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Abstract. We study the effects of the interaction of 40-fs Ti-sapphire laser radiation at 800 nm with biological materials—proteins or intact *Bacillus* spore, dissolved or suspended in pure water, respectively. The estimated laser intensity at the target is 10^{13} W/cm². On the molecular level, oxidation of solvent-accessible parts of proteins has been observed even after a single femtosecond laser pulse, as demonstrated by mass spectrometry. A remarkable morphological effect of the femtosecond laser radiation is the complete disintegration of extremely refractive cells such as bacterial spores, evidenced in scanning electron micrographs. After 500 laser pulses, all suspended spores in the irradiated volume are completely destroyed, which makes them nonviable. Characteristic spore biomolecules, e.g., small acid-soluble spore proteins, are extensively oxidized after several laser pulses. In comparative studies, no effects have been observed when irradiating the same samples with 10-ns laser pulses at the same laser wavelength and fluence. We demonstrate that the laser power density (irradiance), resulting in different amounts of total deposited energy, determines the types of effects for femtosecond laser interactions with biological matter. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: [10.1117/1.OE.53.5.051510](https://doi.org/10.1117/1.OE.53.5.051510)]

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1 Introduction

Studies of the interactions of femtosecond laser radiation with dielectrics, including biological materials, encompass macroscopic effects (e.g., laser ablation^{1–3}), and molecular-level phenomena (such as femtochemistry and fast spectroscopy to elucidate both molecular structure and dynamics⁴). It is well established that femtosecond-laser interactions in condensed matter differ markedly from nanosecond laser interactions.^{1,5} It has been established that intense femtosecond-laser pulses can efficiently ionize polyatomic systems, such as benzene and other polyaromatic molecules, with negligible fragmentation compared to nanosecond-laser pulses.⁶ A number of mass spectrometry (MS) studies demonstrate efficient desorption of large intact biomolecules in the gas phase by femtosecond-laser pulses.^{7,8} Recently, femtosecond UV laser pulses have been shown to induce covalent intramolecular and intermolecular cross-linking that may be useful for structural studies of, e.g., protein-nucleic acid complexes.⁹ Large-scale molecular dynamic simulations have been employed to model material ejection processes under femtosecond laser irradiation.¹⁰ The applications of femtosecond lasers are expanding considerably and range from precise material processing (microfabrication) to biology and medicine.¹¹ For example, femtosecond laser pulses have been used to perforate the membranes of intact mammalian cells, allowing their efficient transfection with DNA, without perturbing the cell structure and function.¹² A comprehensive study on transfection efficiency and cell viability of individual Chinese hamster ovary cells in photoporation by 800-nm femtosecond

Ti-sapphire laser has been reported.¹³ Femtosecond lasers have been developed for clinical applications in dental or eye surgery with submicron resolution.¹⁴ A claim has been made that human immunodeficiency virus is selectively inactivated by sub-picosecond near-infrared laser pulses at moderate laser power densities that do not affect larger cells, such as human red blood cells and mouse dendritic cells.¹⁵ Interestingly, an independent study of the interactions of 90-fs laser pulses (850 or 425 nm) with buffer/water, DNA, protein, M13 bacteriophage, or *Escherichia coli* could not confirm these observations.¹⁶ Very recently, the application of intense ultra-short infrared laser pulses for cancer radiotherapy has been discussed.¹⁷ With this technique, a large energy density can be deposited at unprecedented dose rates precisely in a microscopic volume without exposing the adjacent tissue. As a result of laser filamentation, the deposited energy both spatially and temporally exceeds, by orders of magnitude, parameters characteristic of any current clinical radiotherapy system.

In order to further understand the mechanisms of femtosecond-laser biomaterial interactions in aqueous media, we irradiated proteins or intact *Bacillus* spore in pure water with 40-fs Ti-sapphire laser pulses at 800-nm wavelength. The estimated laser intensity at the target is 10^{13} W/cm². We utilized the MS to interrogate molecular level effects—modification of individual proteins or characteristic cellular biomarkers—induced by the femtosecond laser. Oxidation of solvent-accessible parts of the molecules is observed even after a single femtosecond-laser pulse. A remarkable morphological effect of the femtosecond laser irradiation in water is the complete degradation of extremely refractive cells, such as bacterial spores, evidenced in scanning electron micrographs. After 500 laser pulses, all suspended spores in

