) is one.

The type of dye used significantly affected the energy flux. Rhodamine 6G is an extremely easy dye to lase. The efficiency of conversion is very high. For this investigation, it was found that the kilovolt lasing threshold of rhodamine 6G was approximately one-half that of coumarin. Knowing that energy flux varies with the square of the input voltage, \( D_Y \) should be set to 1.0 for rhodamine 6G and \((0.5)^2\) or 0.25 for coumarin.

The concentration of the dye solution affected lasing conditions. A later discussion on dye solutions will detail this relationship. For the present, it can be stated that a dye concentration of \(3.5 \times 10^{-4}\) was
the optimal although concentrations between $3 \times 10^{-4}$ M and $4 \times 10^{-4}$ M did not require an attenuation factor. Concentrations other than this range required a factor equal to the square of the ratio between the optimal concentration voltage threshold and non-optimal concentration voltage threshold.

The back end optics ratio, $\left(\frac{R}{.9}\right)^n$, depended on the number of times the beam reflected off the back end mirror. Dr. Forbes Dewey of MIT indicated that six passes through an organic laser dye cell was expected for normal lasing conditions. The number of reflections would therefore be three. Thus, $n$ was set equal to three.

When the focused laser beam method was used to irradiate the droplet, several factors had to be considered. If the focused beam is smaller than the droplet size, the area of the beam should be divided by the area of the droplet. Proceeding along the focused beam it is clear that the energy flux (energy per unit cross section) increases as the cross-section of the beam decreases, as such the focus factor, $f_o$, should be made equal to the ratio of the cross-sectional area of the beam before focusing to the area of the beam at droplet contact. When the beam is not focused, $f_o$ should be made equal to 1.0. The final energy flux equation is now

$$E_L = \frac{4\pi V^2 \phi_L P}{4\pi d_d} \left(\frac{4}{10}\right) \frac{V}{k} \frac{5}{d_f} \left(\frac{d}{3}\right) D_Y \left(C_D\right) \left(M_R\right)^{3} \left(\frac{1-M}{.9}\right) f_o \quad (2-18)$$

Equation (2-18) gives the total energy flux output of lasing during an irradiation run. The experimental results will also yield a value for the fraction survival, $\frac{x}{x_o}$, of the irradiated organism. Looking at equation (2-13), we now know $\frac{x}{x_o}$ and $\Psi$. $k_L$, the microbiological constant can therefore be calculated. It will be of
interest to compare the value of \( k_1 \) obtained in the laser irradiated experimental runs with an analogous \( k_c \) for conventional light irradiation. Such a comparison can be made through the use of equation (2-15). In equation (2-15), the exposure, \( I_c t_L \), is analogous to \( E_L \), the total energy flux emitted by the laser. Letting \( I_c t_L \) equal \( E_c \), the total energy flux through conventional irradiation, we then have

\[
\frac{X}{X_0} = e = e \quad (2-19)
\]

Equation (2-19) is now of the form of equation (2-13), the theoretically derived laser irradiation model:

\[
\frac{X}{X_0} = e = e \quad (2-20)
\]

the factor of 1000 being necessary because \( E_c \) is measured in milli-joules/cm\(^2\) and \( E_L \) in joules/cm\(^2\). When the effect of laser radiation is later contrasted to conventional light, the actual comparison will be between the effective kill factors, 0.398 and 1000 \( k_1 \). Equation (2-20) can now be written as follows:

\[
\frac{X}{X_0} = e = e \quad (2-21)
\]

where \( k = 1000 k_1 \).
LITERATURE CITED


32. Han, Youn, private communication.


CHAPTER III
EXPERIMENTAL WORK

1. General Plan of Work

An overview of the entire project is presented in Figures 9A and 9B. Figure 9A, the work accomplished in this dissertation, covers the areas up to June 1971. The basic fundamental work will continue up to June 1972 when attention will be shifted to the actual development of a pilot-plant process of sterilization. As mentioned earlier, succeeding graduate students will continue the work, as the final goal of obtaining a working chemical engineering process of sterilization or catalysis is at least two to five years in the future.

The objective of this investigation as contained in this dissertation was to obtain tangible evidence supporting the feasibility of utilizing a laser for sterilization or catalysis. The product of this dissertation should be fuel for the acquisition of a more substantial grant to develop actual pilot-plant and full-scale commercial processes.

2. Synthesis of Experimental Apparatus

Early in the project, the concept of a tunable laser stood out as the optimal laser for the type of investigation anticipated. In March of 1970, an acceptable continuously tunable laser was not yet on the market. The only forms of tuning possible at this time were:
FIGURE 9A. Overview of Project on the Use of Laser Radiation for Sterilization and Catalysis of Microbiological Systems

- Literature survey on laser technology
- Areas for further investigation
  - LASER TECHNOLOGY
    1) laser construction
    2) tunable lasers
    3) uv lasers
  - RADIATION MICROBIOLOGY
    1) sterilization
    2) computer kinetics
    3) catalysis
  - GRANT SEARCH
    1) saturation correspondence
    2) industrial survey
    3) evaluate feelers
  - MICROBIOLOGICAL SYSTEM
    1) decide on microbiological system
    2) learn analytical techniques
  - MATHMATICAL MODELS
    1) static model
    2) dynamic model

- Buy or build a tunable laser
  - BUY
    No, none on the market at this time
  - BUILD
    Yes, order hardware and optics
- Obtain initial grant
- Obtained initial grant
- Obtained wavelength and energy flux measurements
- Irradiation of E. coli with visible laser radiation
- Test the validity of mathematical model

- Areas for further investigation
  - E. coli in nutrient media selected as system
  - Decision to use microbiological batch reactor model
  - Obtaining initial grant
  - Literature survey on laser technology

- Areas for further investigation
  - Decision to use microbiological batch reactor model
  - Irradiation of E. coli with visible laser radiation
  - Achieve tunable lasing
  - E. coli in nutrient media selected as system
  - Decision to use microbiological batch reactor model
  - Obtain wavelength and energy flux measurements
  - Test the validity of mathematical model

- Areas for further investigation
  - Obtain wavelength and energy flux measurements
  - Irradiation of E. coli with visible laser radiation
  - Achieve tunable lasing
  - E. coli in nutrient media selected as system
  - Decision to use microbiological batch reactor model
  - Obtain wavelength and energy flux measurements
  - Test the validity of mathematical model

- Obtain a working math model that can predict the survival of E. coli after visible laser irradiation
FIGURE 9B: Overview of Project on the use of Laser Radiation for Sterilization and Catalysis of Microbiological Systems

Using the data of PART I, obtain additional funding

**LASER DEVELOPMENT**
- Modify laser system to obtain quicker risetimes and power capability to 200 joules

- Achieve frequency doubling

**MICROBIOLOGICAL SYSTEM**
- Irradiation of other bacteria
- Investigate selective sterilization and catalysis and determine optimal kill wavelength of selected bacteria

**COMPUTER MATH MODEL**
- Develop general math model incorporating time
- Computer applications of generalized model

1) Formulate plans for construction of pilot plant
2) Decide on microbiological process

Construct and de-bug pilot plant

Formulate plans for construction of commercial-scale process

Construct and de-bug commercial-scale laser irradiation process
a) Discrete tuning: for example, RCA had a model that could emit radiation at 3507, 3564, 4762, 4825, 5208, 5682, and 6471 angstroms, but only in these discrete lines; furthermore, the cost was $19,000.

b) Temperature tuning: Tiffany\(^1\) reported the use of liquid nitrogen to cool a ruby laser to 77°K and thereby obtain a tuning range from 6943\(^\text{Å}\) to 6934\(^\text{Å}\); however, this range of 9\(^\text{Å}\) was hardly acceptable ... desired was a tuning range in thousands of angstroms.

Several other means of tuning have been reported in the literature. Of these, the two most promising were the parametric oscillator system and the organic dye laser. Unfortunately, none of these lasers were yet for sale. The only reasonable alternative then was to construct one. Contact was made with Dr. Stephen Harris of Stanford University, the developer of the parametric oscillator system that was soon to be marketed. Although encouragement was given by Dr. Harris, the complex theory involved combined with the predicted exorbitantly high costs quickly cast aside any thoughts of building a laser of this type. In the meantime, communications with developers of organic dye lasers were encouraging enough to decide to build a tunable liquid organic dye laser. The estimated costs were within the $10,000 budget limit and the tremendous progress in this area gave promise for the possibility of attaining tunable ultraviolet laser radiation.

Although the basic design of the laser was tailored after the Lankard scheme,\(^2\) significant help came from Boris Kim of The Johns Hopkins University (APL)\(^3\) who sent information on a high efficiency laser cavity reflector, and C. Richard Panico of XENON Corporation\(^4\) who offered manifold suggestions on assorted aspects of the construction. The three main areas of the construction were the overall system design, alignment, and measurement.
a. Overall System Design

Figure 10 shows that the organic dye laser consists of an amplifying tube, called the dyecell, through which the dye solution is circulated; a second tube, the flashlamp, which radiates intense light into the dyecell; an elliptical reflective cavity which concentrates the flashlamp light into the dyecell; a pair of plane mirrors that are aligned parallel to each other and perpendicular to the optical axis of the dyecell; and a switching-power supply unit which excites the flashlamp. Figure 11 is a photograph of the above assembly and Figure 12 is a photograph of the entire dye laser system including alignment laser, circulation pumps and micropulser.

The dyecell and flashlamp are located at the foci of the elliptical reflector. Maximum concentration of light into the dyecell is achieved through this means as from a theoretical standpoint all the light that leaves the flashlamp reflects back into the dyecell.

A solution of fluorescent dye in alcohol passes through the dyecell. When excited by light, some of the dye molecules absorb the light and are raised to an excited singlet state. A fraction of a second later these molecules emit the excess energy as light. Quite by chance some of these emitted light photons head toward one of the mirrors, which reflects the photons back into the amplifier tube. Passing back through the dyecell the photons stimulate other excited molecules. The resulting emission is identical in wavelength and direction of motion. Continuing its path, the amplified light is reflected off the other mirror and reinforces itself by repeating the process. As the light wave is reflected back and forth through the

Dye solution reservoir, heat exchanger, and pump

Dye in

Dye out

100% reflecting mirror

Elliptical polished reflector

Partially transmitting mirror

Laser beam

Flashlamp

Cathode

Anode

Cooling water in

Cooling water out

Power supply

0-10KV

115VAC

Cooling water reservoir, heat exchanger, and pump
FIGURE 11. Organic Dye Laser Cavity
FIGURE 12. Organic Dye Laser and Peripherals
tube it accumulates energy. One of the mirrors is partially transmitting and lets pass a portion of the emitted radiation. This transmitted light is the laser beam.

Several requirements are necessary for the events just described to occur. First, the flashlamp light energy must be intense enough to excite the dye, and quick enough to avoid depopulation of the excited state before population inversion can occur. Minimum limits on these requirements will be discussed later when dyes are considered. Secondly, the flashlamp must be physically constructed such that the bore diameter is equal to or larger than the dyecell bore. If this criterion is not met, lasing can be achieved only with great difficulty, and even if achieved, the output beam profile will be annular shaped. The flashlamp arc length should be close to the dyecell length. However, it has been reported that adverse thermal effects can be prevented if the arc length is slightly shorter than the distance from window to window of the dyecell.

All transmitting parts should be made of fused quartz, Spectrosil grade being superior to Vitreosil (Thermal American Fused Quartz Company trademarks*). The reason for this requirement is that the organic dye is more easily excited by the ultraviolet portion of the flashlamp output. Most types of glasses do not effectively transmit ultraviolet radiation. Figure 13A represents the spectral output of the xenon filled flashlamp used in this investigation and Figure 13B the transmission versus wavelength curves for window glass, pyrex, and fused quartz.

* Thermal American Fused Quartz Company, Rt. 202 and Change Bridge Rd., Montville, New Jersey. (See Appendix H for a list of pertinent vendors).
FIGURE 13A. Spectral Output of a Xenon Filled Flashlamp. (Xenon Corporation, 1970.)

FIGURE 13B. Transmission Versus Wavelength Curves for Various Transparent Materials. (Thermal American Fused Quartz Company and Koller, 1965.)
The requirements on the cavity reflector are not as critical as those above. Anything from close-coupled aluminum foil to white paper suffices, the object being to keep the flashlamp radiation within the cavity. For the highest efficiencies an elliptical mirror reflector is ideal. Dr. Boris Kim of the Applied Physics Laboratory of The Johns Hopkins University, suggested the use of an ellipse cross-section with the axes close to five inches to take advantage of optimal eccentricity conditions. An elliptical mirror reflector is most easily constructed by cutting a five inch I.D. aluminum pipe to the correct length and squeezing in a vise to the correct minor axis diameter. It is not clear in the literature, but a convenient means for calculating the proper dimensions of the ellipse is as follows: (See Figure 14)

a) Position the water-jacketed flashlamp and dyecell parallel and as close as operationally possible to each other. Measure the cross-sectional center-to-center distance between the dyecell and flashlamp. This measurement will be $F_0$, the distance between the foci. From mensuration tables for the ellipse,

$$F_0 = 2\sqrt{a^2 - b^2}$$

(3-1)

where

$a$ = one-half the major axis

$b$ = one-half the minor axis

b) The circumference of the ellipse is exactly equal to the circumference of the circular pipe:

$$C_E = C_c = \pi D_c$$

Where subscript $E$ refers to the ellipse and $c$ to the circular pipe. The circumference of the ellipse is also approximately equal to the following:
c) Since $F_0$ and $D_c$ are known quantities, solving equations (3-1) and (3-2) simultaneously yields,

\[ b = \frac{1}{2} \sqrt{\frac{D_c F_0^2}{2}} \quad (3-3) \]

\[ a = \sqrt{\frac{D_c^2}{2} - b^2} \quad (3-4) \]

The circular pipe is squeezed until the major axis, $2a$, and minor axis, $2b$, measure to be the values calculated. As a convenient check, the latus ractum, $L_R$, can be physically measured and compared to the calculated value as follows,

\[ L_R = \frac{2b^2}{a} \quad (3-5) \]

If the ellipse is correctly constructed, all the light emitted at $F_1$ will either pass directly through $F_2$, or reflect off the elliptical mirror and pass through $F_2$.

The inside of the elliptical aluminum pipe should be lined, coated, or polished. Since the flashlamp spectral output is mostly between 2500Å and 4000Å, the ideal material for this lining is aluminum. Figure 15 shows that aluminum is superior to silver for this particular application. The reflector lining used was aluminum specular lighting sheet. Figure 16 is a photograph showing the side view alignment of the dyecell and flashlamp without the reflector and Figure 17 is an endview showing the cavity but minus one end.

The dyecell can be purchased from most outfits specializing in quartzware for approximately $90. An equivalent dyecell can be
FIGURE 15. Percent Reflectivity Versus Wavelength for Aluminum and Silver. (Koller, 1965.)
FIGURE 16. Side View of Laser Cavity Showing Flashlamp and Dye Cell Configuration (Minus Elliptical Reflector)
FIGURE 17. End View of Laser Cavity Showing Flashlamp, Dye Cell, One End, and Elliptical Reflector
constructed for less than $2 if glass windows are used and for $4 if quartz windows are required. A special type of glue containing polyvinyl chloride resins and methyl isobutyl ketone solvent proved to be best suited for the solvent conditions. Epoxy was found to be completely unacceptable as the alcoholic dye solution dissolved the bonding in time. Figure 18 is a drawing of the 3mm dyecell used in this investigation. The home-made and purchased varieties performed equally well with regard to efficiency.

The available literature recommended the use of a linear flash-lamp system with a very quick flash risetime on the order of one microsecond. Several commercial sources were investigated and one package stood out as the most suitable. The flashlamp, micropulser, and voltage supply were all purchased from the Xenon Corporation. The Xenon system promised a risetime of 0.7 microsecond and capability for increasing the power input from the initially available 10 joules up to 100 joules.

