

## Brief Report

# In Vitro Production of Viable Bacteriophage in a Laser Plume

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Bacteriophage  $\phi$ X 174, present at high concentrations in an agar overlay in vitro system, were used as a target for pulsed 2.94  $\mu$ m (Er:YAG) laser ablation. In this preliminary experiment, the potential for transport of viable viruses in the photoablation plume was investigated for laser energy densities of approximately 3.5 J/cm<sup>2</sup> per pulse in exposures of 75-600 laser pulses. Transport of relatively few numbers of viable viruses from the ablation site to the plate assay detector, a distance of approximately 2 cm, was observed.

**Key words:** Er:YAG laser, virus aerosols, laser surgery

## INTRODUCTION

The potential hazards of aerosols produced by surgical devices interacting with human tissue and fluids are a source of concern to surgical staff and patients. Typical devices and procedures that can generate airborne debris are bone saws, surgical drills, electrocautery procedures, and laser surgery. In addition to anecdotal reports appearing in the popular medical press of plume-mediated disease [1,2], a growing number of researchers have begun gathering data on the nature of laser plumes. Results to date indicate that laser plumes can contain intact cells [3], papillomavirus DNA molecules [4], viable bacterial cells [5], and spores [6]. In addition, animals exposed to laser plumes have demonstrated various impaired lung functions [7,8].

The chemical composition and physical attributes of laser-generated plumes depend on the target tissue and the surgical technique, as well as on laser parameters such as wavelength, pulse-width, repetition rate, and power density [6,8-10]. However, the interrelationship of these factors that affect the behavior and composition of ablation plumes is not well characterized. We

have begun studies to investigate the potential for viable virus transport via ablation plumes using the bacteriophage  $\phi$ X 174 in an agar overlay system as a model target. Reported here are the results of a preliminary experiment configured to optimize the probability of obtaining viable  $\phi$ X 174 in laser plumes.

## MATERIALS AND METHODS

### Cultures

The bacteriophage  $\phi$ X 174 (ATCC #13706 B1) was selected as a model for submicron-sized viruses that may be encountered in surgical laser plumes. The host cell for this phage is *Escherichia coli*, strain C (ATCC #13706). Cells were grown and maintained in tryptone broth (5 g KCl, 10 g tryptone per liter of medium). The substrate nutrient medium for both target and detector cultures contained 2.5 g NaCl, 10 g tryptone, and 10 g purified agar per liter of medium. The overlay, which suspended and immobilized cells or bacteriophage above the substrate, contained 5 g NaCl, 10 g tryptone, and 8 g purified agar per liter of medium. To facilitate adsorption of bacteriophage to host cells, 6 mM CaCl<sub>2</sub> and 1 mM NaOH were added to both agar media before solidification.

Bacterial detector (*E. coli*) plates were prepared by mixing 0.1 ml of tryptone broth bacterial culture ( $\sim 2 \times 10^8$  cells) with 3 ml soft (45°C) agar and pouring the mixture over the surface of the solid substrate. A single, viable  $\phi$ X 174 particle landing on this surface will become visible as a circular, cleared area (plaque) in a hazy, confluent bacterial lawn.

Target plates were prepared by mixing 0.1 ml  $\phi$ X 174 suspension ( $4 \times 10^3$ ) with 0.1 ml bacterial culture ( $\sim 2 \times 10^8$  cells) in 3 ml agar overlay and incubating overnight at 37°C. Assuming each cell becomes infected and that infected cells yield an average of 500 phage/cell [11], the total number of  $\phi$ X 174 in the agar overlay is  $\sim 10^{11}$ . This in situ amplification step results in phage

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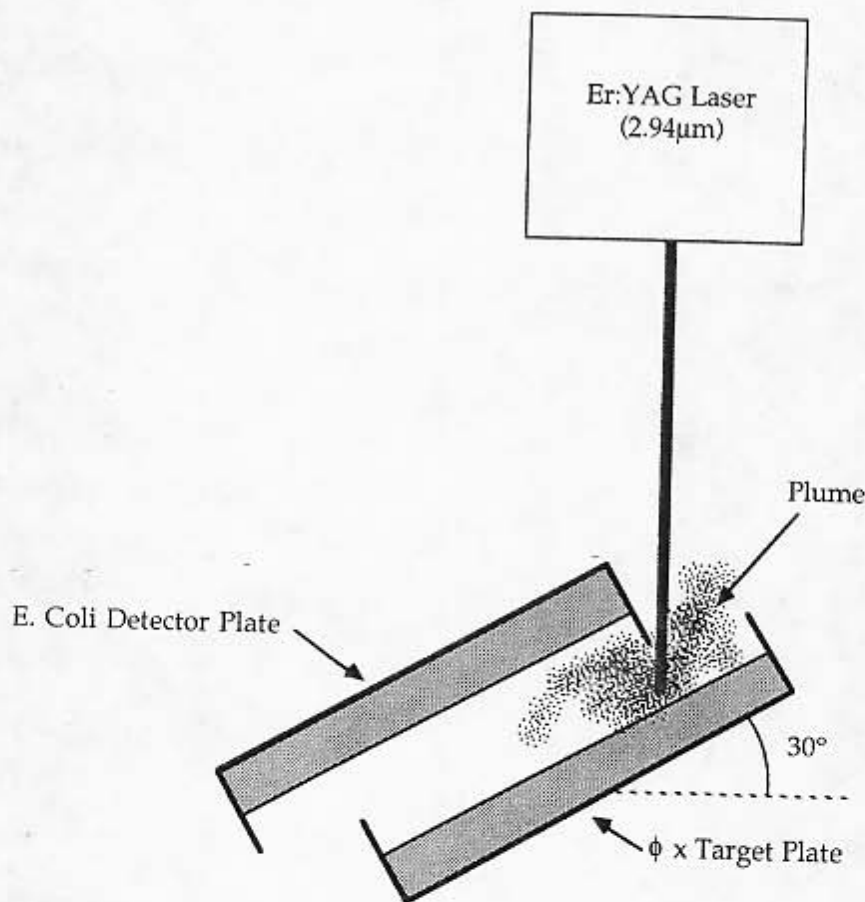