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the irradiated volume are completely destroyed, which obviously renders them nonviable. Characteristic spore biomolecules, e.g., small acid-soluble spore proteins (SASP), are extensively oxidized after several laser pulses, as monitored by the MS. In comparative studies, no effects have been observed when irradiating the same samples with 10-ns laser pulses at the same laser wavelength and fluence. The capability to rapidly and covalently label solvent-exposed sites of biomolecules in water without additional reagents by femtosecond laser irradiation can be utilized in structural biology for biomolecular foot-printing.¹⁸

2 Methods

2.1 Femtosecond Laser Set-Up

A 40-mJ Ti-sapphire laser (Coherent Inc., Santa Clara, California) at 800-nm wavelength was used to irradiate the liquid samples at two different pulse widths [full-width half-maximum (FWHM)]: ~40 or 10 ns. The laser gain medium is Ti-doped sapphire. The femtosecond laser source is a Kerr lens mode-locked oscillator (Mantis, Coherent Inc., Santa Clara, California)¹⁹ that seeds a chirped-pulse amplification system²⁰ composed of a grating stretcher, regenerative amplifier, multi-pass power amplifier, and grating compressor (Hydra, Coherent Inc., Santa Clara, California). The FWHM duration of the measured single-shot autocorrelation signal is 56 fs, corresponding to the time duration of 39 fs for an ideal Gaussian temporal profile (Fig. 1). From the extended “wings” of the autocorrelation signal, it is evident that the typical pulse does not have a truly Gaussian shape (due to, e.g., spectral broadening and high-order dispersion in optics such as the $f = 1$ m lens). Blocking the oscillator and allowing the regenerative amplifier to Q-switch resulted in 10-ns pulses at the same wavelength. Variable doses—from a single laser pulse up to a 1000 pulses—were delivered to a cuvette at 1- to 10-Hz rep rate. The estimated laser fluence for both the femtosecond (short) and nanosecond (long) pulse was 0.5 J/cm^2 , as determined by patterns (3.5-mm-beam diameter) on laser burn paper and a pyroelectric energy meter (Coherent LabMax, Santa Clara, California). The laser pulse was weakly focused with a 1-m focal length lens into the sample irradiation chamber. At the entrance to the chamber, the femtosecond and nanosecond laser irradiance are 10 TW/cm^2 and 40 MW/cm^2 , respectively. Approximately

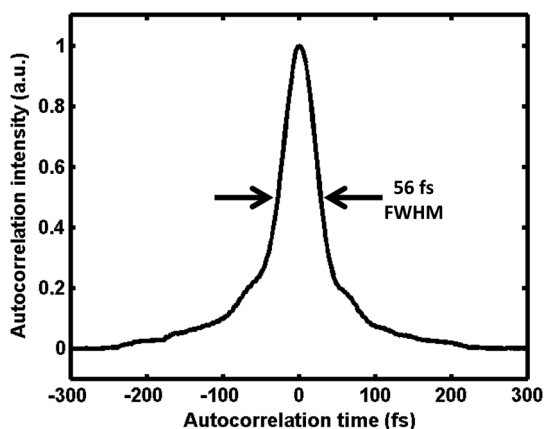


Fig. 1 Single-shot femtosecond laser pulse shape.

1% or 35% of the overall nanosecond or femtosecond laser pulse energy, respectively, was absorbed by water for the specified sample (10-mm optical length), as determined by energy meter measurements. This indicates a nonlinear absorption mechanism for the femtosecond laser pulse (*vide infra*).

2.2 Sample Preparation and Irradiation

All chemicals were commercially available, and were obtained from Sigma Aldrich, St. Louis, Missouri. All solvents were purchased from Thermo Fisher Scientific, Waltham, Massachusetts, at LC-MS grade or higher. Bovine ubiquitin samples were dissolved in water at $20\text{-}\mu\text{M}$ concentrations. *Bacillus globigii* (*B. globigii*, also labeled *Bacillus atrophaeus*) spores were obtained from the US Army Laboratories (Dugway Proving Ground, Utah) and dissolved in plasma grade water at a concentration of 1 mg/mL. The matrix-assisted laser desorption/ionization (MALDI) matrix, α -cyano-4-hydroxycinnamic acid, CHCA, (Fluka Analytical), was prepared at 10 mg/mL in acetonitrile/0.1% trifluoroacetic acid (TFA) (70/30, v/v).