The original micropulser was rated at 10 joules and the compatible flashlamp was the Novatron 790 with a 3mm bore diameter. Subsequent ten-fold upgrading of the energy capacity was obtained by replacing the 0.2 microfarad capacitor with a 2.0 microfarad capacitor. The compatible flashlamp for the 100-joule system was the Novatron 851 with a 7mm bore. To maximize the power density, a Novatron 850 with a 5mm bore was used. The energy density of a flashlamp is inversely proportional to the square of its bore diameter. As such, a 5mm bore flashlamp is approximately twice as intense as a 7mm bore flashlamp with the same total energy output.

As mentioned earlier, for maximum utilization of the flash, the
a) All parts constructed of quartz, Spectrosil grade preferred.
b) Windows should be as closely parallel to each other as possible.
c) Windows should be set perpendicular to the optical axis.
d) Windows can be fused or cemented to dye cell body.
flashlamp should have exactly the same bore diameter as the dyecell. However, to lower the lasing threshold, a smaller bore dyecell works better as the flashlamp radiation need not pass through as much dye solution. Furthermore, mechanical construction inaccuracies are minimized by keeping the dyecell bore slightly smaller than that of the flashlamp.

Cooling water was required to remove the heat generated by the flashlamp. The very high voltages necessitated the use of distilled water. If regular tap water was used the ions in the water would have conducted some of the current and thereby reduce the flashlamp intensity. An improvement in voltage across the flashlamp from 8.5 kilovolts to 10 kilovolts was experienced when distilled water replaced tap water. Chilled water at 50-55°F proved optimal; higher temperatures shortened the life of the flashlamp and lower ones reduced the efficiency of the flashlamp.

The optics involved in the investigation proved to be the one most irritating source of trouble. The early literature recommended the use of aluminum coated mirrors and diffraction gratings. As later learned from experience, aluminum coated optics were inadequate as they tended to burn under high light intensity operation and absorbed several percent of the radiation in transmission. Fortunately enough, silvered mirrors were not used as they absorb as much as 15% of the radiation at the lasing wavelengths of rhodamine 6G and 25% at the coumarin lasing level. Hard dielectric coatings were later found to be the best available. These coatings absorbed only about 0.1% of the radiation and were shown to be far superior to aluminum in withstanding intense radiation. It has been reported that
radiant flux densities up to one megawatt/cm$^2$ can be tolerated by
dielectric coatings.

The section on alignment will provide the details of each
particular optical component. For the present it is sufficient to
state that the reflective parts consisted of a totally reflecting
surface and a partially reflecting surface. The totally reflecting
surface was either a broadband 100% reflecting mirror or a reflecting
diffraction grating, the former for broadband outputs and the latter
for narrowband tuning. The transmitting surface was a dielectric
coated mirror which reflected from 40% to 91% of the incident light,
depending on the mirror and wavelength. Since the coating absorbed
only 0.1% of the energy, transmittancies of from 8.9% to 59.9% were
typical. If the 90% reflectance mirror was used, this meant that
approximately 9.9% of the light was transmitted as the laser beam
and 90% was reflected back into the cavity to generate gain. Again,
an optimal relationship determined output power. As the reflectivity
of the mirror increased, the dye could be more easily lased, i.e.,
with lower voltages, but the percentage transmission dropped. As
the reflectivity decreased, more of the cavity energy was transmitted;
however, a higher threshold voltage was required to induce lasing.

b. Dye Solution

The characteristics desired for the dye solution used in an organic
dye laser were reported in Chapter II. In recapitulation, the dye
must easily be excited into the lowest singlet state without
experiencing much intersystem crossing into the triplet state and with
sufficiently long lifetimes in the singlet state. The dye should be as
pure as chemically possible and dissolved in a solvent of the proper transmission properties. The solution should not contain bubbles or contaminant particles. Seemingly innocuous impurities can seriously decrease fluorescence yields. A filter in series with the flow was used to eliminate errant dust particles.

As for the laser flow apparatus, all materials touching the dye solution had to be of a type which had no plasticizers in it. In more flexible tubing like Tygon, there are softening agents which are leached out by the solvent and thus constitute an impurity in the solution. All tubing used in the apparatus was of polyethylene.

Dye performance varied with temperature, solvent, concentration, and pH. In general, colder temperatures improved lasing action and very hot solutions prevented lasing from even occurring. Methanol, ethanol, and distilled water were the three most universal dye solvents. Methanol worked better for rhodamine 6G than ethanol; the reverse was true for the dye 7-diethylamino 4-methylcoumarin. The optimal concentration was found by determining the threshold lasing condition for each system. For example, using a 20 joule capacity system, 90% reflecting mirror, 3mm bore flashlamp and dye cell, the curve in Figure 19 was obtained for rhodamine 6G in 100% methanol.

The optimal concentration varied with the lasing system. For rhodamine 6G in methanol, the system that was used in the biological irradiation experiments, the optimal concentration was found to be between $3 \times 10^{-4}$M and $4 \times 10^{-4}$M.

Three dyes were found to lase very well with the constructed system. They were rhodamine 6G, rhodamine B, and 7-diethylamino 4-methylcoumarin, with the first-mentioned the easiest to lase and the
FIGURE 19. Curve to Determine the Optimal Lasing Concentration of Rhodamine 6G.

CONCENTRATION OF RHODAMINE 6G, Molarity
last-mentioned the most difficult. A very important dye, disodium fluorescein salt could not be lased. This dye normally lases close to 5300Å. This wavelength is of great significance to the project because 5300Å frequency doubled is 2650Å, the optimal bactericidal wavelength. Furthermore, the KDP crystal used to generate the second harmonic was cut for 5300Å.

The dye 4-methylumbelliferone has been reported in the literature to be tunable from 3650Å to 5740Å by merely adjusting the pH. However, the dye was pumped by a nitrogen laser with a pulse risetime 100 times quicker than the flashlamp used in this project. In fact, it has been suggested that the slow risetime of the flashlamp was responsible for failure to lase dyes like fluorescein and 4-methylumbelliferone.

The dye known to lase with the shortest wavelength is p-terphenyl in dioxane, which lases in the area of 3400Å. One of the longer emitted wavelengths, 8300Å, comes from scintillating 3-ethyl-2-[7-(3-ethyl-2-benzoazolinylidene)-1,3,5-heptatrienyl]-benzoazolium iodide. Thus, if a flashlamp capable of emitting 100 joules in a 10 nanosecond pulsewidth can be obtained, the spectrum can be continuously traversed from the near ultraviolet into the infrared by merely tuning with a diffraction grating and changing the dye solution where appropriate. With frequency doubling, the portion of the spectrum down to 2500Å can be reached.

c. Alignment Procedure

The discovery of the proper method of aligning the system optics proved to be the key to achieving lasing action. Several methods
were reported in the literature, but none of them worked too well with the system on hand. A maximum of four degrees of freedom were required to properly align the optical components. The mirror mounts were the simplest of all as only alignments to position a mirror in a plane perpendicular to the optical axis, that is, with two degrees of freedom, were necessary. The diffraction grating and KDP crystal mounts required two more adjustments to rotate the optics around the optical axis (C) and another adjustment to rotate in the plane of the component, but also perpendicular to the optical axis (D). Figure 20 is a schematic of the adjustments. In Figure 20, all the indicated axes are in the plane of the diagram. Figures 21 and 22 are photographs of the mounts used.

A great amount of correspondence was generated searching for the proper optical mounts. Late in 1969 acceptable laser mounts had not yet reached the market. Ardel Instrument Company took special pains to specifically design the mounts tailored for the needs of the project.

The mirror mounts adjustable to within half a minute of an arc were available as was a rotating table which had a resolution of five seconds of an arc. Special adaptors had to be constructed to combine the degrees of freedom of the mirror mount with the additional rotational degree of freedom of the rotating table.

The next three sections will detail the correct methods for aligning three different types of optical components. In all cases an Edmunds Model 79004 helium-neon laser was used as the alignment tool.
FIGURE 20. Schematic of Optical Mounts.

Adjustments for Mirrors

Adjustments for Diffraction, Grating and KDP Crystal
FIGURE 21. Optical Mount with Two Degrees of Adjustment (Ardel Instrument Company)
FIGURE 22. Optical Mount with Four Degrees of Adjustment
(Ardel Instrument Company)
1) Mirrors and dye cells

The diagrams in Figure 23 show how best to align the organic dye laser using a laser as the alignment tool. In part A, it is important that the dye solution flows at the normal flowing rate, which for the investigation was about a liter per minute. The dye cell was positioned so that the laser beam passed through the tube of the cell. Theoretically, the tube was now colinear with the alignment laser beam. Since the helium-neon beam diameter was 2 mm, tube diameters smaller than 2 mm caused some optical distortion and dispersed the alignment laser beam. Fortunately, the distortion was not serious enough to prevent alignment. Tube diameters excessively larger than 2 mm also were generally harder to align, as the 2 mm beam could theoretically pass through the dye cell and keep a circular spot without properly aligning the dye cell. Again, fortunately, the largest diameter dye cell used was 5 mm which was not quite large enough to seriously hinder alignment. Parts B and C of Figure 23 diagrammatically show how the two mirrors were aligned parallel to each other and perpendicular to the dye cell and alignment laser beam. It was important for the alignment laser to be kept a good distance away from the dye laser as longer distances made for more sensitive alignment. The accuracy required for acceptable alignment was reported to be two minutes of arc. As explained earlier, the reason for this sensitivity was that for amplification to occur in the cavity, the beam had to reflect back and forth through the dye solution. Inaccurate alignment would have caused the beam to "walk off" the mirror.
FIGURE 23. -Mirror Alignment Procedure.

A. First align the dye-cell so that a perfectly circular spot is achieved on a white surface.

B. Then locate the totally reflecting mirror in place and align the mirror so that the laser beam emanating from the He-Ne laser reflects back through the dye-cell into the originating spot. In effect, the beam traces itself back.

C. Place the partially transmitting mirror in place and align the laser beam reflecting off the transmitting mirror back into the originating spot. The spots from both mirrors should now be superimposed.
2) Diffraction Grating

The diffraction grating replaced the totally reflecting mirror in order to obtain tunability. Appendix G should be consulted for details on the theory behind diffraction grating operation.

The physical alignment of the grating involved one extra step; the grooves of the grating had to be aligned parallel to the axis which was perpendicular to the optical axis and in the plane of the diffraction grating. Figure 24 is a schematic which shows the method used to align a diffraction grating. Step A was performed exactly like in the mirror alignment procedure. Steps B, C, and D were needed to align the grating grooves in the correct direction. Step E purposely lost the correct spot setting as the wavelength at which rhodamine 6G lased ranged between 5575Å and 6200Å. The helium-neon laser had a wavelength of exactly 6328Å. Only if the dye lased at exactly 6328Å would step D have been unnecessary.

The angle at which adjustment D was set determined the lasing wavelength of the tunable dye laser. Table 2 gives lasing wavelengths for angular settings on a Bausch and Lomb 1800 grooves per millimeter grating blazed at 26°45' for 5000Å and an Edmunds 1200 grooves per millimeter grating blazed for 4000Å for rhodamine 6G and 7-diethylamino - 4-methylcoumarin.

Monochromaticity and tunability were both obtained when a diffraction grating was used in the laser cavity. Table 2 shows the ranges of tunability that were possible with two different dyes. The literature reported that an 1800 grooves per millimeter grating results in a bandwidth of less than one angstrom and a 1200 grooves

A. Initially align the grating like a mirror using adjustments A and B.

B. Rotate in D direction until the first order can be aligned unto spot at He-Ne laser with adjustment C.

C. Rotate in D direction so that the grating is perpendicular to the laser beam. If reflection spot is not on the source, use A and B adjustments to bring the spot back to the correct location.

D. Rotate in D direction and repeat step "B". By trial and error repeat step "C" until reflected spots both trace back to the source of the beam.

E. After the diffraction grating has been properly aligned, rotate in D direction to the correct angular setting.
Table 2
Diffraction Grating Angular Setting to Determine Lasing Wavelength

Angular Setting on Diffraction Grating

<table>
<thead>
<tr>
<th>Bausch &amp; Lomb</th>
<th>Edmunds</th>
<th>Rhodamine 6G Lasing Output, Angstroms</th>
</tr>
</thead>
<tbody>
<tr>
<td>30°52'</td>
<td>20°0'</td>
<td>5700</td>
</tr>
<tr>
<td>31°28'</td>
<td>20°18'</td>
<td>5800</td>
</tr>
<tr>
<td>32°6'</td>
<td>20°41'</td>
<td>5900</td>
</tr>
<tr>
<td>32°41'</td>
<td>20°59'</td>
<td>6000</td>
</tr>
<tr>
<td>33°22'</td>
<td>21°24'</td>
<td>6100</td>
</tr>
</tbody>
</table>

Angular Setting on Diffraction Grating

<table>
<thead>
<tr>
<th>Bausch &amp; Lomb</th>
<th>Edmunds</th>
<th>Coumarin Lasing Output, Angstroms</th>
</tr>
</thead>
<tbody>
<tr>
<td>22°46'</td>
<td>14°57'</td>
<td>4300</td>
</tr>
<tr>
<td>23°20'</td>
<td>15°18'</td>
<td>4400</td>
</tr>
<tr>
<td>23°53'</td>
<td>15°40'</td>
<td>4500</td>
</tr>
<tr>
<td>24°27'</td>
<td>16°1'</td>
<td>4600</td>
</tr>
<tr>
<td>25°1'</td>
<td>16°23'</td>
<td>4700</td>
</tr>
<tr>
<td>25°36'</td>
<td>16°44'</td>
<td>4800</td>
</tr>
<tr>
<td>26°10'</td>
<td>17°6'</td>
<td>4900</td>
</tr>
</tbody>
</table>
per millimeter grating of one to two angstroms. Also very significantly, tuning the laser along the spectrum did not result in the type of inefficiency present in a conventional monochromator. That is, a monochromator, through the use of filters, blocks out all but a fine band of wavelengths. The resulting output energy is only a small fraction of the total energy imparted by the energy source. A tunable dye laser has the property of capturing approximately 75% of the total energy anywhere along the spectrum. A quantum electronic explanation of this phenomenon is given in Appendix G.

3) KDP Crystal

The KDP crystal is originally lined up exactly the same as a mirror. Adjustment C is initially a close guess at best. This relative non-precision is permissible since the angle of polarization is not critical for frequency doubling. Once frequency doubling has been obtained, the adjustment in the C direction can be performed to find the maximum ultraviolet output.

Rotational alignment D is extremely sensitive for the KDP crystal. For discrete outputs, an inaccuracy of 5 seconds can inhibit frequency doubling from occurring. Broadband inputs need not satisfy this last criterion. For this reason, a broadband rhodamine 6G input was used during initial frequency doubling experiments.

Since the KDP crystal was cut for 5300Å, the ideal input to the crystal should have been a narrowband 5300Å output as resulting from lasing disodium fluorescein salt using a diffraction grating. This particular set-up would have been optimal for three reasons: 1) efficiency of SHG increases as the inverse square of the wavelength,
2) narrowband power is five to ten times higher in efficiency than broadband power, and 3) since the crystal was cut for 5300Å, anything but 5300Å materially reduces the efficiency.

Comparing rhodamine 6G and fluorescein, the efficiency is improved by at least a factor of ten with the latter solution. However, this condition is true only if the output power of fluorescein is equal to that of rhodamine. Since the output power of rhodamine was considerably higher than the other dyes lased, and since frequency doubling efficiency increases with the square of the incident power, rhodamine 6G was used in attempts at inducing generation of the second harmonic.

d. Checkout and Measurement of System Parameters

1) Wavelength

It is well documented in the literature that rhodamine 6G lases between 5700Å and 6100Å, rhodamine B between 6050Å and 6350Å, and 7-diethylamino-4-methylcoumarin between 4300Å and 4900Å. The actual lasing bandwidth of a particular broadband lasing concentration is only 50Å to 150Å wide. As the concentration is changed the wavelength shifts. It was found that all the solutions tested here lased at shorter wavelengths when the dye concentration was decreased. A "wavelength" meter would have been able to measure the relative power output in the wavelength lasing band, i.e., relative power as a function of wavelength.

