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Selective deactivation of viruses using femtosecond laser pulses

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INTRODUCTION

Viruses can be described as acellular organisms whose genome consists of nucleic acid that replicate inside living cells (host cells) using their cellular synthetic machinery, and cause the synthesis of specialised elements called virions, that can transfer the genome to other cells. M13 bacteriophage (virus which infects only bacteria) is a filamentous virus that is about 1 µm long and 5-6 nm in diameter. Its host Escherichia coli (E.coli), is approximately 2-6 µm long and 1-1.5 µm in diameter, see **Figure 1** below.

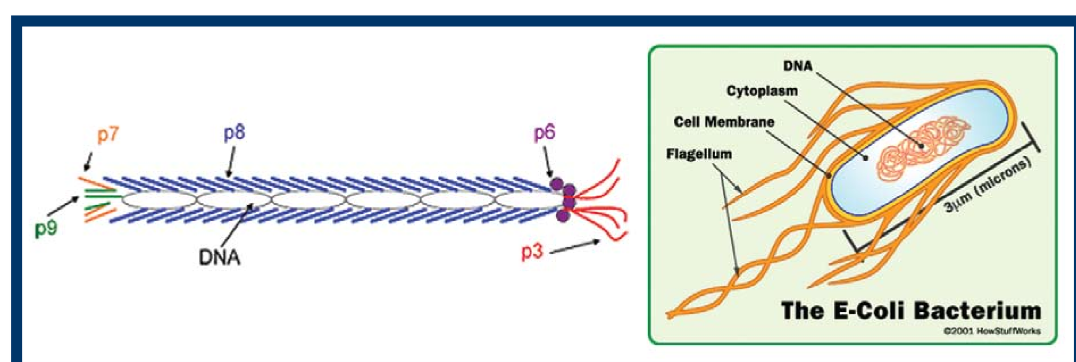

Figure 1: Schematic representations of M13 bacteriophage and its host E.coli

While some viruses are useful and used in therapy, others can cause disastrous diseases and hence tremendous efforts have been made to eliminate them. Most antiviral and antibacterial treatments are only partially successful and may evoke problems of drug resistance and unwanted side effects.

An effective method of deactivating these viruses while leaving the sensitive material such as the host cell unharmed, have been demonstrated by Tsen *et al.* [1, 2]. In these studies, the weak bonds on the protein shells of the viral particles were targeted. By tuning to the appropriate laser power density, the feasibility of damaging the protein shells of the viral particles hence leading to inactivation, without harming the host cells was demonstrated. Virus deactivation was monitored by plaque count method.

In this work, we report preliminary experimental results in a proof of principle comparative experiment using the M13 bacteriophage in E. coli host cells. We will focus on the deactivation intensity threshold and compare this to the damage threshold for the host cell. We envisage that such a study will ultimately lead to a better understanding of the mechanism of virus deactivation using femtosecond laser pulses, which is currently still not understood. This understanding of the physical phenomenon will be useful for practical application development in a wide variety of possible scenarios, and is not limited to specific viruses or host cells.

EXPERIMENTAL PROCEDURE

Samples and Assays for M13 bacteriophage

To determine the infectivity and the titre of the bacteriophage M13, we diluted the phage with LB medium (10 g tryptone, 5 g yeast extract, 5 g NaCl). Serial dilutions were made to choose the optimal phage concentration to use for infection with E.coli to obtain countable plaques. A total of 0.1 ml of these different phage concentrations were added to 0.3 ml of E.coli JM101 strain (overnight culture). This solution was then added to top agar (2.5 ml) and poured evenly onto plates with solid media (10 g tryptone, 5 g yeast extract, 5 g NaCl and 7 g agar). The plates were incubated at 37 °C overnight and plaques were counted on the next day.

