Combining technologies for food decontamination and extending the shelf-life of fruit and vegetables

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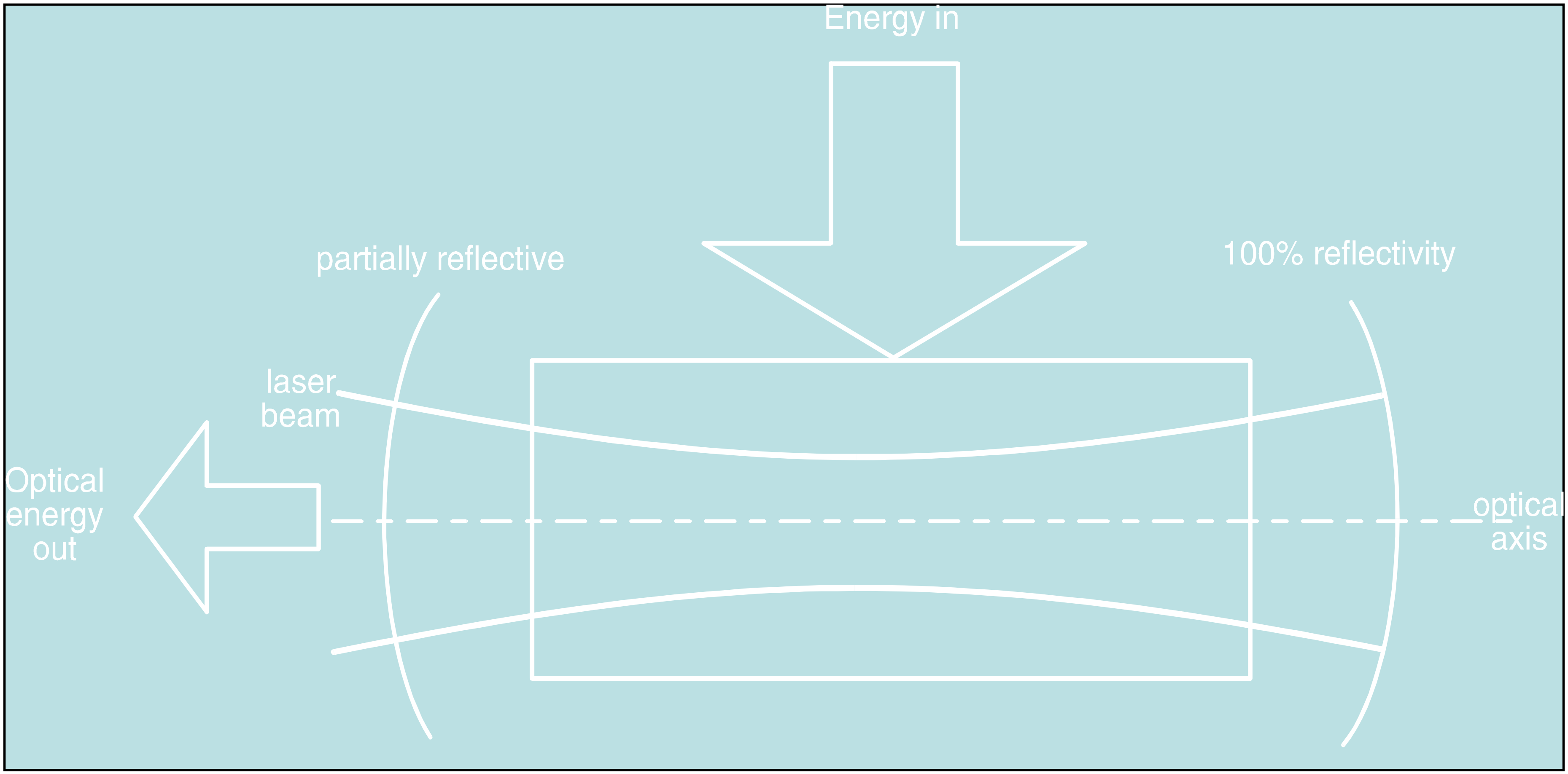
Contents

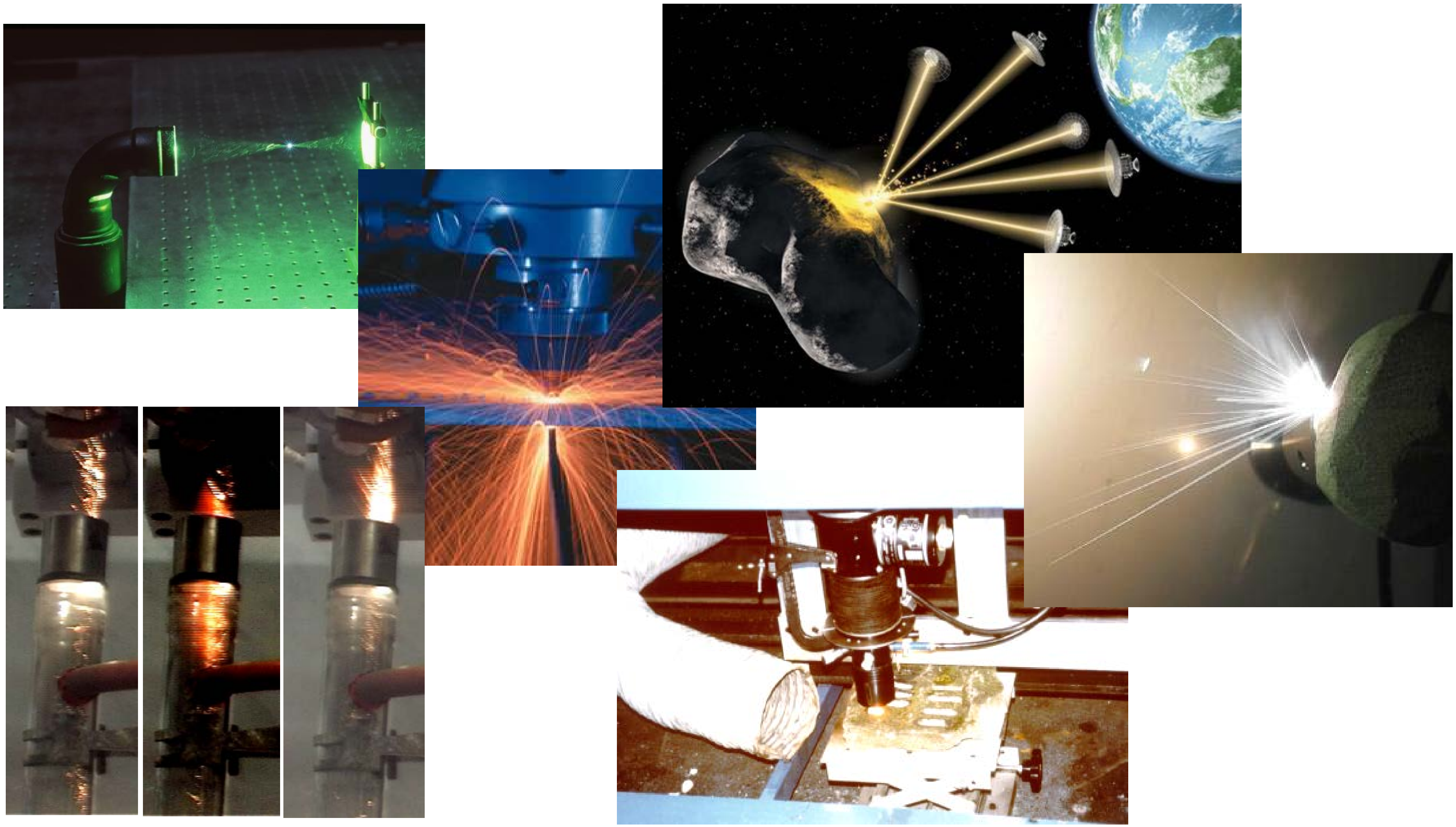
Introduction

- Consumers need safe food with a reasonable shelf-life
- Food poisoning costs the USA alone an estimated $152B
- Kills 5000 people per year in the USA.
- Globally, between 1.2-2.0 Billion tonnes of food produced each year is wasted, in part due to limited shelf-life.
- Solutions?
- Systems have been designed, built and evaluated at the University of Glasgow, UK, utilising a diverse range of technologies; from lasers, microwaves, ultrasonic, pulsed light, UV, and chemical methods.
- These technologies have been combined to reduce levels of contamination on different produce and extend their shelf-life. Some specific examples of these different treatments and systems will be given.
- Real time detection technologies developed

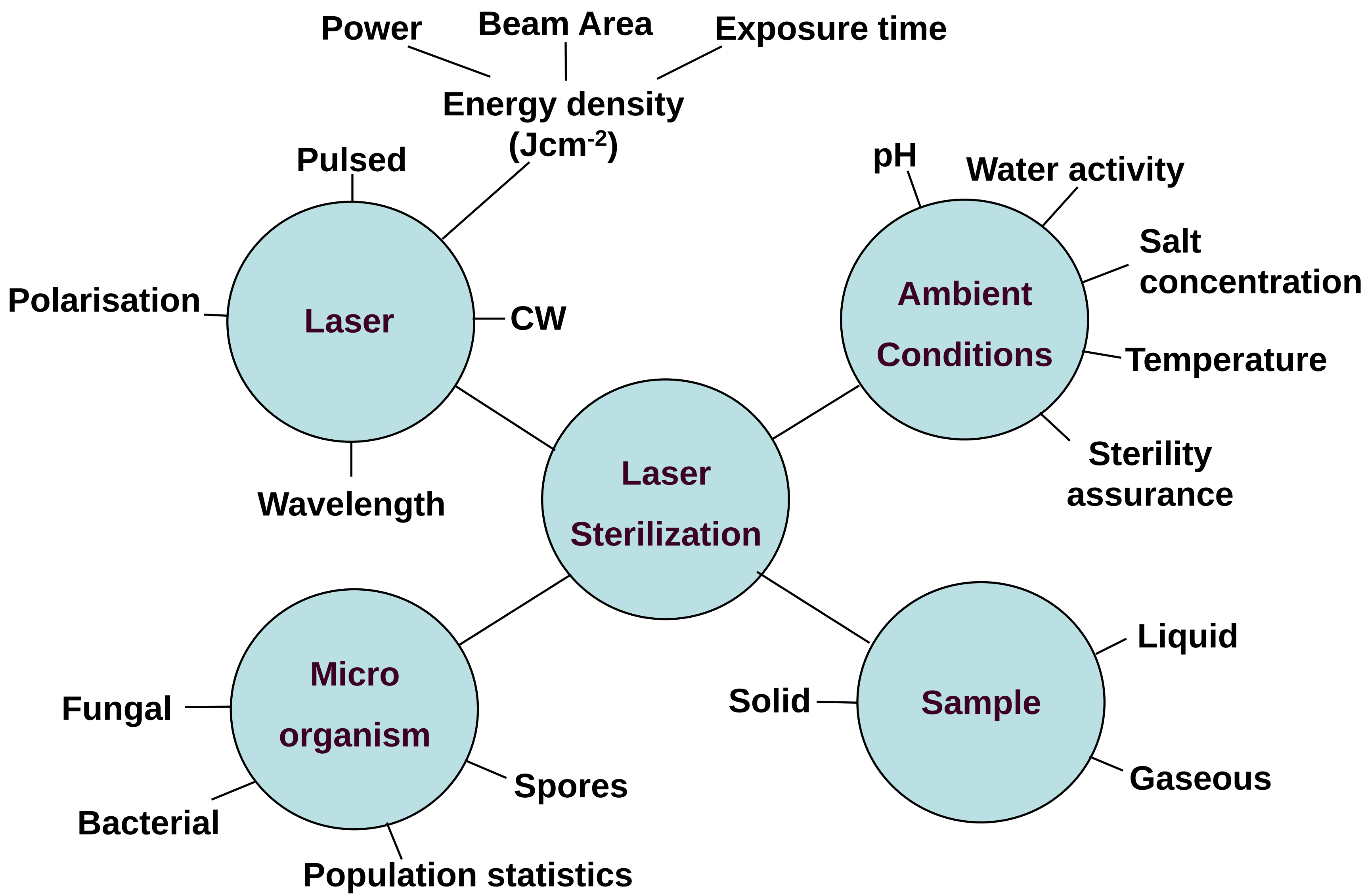
Introduction



Lasers, what they can do!