Since laser performance exactly follows the well-defined rules of optics, the precise lasing wavelength could be conveniently calculated for the case where a diffraction grating was used in place
of the totally reflecting mirror. Table 2 gives these values. Since typical wavelength meters commercially available measure with an accuracy of only 50Å, and the calculated values were at least within 10Å of the actual, a meter was not required for measuring narrowband outputs. The utility of a meter would have been to obtain the peak wavelength for broadband outputs and to verify the calculation for narrowband outputs.

In actual tunable lasing operations, rhodamine 6G was tuned from 5567Å to slightly over 6100Å. The 5567Å output, which is actually well below the reported minimum wavelength, was obtained with a solution that was eight times more dilute than the optimal lasing concentration. Lower concentrations would almost assuredly have resulted in even shorter wavelengths. Coumarin at 3x10^-4 M in ethanol was tuned between 4410Å and 4750Å. Again, higher and lower concentrations would have extended the tunability. Very little tuning was attempted with rhodamine B, but a range between 6130Å and 6349Å was obtained with a 5x10^-4 M ethanolic solution.

A most interesting combination of 10^-5 M rhodamine 6G and 5.9x10^-4 M coumarin lased at approximately 5500Å. This suggests that a synergistic effect must have occurred as 10^-5 M rhodamine 6G is too low a concentration to lase by itself and coumarin lases at no longer than 4900Å. The combination lased at a deep green color in the area of 5500Å.

2) Energy Flux

Adequate energy measurements were obtained from the tunable laser output using an energy measurement system consisting of a Korad K-J2 laser calorimeter, Keithley 150A microvoltmeter, and EAI 1130
Variplotter. The terms powermeter and energy meter are used synonymously in the laser measurement field. The power can be calculated by dividing the energy reading by the pulsing rate. Figures 25, 26, and 27 represent typical responses at the indicated lasing conditions.

The laser calorimeter was calibrated to read 0.059 joules per microvolt. The total energy in joules absorbed by the calorimeter was obtained by extrapolating the recovery portion of the response curve back to time zero. The parameter of interest, energy flux in millijoules per cm$^2$ per pulse, was calculated from the extrapolated microvoltmeter reading by using the following equation:

$$E_p = \frac{(\text{microvolt reading})(.059)(1000)}{(\text{number of pulses})(\text{cross-sectional area of beam, cm}^2)}$$ (3-6)

For the following three output curves, the appropriate energy fluxes were evaluated as:

- Figure 25: 24.2 mj/cm$^2$/pulse
- Figure 26: 201 mj/cm$^2$/pulse
- Figure 27: 161 mj/cm$^2$/pulse

The powermeter was not operating during the time of the E. coli irradiation runs. However, a subsequent check of the energy flux at the conditions under which irradiation occurred substantiated the applicability of the comparative energy equation (2-18).

As mentioned earlier, great difficulty was experienced attempting to stabilize the microvoltmeter at the most sensitive one microvolt setting. Since the calorimeter was originally purchased by the LSU Chemistry Department to measure the output of a ruby laser, which emits in the joule range, certain procedural modifications had to be
FIGURE 25. Laser Calorimeter Response of Five Pulses at 8 Kilovolts, Using 100% and 62% Reflectance Mirrors, 3mm Dyecell, 5mm Flashlamp, and Rhodamine 6G at $4 \times 10^{-4}$M in Methanol
FIGURE 26. Laser Calorimeter Response of Five Pulses at 10 Kilovolts, Using 90% and 50% Reflectance Mirrors, 3mm Dyecell, 5mm Flashlamp, and Rhodamine 6G at $4 \times 10^{-4} M$ in Methanol.
FIGURE 27. Laser Calorimeter Response of Five Pulses at 10 Kilovolts, Using an 1800 Grooves/mm Diffraction Grating Set for 5800 Angstroms and 50% Reflectance Mirror, 3mm Dye-cell, 5mm Flashlamp, and Rhodamine 6G at $4\times10^{-4}$M in Methanol.
made to enable the powermeter system to read accurately in the millijoule area. It was experimentally determined that three pulses gave three times the energy reading of one pulse, etc. Therefore, to increase the extrapolation accuracy, five pulses were utilized.

As a check, the meter was used to measure the response of the laser when the mirrors were purposely misaligned. This non-lasing condition did not register on the meter. The consequence of this test was that the energy measurement obtained under lasing conditions could be taken to be 100% laser energy.

The radiant flux density was also "measured" using a polaroid negative. It is an unofficial standard in the laser measurement field that burning of a polaroid negative is caused by a radiant flux density between 500,000 and 1,000,000 watts/cm². Using the lasing components described in Figure 26 in conjunction with a 10 inch focal length converging lens, the polaroid negative burned along a 2\(\frac{1}{4}\) inch path in the optical axis straddling a point 13\(\frac{1}{2}\) inches from the lens. In fact, burning was experienced even after the focused beam passed through the microbiological droplet.

Two significances of the previous statements are: 1) point focus at 13\(\frac{1}{2}\) inches away from a 10 inch focal length lens means that the laser beam was diverging at a fairly substantial rate; if the microbial droplet is placed at a considerable distance from the tunable laser the power density will suffer accordingly; 2) the fact that the negative burned after the beam passed through the droplet indicated that the droplet was absorbing only a very small fraction of the incident radiation. These two power-related incidents will be discussed in a later section analyzing the effect of energy and power.
on the survival fraction of the irradiated organism.

The results of the powermeter readings indicated that the maximum laser efficiency was in the order of 0.01 to 0.02 percent. Relative to efficiencies reported in the literature, the above range was consistent with data reported by manufacturers of linear flash-lamp-pumped dye lasers.

3) Beam Intensity Profile

For the most part the beam intensity profile approximated a Gaussian output. The purest mode, termed TEM$_{00}$, is the profile expected with an efficient cavity. See Figure 20 for a pictorial representation of mode structures. The laser beam profile observed had a skewed effect because of parallel flashlamp pumping. Coaxial flashlamp pumping would have produced maximum intensity at the center. Unfortunately, this off-center condition was not detected until after several inconsistent inhibition runs were consummated. The beam profile could be observed only with the aid of the laser safety goggles.

A Gaussian mode structure was not the only profile experienced. If the dyecell was very badly misaligned, an annular profile resulted, with nearly zero intensity, or null, at the center and edges. This was a combination of TEM$_{10}$ and TEM$_{01}$ modes. When the 7mm dyecell was used on automatic at 2 pulses per second, the mode structure changed with each pulse, varying from TEM$_{01}$ to TEM$_{10}$ to TEM$_{11}$.

Clearly, the only mode acceptable for irradiation operations was TEM$_{00}$ as the intense portion matched well with the thickest part of the hemispherical droplet. Some of the poor inhibition runs

**TEM\(^{00}\)**

**TEM\(^{10}\)**

**TEM\(^{10}\) and TEM\(^{01}\)**

**TEM\(^{01}\)**: Same as TEM\(^{10}\), but shifted 90°

**TEM\(^{11}\)**
experienced were caused by the multi-mode beam profile.

4) Micropulser and Laser Pulse Risetime

As a general statement of fact, quicker risetimes were desired in the laser pulse response. Since 100 joules were pumped into the flashlamp, and peak output power in watts is equal to energy in joules divided by pulse length in seconds, shorter pulses resulted in higher power densities. The controlling factors in the length of the pulse were the capacitor and micropulser system. Lower inductances resulted in quicker pulses.

The organic dye laser is characterized by a pulse which very closely follows the behavior of the energizing source. Figure 29 is a photograph of the laser beam response using a CRC 931-A photomultiplier tube, a type 561A Tektronix oscilloscope, and a 197A Hewlett-Packard oscilloscope camera. The pulse was obtained by lasing a 3x10^-4 M rhodamine 6G solution in methanol at 6.5 kilovolts using a 5mm bore flashlamp and 3mm bore dyecell. The input was 42 joules. Since each division was equal to one microsecond, the response of the laser pulse was in the order of 0.7 microseconds. The dotted line was added as the film did not pick up the beginning of the pulse. Figure 30 is an electronic schematic of the measurement system utilized as suggested by Mr. Les Edelen of the LSU Physics Department.

In general, the laser pulse risetime increased with increasing size of the capacitor and decreased with increasing voltage, everything else kept constant. The 2.0 microfarad rectangular metal case capacitor used to generate 100 joules had an inductance of 30 nanohenries.
FIGURE 29. Oscilloscope Camera Photograph of the Response of CRC 931-A Photomultiplier Tube to Laser Light (The dotted line and grid were added to aid definition)

DYE: \(3 \times 10^{-4}\) M rhodamine 6G in methanol
INPUT: 42 joules (6.5 KV, 2.0 microfarad capacitor)
FLASHLAMP: NOVATRON 850 (5 mm)
DYECELL: XENON (3 mm)
GRID: 1.0 microsecond/division
FIGURE 30.—Electronic Circuit Used to Measure Pulse Rise Time.
Frequency doubling and the lasing of ultraviolet dyes both require relatively quick risetimes in the order of 100 nanoseconds or less. It has been reported that risetimes of as short as 10 nanoseconds were obtained with a specially designed system and pulse risetimes in the picosecond area through mode-locking.

4. Irradiation of *Escherichia coli*

Several conventional microbiological methods were tried, and the irradiation procedure that was finally utilized evolved from these early attempts. This final microbiological irradiation system was developed largely through the efforts of Dr. George Dimopoullos of the LSU Department of Veterinary Science.

a. Materials and Methods

The microorganism used for the study was *Escherichia coli* (0127-08). The bacteria were grown on a Bio Cert Tryptic Soy agar slant and refrigerated at 4°C. Using aseptic techniques, a small quantity of the bacteria was transferred to 20 milliliters of nutrient broth and incubated at 37°C for twenty four hours. When an exogenous photosensitizer was used, 5 microliters of 2x10^{-2} M dye in distilled water was added at the time of the initial inoculation. The resultant dye concentration was 5x10^{-6} M.

Using a 10 microliter syringe, either identical 0.2 microliter or 0.5 microliter droplets of the organism in nutrient broth were deposited on two microscope coverglasses. A 0.2 microliter sample was used with focused beam irradiation and a 0.5 microliter sample was used when the beam was not focused. A 0.2 microliter droplet had
a deposited diameter of 1.4mm while a 0.5 microliter sample had a
diameter of 2.0mm. Since the laser beam diameter was at least 3mm,
total droplet coverage was possible.

The drop on the coverglass was then placed on a lucite mount,
which when properly moistened, sealed the drop in an air-tight cavity
to prevent drying. Although during irradiation runs the average
droplet time within the cavity amounted to no more than 15 minutes,
and normally less than 5 minutes, tests did show that the drop remained
in the liquid state for several hours.

One sample was not irradiated and served as the control. The
other sample was irradiated through the coverglass. A 100%
reflecting mirror was located behind the mount to reflect back the
beam that passed through the drop. Figure 31 is a photograph of the
coverglass on a mount with a 100% reflecting mirror to reflect the
laser beam which comes in from the left.

After exposure to a pre-determined total energy level that
varied from 0.08 to 130 joules, the drop-coverglass was removed from
the mount and dropped into a sterilized test tube containing 10
milliliters of sterile distilled water. The test tube was shaken
following standard techniques. When a 0.2 milliliter sample was used
a 0.2 milliliter pipette was used to pipette 0.2 milliliters into
each of replicate petri dishes. It was found that three or four
replicates were necessary to obtain a meaningful average.

When a 0.5 microliter drop was used, a 0.1 milliliter pipette
was used for the above procedure. The object of pipetting at these
volumes was to insure that the final plate count ranged between 30
and 300 colonies. Counts as high as 500 were tolerated. Counts below
FIGURE 31. Microbiological Irradiation System
Aside from the exposure to laser irradiation, the controlled sample went through the identical procedure: into 10 milliliters of distilled water, shaken to disperse the droplet, and pipetted into replicated petri dishes. It was important that the irradiated and controlled samples remained in distilled water for the same amount of time as it was found that distilled water adversely affected the survival of *E. coli*. Although ten minutes did not materially alter survival, an hour did. Cell lysis was caused by an osmotic pressure differential between the cell and the distilled water.

To each 0.1 or 0.2 milliliter sample in the petri dish was then added approximately 15 milliliters of McConkey agar. McConkey agar was used because it is the medium used in plating for coliforms in the standard methods for the examination of water and sewage. This medium differentially allowed the gram-negative *E. coli* to grow while reducing the chances for the growth of gram-positive contaminants. Furthermore, *E. coli* had the particular property of fermenting the lactose in the medium and producing acids which reacted with the precipitated bile resulting in absorption of the neutral red dye that was part of the agar composition. The brick-red growth made cell-counting sure and easy.

The agar was prepared by adding 50 grams to 1000 milliliters of distilled water, after which it was boiled and sterilized in an autoclave at 121°C for 20 minutes. The agar has a melting point of 100°C, and solidification point of 40°C. Once melted at 100°C, the agar remains liquid until the temperature drops below 40°C. An automatic water-bath was used to keep the agar at 45°C, ± 0.5°C.
Temperatures of 50°C and over deleteriously affected the organism, and temperatures below 40°C solidified the agar.

The inoculated agar plates were allowed to cool and were placed in a 37°C incubator for a minimum of fifteen hours. An incubation period of 20 to 24 hours was found to be optimal for plate counting. Longer periods resulted in cell overgrowth and shorter periods made plate counting difficult. The plates were counted by tallying the total number of colonies. Figure 32 is a photograph of replicated plates showing the controlled and irradiated samples. The top six plates are replicates of the control and the bottom six are replicates of a 0.5 microliter droplet that was irradiated with a $3 \times 10^{-4} \text{M}$ lasing solution of rhodamine 6G in methanol. The top six plates contained a grand total of 2846 *E. coli* colonies and the bottom six plates contained 548. The resultant survival ratio was then 0.193.

**b. Irradiation Procedure**

The laser beam was superimposed on the drop by physically adjusting the drop so that a visual check could ascertain coverage. It was determined that nearly perfect alignment of the laser beam on the droplet was necessary for inhibition. The reason for this requirement was that the energy profile, normally Gaussian, fell very sharply away from the center of the intense portion of the beam. Early runs were inconsistent because alignment of the beam on the droplet was attempted by visually locating the drop in the middle of the laser beam. Since the beam spot was extraordinarily bright, it was not always possible to pick out the intense portion of the beam. At this time it was assumed that the beam energy profile was exactly
FIGURE 32. Six Replicates Each of Control and Irradiated Samples of *E. coli* in McConkey Agar Pour Plates After Twenty Hours of Inoculation at 37°C
Gaussian with the most intense spot at the center. Unfortunately, a later check with laser safety goggles showed that the intense portion was offset. Subsequent alignment was accomplished with the use of goggles and proved to be adequate.

Mere alignment of the beam on the drop did not always insure perfect irradiation. Thermal gradients within the dyecell had a tendency to cause the laser beam spot to move unpredictably. Movement of as much as the diameter of the droplet was experienced. Automatic pulsing operations alleviated the problem to some degree. Sometimes, however, on automatic pulsing, the mode structure of the beam changed with every pulse. Although Gaussian profiles were obtained with manual pulses, automatic pulsing, which was constrained by a pulsing rate of 2 pulses per second, caused the flowing medium to heat unequally, thereby upsetting the ideal lasing conditions and resulting in impure mode performance. As learned earlier, imperfect mode structures produced beam patterns that did not properly cover the droplet. For example, a Gaussian, or TEM$_{oo}$ mode, suitably covers the hemispherical drop, as the intense portion of the beam passes through the thickest portion of the drop. A combination of TEM$_{01}$ and TEM$_{10}$ modes forms an annular profile and is almost totally useless for irradiation operations as the null portion passes through the thickest part of the drop and the intense ring through the thinnest.