Typically, $55 \mu\text{L}$ of dissolved protein or suspended spore samples were transferred into a narrow, optically transparent quartz cuvette with internal dimensions of $2 \text{ mm(W)} \times 10 \text{ mm(L)} \times 4 \text{ cm(H)}$ (International Crystal Laboratories, Garfield, New Jersey). The filled cuvettes were placed in the collimated beam (typical cross-section beam diameter 2 mm) for irradiation. These parameters ensure that the entire sample volume is irradiated with a single laser shot. The spore suspension was manually shaken after every 100 shots to avoid spore sedimentation. Irradiated aliquots were taken at different exposure times for subsequent MS analysis. A schematic of the irradiation set-up is given in Fig. 2.

2.3 Sample Analysis

Irradiated and nonirradiated (control) ubiquitin samples were diluted in 50:50 (methanol:water with 2% acetic acid) and loaded in nanospray emitters (New Objective, Woburn, Massachusetts). The samples were electrosprayed in positive ion mode and analyzed by high resolution MS and MS/MS on an LTQ-Orbitrap Velos (Thermo Fisher Scientific, Waltham, Massachusetts). Tandem MS and standard bioinformatics software were employed to characterize the modification site of the oxidized protein.

Positive ion MALDI mass spectra were acquired on an Autoflex tandem time-of-flight (TOF/TOF) instrument (Bruker Daltonics, Billerica, Massachusetts). Ions were desorbed by a N_2 laser (337 nm). Standard accelerating voltage (20 kV) and delayed extraction (150 ns) conditions were

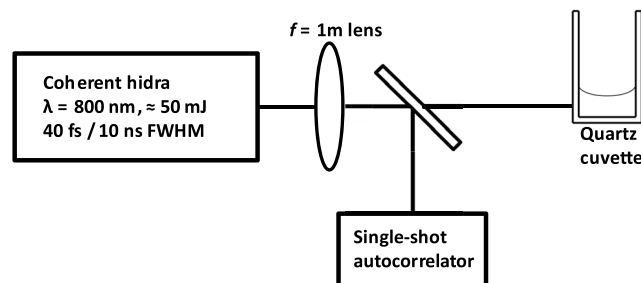


Fig. 2 Experimental laser irradiation set-up.

used in positive ion linear mode.²¹ The mass spectrometer was calibrated using peptide and protein standards (average mass error in linear TOF mode <3 Da across the mass/charge range below 10,000). For MALDI MS analysis, 0.5 μL of the protein solution was pipetted onto the MALDI stainless steel target, followed by an equal volume of CHCA. Prior to matrix addition, *B. globigii* spores were treated on the target with 0.5 μL of 10% TFA.²² The laser beam was manually rastered across the entire sample (3-mm diameter). For each spectrum, several hundred individual traces from single laser shots were accumulated, averaged and processed using the software provided with the instrument.

For scanning electron microscopy (SEM), both laser-irradiated and control spore suspensions were deposited on microscope grids and allowed to dry before analyzing them on an Hitachi S4700 scanning electron microscope (Hitachi High Technologies America, Dallas, Texas).

3 Results & Discussion

3.1 Intact Protein Irradiation

It is well established that femtosecond laser excitation of dielectric materials, such as proteins and other biologically related species, differs markedly from nanosecond laser excitation.²³ Recently, we reported on a novel effect in the interactions of femtosecond lasers with biomolecules in water—oxidation of solvent-accessible sites (e.g., amino acids in proteins).²⁴ This is an entirely femtosecond laser-induced oxidation/reduction chemistry effect that is not observed