Laser and environmental parameters for inactivation



General Materials and Methods

- Produce overnight culture
- Inoculate onto sample medium – agar plates, small sample disks, food sample, or place in aerosol generator for air decontamination
- Perform scoping experiments to determine treatment levels
- Devise parameters settings e.g. laser, microwave…
- Treat with system – laser, UV, pulsed light, microwave, chemical, combination
- Recover counts
- Analyse data

Example of treatment on agar plates
Excimer Laser (248 nm, KrF) on surfaces

(a) (b) (c)

Agar plates lawned with *B. globigii* spores and treated with excimer laser of fixed power for a) 10 seconds, b) 1 minute and c) 10 minutes at 100 Hz PRF

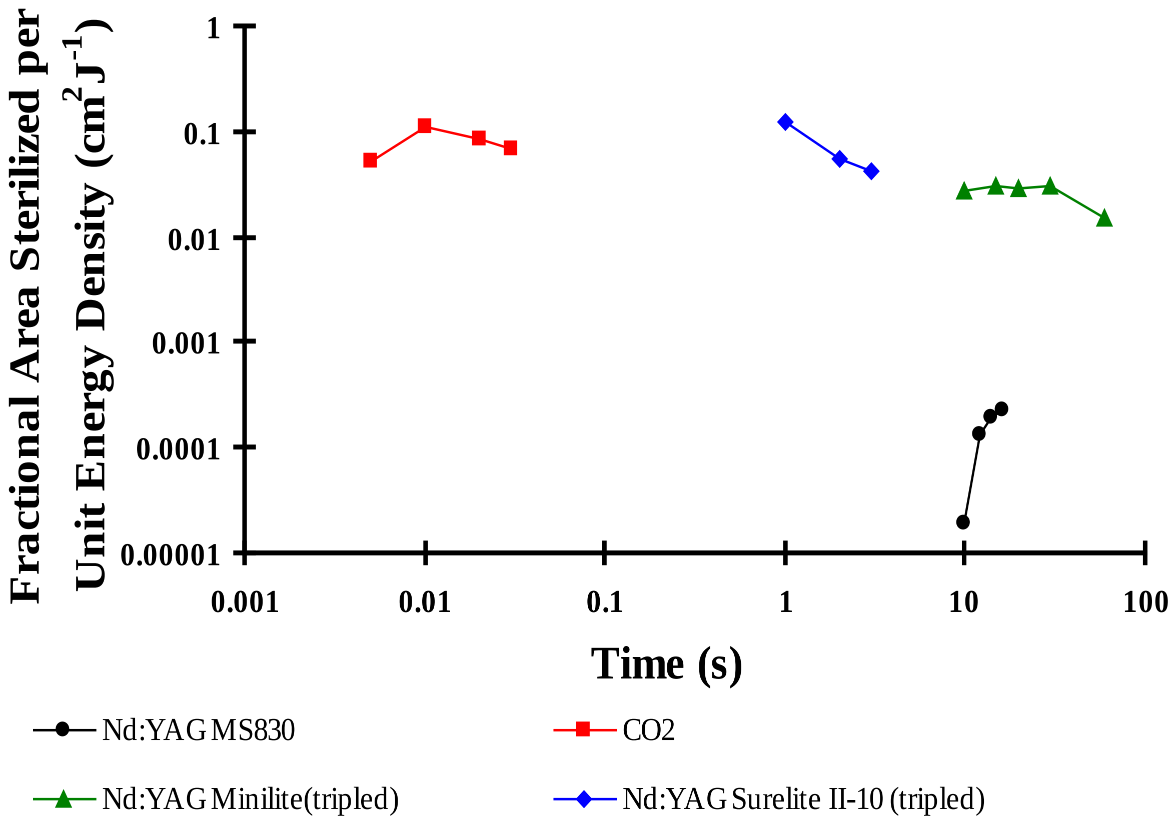
(a) (b) (c) (d) (e)

Agar plates lawned with *B. globigii* spores and treated with a different number of pulses from the excimer laser a) 1 pulse, b) 2 pulses, c) 5 pulses, d) 10 pulses and e) control (no laser radiation)


B. globiggi , 10min

Effect of laser wavelength

Exposure times to generate zones of clearing, normalized to the laser beam area and applied energy density, for each laser which demonstrated a biocidal capacity against *E. coli* lawned on nutrient agar culture plates



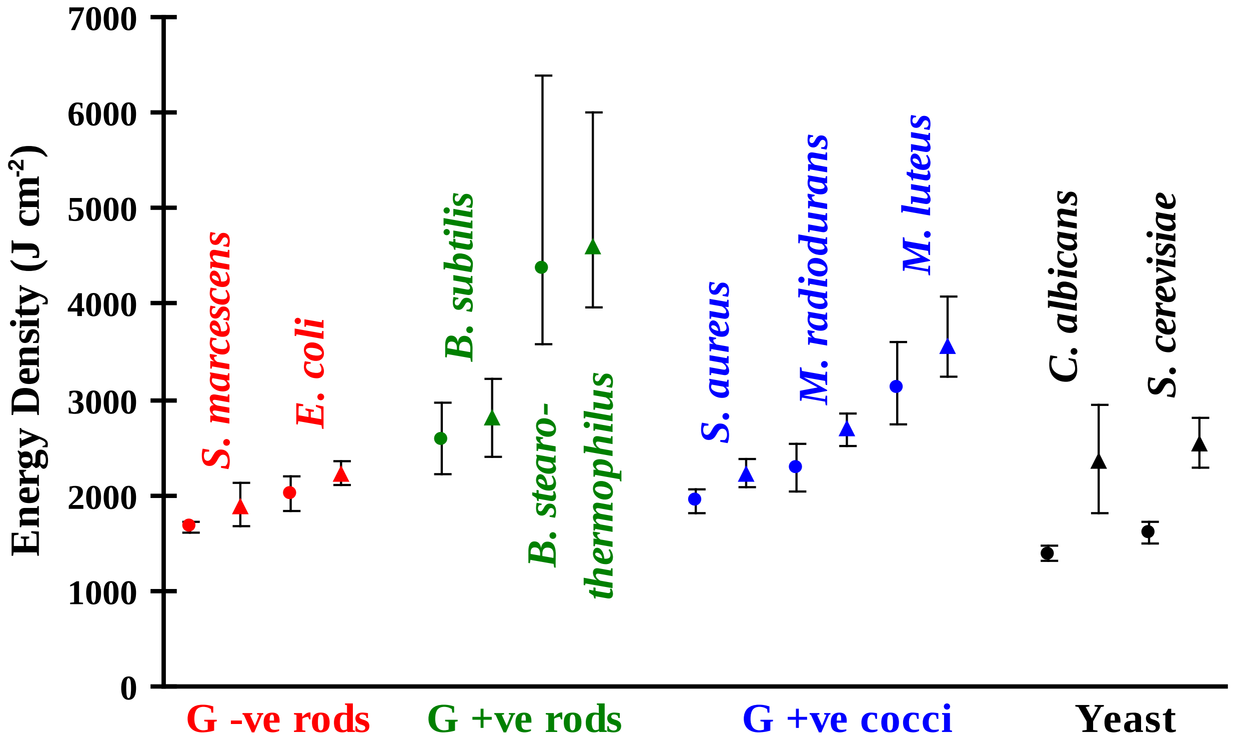
10.6μm, 1.06 μm, 355nm

No inactivation with:
FIR laser(118μ, 7.96 J/cm^2),
Ar ion (488nm, 2210 J/cm^2)

Effect of microorganism

Comparative sensitivities of seven bacterial and two yeast strains to Nd:YAG laser light (10J, 8 ms, 10Hz).

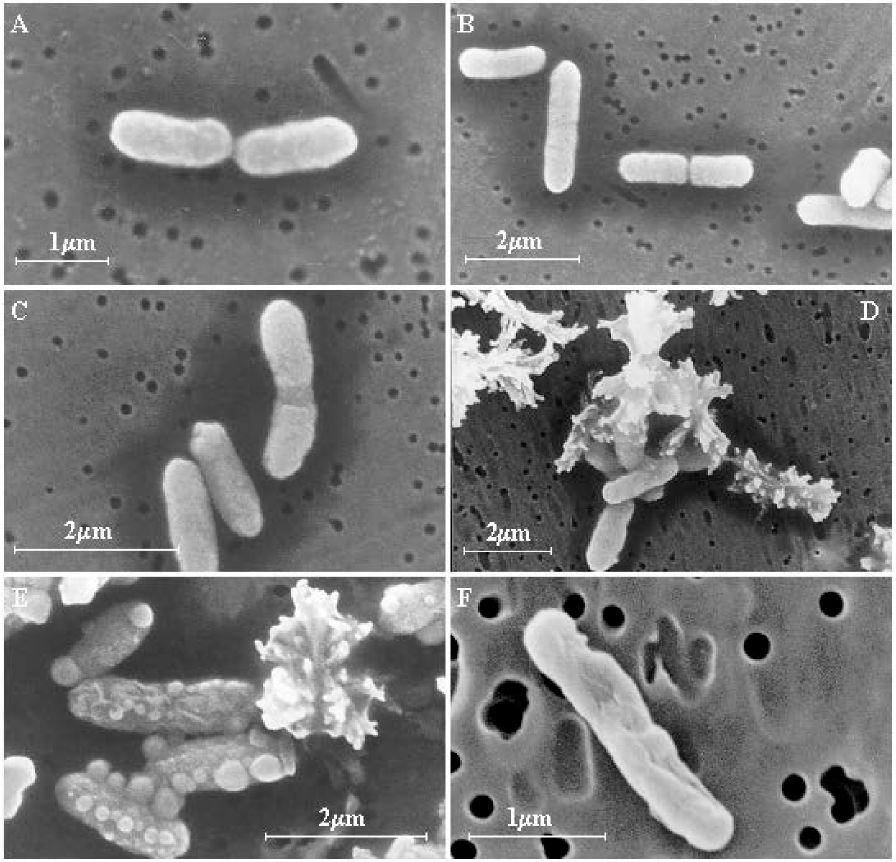
95% confidence limits of the energy densities required to sterilize an area of 0.825 cm^2.