It was found that impure modes almost always occurred when 7mm flashlamp was used. Since most of the later experiments used a 5mm flashlamp, which had a higher energy density, multi-mode outputs did not seriously affect the results.

Several other inconsistencies made reproducibility often
difficult to achieve. Each 5mm bore diameter flashlamp was different. At the lowest automatic pulse rate setting on the micropulser, pulsing rates differed between flashlamps from 2 to 4 pulses per second. If the rate was at 2 pulses per second, a 10 kilovolt setting resulted in each flash occurring at 10 kilovolts. However, at 4 pulses per second, even though the power supply was set at 10 kilovolts, each flash occurred at 9 kilovolts. Furthermore, the output power of each pulse varied somewhat from pulse to pulse. In addition to the above, the dye solution itself degraded with time. Coumarin solutions in particular had to be replaced as frequently as every two daily irradiation runs. Rhodamine 6G solutions, however, remained usable for several weeks.

In summary, although consistency in laser pulse performance was not always achieved, the use of 60 to 250 irradiation pulses was sufficient to compensate for the variation in energy flux from one experimental run to the next. The system and procedures used to irradiate the E. coli organism proved to be quite satisfactory for demonstrating the efficiency of visible laser light for purposes of sterilization and catalysis.


8. Swagel, M., private correspondence.


CHAPTER IV
EXPERIMENTAL RESULTS

Irradiation runs were conducted by varying the following parameters: 1) lasing dye, rhodamine 6G or 7-diethylamino-4-methyl-coumarin, 2) beam, focused or unfocused, 3) energy flux, 4) spectral output, broad or narrow band, and 5) exogenous photosensitizers, presence or absence. Since the main purpose of the investigation was to confirm the feasibility of utilizing laser radiation for sterilization, the majority of the experimental runs were confined to straight rhodamine 6G lased runs without the aid of exogenous photosensitizers.

To ascertain that inhibition was caused by laser radiation and not stray light from the flashlamp, several tests were executed with either the front end mirror slightly misaligned to prevent lasing or with no dye in the flowing alcohol. Table 3 is a summary of these non-lasing runs.

A grand total of 209 irradiation runs were made from April 28, 1971, to June 26, 1971. However, the first 55 runs were discarded because of assorted microbiological inconsistencies. Stability with respect to technique was achieved from the May 26 runs.

Subtracting 25 acridine orange runs, because they almost all showed an unexpected increase in population after irradiation, the remaining 129 runs averaged 40.6% kill at an average energy flux of 24.27 joules/cm². Therefore, the E. coli fraction survival for these runs was 0.594. Out of the 129 runs, 22 runs were invalidated
due to various microbiological and procedural deficiencies. The omission of these spoiled runs reduced the \textit{E. coli} fraction survival to 0.505. The average energy flux remained about the same. Table 4 is a chronicle of these 107 valid runs.

Figures 33, 34, and 35 summarize the effect of energy flux on the fraction survival of \textit{E. coli}. Tables 5, 6, and 7 tabulate the effect of energy flux on the fraction survival of \textit{E. coli}.

Figures 33, 34, and 35 represent the mean, maximum, and minimum fraction survivals at the indicated level of energy flux. The circle represents the mean. The number located above each bar indicates the number of irradiation runs for that particular energy flux. Energy flux was measured in joules/cm\(^2\).
FIGURE 33. The Effect of Energy Flux on the Fraction Survival of *E. coli*.

LASING DYE : Rhodamine 6G

BEAM : Unfocused

PHOTOSENSITIZER : None

ENERGY FLUX, JOULES/CM²

FRACTION SURVIVAL
FIGURE 34. The Effect of Energy Flux on the Fractional Survival of E. coli.

Lasering Dye: Rhodamine 6G
Beam: Focused
Photosensitizer: None

<table>
<thead>
<tr>
<th>Fractional Survival</th>
<th>Energy Flux, Joules/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>25</td>
</tr>
<tr>
<td>0.6</td>
<td>50</td>
</tr>
<tr>
<td>0.4</td>
<td>75</td>
</tr>
<tr>
<td>0.2</td>
<td>100</td>
</tr>
</tbody>
</table>

1 Total number of runs
- Maximum survival
- Mean survival
- Minimum survival
FIGURE 35. - The Effect of Energy Flux on the Fraction Survival of E. coli.

LASING DYE : Rhodamine 6G
BEAM : Focused and Unfocused
PHOTOSENSITIZER : Toluidine Blue, $5 \times 10^{-6}$M

![Graph showing the relationship between energy flux and fraction survival. The x-axis represents energy flux in joules/cm$^2$, and the y-axis represents fraction survival. The graph includes data points and error bars indicating total number of runs, maximum survival, mean survival, and minimum survival.]
Table 3

Effect of "Non-lasing" Irradiation Runs on the Fraction Survival of *E. coli*

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Number of Samples</th>
<th>Multi-Plate Count Control</th>
<th>Irradiated</th>
<th>Fraction Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>only methanol</td>
<td>2</td>
<td>9317</td>
<td>8778</td>
<td>0.942</td>
</tr>
<tr>
<td>rhodamine 6G, but mirror misaligned</td>
<td>5</td>
<td>31840</td>
<td>30307</td>
<td>0.952</td>
</tr>
<tr>
<td>coumarin, also misaligned</td>
<td>2</td>
<td>9043</td>
<td>8798</td>
<td>0.973</td>
</tr>
</tbody>
</table>
Table 4
Daily Average of the Fraction Survival of *E. coli*.

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of Runs</th>
<th>Multi-Plate Count Control</th>
<th>Multi-Plate Count Irradiated</th>
<th>Fraction Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 26</td>
<td>3</td>
<td>1489</td>
<td>233</td>
<td>.156</td>
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<tr>
<td>28</td>
<td>4</td>
<td>1655</td>
<td>637</td>
<td>.385</td>
</tr>
<tr>
<td>30</td>
<td>7</td>
<td>3918</td>
<td>1855</td>
<td>.473</td>
</tr>
<tr>
<td>June 1</td>
<td>5</td>
<td>4605</td>
<td>2678</td>
<td>.582</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>6165</td>
<td>3179</td>
<td>.516</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>3119</td>
<td>1957</td>
<td>.627</td>
</tr>
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<td>8</td>
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<td>5798</td>
<td>1712</td>
<td>.295</td>
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<td>10</td>
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<td>10945</td>
<td>6862</td>
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<td>.419</td>
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<td>3955</td>
<td>1944</td>
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</tr>
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<td>3</td>
<td>4772</td>
<td>2676</td>
<td>.561</td>
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<td>4</td>
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<td>.426</td>
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<td>3</td>
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</tr>
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<td>6</td>
<td>4134</td>
<td>3193</td>
<td>.772</td>
</tr>
<tr>
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<td>7</td>
<td>4520</td>
<td>1777</td>
<td>.393</td>
</tr>
<tr>
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<td>4</td>
<td>4127</td>
<td>1880</td>
<td>.456</td>
</tr>
<tr>
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<td>4</td>
<td>2109</td>
<td>1183</td>
<td>.561</td>
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<td>6</td>
<td>4307</td>
<td>2344</td>
<td>.544</td>
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<td>1</td>
<td>1027</td>
<td>623</td>
<td>.607</td>
</tr>
<tr>
<td>25</td>
<td>4</td>
<td>1515</td>
<td>606</td>
<td>.400</td>
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<td>26</td>
<td>20</td>
<td>12367</td>
<td>6009</td>
<td>.486</td>
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Table 5
Effect of Energy Flux on the Fraction Survival of E. coli with Radiation from the Lasing of Rhodamine 6G

<table>
<thead>
<tr>
<th>Unfocused Energy Flux</th>
<th>Number of Samples</th>
<th>Mean Fraction Survival</th>
<th>Maximum Fraction Survival</th>
<th>Minimum Fraction Survival</th>
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<tr>
<td>.41</td>
<td>2</td>
<td>.682</td>
<td>.775</td>
<td>.572</td>
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<td>.89</td>
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<td>.953</td>
<td>.633</td>
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<tr>
<td>2.01</td>
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<td>.832</td>
<td>.794</td>
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<td>.548</td>
<td>.692</td>
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<td><strong>Focused Energy Flux</strong></td>
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<td>.082</td>
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<td>6</td>
<td>.426</td>
<td>.691</td>
<td>.060</td>
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<td>130.40</td>
<td>3</td>
<td>.166</td>
<td>.279</td>
<td>.076</td>
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</tbody>
</table>
Table 6
Photodynamic Effect of Energy Flux on the Fraction Survival of *E. coli* with Radiation from the Lasing of Rhodamine 6G

<table>
<thead>
<tr>
<th>Energy Flux</th>
<th>Number of Samples</th>
<th>Mean Fraction Survival</th>
<th>Maximum Fraction Survival</th>
<th>Minimum Fraction Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluidine Blue</td>
<td>.08</td>
<td>2</td>
<td>1.008</td>
<td>1.113</td>
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<td>6.40</td>
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<td>.501</td>
<td>.743</td>
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<td>.390</td>
<td>.714</td>
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<td>.306</td>
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<td></td>
<td>24.50</td>
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<td>.732</td>
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<td>49.00</td>
<td>2</td>
<td>.490</td>
<td>.717</td>
</tr>
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<td></td>
<td>65.20</td>
<td>5</td>
<td>.384</td>
<td>.865</td>
</tr>
<tr>
<td></td>
<td>98.00</td>
<td>2</td>
<td>.050</td>
<td>.057</td>
</tr>
<tr>
<td>Acridine Orange</td>
<td>.08 (F)*</td>
<td>2</td>
<td>1.510</td>
<td>2.214</td>
</tr>
<tr>
<td></td>
<td>16.36</td>
<td>2</td>
<td>2.092</td>
<td>2.208</td>
</tr>
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<td></td>
<td>15.10</td>
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<td>1.324</td>
<td>2.708</td>
</tr>
<tr>
<td></td>
<td>20.15</td>
<td>3</td>
<td>1.140</td>
<td>1.305</td>
</tr>
<tr>
<td></td>
<td>22.50</td>
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<td>1.587</td>
<td>1.936</td>
</tr>
<tr>
<td></td>
<td>49.00 (F)</td>
<td>3</td>
<td>.512</td>
<td>.696</td>
</tr>
<tr>
<td></td>
<td>65.20 (F)</td>
<td>4</td>
<td>.668</td>
<td>1.009</td>
</tr>
</tbody>
</table>

*(F) refers to focused irradiation
Table 7
The Photodynamic and Ordinary Effect of Energy Flux on the Fraction Survival of E. coli with Radiation from the Lasing of 7-diethylamino-4-methylcoumarin

<table>
<thead>
<tr>
<th>Energy Flux</th>
<th>Number of Samples</th>
<th>Mean Fraction Survival</th>
<th>Maximum Fraction Survival</th>
<th>Minimum Fraction Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>.51</td>
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<td>.271</td>
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<td>5.03</td>
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<td>11.40</td>
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<td>.474</td>
</tr>
<tr>
<td>Acridine Orange</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.03</td>
<td>4</td>
<td>1.347</td>
<td>2.740</td>
<td>.264</td>
</tr>
<tr>
<td>13.00 (F)</td>
<td>2</td>
<td>.204</td>
<td>.251</td>
<td>.131</td>
</tr>
</tbody>
</table>
CHAPTER V
DISCUSSION OF RESULTS

1. General

A qualitative discussion will evaluate the possible sources for error in the experimental run. These occurred in two main areas, microbiological analysis and laser functioning.

The microbiological errors will be analyzed in the order that the experimental procedure occurred. The 10 microliter syringe was graduated to read in tenths of a microliter. The physical distance moved by the plunger to obtain a 0.2 microliter sample was only 1mm. Suspected were pockets of varying concentration within the E. coli inoculant. Control samples drawn from the same inoculant generally were within 15% of each other. It was important that the control and irradiated droplets were taken as close as possible to each other from the same syringe extraction because of the presence of these aforementioned concentration pockets. Some variation occurred in the replicated plate counts. Although the test tube was shaken following standard water treatment practices, replicated 0.1 ml samples from the same test tube varied in plate count by as much as factors of two and three in some instances. As such, it was important that the sum of the replicated plates be used in the comparative analysis as the average of the replicates gave a meaningful figure.

There was some inconsistency in reference to laser beam movement, varying mode structure, and fluctuating energy densities.
Since a 0.2 microliter droplet had a deposited cross-sectional area of approximately .015 cm$^2$, or a diameter of 1.4 mm, some difficulty was experienced trying to align the 3mm beam diameter to cover the droplet. It would have been relatively easy had the beam diameter been of a constant energy profile. Unfortunately, the beam had a Gaussian profile, and the alignment of the intense portion of the beam on the drop was a good guess at best. It was imperative that the intense portion passed through the droplet as tests indicated that a slight misalignment of only 1mm practically negated the effect of the beam. There was strong evidence to support the contention that misalignment of the beam was the primary cause of several unexpectedly high fraction survivals.

In addition to beam alignment, two other minor shortcomings were experienced. First, the beam itself did not always hit the same spot with every pulse. Movement of several millimeters was sometimes observed. Fortunately, automatic pulsing stabilized the temperature effects responsible for beam movement. Almost no movement was observed under automatic pulsing. A consequence of automatic pulsing was mode structure variation. Since the micro-pulser had a minimum automatic pulsing rate from 2 to 4 pulses per second, depending on the flashlamp, the flowing dye solution could not recover from the temperature effect fast enough. Temperature profiles within the dye solution impaired the "pure" lasing condition and mode structures of $\text{TEM}_{10}$, $\text{TEM}_{10} - \text{TEM}_{01}$, $\text{TEM}_{01}$, and $\text{TEM}_{11}$ resulted (see Figure 28 in Chapter III for a description of these modes). Fortunately, too, this condition was experienced only with the 7mm bore flashlamp.
Despite these irritating random variables, it was positively determined that tunable organic dye laser radiation could be utilized to inhibit the growth of B. coli. A sterilization figure of close to 50% was obtained from 107 runs at an average energy flux of about 25 joules/cm$^2$. There were also indications that growth promotion was experienced as an increase in survival of 150% was obtained with 9 acridine orange runs at an average energy flux of approximately 16.5 joules/cm$^2$.

2. Analysis with Respect to Energy Flux

The terms energy flux and radiant flux density are virtually interchangeable in this discussion. Energy flux refers to the energy per unit cross-sectional area while radiant flux density is the power per unit cross-sectional area. Energy flux is equal to the radiant flux density times the length of the laser pulse. Since the length of the laser pulse was constant, a change in the radiant flux density resulted in a proportional change in the energy density. It was found to be more convenient to analyze with respect to energy flux because the laser calorimeter measured in terms of energy.

Table 8 summarizes the relative effect of the varying irradiation conditions. It can be seen that all the mean fraction survivals were under 0.67 except for the two unfocused acridine orange conditions. Interestingly enough, the focused acridine orange samples showed definite kill. Unfortunately, Table 8 cannot clearly show the relative effect of energy flux on survival because of the
Table 8

The Relative Effect of A Variety of Laser Irradiation Conditions on the Fraction Survival of *E. coli*

<table>
<thead>
<tr>
<th>Lasing Dye</th>
<th>Beam Condition</th>
<th>Exogenous Photosensitizer</th>
<th>Total Number of Runs</th>
<th>Average* Energy Flux</th>
<th>Mean Fraction Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine 6G</td>
<td>Unfocused</td>
<td>None</td>
<td>46</td>
<td>8.4</td>
<td>.49</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Focused</td>
<td>None</td>
<td>21</td>
<td>55.7</td>
<td>.47</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Unfocused</td>
<td>Toluidine Blue</td>
<td>11</td>
<td>13.7</td>
<td>.44</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Focused</td>
<td>Toluidine Blue</td>
<td>15</td>
<td>54.9</td>
<td>.48</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Unfocused</td>
<td>Acridine Orange</td>
<td>9</td>
<td>16.5</td>
<td>1.49</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Focused</td>
<td>Acridine Orange</td>
<td>7</td>
<td>58.3</td>
<td>.60</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Unfocused</td>
<td>None</td>
<td>10</td>
<td>5.1</td>
<td>.67</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Focused</td>
<td>None</td>
<td>2</td>
<td>13.0</td>
<td>.55</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Unfocused</td>
<td>Acridine Orange</td>
<td>4</td>
<td>5.0</td>
<td>1.35</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Focused</td>
<td>Acridine Orange</td>
<td>2</td>
<td>13.0</td>
<td>.20</td>
</tr>
</tbody>
</table>

* Average energy flux was obtained by adding the individual run energy fluxes and dividing by the number of runs.
non-linear relationship between the two.

