Lasers in Medical Science 1997, **12**:55–59

Excimer Laser Cleaning of Mold-contaminated Paper: Sterilization and Air Quality Considerations

T.R. FRIBERG[a], V. ZAFIROPULOS[b], M. KALAITZAKI[b], R. KOWALSKI[a], J. PETRAKIS[b], C. FOTAKIS[b]

[a]*University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA*
[b]*Foundation for Research and Technology—HELLAS (FORTH), Crete, Greece*

Correspondence to T.R. Friberg, Department of Ophthalmology, Eye and Ear Institute, 203 Lothrop Street, Suite 824, Pittsburgh PA 15213, USA

Paper received 18 August 1996 and accepted 7 November 1996 (Irvine)

Abstract. The Excimer laser can be used to remove biological debris from underlying substrates. The ability of the krypton fluoride (KrF) Excimer laser to remove *Aspergillus niger* mold, which had been grown on filter paper, was evaluated in the present study. The air in the immediate vicinity of the laser cleaning apparatus was sampled to determine if viable mold fragments were released into the air during treatment. It was found that filter paper could be sterilized with the Excimer laser wavelength with the proper selection of parameters. During this process, viable spores and mold fragments are released into the atmosphere, and pose a relative risk unless proper measures to prevent contamination of the work area are taken.

INTRODUCTION

Recently, lasers have been used to remove biological organisms from humans as well as inanimate objects. Excimer laser irradiation of the interior of cartons and containers before filling is being investigated as a non-chemical method of sterilization which might lead to a longer shelf life for food products (1). Papillomas (caused by human papilloma virus) can be extirpated using the CO_2 laser (2). However, the smoke plume during papilloma (wart) removal poses a potential health hazard to the medical personnel as it may contain viable viruses capable of infecting the personnel in the vicinity (2, 3). Furthermore, DNA from human immunodeficiency virus has been detected in laser smoke of infected individuals (4).

The present authors have reported previously on the use of the Excimer laser to grossly remove molds from the paper substrate of works of art, including maps, posters and manuscripts, in an effort to restore them aesthetically (5). The aims of the present study were to determine whether: (a) paper samples overgrown with molds are sterilized using Excimer laser cleaning techniques; and (b) whether the ambient air in the vicinity of the sample becomes contaminated by viable mold fragments during the cleaning process.

MATERIALS AND METHODS

Very heavy contamination of mold on paper was simulated by growing *Aspergillus niger* fungi on filter paper discs. Whatman #1 filter paper (W and R Balston, UK) was colonized by mixing a pure culture fungal isolate with melted Sabourauds agar, and placing several drops of the agar onto the paper to produce a circle approximately 3 cm in diameter (Fig. 1). The filter paper substrate was then placed in a sterile plastic Petri dish (100 × 15 cm) on which a small dab of rubber cement had been applied. Gentle pressure with a sterile applicator was used to affix the filter paper to the Petri dish. Thirteen plates were prepared, covered and sealed with tape along the edges. The plates were then incubated for 2 weeks before treatment with a krypton fluoride (KrF) Excimer laser at a wavelength of 248 nm. This wavelength had been used previously by the present authors for the removal of *A. niger* mold from contaminted works of art, and their experience was used as guidance in this study (5).