LASER SET-UP

A ClarkMXR model 2110i regeneratively amplified femtosecond laser produces pulses of 150 fs at a repetition rate of 1 kHz, at a wavelength of 772 nm. The pulse energy can be adjusted by an internal rotating linear polarizer plate, from a maximum of 1 mJ down to approximately 30 uJ per pulse, with additional filters which can be used to attenuate further. In this preliminary experiment, we used 120 µJ per pulse, without any focusing, clipping the beam with an aperture slightly to fit the size of the 96-well plate which is 6.4 mm in diameter (96 well plate is a sample holder that is placed on a controllable x, y stage). Transmitted power was measured on a power meter as 120 mW, corresponding to 120 µJ energy per pulse, without any sample in the well plates.

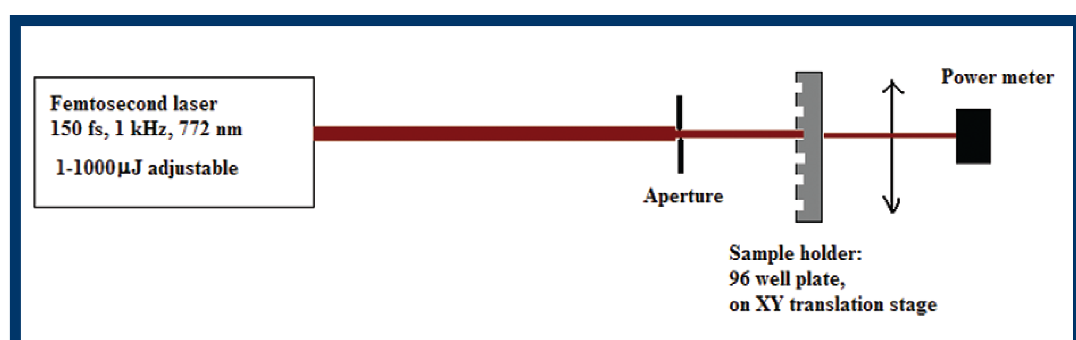

Figure 2: Schematic representation of the laser set up for the irradiation experiments

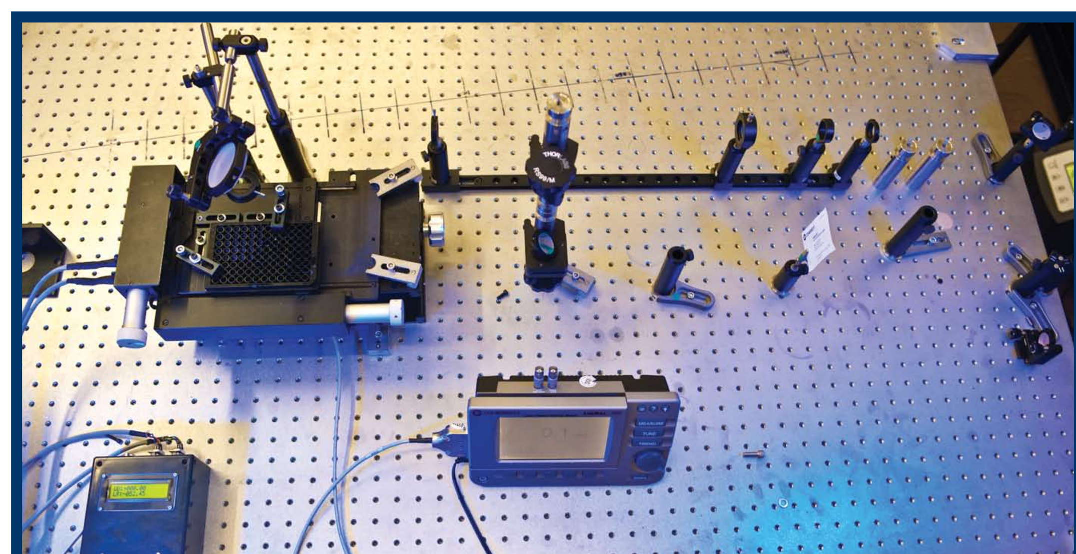

Figure 3 : Actual laser set-up for the irradiation experiments

RESULTS AND DISCUSSION

The bacteriophage titre was found to be 3×10^{12} pfu/ml (see **Figure 4** below). **Figure 5** shows the number of plaques of typical assays without laser irradiation (control). It is expected that the number of plaques should decrease after laser irradiation. In a sample containing 1×10^{3} pfu/ml, ~ 999 were counted in a control and only 3 were seen after laser irradiation (Tsen *et al.*, 2009) indicating the very efficient deactivation of M13 by ultrashort pulsed laser irradiation.