For a direct comparison of the relative inhibitive effect of the various lasing conditions, a computer program was written to calculate $k$ in equation 2-21 given $\lambda$ and $E$; $k$ was previously seen to be a constant dependent on microbiological parameters. Looking at equation 2-21, if the laser irradiation parameters, as represented by $Y(\lambda, E_L)$ are kept constant, as the survival ratio, $\frac{X}{X_0}$, decreases, the value of $k$ increases. That is, the higher the value of $k$ in equation 2-21, the more effective is the particular lasing condition. Table 9 gives the relative kill effect, $k$, of a variety of laser irradiation conditions. A close look at the results shows that the unfocused toluidine blue runs were the most effective in inhibiting $E. coli$.

Table 9 also shows that unfocused runs were significantly more efficient than focused runs. The negative $k$ value for unfocused acridine orange sensitized runs meant that growth was experienced.

There are two reasons advanced for why unfocused runs were more efficient than focused runs. One, beam alignment was more sensitive in focused irradiation as the droplet and beam size were smaller. As a result, the probability of misalignment was greater. Secondly, very intense light seemed to have a photoactivating effect. Focused light irradiation averaged close to 55 joules/cm$^2$, nearly five times more powerful than that of unfocused light. For example, referring to Figure 33 for unfocused rhodamine 6G runs, energy fluxes greater than 15 joules/cm$^2$ actually showed a reversal in the normal exponentially declining curve. In other words, at a certain energy flux point, a type of photoactivation apparently occurs.
Table 9

The Relative Kill Factor, $k$, of a Variety of Laser Irradiation Conditions

<table>
<thead>
<tr>
<th>Lasing Dye</th>
<th>Beam Condition</th>
<th>Exogenous Photosensitizer</th>
<th>Total Number of Runs</th>
<th>Relative Kill Factor, $k$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine 6G</td>
<td>Unfocused</td>
<td>None</td>
<td>46</td>
<td>1.73</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Focused</td>
<td>None</td>
<td>21</td>
<td>0.60</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Unfocused</td>
<td>Toludine Blue</td>
<td>11</td>
<td>1.87</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Focused</td>
<td>Toludine Blue</td>
<td>15</td>
<td>0.47</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Unfocused</td>
<td>Acridine Orange</td>
<td>9</td>
<td>-3.92</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Focused</td>
<td>Acridine Orange</td>
<td>7</td>
<td>0.28</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Unfocused</td>
<td>None</td>
<td>10</td>
<td>1.13</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Unfocused</td>
<td>Acridine Orange</td>
<td>4</td>
<td>-1.98</td>
</tr>
</tbody>
</table>
The coumarin runs followed the same pattern as those for rhodamine 6G. For the unfocused conditions, straight run irradiation showed inhibition and acridine orange sensitized runs indicated growth. Data was insufficient to ascertain the effect of focused irradiation.

One can only speculate as to why high intensity light caused an inhibition reversal. Although the intense light did in fact inhibit, very possibly, the extreme intensity also had an added opposite effect of accelerating the rate of cell division. Since the droplet remained in the liquid state for as long as 8-12 minutes after irradiation, and the E. coli doubling time was 30 minutes, there was sufficient time for added cell growth. There, too, could have been a physical effect of the beam breaking up cell pairs and chains into individual cells. Data was insufficient to model the apparent photoactivating effect.

3. Analysis with Respect to Wavelength

There were eleven rhodamine 6G and three coumarin runs attempted with narrowband radiation. Tuned outputs at 5800Å, 5900Å, 6000Å, and 4500Å were used to irradiate the droplet. The results were inconclusive with respect to wavelength. Table 11 is a summary of these tuned outputs.

Figure 7 in Chapter II shows that at the same energy flux, coumarin at 4500Å is twice as effective as that of rhodamine 6G at 5900Å. Considering the rhodamine 6G effective lasing wavelength band alone, radiation at 5700Å is only 1.1 times more effective than
Table 10

The Effect of Tuned Narrowband Outputs on the Fraction Survival of *E. coli*

<table>
<thead>
<tr>
<th>Lasing Dye</th>
<th>Wavelength (Å)</th>
<th>Beam Condition</th>
<th>Total Number of Samples</th>
<th>Average Energy Flux joules/cm²</th>
<th>Mean Fraction Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine 6G</td>
<td>5750</td>
<td>Unfocused</td>
<td>1</td>
<td>2.3</td>
<td>.52</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>5800</td>
<td>Unfocused</td>
<td>2</td>
<td>7.1</td>
<td>.49</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>5900</td>
<td>Unfocused</td>
<td>1</td>
<td>2.3</td>
<td>.69</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>6050</td>
<td>Unfocused</td>
<td>1</td>
<td>2.3</td>
<td>.52</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>5800</td>
<td>Focused</td>
<td>3</td>
<td>45.7</td>
<td>.38</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>6000</td>
<td>Focused</td>
<td>3</td>
<td>45.7</td>
<td>.61</td>
</tr>
<tr>
<td>Coumarin</td>
<td>4500</td>
<td>Focused</td>
<td>3</td>
<td>11.4</td>
<td>.81</td>
</tr>
</tbody>
</table>
6100\AA. As such, wavelength studies should not be conducted with the same lasing dye. Success might have been found with an intensive effort investigating coumarin at 4500\AA and rhodamine 6G at 5900\AA. However, the primary objective was to ascertain inhibition with broadband and tuned narrowband laser outputs. It was shown that tuned narrowband outputs were approximately as effective as broadband outputs.

4. Comparison of Straight and Photodynamic Runs

Toluidine blue and acridine orange were selected as the exogenous photosensitizers because they each had maximum absorption peaks at the peak lasing conditions of rhodamine 6G for the former and coumarin for the latter. Early microbiological laser irradiation studies \(^1,2\) indicated that exogenous photosensitizers were necessary for the inhibition of microorganisms.

Table 9 shows that toluidine blue sensitive suspensions were slightly more effective than the non-photodynamic runs. This finding supports the MacMillan study.\(^3\) However, the MacMillan group reported that almost no inhibition was observed when toluidine blue was not used. The difference can be attributed to the fact that laser irradiation conditions were not identical. Since a continuous gas laser irradiating at 6328\AA was used in the MacMillan probe, some credence can be attached to extrapolating Harms\(^4\) data into the visible. That is, high dose rates, as obtained from pulsed lasers, are more efficient than an equivalent total energy of many lower doses, as obtained from continuous lasers.
The effect of acridine orange was totally unexpected. Table 8 shows that unfocused laser light at energy fluxes of 16.5 joules/cm² for rhodamine 6G and 5.0 joules/cm² for coumarin caused an increase in the population of E. coli after irradiation. In direct contrast, focused laser radiation caused inhibition. It was earlier noted that focused laser light had a secondary photoactivating affect. This phenomenon was not true for acridine orange cell suspensions. Again the literature does not quite explain why catalysis was experienced. Since acridine orange is known to combine with DNA within the cell, a possible explanation is that with the right amount of light, the acridine orange molecule absorbs and transfers the light energy to the DNA molecule, thus accelerating the reproduction process. Physical cleavage is probably not the reason for the catalytic effect because focused light, which has a greater physical effect than unfocused light, caused inhibition. With very intense light, the acridine orange molecule transferred a critical amount of energy; enough to inhibit the cell.

In summary, the presence of exogenous photosensitizers significantly affected the irradiated microbiological population. Since the E. coli organism did not contain absorbing chromophores in the visible, photodynamic action on dye addition was expected. The purpose of these additives was to illustrate the importance of absorption in inhibition or catalysis. The results obtained proved out this point.
5. Re-evaluation of Math Model

Equation (2-21) relates the known conventionally irradiated \textit{E. coli} equation to the math model representing the laser irradiated droplet. The constant 0.398 was obtained by correlating the effect of 2537$^\circ$ light on the survival of \textit{E. coli} in liquid media. To enable comparison between 2537A and visible laser light, Figure 5 in Chapter II was used to readjust the relative effect of visible light.

From equation (2-21), we had

\[
\frac{x}{x_0} = e^{-0.398 E \chi W(\lambda) - k \chi Y(\lambda, E_L)}
\]  

(5-1)

The experimental runs gave results for $\frac{x}{x_0}$ at $\chi(\lambda, E_L)$. Equation (5-1) was linearized and $k$ was found by least squares technique to best fit the data. Table 11 gives these values for $k$.

In addition to $k$, the computer program also calculated the correlation coefficient $R$. In testing the correlation coefficient, a significance level is first chosen. The correlation coefficient is then calculated and compared with the critical value (as obtained from an appropriate table $^6$) of the correlation coefficient corresponding to the chosen significance level and the proper number of degrees of freedom. If the value of $R$ is larger than the critical value obtained in the table, the model is accepted as that representing the experimental results with a chance of error equal to 1.0 minus the confidence level. Table 11 gives these $k$ and $R$ values.

All runs showed statistically sound correlation coefficients. The unfocused, straight coumarin runs gave a correlation coefficient
Table 11

Computer Solution to a Least Squares Fit of the Experimental Data, where
\[ \ln(\text{fraction survival}) = -k(\text{energy flux}) \]

<table>
<thead>
<tr>
<th>Lasing Dye</th>
<th>Beam Condition</th>
<th>Exogenous Photosensitizer</th>
<th>Total Number of Runs</th>
<th>k</th>
<th>Correlation Coefficient R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine 6G</td>
<td>Unfocused</td>
<td>None</td>
<td>46</td>
<td>1.73</td>
<td>.73</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Focused</td>
<td>None</td>
<td>21</td>
<td>0.60</td>
<td>.90</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Unfocused</td>
<td>Toluidine Blue</td>
<td>11</td>
<td>1.87</td>
<td>.97</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Focused</td>
<td>Toluidine Blue</td>
<td>15</td>
<td>0.47</td>
<td>.99</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Unfocused</td>
<td>None</td>
<td>10</td>
<td>0.68</td>
<td>.53</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Focused</td>
<td>Acridine Orange</td>
<td>7</td>
<td>0.28</td>
<td>.93</td>
</tr>
</tbody>
</table>
of 0.53, which for 9 degrees of freedom meant that the model might be in error 10% of the time. All the other runs might have been in error 1% of the time.

It may be of interest to compare the laser irradiation models to the conventional light model presented in equation (5-1). The resultant models are

a) LASING DYE: Rhodamine 6G
BEAM CONDITION: Unfocused
EQUATION REPRESENTING FRACTION SURVIVAL:
\[ \frac{X}{X_0} = e^{-1.73 \gamma(\lambda, E_L)} \]  
(5-2)

b) LASING DYE: Rhodamine 6G
BEAM CONDITION: Unfocused
PHOTOSENSITIZER: Toluidine Blue
EQUATION REPRESENTING FRACTION SURVIVAL:
\[ \frac{X}{X_0} = e^{-1.87 \gamma(\lambda, E_L)} \]  
(5-3)

c) LASING DYE: Rhodamine 6G
BEAM CONDITION: Focused
PHOTOSENSITIZER: Acridine Orange
EQUATION REPRESENTING FRACTION SURVIVAL:
\[ \frac{X}{X_0} = e^{-0.28 \gamma(\lambda, E_L)} \]  
(5-4)

d) Conventional light equation representing fraction survival:
\[ \frac{X}{X_0} = e^{-0.398 E_c W(\lambda)} \]  
(5-5)

If an assumption can be made that laser light has the same effect as conventional light of the same energy flux and wavelength then
\[ E_c W(\lambda) = \gamma(\lambda, E_L) \] 

Or, on a relative basis, for identical energy flux inputs at the same wavelengths, the fraction survivals will be related by the following:

\[
\begin{align*}
\ln \left( \frac{X}{X_0} \right)_S &= \frac{1}{1.73} \\
\ln \left( \frac{X}{X_0} \right)_{TB} &= \frac{1}{0.87} \\
\ln \left( \frac{X}{X_0} \right)_{AO} &= \frac{1}{0.28} \\
\ln \left( \frac{X}{X_0} \right)_{C} &= \frac{1}{0.398}
\end{align*}
\]

where the subscripts S, TB, AO, and C refer to straight run laser radiation, toluidine blue suspension laser radiation, acridine orange laser radiation, and conventional radiation, respectively.

Simplifying,

\[
0.58 \ln \left( \frac{X}{X_0} \right)_S = 0.54 \ln \left( \frac{X}{X_0} \right)_{TB} = 3.57 \ln \left( \frac{X}{X_0} \right)_{AO} = 2.51 \ln \left( \frac{X}{X_0} \right)_C
\]

For example, if the fraction survival in the conventionally irradiated system is 0.80 the fraction survival for the laser irradiated straight run system will be 0.38, the laser irradiated toluidine blue sensitized system will be 0.35, and the laser irradiated acridine orange sensitized system will be 0.86.

In summary, the above calculations for the specific models considered showed that a toluidine blue sensitized \textit{E. coli} suspension ranked first in ease of kill. The un-sensitized suspension was a very close second. Both laser irradiated systems were more efficient than conventional light irradiation. However, conventional light was more effective than focused laser light on an acridine orange sensitized suspension. In conclusion, the data in Table 11 indicate that laser light is more efficient than conventional light at 5900\AA. 
LITERATURE CITED


CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

As stated in Chapter I, the objective of this investigation was to obtain firm evidence supporting the practicability of using a laser for either a process of sterilization or catalysis. The results of the experimental runs showed that inhibition of E. coli organisms could be achieved with visible laser light. Furthermore, the kill effect was enhanced when the exogenous photosensitizer, toluidine blue, was added to the cell suspension. Interestingly enough, sensitization with acridine orange caused a reverse effect, or catalysis. It could not be ascertained whether the increase in growth of the irradiated samples was a true catalytic effect or a chain-breaking cleavage effect.

This investigation showed that laser radiation was at least as effective as conventional visible light in inhibiting E. coli. There was an indication that pulsed laser light was actually more effective than continuous conventional light.

Since the outcome of this initial probe is actually point of departure for future work in the area of laser induced sterilization and catalysis of microorganisms, several areas for further research are worthy of mention:

a) Since visible radiation was shown to be effective in inhibiting E. coli, and ultraviolet radiation at 2650Å is known to be about 30,000 times more effective than rhodamine 6G radiation at 5900Å, it becomes immediately obvious that an ultraviolet laser
would be ideal for laser sterilization. Since the wavelength effectiveness curve begins tapering off at around 3000Å, frequency doubled 5900Å would be satisfactory for laser irradiation studies; 2950Å radiation is only half as effective as 2650Å. There is a 15,000-fold improvement in frequency doubled 2950Å radiation relative to the primary incident wavelength at 5900Å. There is strong evidence that the present 100 joule/one microsecond lasing system will not be able to efficiently frequency double rhodamine 6G. For this reason, an upgraded system is recommended. Three courses can be taken. The first is to improve the present risetime by at least a factor of 10, with 100 being preferred. Several coaxial flashlamp tunable dye lasers now on the market can satisfy the factor of 10 criterion. Another route is to purchase a sufficiently powerful nitrogen or argon gas laser to obtain laser-pumped organic dye laser outputs. The factor of 100 criterion will be satisfied with this system. With each of the above two improvements should go a correspondent renovation of the frequency doubling accessory. It is suggested that a temperature-controlled ADF crystal be substituted for the angle-controlled KDP crystal. A third path is to purchase a parametric oscillator system capable of furnishing tunable ultraviolet outputs.

b) A second hardware refurbishment suggestion applies to a device which can measure power and wavelength. Several spectroradiometer systems on the market can accomplish both tasks.

c) The radiation of organisms other than E. coli is recommended. This organism did prove that visible laser radiation could inhibit bacteria. However, if research is continued on visible light irradia-
tion, a bacterium containing an endogenous photosensitizer is advisable. Although an intense literature survey seeking visible light absorbing and other more relevant bacteria should be undertaken, the purple photosynthetic bacteria, Chromatium, Thiospirillum, Rhodospirillum, and particularly Rhodospirillum rubrum are mentioned as possibilities. For obvious reasons, it is strongly recommended that efforts be confined to the ultraviolet irradiation of bacteria. This being the case follow-up can be made to the initial industrial survey with the purpose of updating the list of organisms suggested by industry for sterilization, selective sterilization, and catalysis.

d) If perchance the decision is made to more fully exploit the present tunable dye laser, the following emendations should be seriously considered. An attempt should be made to modify the micropulser electronic circuitry to reduce the minimum pulsing rate to one pulse every five seconds. The maximum pulsing rate should be set to five pulses every second. The reflector cavity should be re-designed to enable direct pumping of the dye solution through the dyecell. The mirrors should also be moved closer to the dyecell. Arrangements should be made with XENON for an L- or U-shaped flash-lamp so this maneuvering about can be made possible.