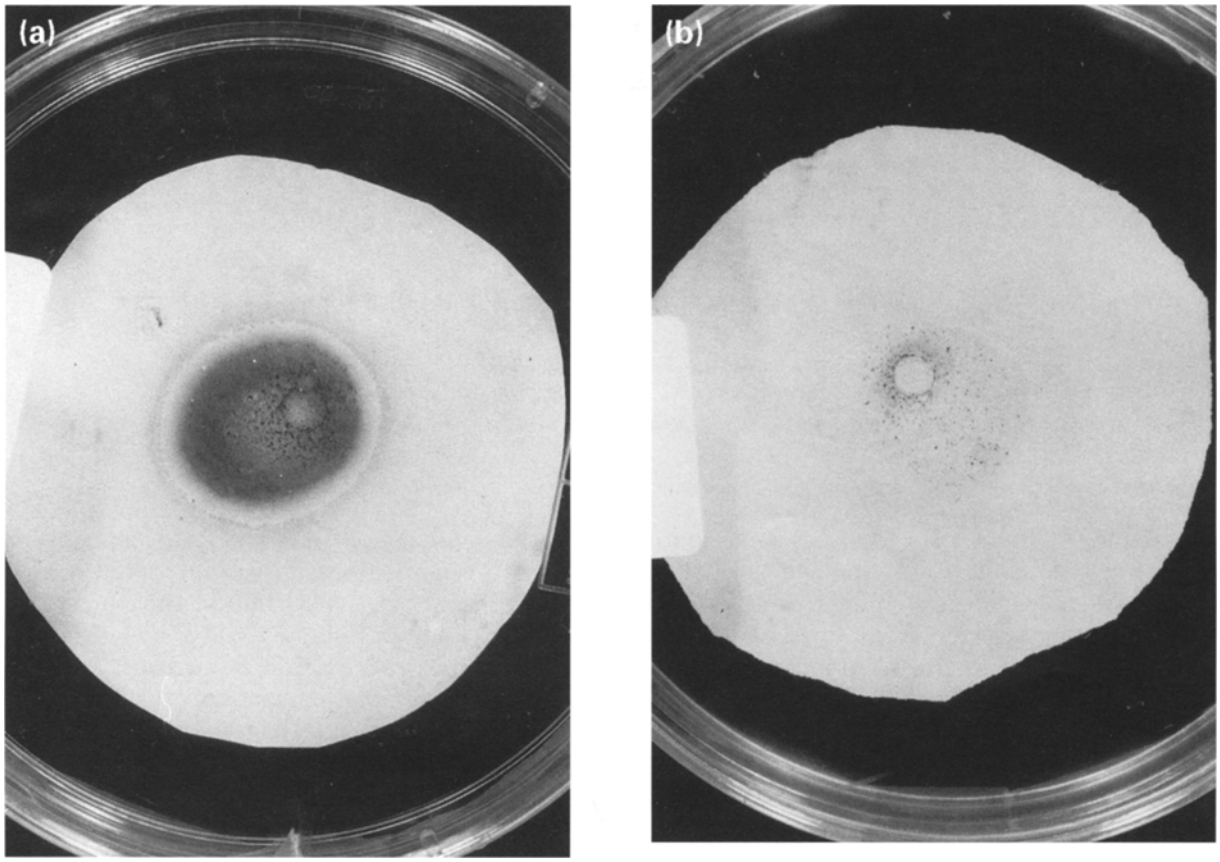

Fig. 1. (a) A filter paper has been overgrown with a luxuriant growth of *Aspergillus niger* and is grossly contaminated. This sample is an untreated control. (b) Excimer laser cleaning has removed most of the mold in Sample #7, but more deeply imbedded hyphae and fruiting bodies are still present. Using different laser parameters, the paper could be completely cleaned with no apparent residual mold remaining upon examination with light microscopy.

The Excimer laser treatment of the samples proceeded as follows. Eleven of the fungus-on-paper cultures were treated, with two additional plates serving as untreated controls. Each of the 11 was mounted in a position perpendicular to the Excimer laser beam on an automated computer-controlled x-y translator which was moved by servomotors. This x-y translator is a precision device which allows reproducible positioning to tolerances of $\pm 100\,\mu$m in the x-y plane. After mounting, the cover of the Petri dish was removed and the KrF Excimer laser was activated. A certain number of pulses per translator step at variable energy fluences were delivered. This was done by focusing the rectangular-shaped (2.5×1 cm) laser beam using a cylindrical lens of 300 mm focal length. Laser pulses were overlapped by 60% in the horizontal direction and 40% in the vertical direction as the mold-contaminated paper was stepped across the beam (Fig. 2). This overlap helps to prevent streaking of artwork when it is cleaned by laser techniques, and re-positioning is accomplished within fractions of a second. The entire surface area in question was treated in only a few minutes.

After treatment, the Petri dish cover was replaced, and the dish was sealed and submitted for microscopic analysis and for re-culture. The quantity of fungal remnants remaining after cleaning was subjectively graded from 0 to 4+ for both hyphae and fruiting bodies, with 0 denoting no organisms found and 4+ denoting the quantity of hyphae and fruiting bodies present in the untreated control samples. Re-culturing was accomplished by placing the laser-cleaned filter paper on Sabaroud's culture media and incubating the paper for 3 weeks.