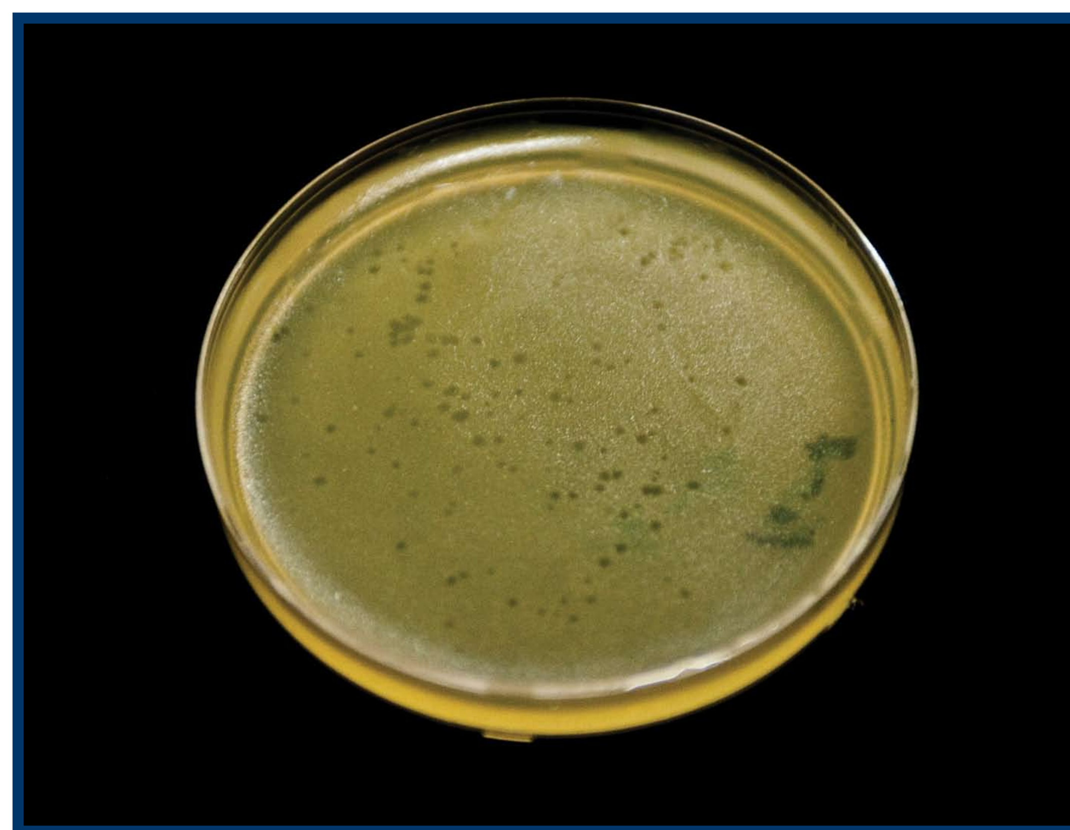

Figure 4: Plaque assay to determine the bacteriophage titre

Figure 5: Showing a typical plaque assay

We are still at a stage of growing bacteria and infecting it with the virus to get countable plaques, but we should be irradiating soon and the process of trying to understand the deactivation mechanism will then follow.

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CSIR scientists are looking into use of femtosecond lasers to selectively deactivate certain viruses.

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