In summary, it is recommended that the present research be extended to include the following items:

(a) A specifically designed and a more precise investigation of the parameters that describe the survival ratio of one organism in a batch reactor. For this application, a digital or hybrid
solution of the competing equations should be conducted to complement the laboratory data.

(b) Extension of the immediate above to a continuous stirred tank reactor.

(c) Another extension to investigate the feasibility of selective sterilization and or catalysis.

Since Figure 9B in Chapter III outlines the long range projections, it is only sufficient to say at this time that the course of this project should be aimed at the eventual development of full scale processes of sterilization and catalysis. In closing, it is not recommended that pioneering research work be conducted at establishing novel lasing schemes. The field of laser technology has been characterized by continuing successions of progress in quantum leaps. It is more important to keep up with this progress and adapt working laser systems to well-designed processes of sterilization and catalysis.
APPENDICES
APPENDIX A

NOMENCLATURE

\[ a \quad \text{one-half the major axis} \]
\[ a(\lambda) \quad \text{absorbance} \]
\[ A \quad \text{amplitude of waveform} \]
\[ \bar{A} \quad \text{angstroms, } 10^{-10} \text{ meters} \]
\[ b \quad \text{one-half the minor axis} \]
\[ B \quad \text{Einstein coefficients} \]
\[ c \quad \text{speed of light} \]
\[ c_s \quad \text{concentration of solute in moles/liter} \]
\[ C \quad \text{capacitance of micropulser, farads} \]
\[ C_1 \quad \text{constant in the radiation formula} = 2\pi hc^2 = 3.74 \times 10^{20} \text{ watts/cm}^2 (\bar{A})^4 \]
\[ C_2 \quad \text{constant in the radiation formula} = \frac{hc}{k} = 1.438 \times 10^8 \bar{A}^0 K \]
\[ C_C \quad \text{circumference of a circle} \]
\[ C_D \quad \text{concentration-threshold factor of dye} \]
\[ C_E \quad \text{circumference of an ellipse} \]
\[ d \quad \text{groove spacing of diffraction grating} \]
\[ d_d \quad \text{bore diameter of dyecell, cm} \]
\[ d_f \quad \text{bore diameter of flashlamp, cm} \]
\[ D \quad \text{dilution rate, or volumetric flow rate divided by the reactor volume} \]
\[ D_C \quad \text{diameter of circle} \]
\[ D_y \quad \text{dye factor} \]
\[ E \quad \text{energy, joules} \]
\[ E(\lambda) \quad \text{validity efficiency} \]
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_C$</td>
<td>total energy flux of conventional radiation, $\text{mJ/cm}^2$</td>
</tr>
<tr>
<td>$E_L$</td>
<td>total energy flux emitted by laser, joules/cm$^2$</td>
</tr>
<tr>
<td>$E_m$</td>
<td>molar extinction coefficient, the optical density at 1M, liters/mole</td>
</tr>
<tr>
<td>$f$</td>
<td>frequency of radiation, $\text{sec}^{-1}$</td>
</tr>
<tr>
<td>$F_0$</td>
<td>distance between the foci of an ellipse</td>
</tr>
<tr>
<td>$g$</td>
<td>probability per unit frequency that an excited molecule emits at the frequency</td>
</tr>
<tr>
<td>$G$</td>
<td>cavity gain</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck's constant $= 6.625 \times 10^{-27}$ erg/sec</td>
</tr>
<tr>
<td>HS</td>
<td>un-ionized substrate concentration, a pH effect, gm/liter</td>
</tr>
<tr>
<td>$I$</td>
<td>transmitted light intensity, mw/cm$^2$</td>
</tr>
<tr>
<td>$I_c$</td>
<td>radiant flux density of laser beam, mw/cm$^2$</td>
</tr>
<tr>
<td>$I_o$</td>
<td>incident light intensity, mw/cm$^2$</td>
</tr>
<tr>
<td>$I_\phi$</td>
<td>radiant flux density of laser beam as measured with a laser calorimeter, mw/cm$^2$</td>
</tr>
<tr>
<td>$k$</td>
<td>Boltzmann's constant $= 1.380 \times 10^{-16}$ erg/deg</td>
</tr>
<tr>
<td>$k'$</td>
<td>microbiological growth constant</td>
</tr>
<tr>
<td>$k_{nr}$</td>
<td>rate constant for radiation less transitions to the ground state</td>
</tr>
<tr>
<td>$k_{s,T}$</td>
<td>rate constant for intersystem crossing into the triplet manifold</td>
</tr>
<tr>
<td>$k_Q$</td>
<td>rate constant for the quenching reaction</td>
</tr>
<tr>
<td>$K$</td>
<td>temperature in degrees Kelvin</td>
</tr>
<tr>
<td>$K_f$</td>
<td>spatial frequency or phase, numerically equal to the number of waves in a distance $2\pi$ centimeters</td>
</tr>
<tr>
<td>$K_i$</td>
<td>inhibition constant, numerically equals the highest substrate concentration at which the specific growth rate is equal to one-half the maximum specific growth rate in the absence of inhibition, gm/liter</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>$K_S$</td>
<td>saturation constant, numerically equals the lowest concentration of the substrate at which the specific growth rate is equal to one-half the maximum specific growth rate in the absence of inhibition, gm/liter</td>
</tr>
<tr>
<td>$L$</td>
<td>traverse length of the light beam through a medium</td>
</tr>
<tr>
<td>$L_o$</td>
<td>decay length</td>
</tr>
<tr>
<td>$L_R$</td>
<td>latus rectum of an ellipse</td>
</tr>
<tr>
<td>$M_B$</td>
<td>efficiency factor depending on optics used at back end of laser cavity</td>
</tr>
<tr>
<td>$M_F$</td>
<td>efficiency factor depending on optics used at front end of laser cavity</td>
</tr>
<tr>
<td>$M_R$</td>
<td>reflectance of mirror located behind the microbiological sample</td>
</tr>
<tr>
<td>$n$</td>
<td>inversion</td>
</tr>
<tr>
<td>$n_o$</td>
<td>threshold inversion population</td>
</tr>
<tr>
<td>$n_M$</td>
<td>number of molecules per unit volume</td>
</tr>
<tr>
<td>$n_D$</td>
<td>order of diffraction</td>
</tr>
<tr>
<td>$n_{2C}$</td>
<td>critical inversion</td>
</tr>
<tr>
<td>$n_T$</td>
<td>the population of the first excited triplet state</td>
</tr>
<tr>
<td>$N_1$</td>
<td>number of atoms in the lower excited or ground state</td>
</tr>
<tr>
<td>$N_2$</td>
<td>number of atoms in the higher excited state</td>
</tr>
<tr>
<td>$N_T$</td>
<td>critical inversion</td>
</tr>
<tr>
<td>$P$</td>
<td>polarization</td>
</tr>
<tr>
<td>$P_u$</td>
<td>number of laser pulses</td>
</tr>
<tr>
<td>$q$</td>
<td>number of quanta of lasing photons in the cavity</td>
</tr>
<tr>
<td>$Q$</td>
<td>concentration of the quenching agent</td>
</tr>
<tr>
<td>$s$</td>
<td>absorption cross-sectional area of molecule</td>
</tr>
<tr>
<td>$S$</td>
<td>residual concentration of substrate nutrient, gm/liter</td>
</tr>
<tr>
<td>$S_0$</td>
<td>ground state</td>
</tr>
</tbody>
</table>
\begin{align*}
S_1 &= \text{first excited singlet state} \\
t &= \text{time, sec} \\
t &= \text{radiation exposure time to laser light, sec} \\
t_c &= \text{cavity lifetime} \\
t_0 &= \text{photon lifetime in the active region of the laser cavity} \\
T &= \text{temperature, } ^\circ\text{K} \\
T_1 &= \text{first excited triplet state} \\
T_2 &= \text{second excited triplet state} \\
V &= \text{voltage across flashlamp, volts} \\
V_K &= \text{power supply voltage, kilovolts} \\
W &= \text{total energy flux, watts/ cm}^2 \\
W(t) &= \text{pumping pulse with Gaussian profile} \\
W(\lambda) &= \text{relative effect of wavelength} \\
x &= \text{space distance along the optical axis, cm} \\
X &= \text{concentration of microorganism, cells/ml} \\
X_0 &= \text{initial concentration of microorganisms, cells/ml} \\
Y &= \text{yield coefficient, gram cells produced per gram nutrient consumed} \\
\delta &= \text{absorption coefficient} \\
\epsilon_T(\lambda) &= \text{triplet-state Einstein coefficient} \\
\eta &= \text{refractive index of the lasing medium} \\
\theta &= \text{angle of incidence measured from the normal} \\
\theta' &= \text{angle of diffraction measured from the normal} \\
\lambda &= \text{wavelength of laser emission, } \AA \\
\lambda_m &= \text{wavelength of laser emission, } \AA \\
\mu &= \text{growth rate, hr}^{-1}
\end{align*}
\[ \mu_L = \text{laser radiation kill rate, hr}^{-1} \]
\[ \hat{\mu} = \text{maximum growth rate in the absence of inhibition, hr}^{-1} \]
\[ \tau_f = \text{fluorescence lifetime} \]
\[ \phi = \text{fluorescence quantum yield} \]
\[ \phi_L = \text{efficiency of laser} = \frac{\text{input energy}}{\text{emitted energy}} \]
\[ \psi = \text{variable dependent on the laser irradiation parameters wavelength and energy flux} \]
APPENDIX B
GLOSSARY OF LASER SCIENCE TERMS

Angstrom: unit of length equivalent to $10^{-10}$ meters.

Bandwidth: the portion of the light spectrum occupied by an electromagnetic wave.

Brewster Angle: that angle of a transparent material at which the reflection coefficient drops to zero and transmission is maximized.

Coherence: the ability of electromagnetic radiation to keep in phase.

Collimation: ability of a beam of light to preserve a constant cross-sectional area.

Divergence: the loss of collimation; a typical laser has an output that diverges from one-half to ten minutes of arc.

Excitation: the transition of a ground state molecule to an excited state; same as "pumping".

Flashlamp-pumped dye laser: an organic dye laser that is excited by a flashlamp.

Frequency doubling: the doubling of the frequency, or halving of the wavelength of light that passes through a crystal having such non-linear properties; also called second harmonic generation.

Gain: the amplification factor characterizing the magnification intensity of stimulated light in a laser cavity.

Giant pulse: extremely intense radiation caused by building up energy and releasing in very short intervals; see "Q-switching".

Ground state: that state in which the energies of rotation, vibration and electronic configuration within the molecule are at the minimum.

KDP crystal: a non-linear crystal used for frequency doubling; potassium dihydrogen phosphate.

LASER: acronym for light amplification by the stimulated emission of radiation.

Laser-pumped laser: an organic dye laser that is excited by another laser.
Lithium niobate: a non-linear crystal used in the parametric oscillator system.

MASER: microwave analog of laser.

Mode: the intensity profile of a laser; TEM\textsubscript{oo} mode indicates a Gaussian profile (very intense at the center of the beam, less intense as the edges are approached).

Monochromatic radiation: electromagnetic radiation of a single wavelength; for practical purposes, a single wavelength is anything close to or less than one angstrom.

Parametric oscillator: an optical system which utilizes a non-linear principle wherein the property of certain crystals is used to create other wavelengths in a tunable laser.

Population inversion: atomic or molecular level condition wherein more atoms or molecules of a lasing media are found in the excited state than the ground state.

Pump: to excite a molecule to a higher energy state.

Q-switch: an optical component that artificially impairs the optical path of a laser with the purpose of delaying the onset of laser oscillations.

Radiant flux: the rate of flow or radiant energy through a surface; typical units are watts or joules/second.

Radiant flux density: intensity of radiation incident on a surface; typical units are watts/cm\textsuperscript{2}.

Risetime: the time required for the light pulse to reach maximum intensity; organic dye laser flashlamps have risetimes in the order of 100 nanoseconds to one microsecond.

Second harmonic generation: same as frequency doubling (SHG).

Singlet state: all electrons are present in pairs in which the spins are opposite so that the resultant spin for each pair is zero.

Spontaneous emission: emission of radiation without external causation.

Stimulated emission: radiation emitted from an atomic system in the presence of external radiation; the phase and wavelength of the stimulated emission are the same as that of the stimulating external radiation.
Triplet state: an excited state in which an orbital electron has an unpaired spin; intersystem crossing from the singlet into the triplet state is a significant source of loss in lasing action.

Tunable laser: a laser whose output wavelength can be changed to suit any desired wavelength condition within the lasing range of the laser.
APPENDIX C

GLOSSARY OF RADIATION MICROBIOLOGY TERMS

Absorption coefficient: the value $k$ in the equation $p = kcx$, where $p$ is the optical density or absorbance of a solution, $c$ is the concentration of the solution, and $x$ is the thickness of the solution.

Absorption cross-section: the relative probability that an incident photon will be absorbed; or in ratio form, the number of photons absorbed divided by the number of photons incident.

Actinometry: a means of measuring the intensity of incident radiation by observing the resultant chemical change.

Action spectrum: the relative response to wavelength.

Bacteria: any of a class (Schizomycetes) of unicellular microscopic plants receiving nourishment autotrophically ($CO_2$) or parasitically; $10^{-5}$ to $10^{-9}$ in size.

Bacteriophage: bacterial viruses that are noted for destroying bacteria; about $10^{-3}$ in size.

Bandwidth: the wavelength range of radiation.

Beer's Law: the relationship represented by the following equation,

$$\text{Transmittance} = \frac{I}{I_0} = 10^{-Emc} = e^{-ns}$$

where,

$E_m$ = molar extinction coefficient, or the optical density under standard conditions of a 1M solution; liters/mole-cm

$l$ = thickness of solution in centimeters

$c$ = concentration of solute in moles/liter

$n_m$ = number of molecules per unit volume

$s$ = absorption cross-sectional area of a molecule.

Chromophore: molecular conjugated structures that are responsible for most organic absorption of radiation.

DNA: the double helix-shaped molecule responsibility for heredity in any living organism and characterized by strong ultraviolet absorption at 2650Å; deoxyribonucleic acid.
Enzyme: complex proteinaceous substance produced by living cells to accelerate reactions; the enzyme itself is not a living organism but its reacting properties can be altered or destroyed by the same factors that cause bacterial death.

Inactivation cross-section: a term that represents the relative probability that an incident photon will inactivate an organism.

Irradiance: the power striking an object per unit area; watts/cm².

Optical density: the absorbancy of a solution; \( \log_{10} \) of transmittance.