upon nanosecond laser irradiation of the same sample at the same wavelength and energy density (Fig. 3). For example, upon femtosecond laser irradiation of ubiquitin (a small 76 amino acid protein with an average molecular weight of 8565 Da) regular peaks uniformly spaced 16 Da above the intact molecular ion peak are observed in its mass spectrum. Up to 14 oxidized sites per ubiquitin molecule are clearly discerned from these characteristic mass shifts based on high resolution MS data (Fig. 3). Oxidation occurs even upon irradiation with a single femtosecond laser pulse. Under our experimental conditions, intact ubiquitin is >60% oxidized after 100 laser pulses. The femtosecond laser–water interaction is akin to the interactions of ionizing radiation with water. As a result of gamma-irradiation²⁵ or electron-irradiation²⁶ of solutions, hydroxyl radical species are formed and subsequently interact with solvent-exposed groups of biomolecules. Similarly, femtosecond laser interactions with water induce the formation of hydroxyl radicals and solvated electrons. Although the photon energy of the femtosecond laser is 1.55 eV, compared to the 6.5-eV ionization energy of a water molecule in liquid,²⁷ the incident laser intensity of 10 TW/cm² is sufficient to enable 5-photon ionization of the water. For this laser intensity, multiphoton ionization has been found to be the dominant free-electron generation mechanism, over tunneling ionization and strong-field ionization (transition to tunneling ionization occurs at slightly higher irradiances of 13 to 26 TW/cm²).²⁸ Subsequent fast (sub-microsecond time scale) secondary reactions with diffusion-limited rates result in accessible side chains of the proteins that are oxidized or reduced. Tandem MS has been utilized to discern the modified amino acid(s) for singly oxidized ubiquitin.²⁴ For a singly oxidized ubiquitin molecules, it is clear that oxidation occurs only on the *N*-terminal amino acid Methionine (Met). This result is in agreement with the reported much higher Met reactivity towards hydroxyl free radicals compared to other amino acids¹⁸ as well as the free and water-exposed *N*-terminal of the molecules. However, for doubly and more highly oxidized ubiquitin, we cannot rule out oxidation sites that are variable across the chain. A very promising application of this femtosecond laser technique for hydroxyl radical generation is for structural biology “foot-printing” of exposed amino acid side chains.¹⁸ Till now, foot-printing has typically required complex and orders of magnitude more expensive equipment than lasers (e.g., synchrotrons¹⁸), or the presence in the solution of non-native reagents (e.g., hydrogen peroxide²⁹) that could perturb the native biomolecular structure.

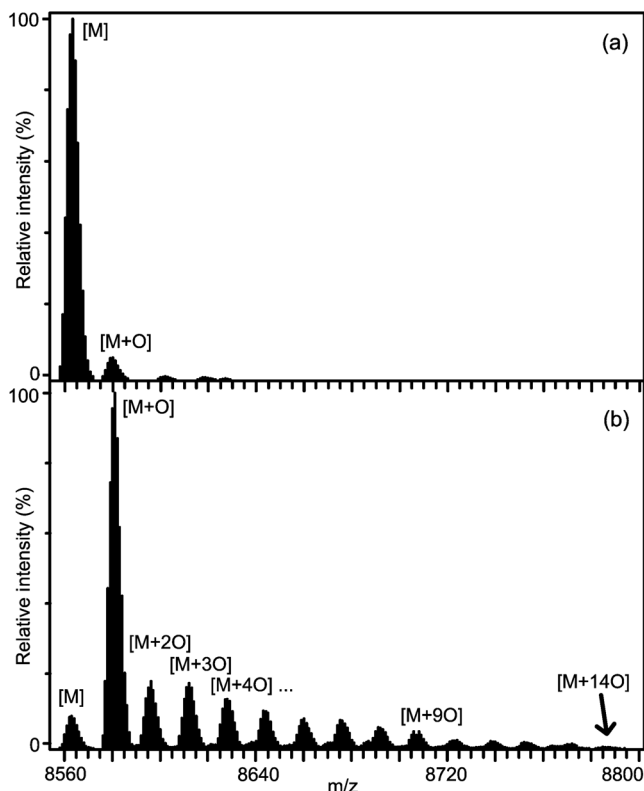


Fig. 3 Comparison of electrospray ionization mass spectra of ubiquitin after irradiation with: (a) 100 shots with the nanosecond laser, and (b) 100 shots with the femtosecond laser. Oxidized ubiquitin with up to 14 oxidation sites is observed after femtosecond laser irradiation.

3.2 Intact Bacillus Spore Irradiation

Bacterial spores, formed when microorganisms encounter nutrient shortages or are exposed to other adverse factors, are some of the most refractory life forms. *B. globigii* (*B. atrophaeus*) spores have been used for decades as a simulant for the biowarfare agent *Bacillus anthracis*.³⁰ Electron micrographs of *B. globigii* spores, obtained before and after exposure of the spore suspension to femtosecond laser irradiation, are shown in Fig. 4. Clearly, the spores have completely disintegrated after irradiation at the current experimental conditions. This effect is in stark contrast to other types of energy deposition to intact spores, e.g., prolonged spore exposure to high voltage high frequency