Effect of Nd:YAG laser radiation on *E. coli* in saline solution

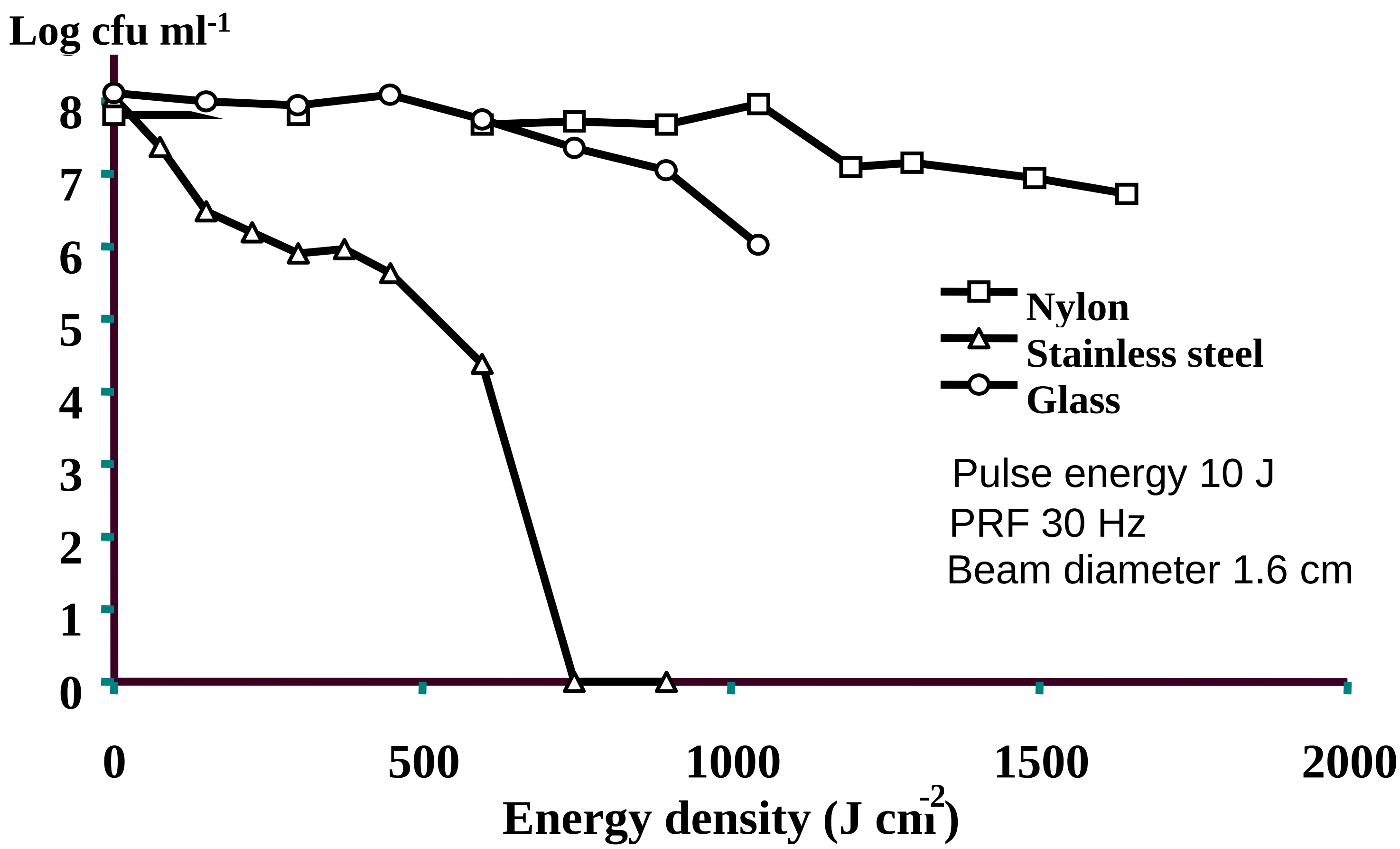
Scanning Electron Micrographs of *E. coli* after various laser exposures and water bath treatments.

A. Control; laser exposure to temperatures (B-E) of 40, 50, 60 and 70°C and F. 100°C in a waterbath.



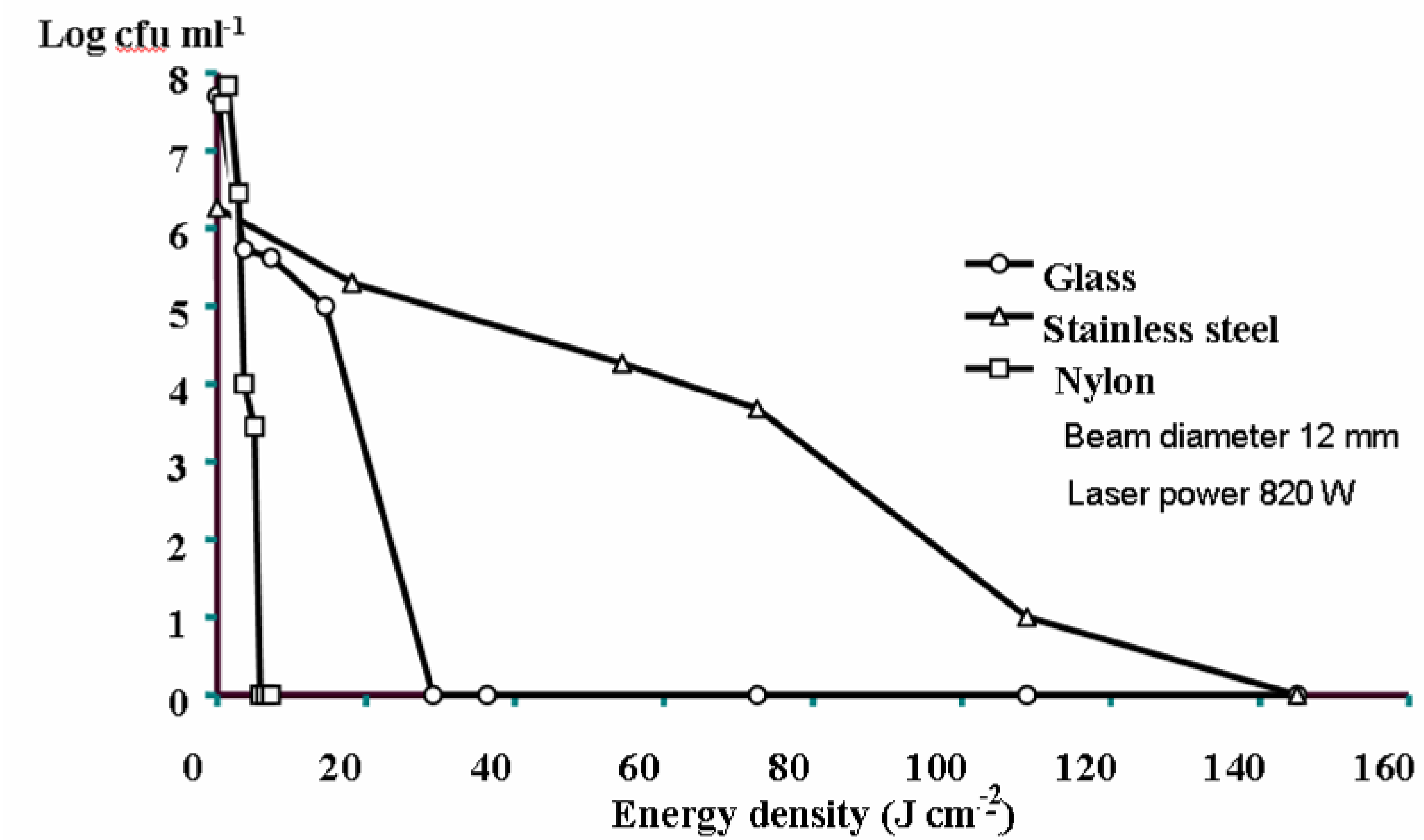
Effect of substrate

Nd:YAG laser killing curves of *S. aureus* films dried on glass, nylon and stainless steel



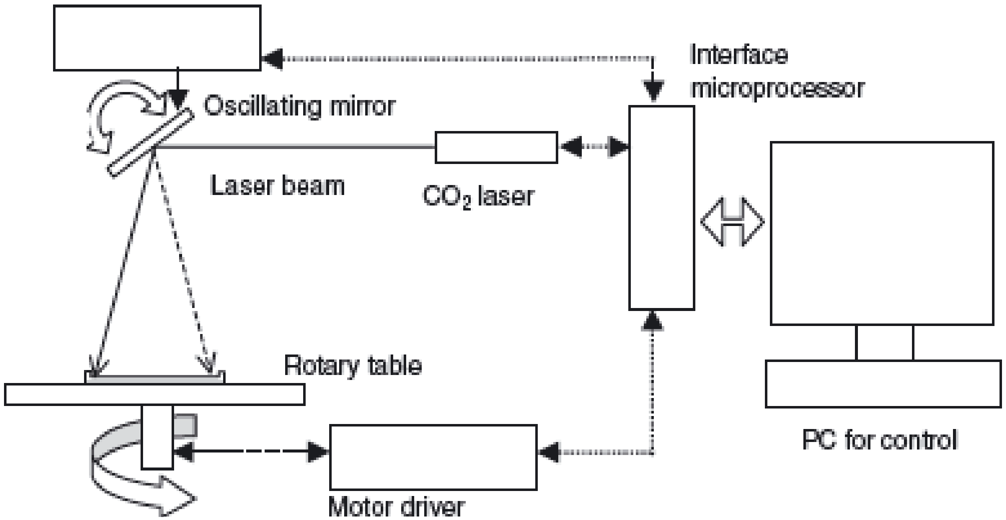
Effect of substrate

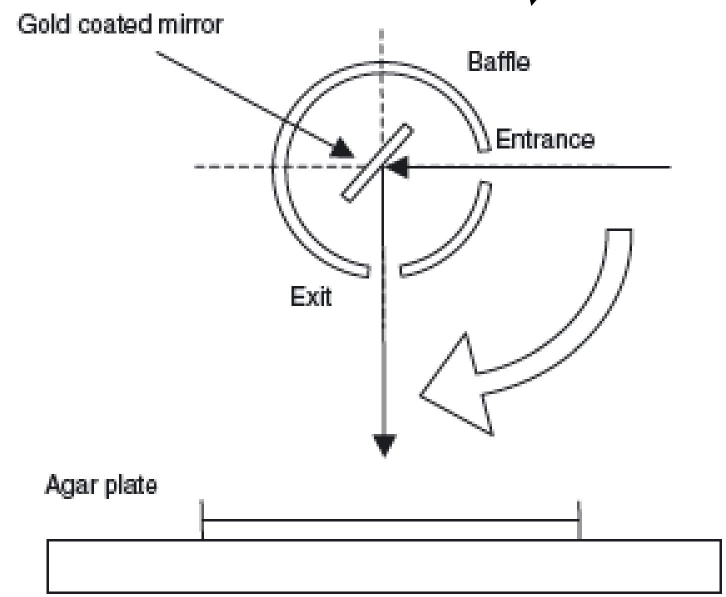
CO_2 laser killing curves of *S. aureus* films dried on glass, plastic and stainless steel