Photosensitizer:
- **Endogenous**: pigments naturally occurring within the cell that absorb light; like for example chlorophyll in a photosynthetic organism.
- **Exogenous**: pigments that can be absorbed by a cell; like for example the dye toluidine blue that is artificially added to a growing medium.

Radiant flux density: the power crossing a unit area normal to the beam; watts/cm².

Reciprocity of time and dose rate: the effect of radiation is only a function of the total dose, and not of the time during which the irradiation occurs, that is, if the time is doubled and dose rate halved, the effect will be the same.

RNA: another type of nucleic acid; ribonucleic acid.

Thymine: a nucleic acid base most susceptible to irradiation at 2650Å.

Transmittance: the ratio of the energy leaving a sample to the energy incident upon the sample.

Ultraviolet:
- **Near ultraviolet**: 3800Å-3000Å
- **Far ultraviolet**: 3000Å-1900Å
- **Vacuum ultraviolet**: 1900Å-1000Å

Virus: either plant or animal of very small size \((10^3 \text{ Å})\) which does not exhibit characteristics of living materials yet can invade cells and multiply intracellularly.

Visible light: radiation between 4000Å and 7000Å.

Yeast: a term of convenience referring to nonfilamentous *Ascomycetes*; yeasts are somewhat larger than bacteria.
APPENDIX D

RADIATION MICROBIOLOGY MEASUREMENT UNITS

Photobiology is the study of the effect of nonionizing radiation on any biological material. Radiobiology is generally thought to be biological study with ionizing radiation. Although the present investigation worked with nonionizing radiation the ultimate process can very well utilize some form of ionizing laser beam. For this reason the term radiation microbiology was used because the expression covered both ionizing and nonionizing radiation, and for the particular interest of the project, alluded to microbial organisms.

Radiometry uses the c.g.s. system of measurement and applies to all electromagnetic radiation. Photometry uses the international candle and lumens as units and measures the intensity of light in terms of eye sensations. In photometrical terms, monochromatic light is difficult to define and ultraviolet light has a zero intensity. All terminology used in the investigation refer to radiometry units.

A beam of radiation is measured in terms of radiant energy, or joules. The rate of flow of the beam of radiation is called radiant flux, or analogously, radiant power, and is measured in watts. The radiant flux crossing a unit area normal to the beam is called the radiant flux density and is measured in watts/cm². Another common term is energy flux which is measured in joules/cm² and normally refers to the total amount of energy absorbed by a medium per unit cross-section.
Terms relating to a point source of radiation will be avoided as radiant intensity and radiance refer to power per steradian. A laser beam is well collimated and for the completed experiments experienced negligible divergence.

A term of great use is irradiance, which is the power striking an object per unit area. In biological applications, the irradiance is normally expressed in microwatts/cm$^2$.

Intensity is a universal term indicating power per unit area. However, to avoid misdirection, power per unit area will be expressed by the conventional radiometry term irradiance when a struck object is intended and radiant flux density when an emitted beam of radiation is the designation.
Although all three sections are closely related, the following discussion will be split into the quantum mechanics of laser action, a special detailed presentation on organic dye lasers, and second harmonic generation. The information contained in this appendix is a condensation of several articles on the subject and is presented as an aid for the reader interested in the mathematics behind laser theory.

The coupled differential equations describing the laser process are as follows:

\[
\frac{dn}{dt} = W(t) - \left( \frac{n}{n_0} \right) \left( \frac{q}{t_c} \right) - n \left( \tau_f^{-1} + k_{nr} \right) - nk_s \tau_T \tag{E-1}
\]

\[
\frac{dq}{dt} = \left( \frac{q}{t_c} \right) \left( \frac{n}{n_0} - 1 \right) - \frac{q n_T}{t \delta t_c n_0} \tag{E-2}
\]

\[
\frac{dn_T}{dt} = nk_s \tau_T - k_Q n_T \tag{E-3}
\]

\[
W(t) = W_{\text{max}} \exp \left\{ -\left[ \frac{t - T_1}{(\ln 2)^{1/2}} \right]^2 \right\} \tag{E-4}
\]

where

\[ n \] the inversion, and to a good approximation represents the population of the first excited singlet state

\[ q \] the number of quanta of lasing photons in the cavity

\[ n_T \] the population of the first excited triplet state

\[ t \] the time

\[ W(t) \] the pumping pulse that is assumed to have a Gaussian shape
with a maximum at $t = 0$

$n_o$ = the threshold inversion population

$\tau_f$ = the fluorescence lifetime

$k_{nr}$ = the rate constant for radiationless transitions to the ground state (assigned value of 0 for all calculations)

$k_{ST}$ = the rate constant for intersystem crossing into the triplet manifold

$\delta$ = the ratio of the singlet-singlet extinction coefficient to the triplet-triplet extinction coefficient at the laser wavelength

$T_1$ = the half-width at half-height of the pumping pulse

$N$ = the total number of absorbed photons = $\int_{-\infty}^{\infty} W(t)dt$

$k_Q$ = the rate constant for the quenching reaction

$Q$ = the concentration of the quenching agent

$k_{Q}^{-1}$ = the reciprocal of the triplet lifetime ($\tau_p$).

The above equations were derived by Peter Sorokin, et al. Using the Runge-Kutta method and solving for particular sets of parameters, relationships between the concentrations of the excited singlet state, excited triplet state, and photons in the cavity were obtained.

However, making a steady-state approximation and solving the resulting set of simultaneous equations, the following solution can be obtained:

$$q(X) = t_{\text{c}} W_{\text{max}} \left[ \left( \frac{N}{n_o} \right) R(X) \left( 1 - \frac{k_{ST}}{\delta k_Q} - US \right) / S \right]$$  \hspace{1cm} (E-5)

$$n(X) = \frac{NR(X)}{\left( \frac{S_q(X)}{t_{\text{c}} W_{\text{max}}} + U \right)}$$  \hspace{1cm} (E-6)

$$n_T(X) = k_{ST} \tau_p n(X)$$  \hspace{1cm} (E-7)
where
\[ R(X) = \frac{t_c}{T_1} \left( \frac{1n2}{\pi} \right)^{\frac{1}{2}} \exp\left\{ -\left( \frac{Xt_c}{T_1} \right) \left( \frac{1n2}{\pi} \right)^{\frac{1}{2}} \right\} \] (E-8)

\[ S = \frac{N}{n_o} \frac{t_c}{T_1} \left( \frac{1n2}{\pi} \right)^{\frac{1}{2}} \] (E-9)

\[ U = \frac{t_c (T_{nr} + T_f)}{\tau_{nr} + T_f t_c} + k_{s'T} t_c \] (E-10)

\[ X = \frac{t_c}{\tau_c} \]

By integrating equation (E-5) over the range of its validity, efficiency \( E \) can be found as

\[ E = \frac{1}{T_1} \left( \frac{1n2}{\pi} \right)^{\frac{1}{2}} \int_{-A}^{A} \frac{q(X)}{W_{max}} dx \] (E-11)

An approximate solution for the efficiency is

\[ E \approx \left( 1 - \frac{k_{s'T}}{6k_{Q}} \right) \text{erf}(A') \] (E-12)

where

\[ A' = \left\{ \ln\left[ \left( 1 - \frac{k_{s'T}}{6k_{Q}} \right) \left( \frac{S}{U} \right) \right] \right\}^{\frac{1}{2}} \] (E-13)

In equation (E-11) \( A \) and \(-A\) represent the time of extinction and initiation of the laser. The error function used in (E-12) is defined as

\[ \text{erf}(\xi) = \frac{2}{\sqrt{\pi}} \int_{0}^{\xi} e^{-t^2} dt \] (E-14)
No attempt was made by this investigation to formulate nor find the solution to the above since they already existed and the chief interest with these equations was to extract information necessary to fabricate a working laser system. As it was, these solutions were not used to actually design a novel system; they were used as checks in determining which parts of which extant systems to incorporate.

Since it is very difficult to get a feel for the physical significance of the above solution, a simplified treatment working with the critical inversion, $n_{2c}$, and cavity gain, $G(\lambda)$, is appropriate. Most of the following was adapted from the analysis by Snavely and Peterson. The start oscillation condition for critical inversion is

$$n_{2c} = \frac{8\pi\tau V^2}{3c^3g(V)t_0} \quad \text{(E-15)}$$

where

- $\tau$ = spontaneous emission decay time for the singlet transition ($2 \rightarrow 1$ in Figure 2)
- $V$ = oscillation frequency of the laser transition
- $t_0$ = photon lifetime in the active region of the laser cavity
- $g(V)$ = probability per unit frequency interval that an excited molecule emits at the frequency
- $c$ = velocity of light

Since it is easier to work with the measured fluorescence, $E(\lambda)$, rather than $g(V)$, the following identity can be used:

$$\int_{0}^{\infty} g(V)dV = \phi = \int_{0}^{\infty} E(\lambda)d\lambda \quad \text{(E-16)}$$
\( \phi \), the fluorescence quantum yield, is defined as the ratio of the number of fluorescent photons emitted to the number of excitation photons absorbed by a large number of dye molecules, that is,

\[
\phi = \frac{\text{wanted fluorescence}}{\text{unwanted decay process losses}}
\]  

(E-17)

Although \( \phi \) varies with the dye, solvent, temperature, and other experimental conditions, the value is normally between 0.5 and 1.0.

Knowing that \( c = \lambda V \) and integrating equation (E-16), we obtain

\[
E(\lambda) = \frac{c g(V)}{\lambda^2}
\]  

(E-18)

Thus, the critical inversion becomes

\[
n_{2c} = \frac{8\pi}{\lambda^4 E(\lambda) t_o}
\]  

(E-19)

The terms \( E(\lambda) \), \( \tau \), and \( \lambda \) can be obtained experimentally. If the dye fluorescence spectrum and the fluorescence decay time are known, and the cavity lifetime specified, equation (E-19) can be used to determine the inversion required for stimulated emission at any arbitrary wavelength.

Unfortunately, equation (E-19) does not work too well for dye lasers as optical losses due to triplet absorption have been neglected. Without getting too involved, an expression can be developed for the critical inversion that includes triplet-state losses.

Utilizing the standard laser paradigm, cavity with flashlamp, dyecell and two mirrors as shown in Figure 15, consider the gain,
$G(\lambda)$ of a laser at wavelength $\lambda$. $G(\lambda)$ is equal to the number of photons produced by stimulated emission minus the number lost as a radiation pulse of intensity $u(\lambda)$. Therefore,

$$G(\lambda) = u(\lambda)N_2B_2V_2 \cdot 2L_1 - u(\lambda)N_0B_0V_0 \cdot 2L_2$$

$$- u(\lambda)N_3B_3V_3 \cdot 2L_1 - u(\lambda)(1-r_1r_2)$$

(E-20)

where

- $N_2, N_0, N_t$ = density of molecules at $S_1, S_0, T_1$, respectively
- $B_0, B_2, B_3$ = Einstein B coefficients for singlet, emission, and triplet absorption, respectively
- $V_0, V_2, V_3$ = appropriate density-of-state factors

Capitalized terms are factors used to distinguish the previous calculation from the ensuing.

The products in equation (E-20) can be combined if one notes that $B_0V_0$ is the probability per unit time $A(\lambda)$ that a molecule in the ground state absorbs radiation at the wavelength $\lambda$, and is equal to the product of the extinction coefficient and $c$ in the medium.

$B_3V_3$ is a similar product for the triplet state term and is equal to $T(\lambda)$, where

$$T(\lambda) = \frac{\epsilon_T(\lambda)c}{\eta}$$

and

$$\epsilon_T(\lambda) = \text{triplet-state einstein coefficient}$$

$$\eta = \text{refractive index of the medium}$$

In their analysis of the laser, Yariv and Gordon demonstrated that the ratio of the induced transition rate to the spontaneous transition rate into a given cavity mode is equal to the number of quanta in the
Thus, 

\[ E_{2V_2} = \frac{E(\lambda)\lambda^4}{8\pi} \] (E-22)

Equation (E-20) now becomes

\[ G(\lambda) = u(\lambda) \frac{\lambda^4}{8\pi\pi} N_2 E(\lambda) 2L_1 - u(\lambda) N_o A(\lambda) 2L_2 \]

\[- u(\lambda) N_t T(\lambda) 2L_1 - u(\lambda)(1 - r_1 r_2) \] (E-23)

Normalizing the gain,

\[ g(\lambda) = \frac{G(\lambda)}{2u(\lambda)} \] (E-24)

and knowing that stimulated emission occurs at the wavelength for which \( g(\lambda) \) is a maximum, differentiation of (E-23) with respect to the wavelength given results in

\[ \frac{dg(\lambda)}{d\lambda} = 0 = \frac{N_2 L_1 \lambda^3}{2\pi\pi} \left\{ \frac{\lambda}{4} E'(\lambda) + E(\lambda) \right\} \]

\[-N_o A'(\lambda) L_2 - N_1 T'(\lambda) L_1 + \frac{1}{2} \frac{d}{d\lambda} r_1 r_2 \] (E-25)

The differentiated terms are denoted by primes. Since all molecules are in the ground state, the excited singlet state, or the triplet state we have the total molecular concentration \( N = N_o + N_2 + N_t \).

Substituting into (E-25) to eliminate \( N_o \), the following results

\[ N_2 = K_1 N \begin{pmatrix} L_2 \\ L_1 \end{pmatrix} + K_2 N_t \] (E-26)

where

\[ K_1 = \frac{A'(\lambda_2)}{\frac{\lambda_2^3}{2\pi\pi} \left[ \frac{\lambda_2^2}{4} E'(\lambda_2) + E(\lambda_2) \right] + A'(\lambda_2)} \] (E-27)
The subscript 2 means that the wavelength can be determined experimentally. At this point it is convenient to digress a bit and define a decay length, $L_o$, as

$$L_o = \frac{t_o c}{N} = \frac{t_o c}{.434}$$

where $N$ is the refractive index of the laser medium as defined by transmission over the path $e^{-1}$. Since $T_o N_2c = t_o n_2c$

$$L_o = \frac{n_2 c t_o c}{N_2c}$$

Furthermore, let us assume that the triplet-state extinction, $\epsilon_t$, can be found in the literature. The triplet state concentration is now equal to the entire absorption in the decay length minus the singlet-state contribution, or

$$N_t = \frac{.434 - a(\lambda)L_o}{L_o \epsilon_T(\lambda)}$$

where $a(\lambda)$ is the absorbance of a one centimeter path due to singlet-state absorption. Finally, we now have the critical inversion by substituting equation (E-31) into (E-26)

$$N_{2c} = \frac{K_1 N (\frac{L_2}{L_1}) - K_2 a(\lambda)/\epsilon_T(\lambda)}{1 - .434 K_2 / \epsilon_T(\lambda) n_{2c} t_o c}$$

where $K_1$ and $K_2$ are defined in equations (E-27) and (E-28).

This relatively "simple" equation will now be used to determine the critical inversion and concentration of the triplet state of
the dye disodium fluorescein salt. Fluorescein was especially chosen because it is known to be extremely difficult to lase and awareness of the populations in the singlet and triplet states can determine if lasing can indeed be induced with the present 100-joule setup. Calculations for rhodamine 6G, the easiest dye to lase, will be included for comparison purposes.

Although an assumption was made earlier in that certain important values had to be found in the literature, if need be, accurate measurements of the absorption and emission spectra can be obtained in the laboratory. For example, a spectrophotometer can measure the optical absorption spectra of fluorescein and fluorescence spectra and quantum yields with a fluorimeter. Fluorescence lifetimes can be determined using fluorescence decay apparatus. As these determinations were far beyond the scope and purpose of this investigation, values for the fluorescence spectra and quantum yields were taken from the flash photolysis study of fluorescein by Lindqvist. 4

Since lasing commences at the shorter wavelength end of the spectrum, the wavelength used for fluorescein is 5360 Å. As such T'(λ) is approximately zero. Therefore, K2 = -K1, and the critical inversion equation is simplified to

\[ N_{2c} = \frac{\frac{L_2}{L_1} + a(\lambda)/\epsilon_T(\lambda)}{(K_1') + 0.434/\alpha \epsilon_T(\lambda)} \]  

where

\[ \alpha = \frac{n_{2c} t_{2c}}{\eta} \]
The result in Table E-1 is obtained when an energy density in the area of 0.1 joules per cubic millimeter and a flashtube risetime of 700 nanoseconds are used.

