18.5 The Effect of Tunable Laser Radiation on Exogenously Photosensitized *Escherichia Coli*,
P. K. Takahashi, *College of Engineering*, and S. Gaines, *School of Medicine, University of Hawaii, Honolulu, Hawaii*

(*15* min)

The literature is relatively void on the macroscopic microbiological effects of laser radiation, particularly with respect to process applications. Excellent progress has occured in micro-chromosome studies and ultramacro skin/retina biomedical investigations. However, cellular microorganism studies have not been widely reported. The paper will recapitulate preliminary laboratory results conducted in the early '70s not previously presented at any conference (partly due to proprietary reasons) and summarize the results of recently completed studies. (Details which follow apply only to the recent investigation.)

The laser used was a modified Synergetics Chromabeam 1070 dye laser with an energy output between 100 and 300 millijoules over a 400 nanosecond pulsewidth. Rhodamine 6G (orange) and 7-diethylamino-4-methycoumarin (blue) were used as the lasing dyes.

The microorganism used for the study was *Escherichia coli* maintained in cystine trypticase agar (CTA). Forty-eight hours before experimental irradiation, the microorganisms were transferred from CTA stock culture into tryptic agar slant. 3×10^7 cells were inoculated into 10 ml of plain nutrient broth solution, then incubated at 37° C for 24 hours. The same procedure was used for 10 ml of photosensitizer-nutrient broth solution. The exogenous dyes used were acridine orange and new methylene blue, the former at a concentration of 1×10^{-5} M/liter and the latter at 5×10^{-5} M/liter.

A 1.0 microliter droplet of the microorganism suspension was deposited on a sterilized 24 × 30 mm microscope coverglass and placed in the irradiation chamber. (A control sample was kept under similar conditions, but away from the laser beam.) After irradiation to a predetermined energy level, the assembly was processed using standard aseptic microbiological techniques and prepared for plate count. (Again, the control, save for irradiation, underwent a similar process.) After incubation for 24 hours at 37° C, the plates (triplicates) were then counted under a stereoscopic microscope.

The mean survival figure was then obtained by averaging the plate count of the irradiated samples and dividing by the average plate count of the control. A mean survival ratio (MSR): 1) greater than one, means catalysis or enhanced growth; 2) one, means no apparent effect; 3) less than one, means sterilization.

The effect was minimal (MSR = 0.95) when no exogenous photosensitizer was used. The following MSR pattern resulted with photosensitizer.

	Exogenous Photosensitizer	
Laser Dye	Acridine Orange	New Methylene Blue
Rhodamine 6G	1.42	0.61
Coumarin	0.70	0.98

When complementary colors were used, for the energy level studied, there was a similar sterilization effect. However, when like colors were matched, the results were most intriguing. First, the coumarin/new methylene blue combination gave mixed results. But when R6G/acridine orange was used as the pair there was an enhancing effect. (This rather surprising reversal only confirms the results of the earlier study. Acridine orange is known to attach to DNA. The stimulation of 580 ± 20 nanometer radiation under acridine orange photosensitization increases growth.)

Selected colonies of irradiated organisms then underwent various biochemical and sensitivity tests. Results were generally mixed.

The long term significance of this study applies to fields varying from selective sterilization in the various process industries to total sterilization in sewage treatment to enhancement in pharmaceutical applications. The medical implications are also worthy of speculation.

18.6 Laser Interferometer for Sensing of Respiratory Sounds,
S. Donati and V. Speziali, *Instituto di Elettronica, Università di Pavia, Pavia, Italy*

(*15* min)

Optical stethoscopy is attractive because it overcomes acoustical loading and skin friction noise, limitations encountered in acoustical sensing of respiratory sounds. A new interferometric method has been devised, suitable for measurements on a diffusing surface like skin and requiring no additional optics. The method is based on the induced modulation, both in amplitude and frequency, of the laser cavity field that is produced by backscattering from the examined spot. An adequate dynamic range is obtained, i.e. several wavelengths of amplitude at audio bandwidths, with low power requirements for operation up to 1–2 m object distance.