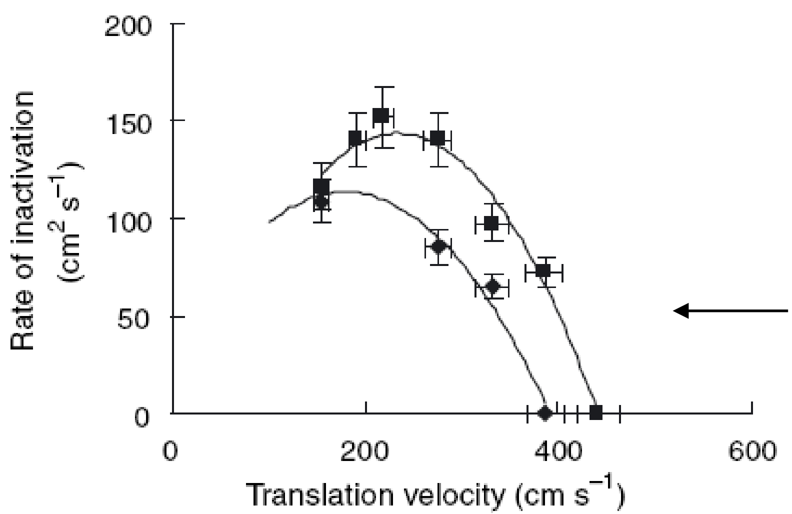


Laser scanning systems

CO_2 laser systems
low power and high power

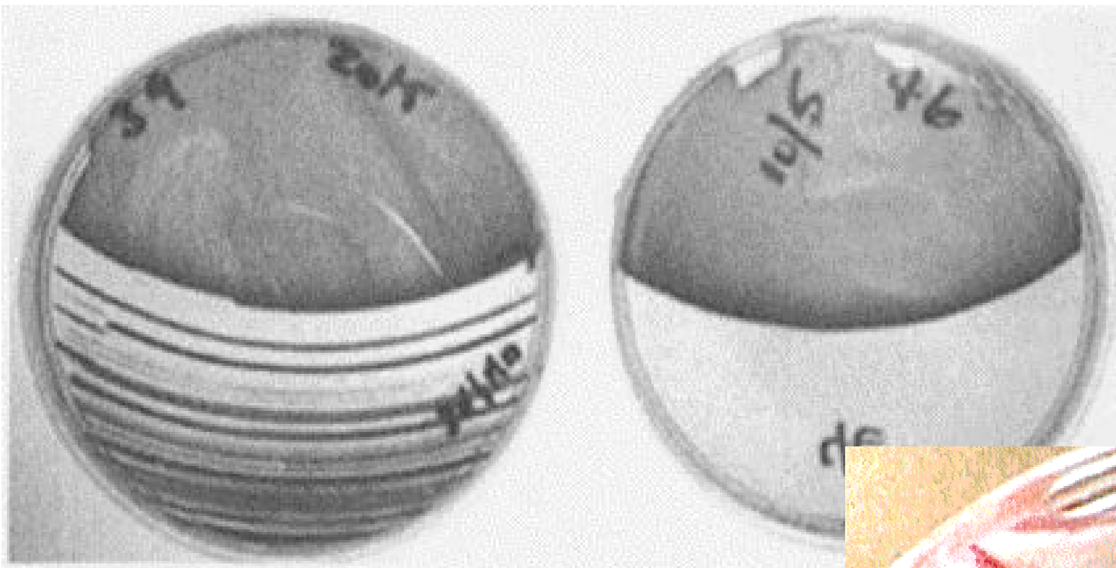






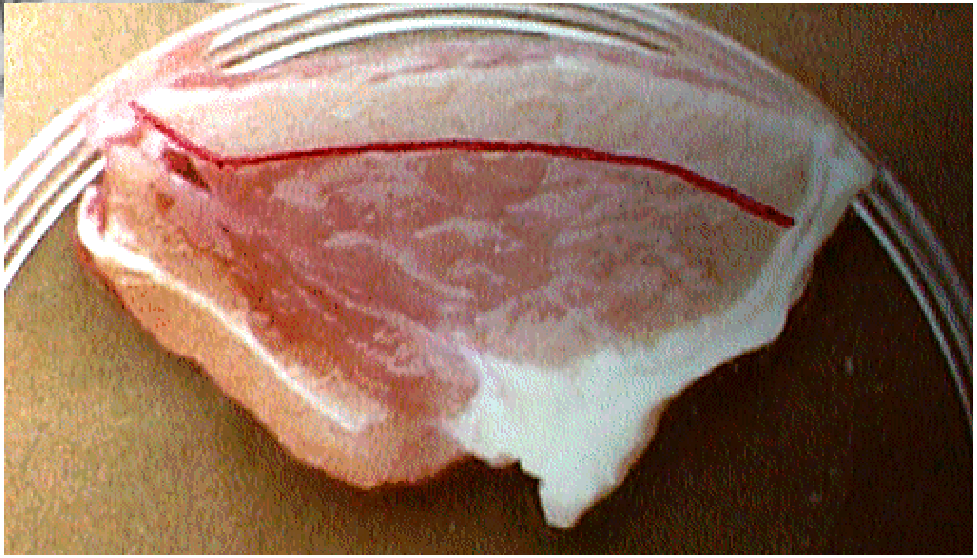
High power system (~1kW), *S. aureus* on collagen (square) and agar (diamond), 10 mm beam diameter

Low power laser scanning systems

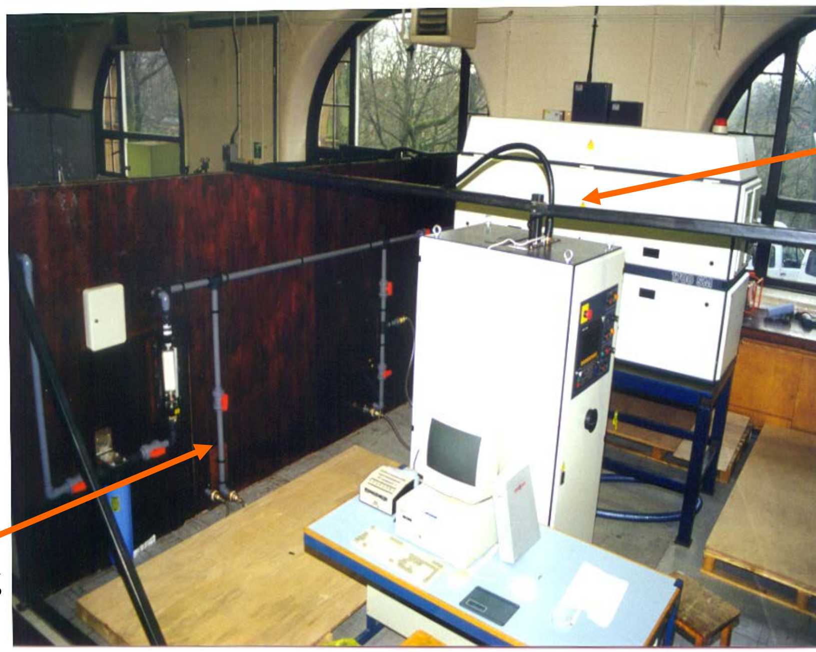


Optimal scanning speed

Scanning speed too slow and/or insufficient power



Combined inactivation systems (EU funding – carrots and potatoes)