While laser mold removal was taking place, the ambient air was sampled by drawing a vacuum through the tip of a sterile 22 μm filter (Millipore Millex-GS, Bedford, MA., USA), and mounting the male tip of the filter 3 cm from the fungus-paper laser beam target. These filters were photographed to document the degree of gross contamination (Fig. 3), and

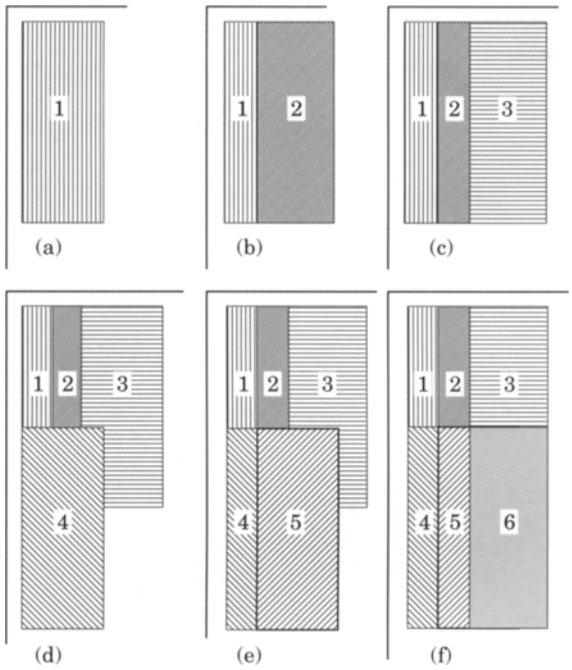

Fig. 2. (a) The rectangular laser beam (Position 1) has been focused onto the upper left-hand corner of a paper target. (b) The x-y translator has moved the paper in the x direction so that the laser beam, now in Position 2, now overlaps the last treatment zone by 60%. (c) The laser beam is in Position 3 with respect to the paper, and in turn overlaps Position 2 by 60%. In reality, the laser beam is stationary, and the paper is translated. After a complete pass in the x direction has been completed (here after only three zones have been 'cleaned'), the x-y translator is stepped in the y direction as in (d). The paper is moved so that the beam is in Position 4, and it now overlaps the treated strip above it by 40% in the y direction. (f) A rectangular area has been treated using a total of 6 pulses. As the laser beam dimensions are quite small, many overlapping treatment zones are required to treat a large piece of artwork.

the photographs were graded to determine grossly the density and surface area of the mold collection along the filter surface. The fungi within each filter was sampled for mold viability by injecting 2 ml distilled water through the contaminated filter, and plating the fluid washings on a Sabouraud's agar culture plate.

A brief experiment was also performed where each of two plastic Petri dishes containing fungal samples were struck on the periphery of the plastic without the laser pulse hitting the fungal targets at all. This was done to determine whether the shock wave of the laser had sufficient energy to dislodge viable fungi into the atmosphere around the laser cleaning apparatus. Prior to striking these Petri dishes with the laser, the air over the fungi was sampled as a control.

RESULTS

Of the 11 samples treated, two samples exposed to energy fluences of 0.81 J cm^{-2} and 6–8 pulses showed no spores or hyphae upon light microscopic analysis performed by R.K., a microbiologist. Furthermore, no fungi could be isolated on re-culture on these treated samples, indiating that the Excimer laser could indeed sterilize fungus-contaminated filter paper. At lesser fluences, some spores or hyphae remained, some of which were viable (Table 1).

It was found that when striking the plastic dish with 6 pulses at a fluence of 0.81 J cm^{-2}, viable fungi could be collected 3 cm from the centre of the laser target. No fungi was collected at the same sampling location when two fungal targets were placed in the fixture without shocking the containers with Excimer pulses.

The air in the vicinity of the fungal target was grossly contaminated with fungi as per gross inspection of the air filter targets (Fig. 3). Air filters used during the laser cleaning of Samples 1–11 were all contaminated and grew viable fungi on culture (Table 1). Only the two controls, which sampled the air above untreated fungal targets, were culture negative.