A 0.5 mW He-Ne source[1] was frequency stabilized by Zeeman splitting the random polarized mode into two orthogonal, linearly polarized modes. A transverse magnetic field, 300 G applied to a 20 cm section of the tube, produces gain line narrowing and a wide single mode region around the atomic line center.[2] The orthogonal modes are separated in frequency by pulling toward the centers of the Zeeman shifted atomic lines, and their beat is detected with a photodiode, using a polarizer oriented at 45° respect to the modes' polarizations. Since pulling frequency depends on cavity detuning, frequency stabilization is achieved by controlling the cavity length against beat frequency variations.[2] Through frequency-to-voltage conversion and integration, a signal is fed to a coil wound on the plasma tube (Fig. 1) which produces thermal expansion of the cavity.

In this single, split-mode operation, the output power is 0.45 mW and the beat frequency is

[1] Spectra Physics model 155.

[2] R. H. Morris, J. B. Fergusson and J. S. Warniak, *Appl. Opt.*, vol. 14, p. 2808, 1975.

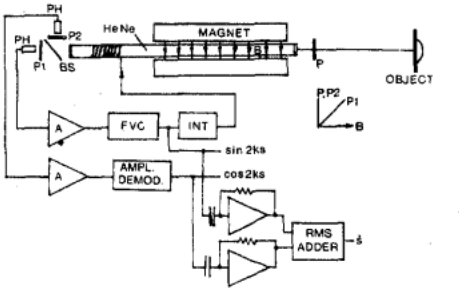

Fig. 1.

stabilized at 50 kHz with typical drifts of 2 Hz/s.

With polarizer P inserted in the output beam (Fig. 1) so as to transmit only one of the modes to the target surface, the beat frequency exhibits a strong effect of frequency modulation due to the spot falling on a remote surface; in addition, if polarizer $P1$ is rotated 45° so as to align it with P, an amplitude modulation is found. A simple explanation of these effects is the following. The field E_b backscattered from the remote surface into the laser cavity adds coherently to the unperturbed field, under a readily satisfied far-field condition. The cavity field E can be written as a slowly varying function, $E = E_o \exp(i\Phi)$, and the backscattering field can be expressed as $E_b = \alpha E \exp(i2ks)$, where α is the field attenuation for an object distance s. If the cavity relaxation time is $\tau = Q/\omega$ (Q is the quality factor), the cavity field E will vary at a rate $\dot{E} = E_b/\tau$, ignoring saturation effects. By inserting the above expressions in this equation, and equating real and imaginary parts separately to zero, one obtains:

$$\dot{\Phi} = \frac{\alpha}{\tau} \sin 2ks \tag{1}$$

$$E_o = \frac{\alpha}{\tau} E_o \cos 2ks \tag{2}$$

(1) displays the phase-to-frequency conversion achieved in a laser cavity for the mode whose polarization is transmitted by the output polarizer P. The orthogonally polarized mode is however unaffected, and serves as a reference oscillator for heterodyne detection of the distance-dependent term. If ω_p is the unperturbed beat frequency, the signal detected using a polarizer oriented at 45° with respect to the output polarizer is:

$$V = \dot{E}_o^2 \cos\left(\left[\omega_p + \frac{\alpha}{\tau} \sin (2ks)\right] t\right),$$

and the interferometric information contained in the modulating term can be recovered by frequency demodulation.

The amplitude modulation of the propagated mode is shown in (2). Though smoothed by gain saturation, the amplitude contains a second, in-quadrature term which can be detected separately using a second detector, that is oriented parallel to the output polarizer.

After demodulation (Fig. 1), the signals sin $2ks$ and cos $2ks$ are differentiated through operational amplifier circuits and the result added in a rms hybrid module to yield the velocity signal $\dot{s}$. Examples of operation will be reported and discussed.