Electrical connection

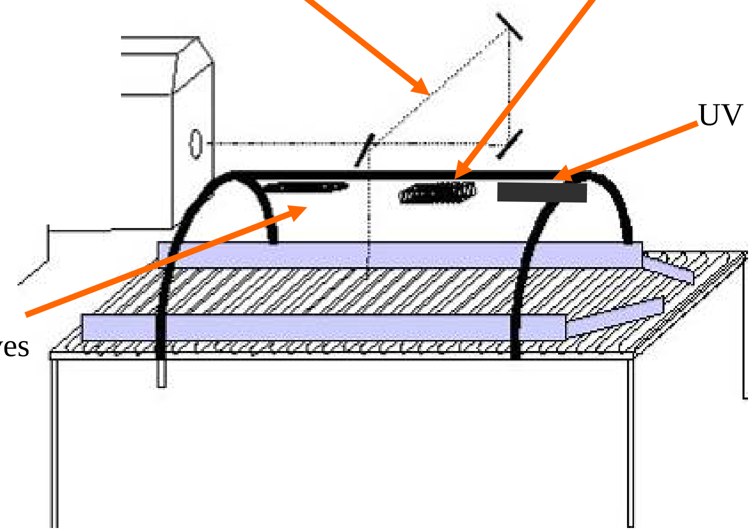
Water cooling connections

Laser Beam

UV Flashlamp

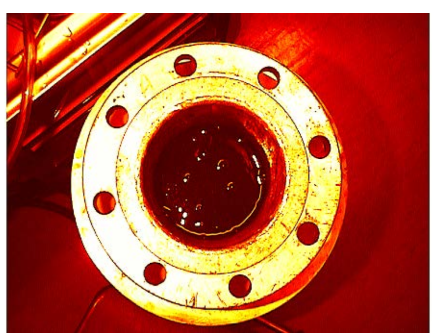
UV cw lamp

Microwaves

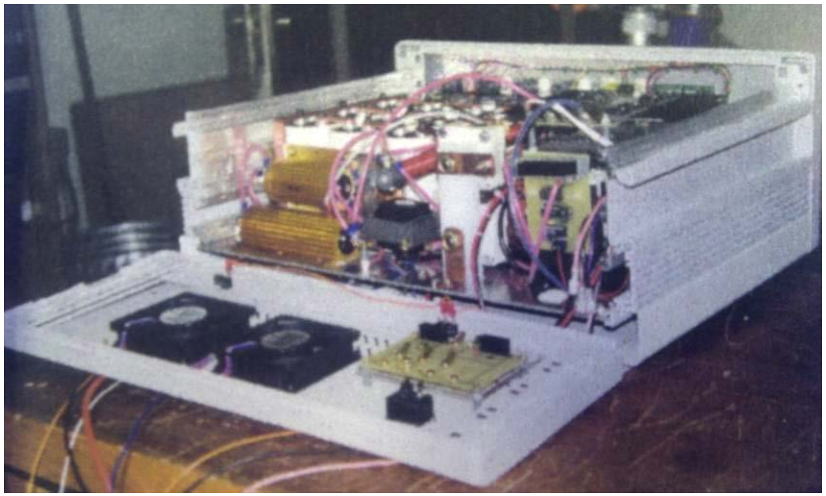


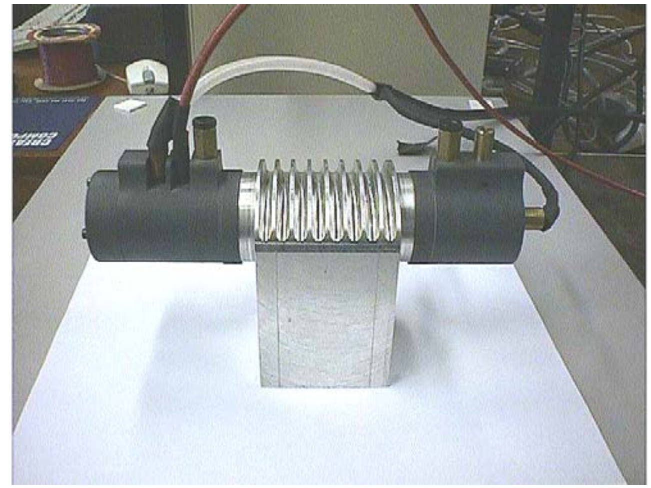
Radiation enclosure

Ultrasonic system

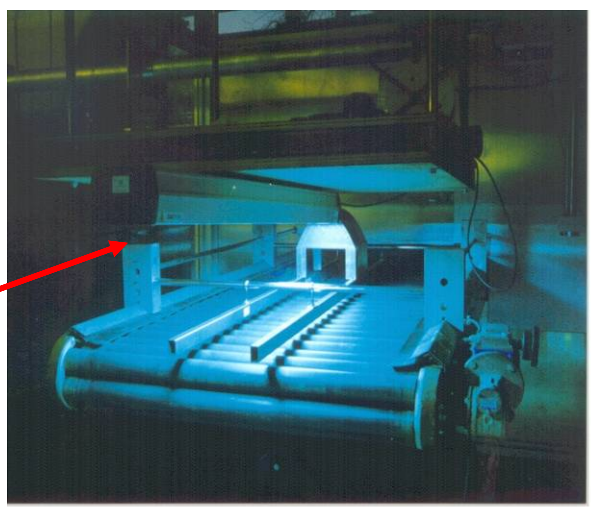


Pulsed light systems

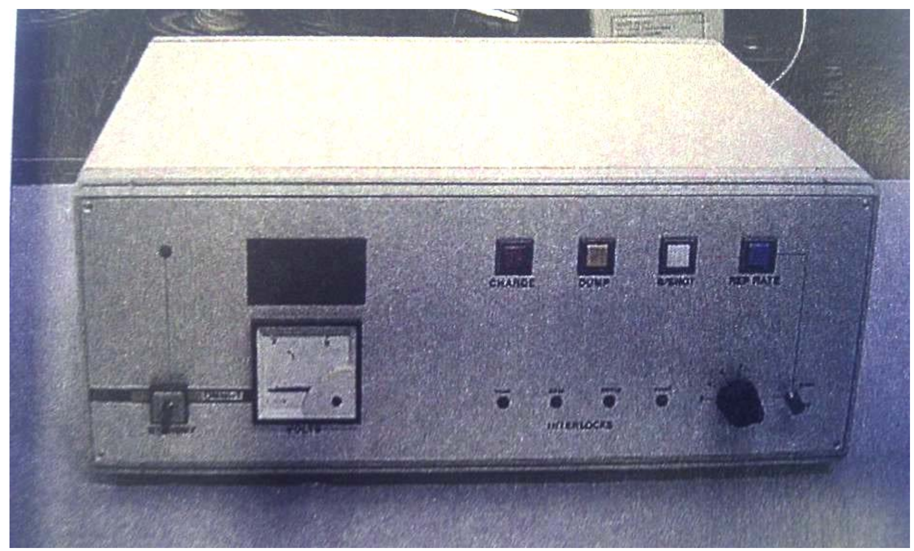




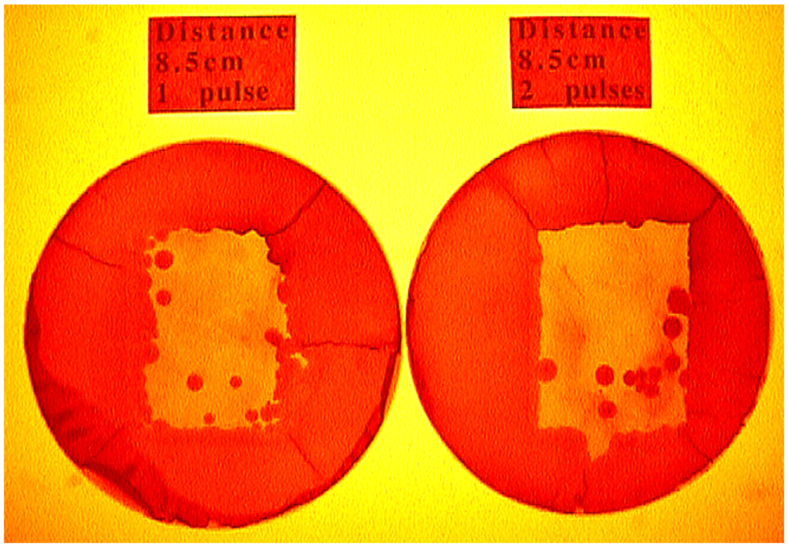
CW UV lamp

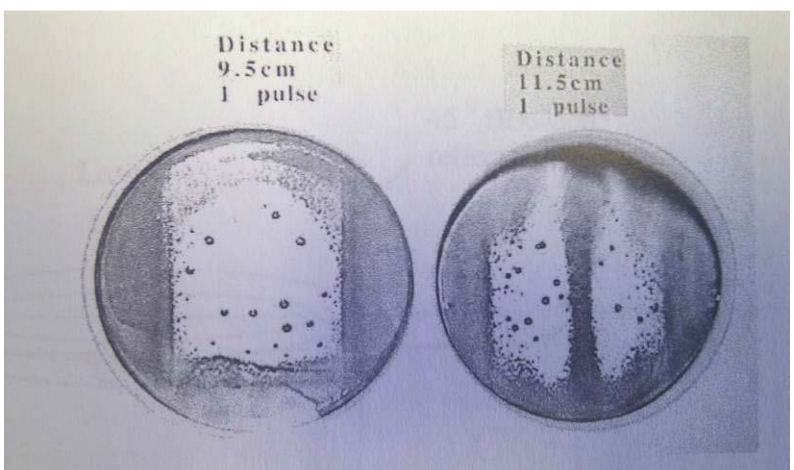


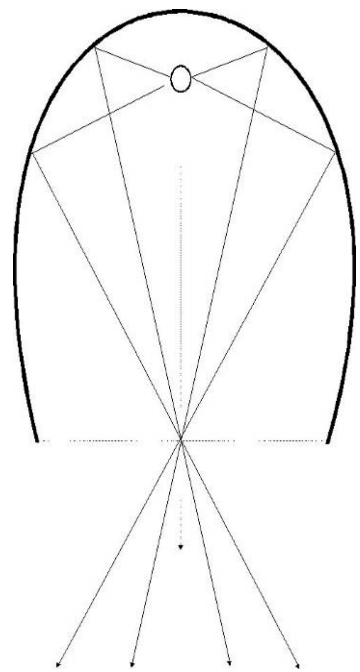
UV system, installed above roller table



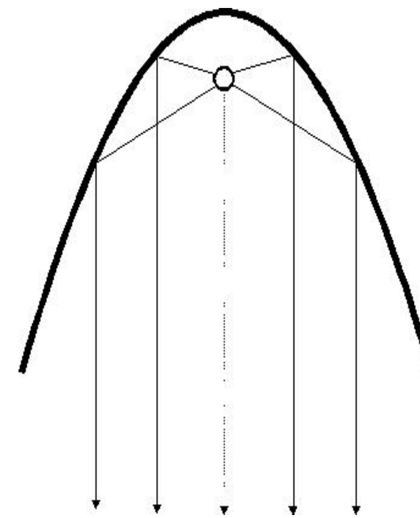
Pulsed light (*B. cereus* spores)







Elliptical pulsed light head



Parabolic UV pulsed light head

Combined inactivation systems

Shelf life extension of carrots and potatoes:
A comparison of H_2O_2, laser, UV, and microwave treatments

UV Treatment

Delivered via 210 W, UV source (254 nm)
Lamps warmed up for 15 min/until stabilised
irradiated area ~0.48 m^2
Produce passed through system at ~0.21 ms^{-1}
After treatment, produce stored in autoclave plastic bags

Laser Treatment

1kW CO_2 laser (Rofin MS1700, UK)
Samples rotated at 4 rps and exposed for 4s

Microwave Treatment

Variable power microwave source (max.1kW)
Operated at 800 W for 5 s

H_2O_2 Treatment

1% H_2O_2 mixed with sterile water 1 minute before treatment process
Mixture prepared in sterile and chemically inert vessel
Carrots and potatoes were immersed in solution for 20s.

Combined inactivation systems

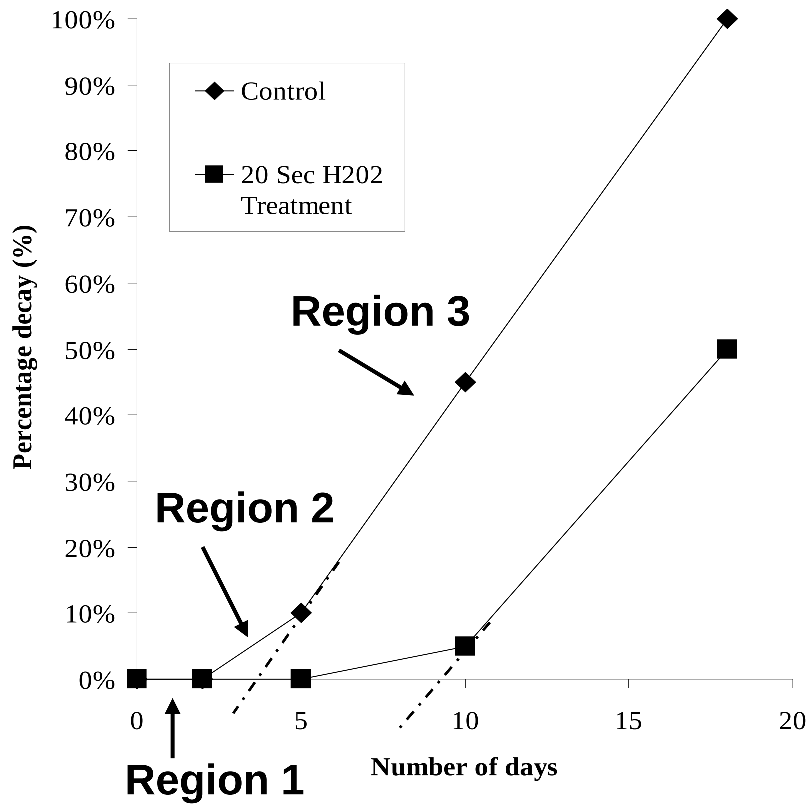
Shelf life extension of carrots and potatoes: A comparison of H_2O_2, laser, UV, and microwave treatments

Control 10 Days

H_2O_2 10 Days

Control 18 Days

H_2O_2 18 Days



Combined inactivation systems

Shelf life extension of carrots and potatoes: A comparison of H_2O_2, laser, UV, and microwave treatments

From **Figures of decay** the approximate rate of decay (%/day) for the regions defined as 2 and 3 can be found. By definition there is no decay in region one. **Table 1** shows the shelf life, obtained by extrapolating back the late phase decay to 0 percent decay, and the early (region 2) and late phase decay (region 3) as % decay/day.