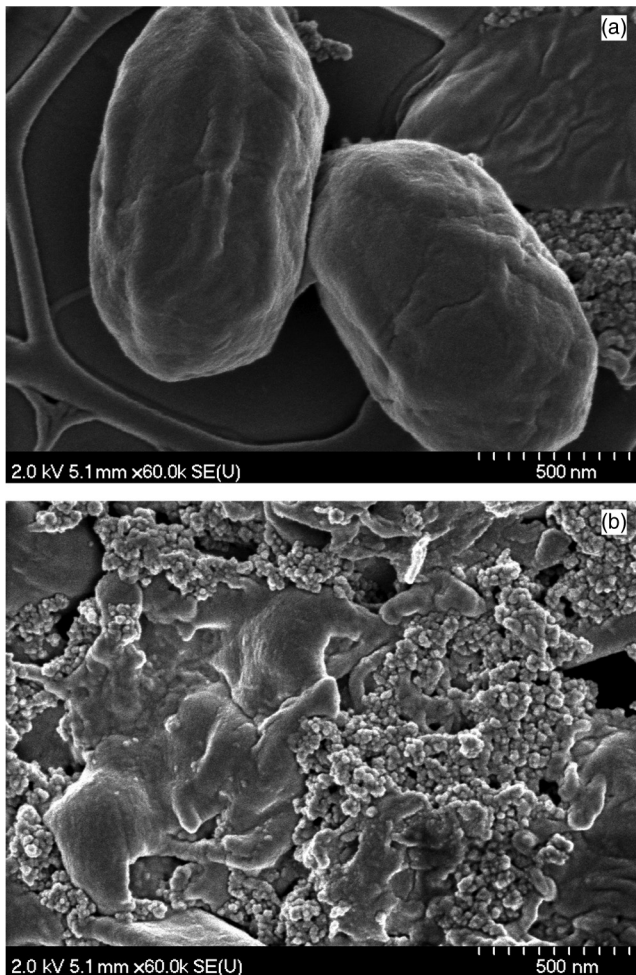


Fig. 4 *Bacillus globigii* spore micrographs (magnification 50,000 \times , typical spore length is 1 μm): (a) control and (b) after 500 laser shots with the femtosecond laser.

atmospheric plasma.³¹ During ultrafast excitation of condensed matter with the femtosecond laser pulse, the irradiated volume cannot transfer significant amounts of the absorbed energy to the surrounding material since phonon relaxation times in condensed matter are ~ 100 fs. Consequently, during the excitation process, electrons absorb the laser pulse energy and release it to the surrounding material at rates characteristic of the material (related to the electron-phonon coupling constant). During these early stages of excitation, various material effects occur. A “direct hit” of the micrometer scale length spores and subsequent phase explosion⁵ may lead to their complete disintegration. We speculate that microscale mechanical shock waves² in water may also cause complete spore disintegration and ultimately their inactivation. Observation of macroscopic bubbling in bacterial suspensions after 10- to 100-fs laser shots lends credence to this notion. Similar bubbling is not observed under our experimental conditions in the fully aqueous protein samples. Macroscopic bubble formation is likely due to spore suspension components that have stronger optical absorption bands and lower ionization thresholds compared to that of pure water.²⁸ For example, a decreased ionization threshold leads to more rapidly ionized plasma that can seed further collisional ionization.²

The dense plasma electrons transfer energy to the heavier ions, resulting in thermoelastic stress-induced shock waves and bubble formation.²⁸

We have examined by MALDI TOF MS the biomarker signatures, characteristic of *B. globigii* spores before and after irradiation—Fig. 5. The predominant species, detected in the MALDI MS of intact spores are the SASP that comprise about 20% of the dry spore weight.²¹ For *B. globigii* spores, the three major SASP have average molecular weights of 7068 Da (SASP1); 7333 Da (SASP2), 8890 Da (γ -SASP).^{21,22} As a result of femtosecond laser irradiation, the SASP2 is quickly oxidized, evidenced by the appearance of satellite peaks 16 Da above the unmodified peak. The degree of oxidation correlates with the number of laser pulses. This effect is similar to the SASP oxidation, observed upon rapid on-target oxidation after spore treatment with 30% hydrogen peroxide solution.²² In this latter experiment, predominantly the Met residues found in the SASPs are oxidized. The masses of Met-containing SASPs increase by $N \times 16$ Da, where N is the number of Met residues per protein. In parallel to this on-target H_2O_2 oxidation, the SASP2 containing two Met is mostly oxidized after femtosecond laser irradiation as well. In contrast, the γ -SASP, which does not contain any Met residues, remains unchanged. These observations suggest that a chemical oxidation reaction takes place as a result of hydroxyl radical formed by the femtosecond laser pulse, similar to what is observed during irradiation of individual proteins (vide supra). Presumably, the mechanical spore destruction releases intact SASP, which are subsequently oxidized upon femtosecond laser irradiation. No oxidation or spore disruption is observed even after irradiation with 1000 pulses by a 10-ns laser at the same wavelength and fluence.