DISCUSSION

Removal of fungi from art pieces is customarily performed using standard conservation methods, including mechanical removal with brushes and suction pipettes. When fungal fruiting bodies have become embedded in paper, fine scalpel blades can be used to remove the contamination, but this method often damages the paper substrate (6, 7). The present authors have performed previous experiments removing fungus from contaminated posters and maps using the 248 nm Excimer wavelength with minimal or no damage to the underlying artwork (5). Other Excimer or Excimer pumped dye lasers emitting at alternative wavelengths could also be used and would probably yield similar results. While this method requires careful set-up and monitoring, it is believed that the Excimer laser can be used where more traditional methods may be difficult or impossible to employ. However, the present authors had not previously conducted any experiments to

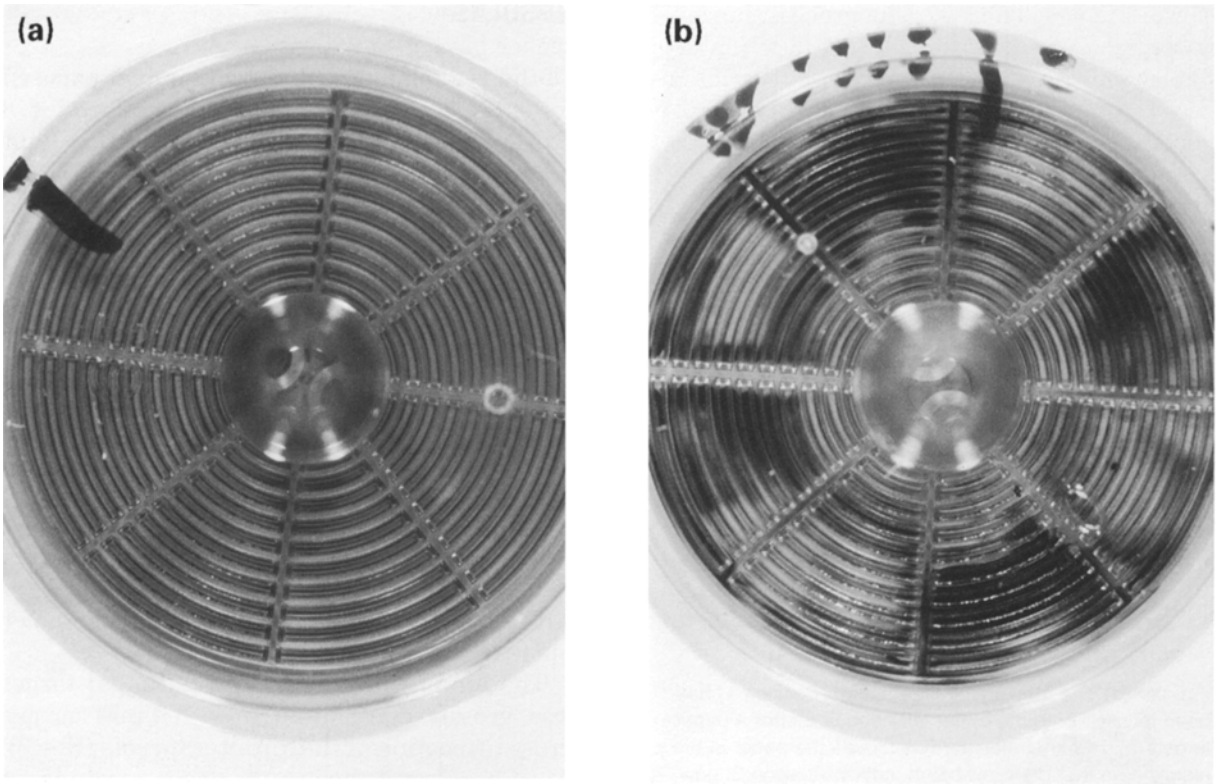

Fig. 3. (a) The air in the region of the laser cleaning was sampled by aspirating air through a sterile Millipore filter, pore size 0.22 μm. (b) After laser cleaning, the air filter is grossly contaminated and harbours viable mold fragments which could be re-grown on culture.

Table 1.