Fig. 1. Schematic of the arrangement of the target and detector plates and their orientation with respect to the laser. The normal axis of the target plate surface is directed away from the laser axis at  $\approx 30^\circ$ . The target and detector plate

surfaces were parallel and separated by approximately 2 cm. Representation of the plume is indicative of the "smoke" visible when the target surface was irradiated by the laser.

concentrations on the target plate considerably higher than could be achieved easily by other methods.

positioned to intercept ejected material, without interrupting the beam path. Separation of the parallel target and detector plates was 2.0 cm.

#### EXPERIMENTAL APPARATUS

The experimental configuration is illustrated in Figure 1. The target plate, which was mounted on a rotation stage, was oriented  $\sim 30^\circ$  with respect to the optical axis of a flashlamp pumped Er:YAG laser ( $2.94 \mu\text{m}$ ), as shown. The output from the laser was incident, without focusing, on the target plate at a point approximately  $R/2$  from the center, where  $R$  is the radius of the culture plate. The pulsewidth of the laser is approximately  $200 \mu\text{s}$ , whereas the repetition rate for this study was 5 Hz. The beam cross section at the target was essentially round with a diameter of approximately 0.25 cm. The detector plate was

#### EXPERIMENTAL PROCEDURE

Since absorptivity of water is quite high at  $\sim 3 \mu\text{m}$  [12], substantial ablation of the agar surface was expected. Tests of surface ablation of the target culture produced readily visible plumes and troughs in the target agar surface. For these experiments, average laser power was maintained at  $1.0 \pm 0.1 \text{ W}$ , which represents an energy density at the target of  $3.5 \pm .35 \text{ J/cm}^2$  per laser pulse. A total of seven exposures were performed in two experimental sessions with identical methodology (two each for 15, 30, and 60 s, and one for 120 s [75, 150, 300, and 600 laser pulses, respectively]). The plate was continuously, slowly ro-

tated to present a fresh surface for each laser pulse. Also, the plate/rotation stage assembly was adjusted laterally after the completion of each exposure, while maintaining the parallel 2 cm separation of the plates. This insured that the ensuing exposure would occur on a fresh "band" of the target plate. A separate detector plate was used for each exposure. The detector plates were subsequently incubated for 4 h at 37°C and then examined for plaques. Viruses were directly pipetted onto a detector plate as a positive control, whereas a negative control was performed by mounting a detector plate in position for 120 s without operating the laser. Also, an environmental control was maintained by placing another detector plate on the optical bench for the duration of a session at a distance of 30 cm from the target ablation site.

## RESULTS AND DISCUSSION

Plaques were seen in the bacterial lawn for six of the seven exposures, indicating that viable viruses had been transmitted in the plume. In all cases, the plaques were grouped near the edge of the plate, in the region where the distance to the ablation site on the target was minimized. One of the 30 s exposures did not yield plaques. This result may be due to the probabilistic nature of particle transport, since relatively few viruses compared with the potential number liberated by ablation were intercepted, as will be discussed later. Numerous plaques were evident on the positive control, whereas none were apparent on either the negative or environmental controls. A photograph of a detector plate exposed to the plume generated by the photoablation of 150 laser pulses is shown in Figure 2. Less than ten individual plaques are readily evident. However, this can be considered only an approximation of the total number of viruses intercepted by the detector since multiple virus landings in close proximity are possible.

In conclusion, it is emphasized that for these experiments target phage concentration was exaggerated to maximize the probability of achieving a positive result. Assuming that approximately  $10^{11}$  bacteriophage particles are evenly distributed within the agar overlay, each laser pulse potentially liberated approximately  $10^7$  phage. Although some of the plume may have escaped the detector plate surface, our results indicate orders of magnitude fewer viable phage in

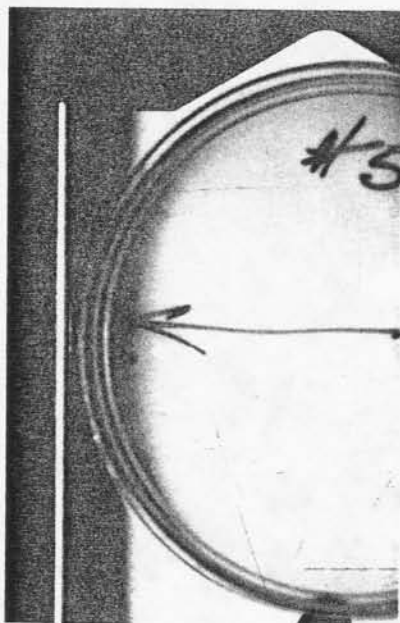


Fig. 2. Detector plate exhibiting several plaques resulting from the transport of viable bacteriophage via a laser-produced plume. The exposure for this plate was 150 laser pulses. Orientation marks indicate that the plaques are grouped around the region of the detector closest to the ablation site in the target plate.

the laser plume than those suspended in the ablated overlay.

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