In a recent study on the interactions of ultra-short pulses (90 fs at 800 nm) with biological molecules and cells, no effects have been observed.¹⁶ In those experiments, the irradiation times are on the order of 1 h, while the individual laser pulse energy is only on the order of 1 nJ (100 MW/cm² on target power density). The integrated incident laser energy for that experiment is ~ 288 J/mL (2-mL total irradiated volume), which is almost 600 times higher than the total dose in our experiment (0.5-J/mL, 50- μL sample volume). We point out that the laser power density for our experiment is five orders of magnitude higher, which indicates that the power density (peak power) rather than total incident energy determines the types of effects for femtosecond laser interactions with biological matter. Thus, effects of strand breaks and uncoiling of DNA plasmids in aqueous media are observed upon irradiation with an 820 nm, 45-fs pulse laser at power densities up to 12 TW/cm².³² Similarly, almost instant skin lesions have been observed in Yucatan mini-pigs irradiated with 810 nm, 44-fs terawatt laser pulses (8.2-mJ total deposited energy for 12-mm spot size).³³

4 Conclusion

Femtosecond laser radiation oxidizes proteins and other cell components in water efficiently without addition of non-native solvents and reagents (e.g., hydrogen peroxide). This is a result of femtosecond laser-induced hydroxyl radical generation in water and subsequent biomolecule-radical oxidation reactions. In comparative studies, no effects have been observed when irradiating the same samples with