Sample no.	Energy fluence (Jcm^{-2})	Total no. of pulses	Fungal remnants remaining after laser cleaning	Ambient air contamination (no. of air filter segments contaminated)
1	0.41	27	3+HS	8
2	0.54	22	3+HS	7
3	0.57	4	2+S	4
4	0.57	8	2+H	8
5	0.57	12	1+H	6.5
6	0.57	14	1+S	8
7	0.62	4	2+HS	8
8	0.81	1	3+S	6
9	0.81	2	3+S	3
10	0.81	6	None	2
11	0.81	8	None	2
Untreated Control A	–	–	4+H, S	0
Untreated Control B	–	–	4+H, S	0

H, hyphae; S, spores.

ascertain whether the Excimer laser could effectively sterilize the paper object in question, or whether any viable debris is present in the laser plume.

The present data demonstrates that with proper selection of laser parameters, it is possible to completely remove fungal organisms from filter paper samples and to sterilize

these samples (Table 1). The process, however, is dependent on several variables, and sufficient energy fluence is necessary to achieve the desired result. The conservator may be constrained by the fact that sterilization thresholds may be higher than the safe laser cleaning threshold (5). That is, damage to the underlying artwork may be produced if complete sterilization is the goal.

This study determined that when thick growths of molds are treated, the shock wave of the laser cleaning by itself can cause fungi to be dislodged from the object of regard, in regions distant from the area being irradiated. Of course, if a contaminated object is shocked in transit or during handling, it is expected that viable components of luxuriant fungal colonies, such as those studied in the present experiment, would also become dislodged.

When performing laser removal of fungi, viable fungal particles can contaminate the air in the vicinity of the cleaning station. For this reason, when cleaning objects which are overgrown by thick colonies of mold, the air in the target area should be collected via a vacuum to prevent unwanted contamination of the surrounding facility. Alternatively, the cleaning process could be performed under a protective vacuum hood or in an enclosed space. The present work focused on the fungus *A. niger* which is an ubiquitous organism. A few spores of this mold are unlikely to pose any biological hazards to most individuals. Different organisms undoubtedly have different thresholds, however, below which complete sterilization might not be achieved reliably. Furthermore, molds at certain stages of growth are probably more suspectible to laser irradiation compared with mature colonies.

In summary, the Excimer laser can be used to sterilize fungal-contaminated paper while simultaneously cleaning the paper when proper laser cleaning parameters are selected. The plasma cloud in the immediate vicinity of the laser target may contain viable organisms, and precautions should be taken to avoid contamination of the work room.

ACKNOWLEDGEMENTS

This work was supported by a NATO Collaborative Research Grant, #950841. Lamda Physik GMBH, a subsidiary of Coherent, Inc, provided the Excimer laser used in this study.

REFERENCES

1 McEntee J. Excimer's pulsed beams sterilize food packaging. *Opto and Laser Europe News* 1996, July:7
2 Gloster JM, Roenigk RK. Risk of acquiring human papilloma virus from the plume produced by the carbon dioxide laser in the treatment of warts. *J Am Acad Dermatol* 1995, **32**:436–41
3 Hallmo P, Naess O. Laryngeal papillomatosis with human papilloma virus DNA contracted by a laser surgeon. *Eur Arch Otorhinolarngol* 1991, **248**:425–7
4 Baggish MS, Polesz BJ, Joret D, Williamson P, Regai A. Presence of human immunodeficiency virus DNA in laser smoke. *Lasers Surg Med* 1991, **11**:197–203
5 Friberg TR, Zafiropulos V, Petrakis Y, Fotakis C. Removal of fungi and stains from paper substrates using laser cleaning strategies in Restauratorenblaller. Vienna, Australia: International Institute for Conservator of Historic and Artistic Works, Verlag Mayer and Co (in press)
6 Szczepanowska HM. Biodeterioration of art objects on paper. *The Paper Conservator, The Institute of Paper Conservation* 1986, **10**:31–9
7 Craig R. Alternative approaches to the treatment of mold biodeterioration—an international problem. *The Paper Conservator, The Institute of Paper Conservation* 1986, **10**:27–30

Key words: Excimer laser; Krypton fluoride (KrF); Mold; Fungus; *Aspergillus niger*; Laser cleaning