Sample	Shelf life (Day)	Early Phase Decay (%/Day)	Late Phase Decay (% / Day)
Control	3.6	3.3	6.9
H_2O_2	9.1	1.0	5.6
Laser	8.0	2.0	5.0
Microwave	5.0	6.2	6.2
UV	5.0	6.2	6.2

Combined inactivation systems

Shelf life extension of carrots and potatoes: A comparison of H_2O_2, laser, UV, and microwave treatments

- Laser treatment darkened potato on first day, didn't worsen (unacceptable)
- Experiments done in ambient conditions, accelerate decay process and shorten shelf life compared to refrigerated conditions
- Laser successfully extended shelf life of the carrots but again discolouration
- No variation in beta carotene or vitamin C

Control 10 Days

Laser 4 Sec 10 Days

Microwave 10 Days

Control 18 Days

Laser 4 Sec 18 Days

Microwave 18 Days

Modelling decontamination on potatoes

Model used to investigate effect of spatial distribution of bacteria before and after treatment

Samples of eyes and smooth skin (~ 1g of potato flesh) were analysed microbiologically for input data

Various assumptions

- no killing on the eyes due to shadowing
- complete killing on the skin
- equal killing on the skin and eyes
- 1 D value reduction on the skin only
- 1 D value reduction on the skin and eyes

Effect of varying weight of potato flesh investigated
Input data included weight and radius of potato.
Assumed potato was round
Different scenarios were run through model to investigate the effect of different number of eyes (and weight) and different size of potatoes

Modelling decontamination on potatoes

Spatial distribution of bacteria over the surface of the potato, in the skin and eye areas was determined experimentally

Found that eyes had ~4x contamination of that of the smooth skin

Data were used as input into the model to estimate the effect of different decontamination treatment on the overall bioburden.

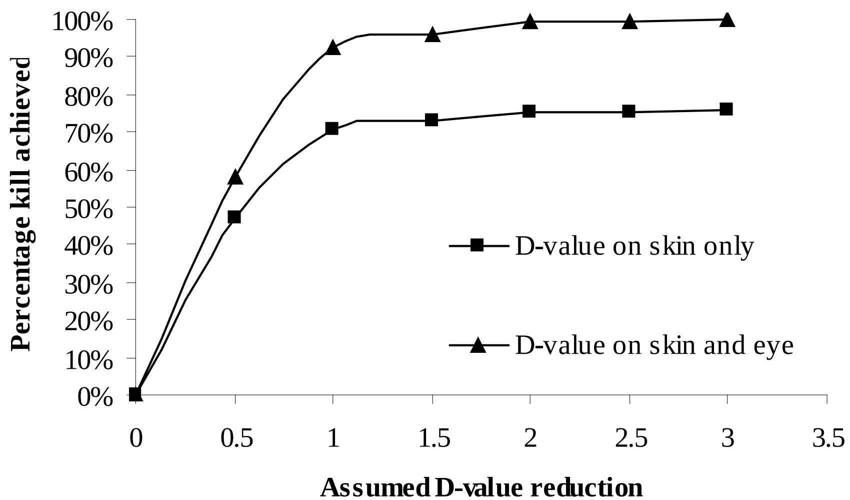
Area	**Cfu/200ml**	**Cfu g^{-1}**
Smooth skin	5.8×10^5	1.4×10^4
Eyes	1.8×10^6	5.8×10^4

Modelling decontamination on potatoes

Calculations based on weight or surface area.

The calculations based on weight gave a greater reduction than those based on area, but with similar trends in the results for each set of assumptions.

Different scenarios were put into the model to investigate the effect of a different number of eyes (and weight) and different size of potatoes.



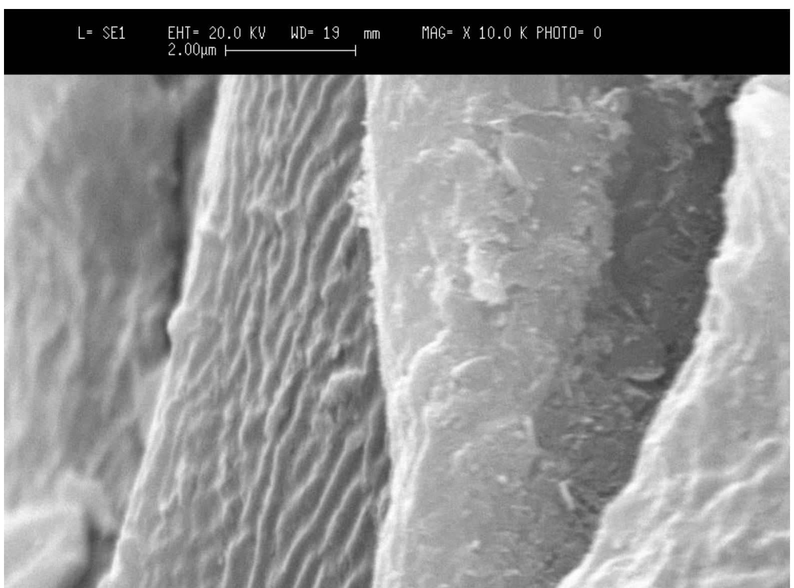
Model of potato inactivation showing percentage of organisms killed after different assumed log reductions on skin and eyes

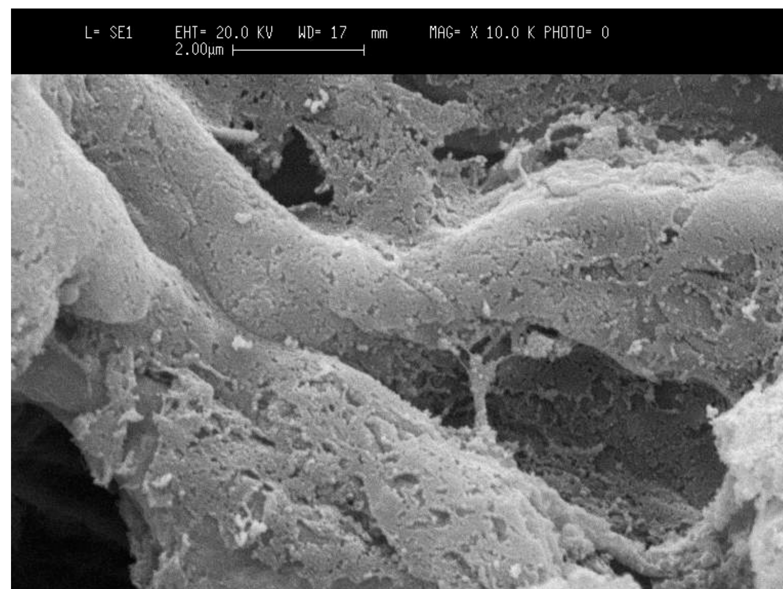
(140g, 30 mm radius, 10 eyes, 1 g/eye)

Surface decontamination affected by surface topology decontamination on potatoes

SEM Analysis

Carrot's fibres arranged in longitudinal direction along circumference of produce
Fibre width about 10 to 15 μm, skin cells on potato about 50 μm
These dimensions are same order as CO_2 laser wavelength (10.6 μm), considerably larger than the UV wavelength (254 nm) and vastly smaller than the microwave wavelength (~12 cm).





Carrot (left) and potato surface at 10000x magnification

Comparison of Vitamin C in Potatoes and Beta-carotene in carrots before/after treatment

No statistical difference between the control or treated samples for β-carotene or Vitamin C concentrations

No adverse effects from treatment that damaged the β-carotene or Vitamin C.

	Carrots (μg/100g)		Potatoes (mg/100g)	
Treatment	β-carotene	Average	Vitamin C	Average
Control	2940	2936.7	14	
Control	2670		13	14.3
Control	3200		16	
Laser	4310	3918.3	11	
Laser	3290		15	15.0
Laser	4155		19	
UV	2815	3535.0	12	
UV	3915		14	13.0
UV	3875		13	
H_2O_2	4025	3435.0	14	
H_2O_2	3300		14	16.0
$H2O_2$	2980		20	
Microwave	4175	3598.3	15	
Microwave	3210		11	12.7
Microwave	3410		12	

Combined inactivation systems

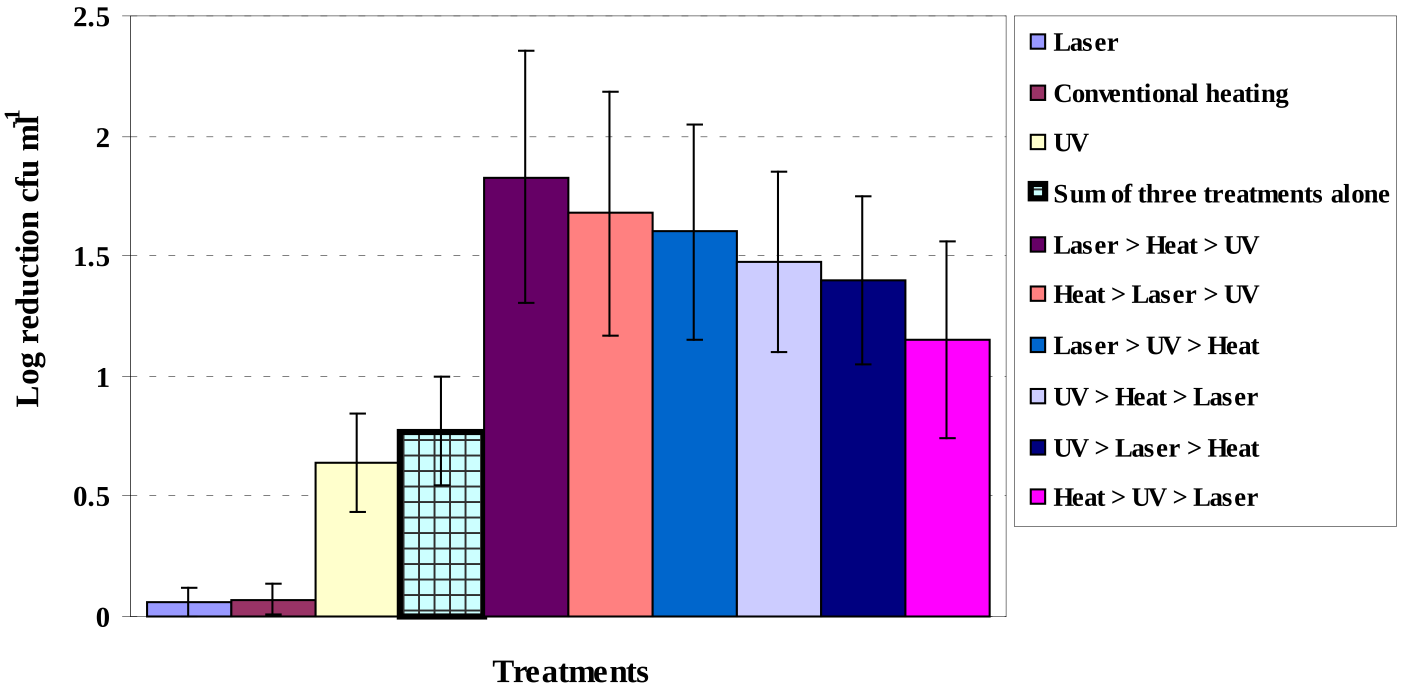
UV, laser, microwave or conventional heating on:

Escherichia coli,
Listeria monocytogenes,
Shewanella putrefaciens,
Pseudomonas fragi
Micrococcus leteus

- Determined optimal treatment energy densities, power and time
- Individual treatments investigated
- Effects of order of sequential treatment studied
- Sum of log kill of individual treatments compared to combined treatments

Combined inactivation systems

Killing effect of different treatments on *E. coli* in saline suspension, errors show the standard error of the mean (n = 3)



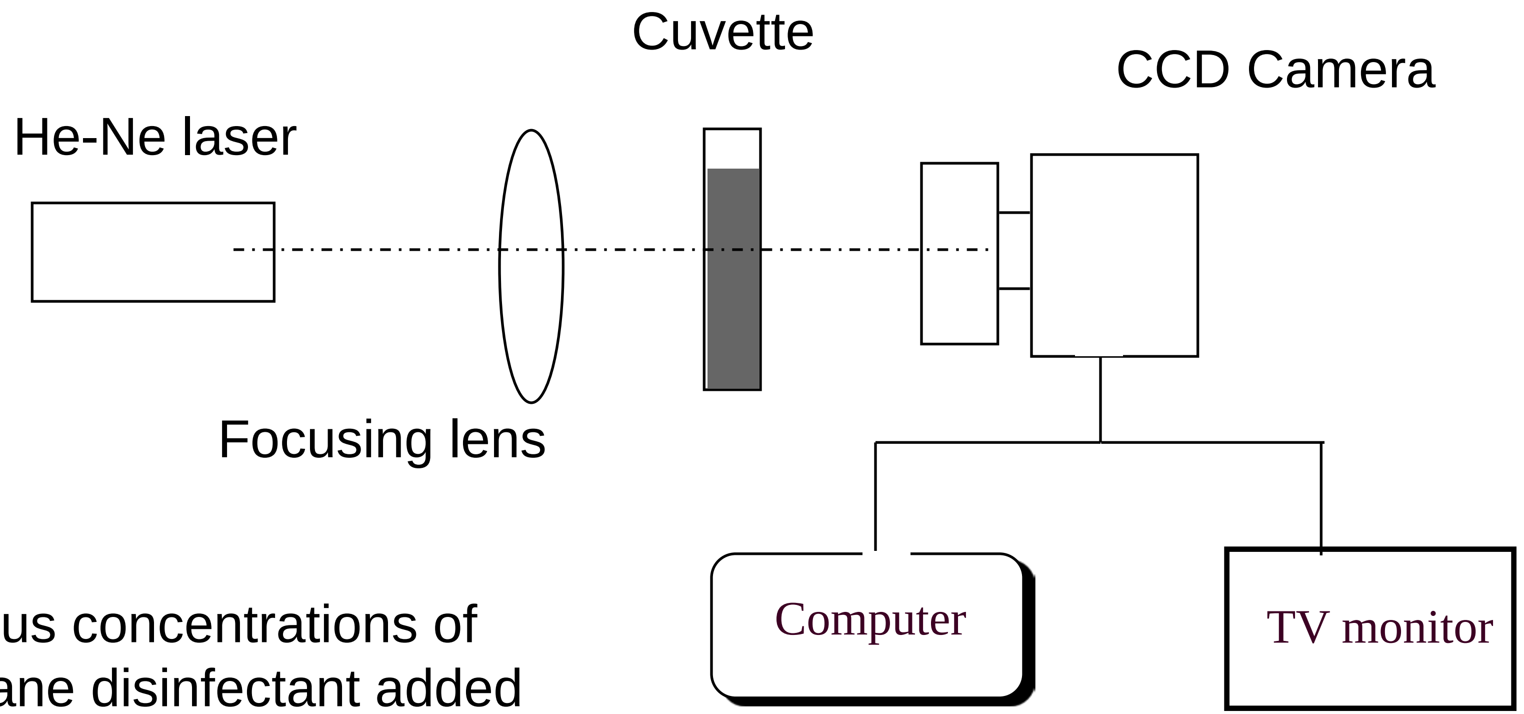
Treatment order important

Laser ➔ Heat ➔ UV more effective than Heat ➔ UV ➔ Laser

Synergistic effect discovered – combined treatment > than sum of treatments alone

Real time detection technologies

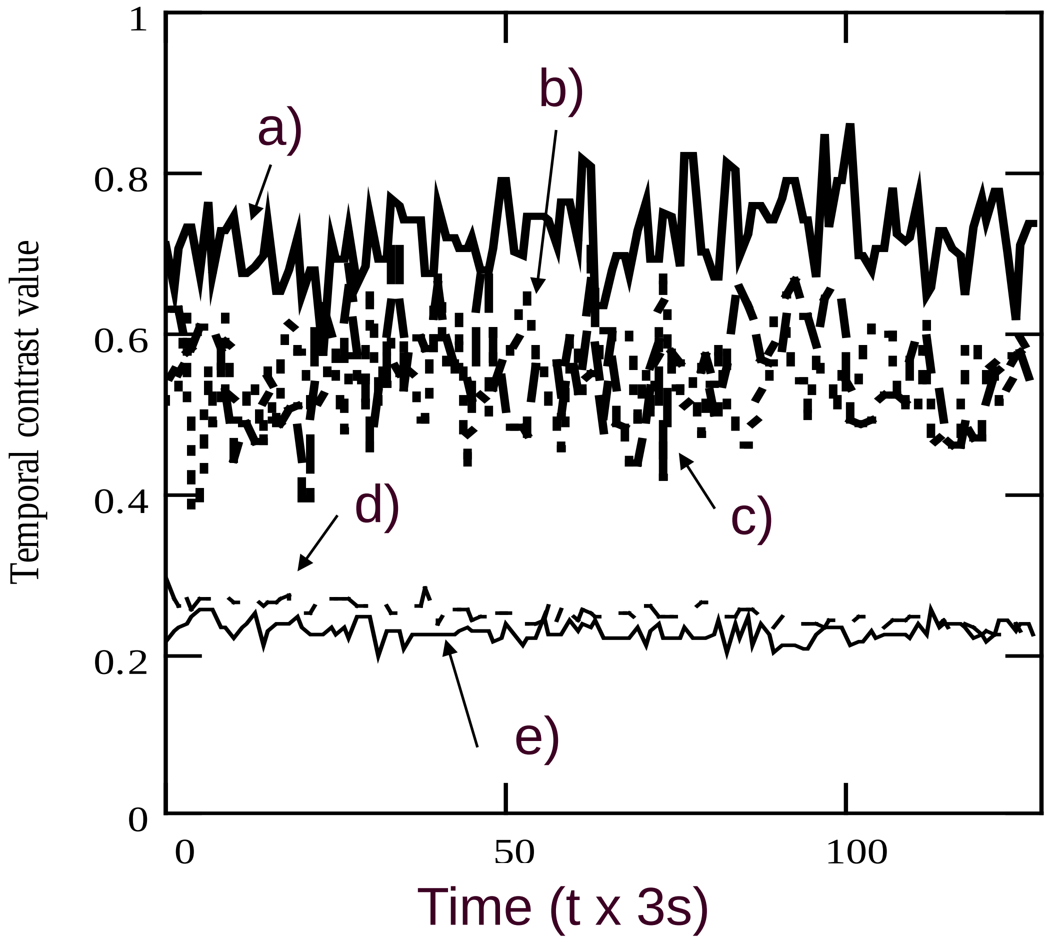
Biodynamic Speckle for real time analysis of bacterial suspensions



Various concentrations of Hibitane disinfectant added to *E. coli* suspension and speckle pattern analysed.

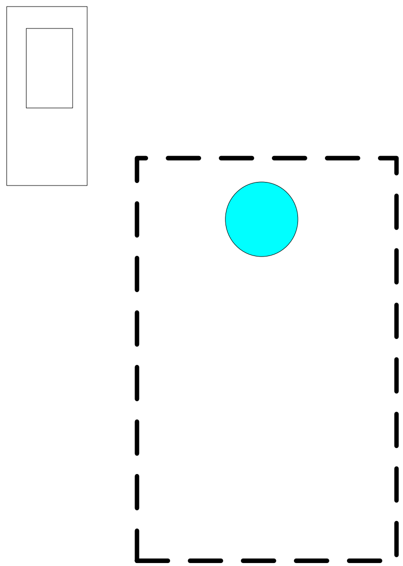
Speckle

Temporal Contrast of
- a) *E.coli* suspension
- b) with 1/6000
- c) 1/4000
- d) 1/40
- e) Neat hibitane disinfectant solution

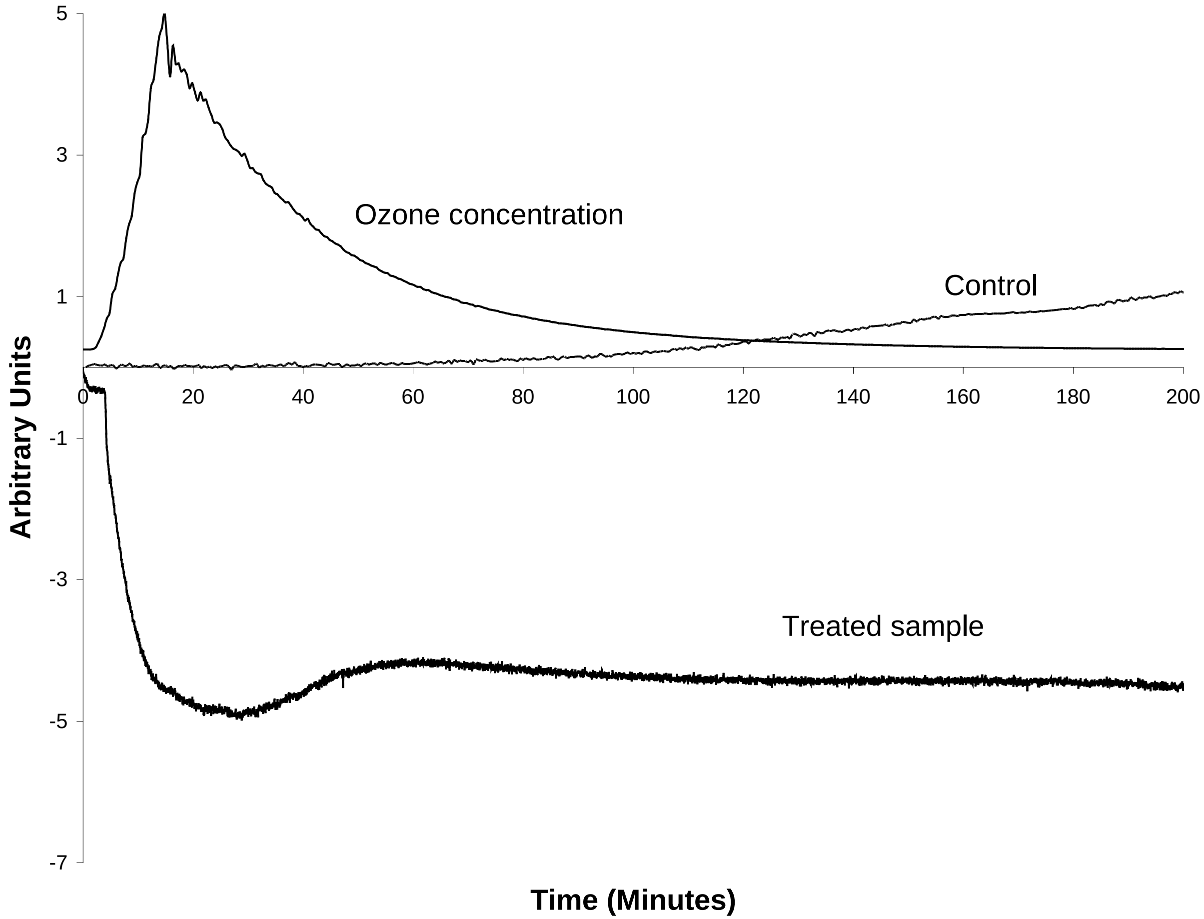


Bioluminescent system for biocide treatment optimization on solid surfaces.