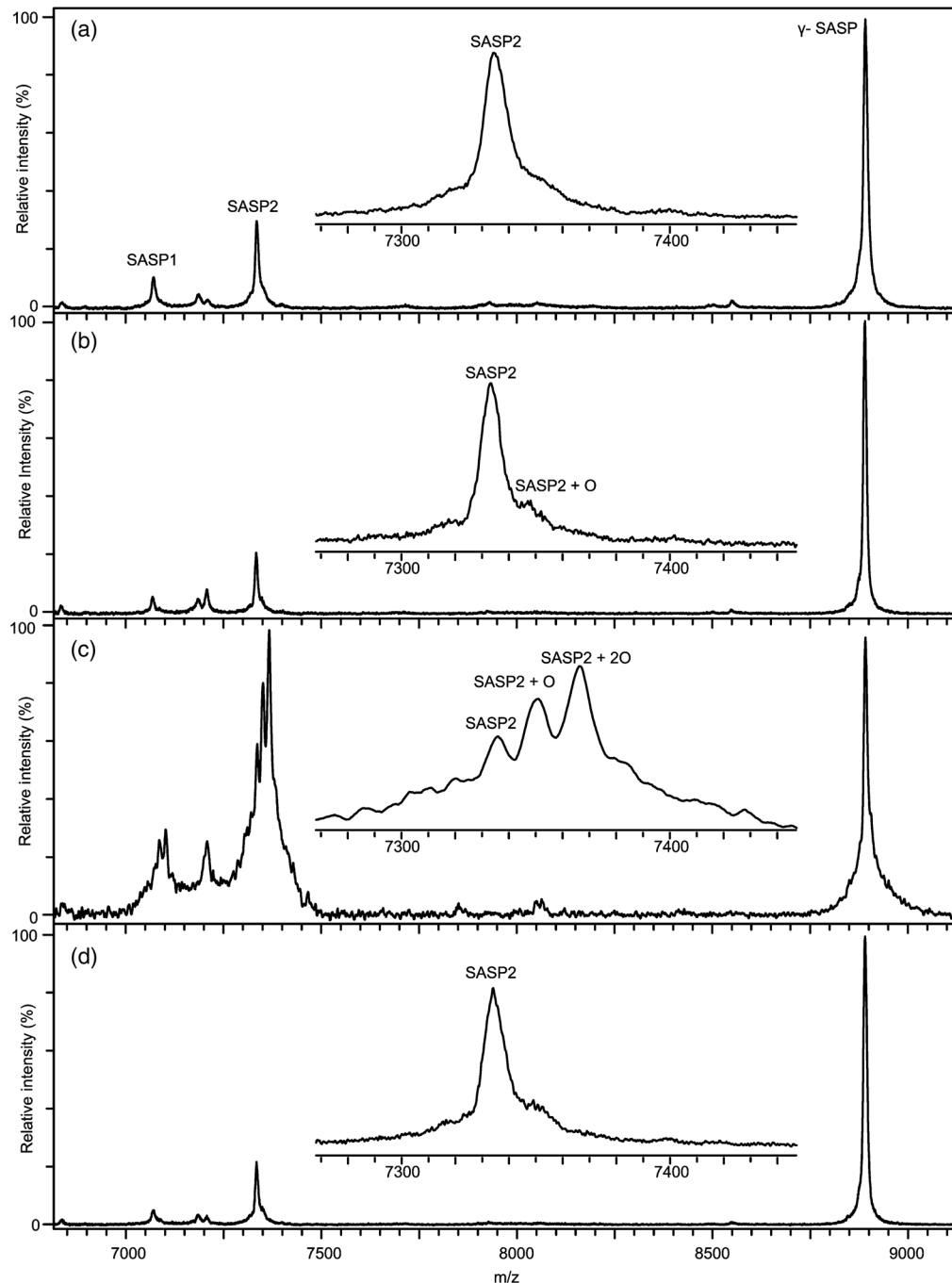


Fig. 5 Comparison of positive ion matrix-assisted laser desorption/ionization mass spectra of *B. globigii* in the small acid-soluble spore protein mass range; (a) control, (b) after 10 shots with the femtosecond laser, (c) after 500 shots with the femtosecond laser, and (d) after 500 shots with the nanosecond laser.

10-ns laser pulses at the same laser wavelength and fluence. We demonstrate that the power density (peak power) rather than total incident energy determines the types of effects for femtosecond laser interactions with biological matter. The observed effect of hydroxyl radical generation has potential applications in structural biology for biomolecular footprinting. We point out that femtosecond lasers may become a highly efficient, less complex, and relatively inexpensive alternative to currently used synchrotron radiation facilities for structural elucidation of biomolecules. Femtosecond laser irradiation of extremely refractive bacterial spores

results in their complete disintegration, as evidenced in scanning electron micrographs. The details of the excitation and disruption processes are not well understood. Furthermore, molecular level effects during spore irradiation with a femtosecond laser are also observed—as a result of the irradiation, characteristic spore biomarker molecules are oxidized. The degree of oxidation is correlated to the number of femtosecond laser pulses. We are continuing work to study effects of femtosecond laser irradiation on other types of biomolecules, such as phospholipids and DNA, as well as microorganisms such as intact viruses and vegetative bacterial cells.