Table E-1 graphically reveals that the concentration of the triplet state for fluorescein is considerably higher than that for rhodamine 6G. This inefficiency of the fluorescein dye is largely responsible for the difficulty experienced in trying to lase the dye. Since the present system has an energy density only one-half that of value used to calculate the data in Table E-1, and the flashtube risetime is at least twice that of the figure used in the example, it is understandable that lasing could not be achieved with the present setup. To induce fluorescein to lase, either significantly higher energy densities or much quicker risetimes is necessary. Another means to aid lasing of fluorescein is the addition of a compound that can quench the triplet state. Such an additive is cyclooctatetraene, which reduces the risetime requirement for lasing xanthene dyes like fluorescein.

A short qualitative discussion was presented earlier on frequency doubling or second harmonic generation (SHG). The origin of SHG can be understood in simple mathematical terms. Assume a one dimensional nonlinear relationship between polarization $P$ and the electric field $E$.

$$\vec{P} = \chi \vec{E}$$  \hspace{1cm} (E-35)

where $\chi$ is the susceptibility, a tensor quantity or

$$P = \chi E(1 + a_2 E + a_2E^2 + a_3E^3 + \ldots)$$  \hspace{1cm} (E-36)

The following oscillating field due to the incident light wave
Table E-1

Critical Inversion and Triplet-state Concentration for Laser Dyes (Snively, 1968)

<table>
<thead>
<tr>
<th></th>
<th>$\lambda_L \ (\text{cm}^{-1})$</th>
<th>$N_{2c} \ (10^{15} \text{cm}^{-3})$</th>
<th>$N_{2c} \ (10^{15} \text{cm}^{-3})$</th>
<th>$N_t \ (% \ \text{of} \ n_{2c})$</th>
<th>$n_{2c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na-fluorescein</td>
<td>5360</td>
<td>1.4</td>
<td>7.6</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>5790</td>
<td>0.84</td>
<td>4.4</td>
<td>3.9</td>
<td></td>
</tr>
</tbody>
</table>


$E = E_0 \sin wt \quad \text{(E-37)}$

will then produce a polarization

$$P = \chi E_0 \sin wt + \chi a_2 E_0^2 \sin^2 wt + \chi a_3 E_0^3 \sin^3 wt + \ldots \quad \text{(E-38)}$$

$$P = \chi E_0 \sin wt + \chi a_2 E_0^2 \frac{1}{2}(1-\cos 2wt) + \ldots \quad \text{(E-39)}$$

$$P = \chi E_0 \sin wt + \chi a_2 E_0^2 \frac{E^2}{2} - \chi a_2 E_0^2 \cos 2wt + \ldots \quad \text{(E-40)}$$

Since $a$ is very small, only if $E_0$ is very large will the second harmonic term $2\omega$ be significant. The second term is not important to the discussion. It stands for the direct current polarization. Obviously, with extremely high intensities, third harmonics are possible.
LITERATURE CITED


APPENDIX F
DYE-SENSITIZED PHOTOAUTOXIDATION

Photoautoxidation, photosensitization, or photodynamic action can occur in a normally transparent cell by the addition of a dye. The induced effect is called exogenous photosensitization or dye-sensitized photoautoxidation. The photodynamic change of subcellular structures results from the selective oxidation of particular component molecules, and of biopolymers like nucleic acids from the selective destruction of particular amino acid or nucleic acid base residues.

The characteristics of photodynamic action are that the mechanism is: 1) dependent on oxygen, 2) independent of temperature, 3) irreversible, and 4) of zero order with respect to the amount of dye. The sensitizer molecule acts as a catalyst.

The kinetic treatment of dye-sensitized photoautoxidations in biological systems requires an examination of the formation and the nature of the excited states of sensitizing dyes, as well as the possible interactions of these excited forms with the various components of the photodynamic system. Qualitatively the electronic mechanism is similar to laser action. In the dark, a dye molecule in solution is in the ground singlet state. When a photon is absorbed, one of the electrons from a filled orbital is transferred to an unfilled higher energy orbital, or an excited state. The state can be either the singlet or triplet. As before, the triplet state is reached by intersystem crossing. It is suspected in the literature that sensitized photoautoxidations have a greater probability of
proceeding by way of the triplet state because the lifetime is larger.

In the simplest case, the exogenously photosensitized system consists of the dye, molecular oxygen, medium, and bacteria. Spikes and Glad\(^1\) summarized the more commonly proposed mechanisms for photodynamic action.

(a) \[ S + h\nu \rightarrow S^* \rightarrow S' \]

\[ S' + RH_2 \rightarrow S + RH_2^* \]

\[ RH_2^* + O_2 \rightarrow \text{products} \]

(b) \[ S + h\nu \rightarrow S^* \rightarrow S' \]

\[ S' + RH_2 \rightarrow SH_2 + R \]

\[ SH_2 + O_2 \rightarrow O_2 \rightarrow S + H_2O_2 \]

(c) \[ S + h\nu \rightarrow S^* \rightarrow S' \]

\[ S' + O_2 \rightarrow S + O_2^* \]

\[ RH_2 + O_2^* \rightarrow \text{products} \]

(d) \[ S + h\nu \rightarrow S^* \rightarrow S' \]

\[ S' + O_2 \rightarrow \text{SOO} \]

\[ RH_2 + \text{SOO} \rightarrow \text{products} + S \]

(e) \[ S + RH_2 \rightarrow SRH_2 \]

\[ SRH_2 + h\nu \rightarrow SRH_2^* \rightarrow BRH_2' \]

\[ SRH_2' + O_2 \rightarrow S + \text{product} \]

In the above five equation sets, \( S \) is the sensitizing dye, \( h\nu \) is a quantum of light, \( S^* \) is the first excited singlet state of the dye, \( S' \) is the triplet state of the dye, \( RH_2 \) is the substrate, \( RH_2^* \) is an excited form of the substrate, "products" represents the oxidized

form(s) of the substrate, R is an oxidized form of the substrate, SH₂ is a photoreduced form of the dye, O₂* is an excited or reactive form of oxygen, SOO is a reactive dye-oxygen complex, SRH₂ is a dye-substrate complex, and SRH₂* and SRH₂' are excited forms of the dye-substrate complex.

Equations (a) and (b) are the only two where the bacterium is the primary reactant with the excited sensitizer. Although the excited dye can react with the bacterium through resonance transfer, by charge transfer complex formation, and by electron transfer, it is generally believed that the latter, as represented by equation (a) is the most probable.
A reflecting diffraction grating is composed of an array of narrow slits, parallel, equidistant, and close together. The gratings used in the investigation were coated with aluminum, as dielectric coated gratings are not manufactured. The more grooves per unit length the greater the spectral narrowing capability of the grating. 1800 grooves per millimeter gratings were found to be optimal for this study.

The most general form of the diffraction grating equation is

\[ n \lambda = d(\sin \theta + \sin \theta') \]  \hspace{1cm} (G-1)

where

- \( n \) = order of diffraction
- \( \lambda \) = incident wavelength
- \( d \) = groove spacing
- \( \theta \) = angle of incidence measured from the normal
- \( \theta' \) = angle of diffraction measured from the normal.

Figure G-1 is a schematic of a typical reflecting diffraction grating.

In the special case, as in Figure G-1, when the grating is illuminated by a laser beam at exactly the blaze angle, that is, in the first order, the mounting is said to be in autocollimation. This setting is popularly known as the Littrow mount. In this instance, the grating equation reduces to

\[ n \lambda = 2d \sin \theta \]  \hspace{1cm} (G-2)

To obtain the angle settings in Table 2, \( \theta \) in equation (G-2) is calculated. For the Bausch and Lomb grating which had a blaze angle
FIGURE G-1. Schematic of Diffraction Grating.
of 26°45' at 5000Å, and was lined for 1800 grooves/mm, \( \theta \) can be calculated by selecting any wavelength, \( \lambda \).

\[
\begin{align*}
  n &= 1 \\
  d &= \frac{10^7}{1800} = 5550\text{Å} \\
  \sin \theta &= \frac{\lambda}{11100}
\end{align*}
\]

where the wavelength \( \lambda \) is in angstroms.

A diffraction grating can tune a dye laser by virtue of its ability to selectively allow only a very narrow bandwidth to undergo gain in the laser cavity. In the normal two-mirror cavity, emission from the dye molecules span a broad spectrum of anywhere from fifty to one hundred and fifty angstroms. The totally reflecting mirror allows all the wavelengths present to amplify themselves. As a result, a two-mirror system imparts only broadband outputs. If the totally reflecting mirror is replaced with a diffraction grating, the angular setting of the grating determines which narrowband wavelength rays can undergo amplification. By preventing wavelengths outside this permitted band to lase, there is relatively little competition for simulated sites and virtually the same energy that was emitted at the two-mirror broadband output results, but in a very narrow wavelength band. Typical bandwidths resulting from the use of a diffraction grating are less than one angstrom with an 1800 grooves/mm grating and one to two angstroms with a 1200 grooves/mm grating.
APPENDIX H
LIST OF MANUFACTURERS

Products from the following companies were used in constructing the organic dye laser. Several of these companies sent material and equipment gratis. A very special acknowledgement is hereby made for this accommodation.

1. Cavity
   a. XENON (see Micropulser)
      (Dyecell and flashlamps)
   b. Whitehead Metals
      Blato, Md.
      (Aluminum Specular Lighting Sheet)
   c. M. R. Prescott
      116 Main Street
      Northfield, Massachusetts 01360
      (Mirrors and dyecell)

2. Chemicals
   a. Eastman Kodak Company
      343 State St.
      Rochester, New York 14650
      (laser dyes)
   b. Aldrich Chemical Company
      940 West St. Paul Ave.
      Milwaukee, Wisconsin
      (cyclooctatetraene)

3. Micropulser and Power Supply
   a. XENON Corporation
      39 Commercial St.
      Medford, Mass. 02155
      (Micropulser and power supply)
   b. Capacitor Specialists Inc.
      Del Dios Highway
      Escondido, Calif. 92025
      (100 joule capacitor)
4. Optics

a. Corning Glass Works
   Corning, New York
   (filters and fluorescent glass)

b. Isomet Corporation
   103 Bauer Drive
   Oakland, New Jersey 07436
   (KDP crystal)

c. PHASE-R-CORPORATION
   Box G2
   New Durham, N. H. 03855
   (1800 grooves/mm diffraction grating)

d. Valpey Corporation
   1244 Highland St.
   Holliston, Mass. 01746
   (partially transmitting mirrors)

e. Esco Products
   171 Oak Ridge Road
   Oak Ridge, New Jersey 07438
   (totally reflecting mirror, quartz windows, filters)

f. Bond Optics
   Etna Road
   Lebanon, N.H. 03766
   (condenser lenses, quartz windows)

g. Special Optics
   Cedar Grove, N. J. 07009
   (totally reflecting mirror)

h. Ardel Instrument Co., Inc.
   P. O. Box 992
   Jamaica, New York 11431
   (optical mounts)

i. Thermal American Fused Quartz Company
   Route 202 & Change Bridge Rd.
   Montville, New Jersey 07045

j. Edmonds Scientific Company
   150 Edscorp Bldg.
   Barrington, New Jersey 08007
   (variety of optics & alignment laser)

k. Southwest Engineering and Equipment Co.
   4242 Richmond Avenue
   Suite 204
5. Powermeters

a. Scientech, Inc.
1724 14th St.
Boulder, Colorado 80302
(Powermeter)

b. Steller Instrument Northwest Inc.
11459 S.E. 266th St.
Kent, Washington 98031
(Powermeter)

c. UV Products
5114 Walnut Grove Avenue
San Gabriel, California 91778
(UV powermeter)

d. HADRON, Inc.
800 Shames Drive
Westbury, New York 11590
(Laser footprint paper)

e. KORAD
2520 Colorado Ave.
Santa Monica, California 90406
(Laser receiver)

f. Keithley Instruments, Inc.
28775 Aurora Rd.
Cleveland, Ohio 44139
(Microvoltmeter)

g. Tektronix, Inc.
13955 S.W. Millikan Way
Tektronix Industrial Park
Beaverton, Oregon 97005

6. Pumps and Fittings

a. The Randolph Company
1018 Rosine Street
Houston, Texas 77019
(variable flow peristaltic pump)

b. Cole-Parmer
7425 N. Oak Avenue
Chicago, Illinois 60648
(Magnetic drive pump)
c. New Brunswick Scientific Company, Inc.  
1130 Somerset St.  
P. O. Box 606  
New Brunswick, New Jersey 08903  
(non-plasticizer flexible tubing)

d. Imperial Eastman  
1440 North 24th St.  
Manitowoc, Wisconsin 54220  
(non-plasticizer flexible tubing)

e. Goodall Rubber Company  
Plastic Division  
2320 E. State St.  
P. O. Box 631  
Trenton, New Jersey 08604  
(non-plasticizer flexible tubing)

7. Safety

a. BIO ENGINEERING  
Box 575  
Lexington, Mass. 02173  
(laser signs)

b. Bausch and Lomb Frame Center  
465 Paul Road  
Rochester, New York 14624  
(laser goggles)
APPENDIX I

TYPICAL LETTER OF INQUIRY

LOUISIANA STATE UNIVERSITY
AND AGRICULTURAL AND MECHANICAL COLLEGE
BATON ROUGE - LOUISIANA - 70803

DEPARTMENT OF CHEMICAL ENGINEERING

COMPANY
ADDRESS

Gentlemen:

I am a chemical engineering graduate student at Louisiana State University and would like your aid in ("helping me select specific bacterial strains of particular significance to your company," for microbiologically oriented companies and "suggesting a means of obtaining continuously tunable laser outputs", for laser manufacturers.)

[THE FOLLOWING APPLIED MOSTLY TO MICROBIOLOGICALLY ORIENTED COMPANIES]

For my dissertation I have decided to study the effect of laser radiation wavelength and intensity on microbiological organisms. The extremely high intensity and tunable monochromatic nature of certain types of lasers are unique characteristics not found in conventional energy sources.

As an aid in sending the needed information, I have enclosed a simple questionnaire which can be filled-in and supplemented with other literature that you feel might be relevant to my dissertation. Essentially, I am attempting to isolate microbiological organisms for studies that can hopefully bring immediate benefits to the industry which can offer an organism or organisms most susceptible to the aims of our investigation. In particular, I am interested in exploiting the possibility of selective sterilization (i.e., binary system wherein the contaminant is destroyed without harming the beneficial bacteria) and catalysis.

In addition to the above, I would appreciate it if you could direct me to anyone or any group that might possibly be interested in funding fundamental research in this area. With optimism that you will be able to help me, I thank you for your time and concern in this matter.

Very truly yours,

Patrick K. Takahashi
Research Assistant
The author was born in Honolulu, Hawaii, on September 6, 1940. He received his B.S. degree in Chemical Engineering from Stanford University in 1962. After six years of experience as a process engineer and computer applications project leader with the Hawaiian sugar industry, he returned to graduate school and in 1969 earned the degree of M.S. in Chemical Engineering at Louisiana State University. The author has always strived to balance his academic interests, and has minored in art, math, and management for his B.S., M.S., and Ph.D. degrees respectively. During his graduate career, he has also maintained a comprehensive auditing program in ecological awareness by participating in more than half a dozen courses dealing with the environmental sciences, economics, law, and population dynamics. He is presently a candidate for the degree of Doctor of Philosophy in Chemical Engineering.
EXAMINATION AND THESIS REPORT

Candidate:  Patrick Kenji Takahashi

Major Field:  Chemical Engineering

Title of Thesis:  Tunable Organic Dye Laser Irradiation of Escherichia coli

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

July 16, 1971