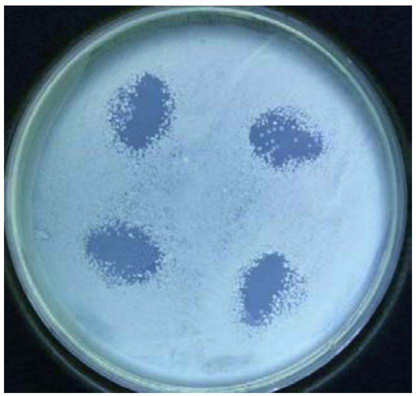
E. coli lux on agar plates placed in one chamber, ozone concentration measured in another. Lux output measured via a photomultiplier, results correlated

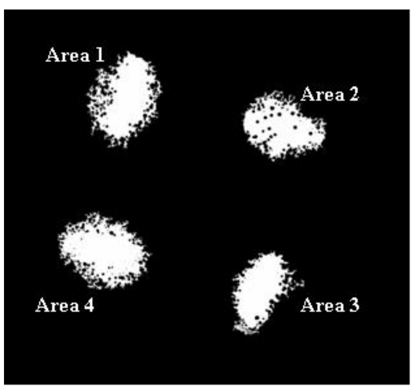


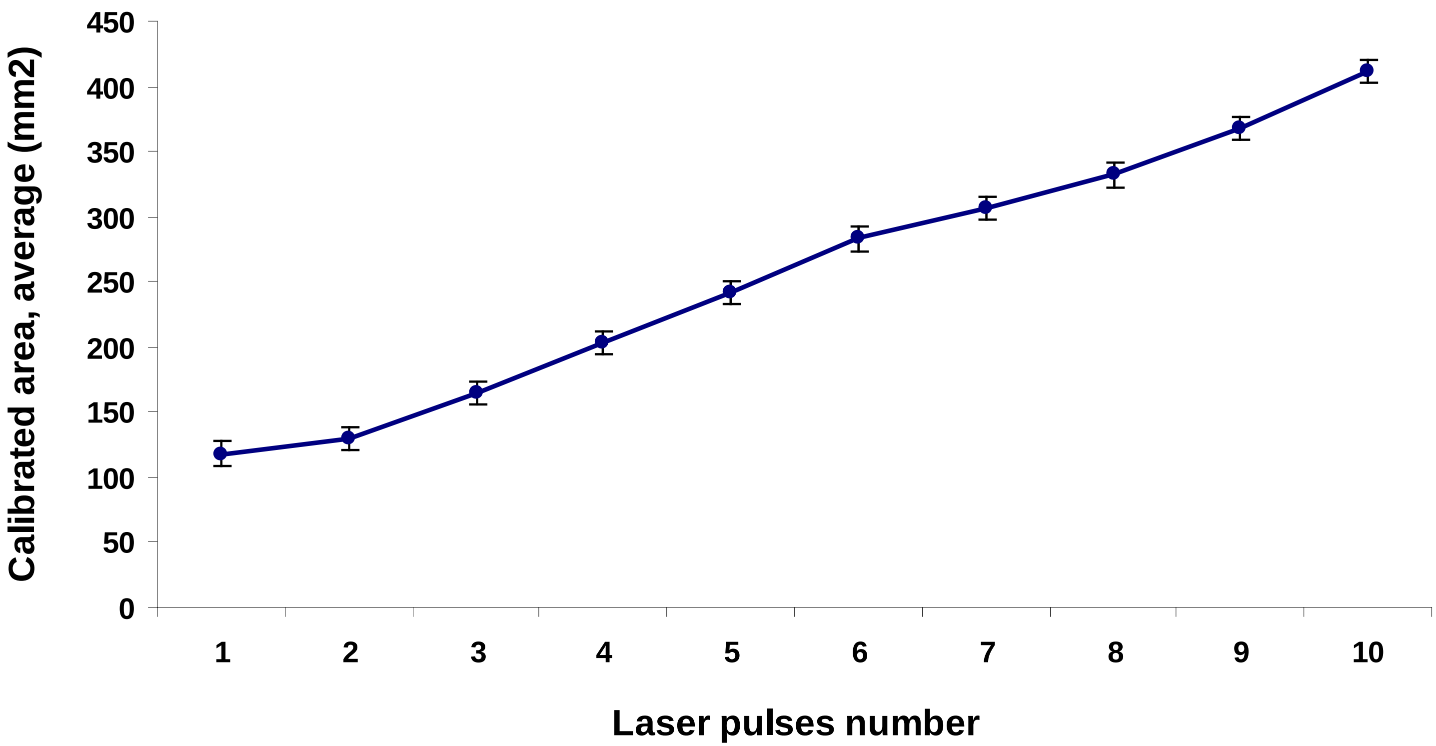
Temporal variation of bioluminescent output from an *E. coli lux* culture with (treated) and without (control) ozone treatment



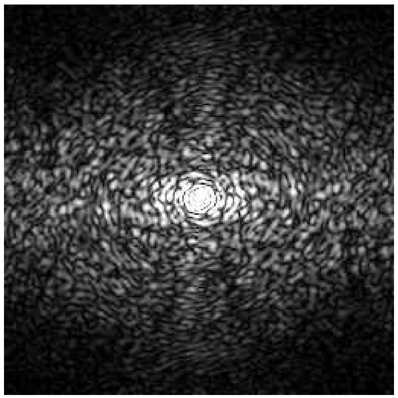
Microscopy and image processing

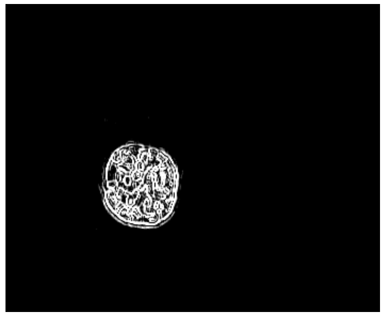


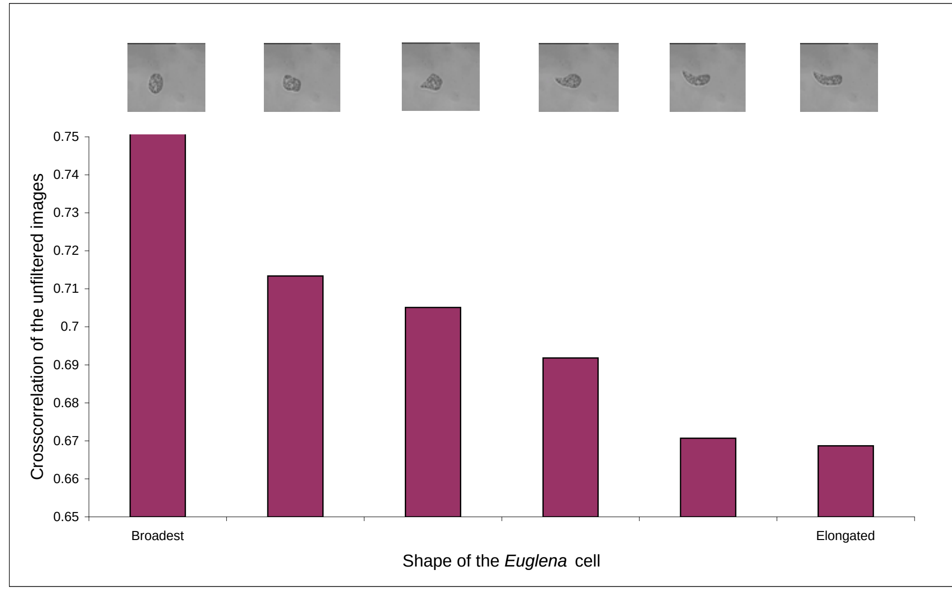


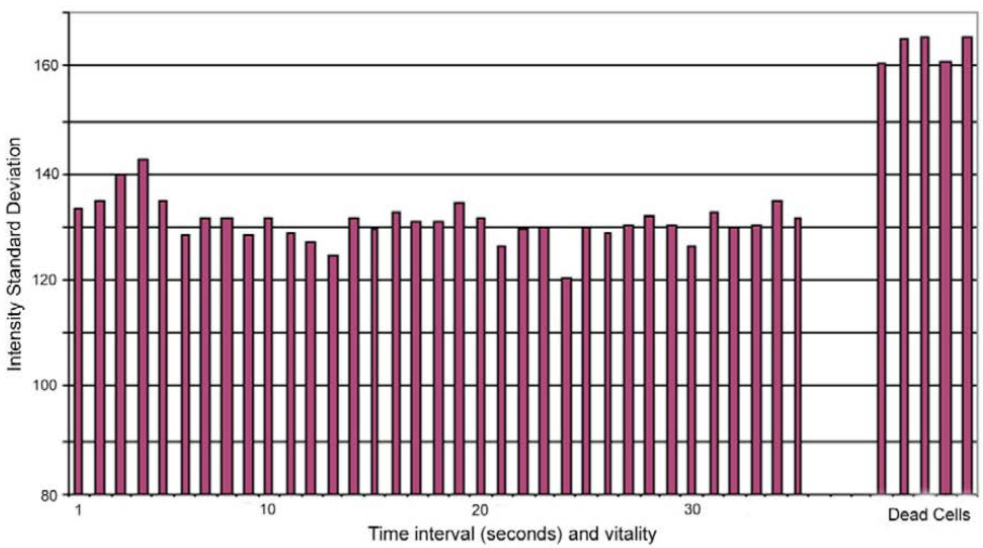


Microscopy and image processing









Conclusions

- Most efficient order for CO_2: nylon, glass, stainless steel.
- Nd:YAG most efficient order: stainless steel, glass, nylon.
- Differences mainly due to properties of materials at 1.06 and 10.6 µm.
- For laser and carrot treatments, all extended shelf life beyond the control (3.6 days), H_2O_2 most efficient treatment (9.1), laser (8.0), microwave and UV (5.0 days).
- Laser successfully extended shelf life of the carrots but discolouration (unacceptable for consumer).
- No adverse effects on β-carotene or vitamin C, possibly an increase.
- The treatment processes induced shoot growth whereas none were evident with the control samples.
- Reduce damage of substrate but still inactivate microorganisms.
- Treatment order important

  Laser ➔ Heat ➔ UV more effective than Heat ➔ UV ➔ Laser
- Synergistic enhancement i.e. combined treatment > than sum of treatments
- Real time detection systems introduced.

Acknowledgements

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Selected References

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