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References

- B. Chichkov et al., "Femtosecond, picosecond and nanosecond laser ablation of solids," *Appl. Phys. A* **63**(2), 109–115 (1996).
- E. G. Gamaly, "The physics of ultra-short laser interaction with solids at non-relativistic intensities," *Phys. Rep.* **508**(4–5), 91–243 (2011).
- P. Balling and J. Schou, "Femtosecond laser ablation dynamics of dielectrics: vases and applications for thin films," *Rep. Prog. Phys.* **76**(3), 036502 (2013).
- A. Zewail, "Femtochemistry: atomic-scale dynamics of the chemical bond," *J. Phys. Chem. A* **104**(24), 5660–5694 (2000).
- P. Lorazo, L. J. Lewis, and M. Meunier, "Short-pulse laser ablation of solids: from phase explosion to fragmentation," *Phys. Rev. Lett.* **91**(22), 225502 (2003).
- M. DeWitt and R. Levis, "Concerning the ionization of large polyatomic molecules with intense ultrafast lasers," *J. Chem. Phys.* **110**(23), 11368–11375 (1999).
- P. Demirev et al., "Matrix-assisted laser desorption with ultra-short laser pulses," *Rapid Commun. Mass Spectrom.* **6**(3), 187–191 (1992).
- J. J. Brady, E. J. Judge, and R. J. Levis, "Non-resonant femtosecond laser vaporization of aqueous protein preserves folded structure," *Proc. Natl. Acad. Sci. U. S. A.* **108**(30), 12217–12222 (2011).
- G. Leo et al., "Ultraviolet laser-induced cross-linking in peptides," *Rapid Commun. Mass Spectrom.* **27**(14), 1660–1668 (2013).
- L. V. Zhigilei, Z. B. Lin, and D. S. Ivanov, "Atomistic modeling of short pulse laser ablation of metals: connections between melting, spallation, and phase explosion," *J. Phys. Chem.* **113**(27), 11892–11906 (2009).
- W. Sibbett, A. A. Lagatsky, and C. T. A. Brown, "The development and application of femtosecond laser systems," *Opt. Express* **20**(7), 6989–7001 (2012).
- U. Tirlapur and K. König, "Targeted transfection by fs laser," *Nature* **418**(6895), 290–291 (2002).
- D. Stevenson et al., "Femtosecond optical transfection of cells: viability and efficiency," *Opt. Express* **14**(16), 7125–7133 (2006).
- S. H. Chung and E. Mazur, "Surgical applications of femtosecond lasers," *J. Biophot.* **2**(10), 557–572 (2009).
- K. T. Tsen et al., "Selective inactivation of human immunodeficiency virus with subpicosecond near-infrared laser pulses," *J. Phys.-Cond. Matter* **20**(25), 252205 (2008).
- J. C. Wigle et al., "No effect of femtosecond laser pulses on DNA, protein, M13, or *E. coli*," *Proc. SPIE* **7897**, 789716 (2011).
- R. Meesat et al., "Cancer radiotherapy based on femtosecond IR laser-beam filamentation yielding ultra-high dose rates and zero entrance dose," *Proc. Natl. Acad. Sci. U. S. A.* **109**(38), E2508–E2513 (2012).
- K. Takamoto and M. Chance, "Radiolytic protein foot-printing with mass spectrometry to probe the structure of macromolecular complexes," *Annu. Rev. Biophys.* **35**, 251–276 (2006).
- D. Spence, P. Kean, and W. Sibbett, "60-fsec pulse generation from a self-mode-locked Ti:sapphire laser," *Opt. Lett.* **16**, 42–44 (1991).
- P. Maine et al., "Generation of ultrahigh peak power pulses by chirped pulse amplification," *IEEE J. Quant. Electron.* **24**, 398–403 (1988).
- P. A. Demirev et al., "Top-down proteomics for rapid identification of intact microorganisms," *Anal. Chem.* **77**(22), 7455–7461 (2005).
- T. J. Cornish et al., "Arrayed time-of-flight mass spectrometry for time-critical detection of hazardous agents," *Anal. Chem.* **77**(13), 3954–3959 (2005).
- A. Vogel et al., "Mechanisms of femtosecond laser nanoprocessing of biological cells and tissues," *J. Phys. Conf. Ser.* **59**, 249–254 (2007).
- S. Varma et al., "Femtosecond laser-induced oxidation of biomolecules for solvent-accessible site mapping," in *Proc. 60th ASMS Conf. Mass Spectrom. Allied Top., TP14/285*, American Society for Mass Spectrometry, Vancouver, Canada (2012).
- X. Tong, J. Wren, and L. Konermann, "Gamma-ray-mediated oxidative labeling for detecting protein conformational changes by electro-spray mass spectrometry," *Anal. Chem.* **80**(6), 2222–2231 (2008).
- C. Watson et al., "Pulsed electron beam water radiolysis for submicrosecond hydroxyl radical protein foot-printing," *Anal. Chem.* **81**(7), 2496–2505 (2009).
- D. N. Nikogosyan, A. A. Oraevsky, and V. I. Rupasov, "Two-photon ionization and dissociation of liquid water by powerful laser UV radiation," *Chem. Phys.* **77**(1), 131–143 (1983).
- A. Vogel et al., "Mechanisms of femtosecond laser nanosurgery of cells and tissues," *Appl. Phys. B* **81**(8), 1015–1047 (2005).
- J. Sharp, J. Becker, and R. Hettich, "Analysis of protein solvent accessible surfaces by photochemical oxidation and mass spectrometry," *Anal. Chem.* **76**(3), 672–683 (2004).
- H. S. Gibbons et al., "Genomic signatures of strain selection and enhancement in *Bacillus atrophaeus* var. *globigii*, a historical bio-warfare simulant," *PLOS One* **6**(3), e17836 (2011).
- Y. Hathout et al., "Identification of *Bacillus* spores by matrix-assisted laser desorption ionization-mass spectrometry," *Appl. Environ. Microbiol.* **65**(10), 4313–4319 (1999).
- J. S. D'Souza et al., "Effect of intense, ultrashort laser pulses on DNA plasmids in their native state: strand breakages induced by *in situ* electrons and radicals," *Phys. Rev. Lett.* **106**(11), 118101 (2011).
- C. P. Cain et al., "Infrared laser damage thresholds for skin at wavelengths from 0.810 to 1.54 microns for femtosecond to microsecond pulse durations," *Proc. SPIE* **6435**, 64350W (2007).

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