

Ultrafast-Laser Interactions with Soft Biological Tissues – a Study with Viable 3-D Hydrogel Cell Cultures

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Abstract: We've developed a 3-dimensional hydrogel cell culture to investigate the effects of ultrafast laser pulses on soft biological tissues. We characterize the physical and histological impact of intense laser irradiation at >100MHz repetition rates.

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1. Introduction

Recent advances in ultrafast laser technology, such as the scaling of pulse repetition rate (> 10 MHz), have made accessible a new regime where the pulse period is comparable to the lifetime of the plasma induced [1]. This regime is especially interesting for medical and surgical applications because it allows one to simultaneously benefit from the precision of ultrafast ablation while producing new control over heat gradients, over longer spatial scales relevant to histological responses in tissues. However, basic investigation of laser pulse interaction with a viable real tissue is complicated by their inhomogeneities and tissue-type differentiation. As a simpler proxy for *in vivo* studies, we have developed an agar-based hydrogel as a 3D tissue model with a consistency comparable to soft tissue and which can be used to perform histological studies on cells following laser irradiation.

2. The hydrogel cell culture

Hydrogels cell cultures are a simple, optically-homogenous model for soft biotissues that facilitate the study of cell response to drug and radiation treatments in an more realistic than flat plated-cell cultures. Since they can be seeded with a wide variety of animal cells, agar-based hydrogels are of particular interest for photodynamic therapy (PDT) drug development as well as for radiotherapy and laser surgery studies. In our case, we selected F98 rat glioma cells to seed our hydrogel model, due to their availability and rapid growth.

Monolayers of the F98 cells were cultured in a flask and incubated at 37° C with 5% CO₂. A solution of DMEM-H21 (GIBCO) supplemented with fetal bovine serum (FBS) and antibiotics was used as the culturing media. The cells were passaged every 48 to 72 hour period, when the confluency in the culturing flask reaches approximately 80%. A viable hydrogel was prepared by mixing a solution of the F98 cells Alpha MEM Media solution (60%) with an autoclaved agar-water solution at 55° C and the mixture was left to solidify at room temperature. The percentage of agar content was carefully chosen so that the consistency of the solidified gel was sufficiently high to maintain physical integrity without compromising the viability of the cells in the gel, both necessary for carrying out the various steps of the experiment, from laser irradiation to confocal microscopy. A number of histological staining protocols were tested to be compatible with our hydrogel model, including a Hoechst 33342 protocol to detect all cells in the gel, a Propidium Iodide protocol to detect necrotic cells, and an Annexin-V protocol to detect cells undergoing apoptosis. The hydrogel viability was confirmed to be >90% live cells, even 24 hours after the gel preparation when incubated. The detailed protocols for gel preparation and staining are described in Ref. [2].

3. Laser-biotissue interaction at >100MHz repetition rates

The experiments were carried out using a custom-designed Nd:glass laser system ($\lambda=1 \mu\text{m}$) producing a pulsetrain burst of ~1ps pulses at 133 MHz with per-pulse energy up to 30 μJ after amplification. The duration of the pulsetrain burst can be varied using a Pockels cell from 100 ns to ~30 μs . The laser pulses were tightly focused onto

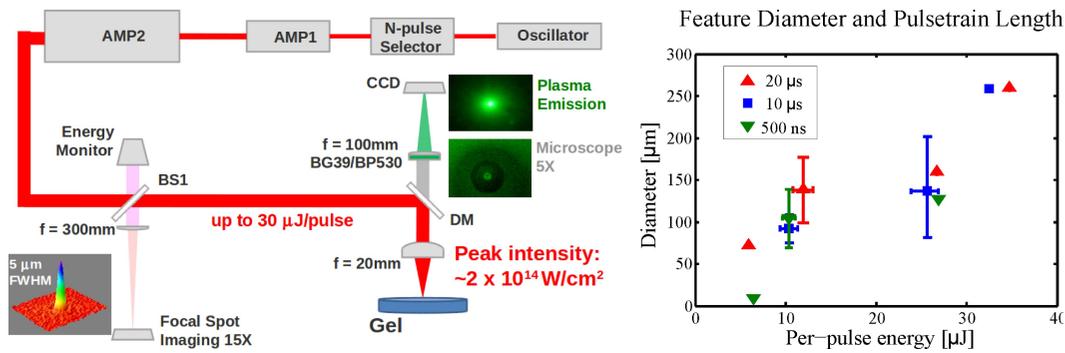


Figure 1: (Left) Simplified diagram of the laser system and experimental set-up. (Right) Scaling of the laser-produced crater with the laser pulse energy.

F-98-embedded hydrogels using an $f=20\text{mm}$ lens producing a focal spot of $5 \mu\text{m}$ FWHM (Fig. 1, left). At maximum per-pulse energies, peak intensities exceeding $2 \times 10^{14} \text{ W/cm}^2$ were attained. Beam splitters and a dichroic mirror sampled the laser beam at various locations to monitor the incident energy, the focal spot on the gel surface, as well as the visible light emitted from the laser-produced plasma during the interaction (Fig.1, left).

We studied the scaling of the laser-induced crater volume as well as the necrosis range for various laser parameters such as the per-pulse energy and pulsetrain length. We observed that the diameter of the laser-produced crater scaled weakly with the pulsetrain length (Fig. 1, right), for any given per-pulse energy, suggesting that only the leading pulses in the pulsetrain contribute to ablation. On the other hand, the crater diameter (and volume) scaled linearly with the per-pulse energy as can be seen in Fig. 1, right. Viable-cell hydrogels irradiated under similar conditions were stained by fluorescent dyes and imaged with a confocal microscope. The reconstructed 3D confocal images (Fig. 2, left) show the distribution of viable and necrotic cells relative to the laser focus, determined as the center of the damage crater. While the laser spot was $5 \mu\text{m}$ FWHM, the diameter of the necrosis zone extended to a diameter of $\sim 200 \mu\text{m}$ for a per-pulse intensity of 10^{14} W/cm^2 (Fig 2.right).

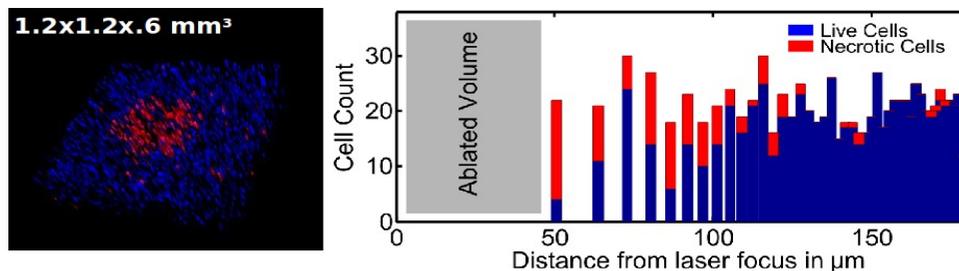


Figure 2: (Left) 3-D reconstruction of confocal images showing live (blue) and necrotic (red) cells after laser irradiation at $\sim 10^{14} \text{ W/cm}^2$. (Right) Histogram of live (blue) and necrotic (red) cell distance from the laser focus point.

4. Conclusion

We have developed a viable 3D hydrogel cell culture to be used as a model to study the interaction of intense ultrafast laser pulses with biological tissues. The hydrogel is compatible with a set of histological assays that allow for detection of cell necrosis and apoptosis. We have used the hydrogel to investigate the physical and histological impact of high-repetition-rate ultrafast pulses on biotissues at intensities relevant for laser surgery. Detailed results will be presented.

5. References

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