

- [1] H. Adachi et al., Jpn. J. Appl. Phys. 42 (2003) L798.
 [2] H. Adachi et al., Jpn. J. Appl. Phys. 43 (2004) L1376.

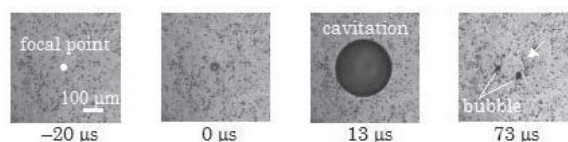


Fig.1 The movement of microbeads by femtosecond laser irradiation

Keywords: protein crystallization, nucleation, laser technology

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Single-molecule visualization on a protein crystal surface

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During elementary growth processes of crystals, such as surface diffusion, adsorption and desorption of molecules at a solution-crystal interface, the behavior of individual molecules that constitute a crystal plays a key role. A single-molecule visualization (SMV) technique allows us to track dynamic behavior of individual molecules. Since SMV requires a fluorescent label attached to a target molecule for visualization, target molecules have to be large enough so that a fluorescent label does not affect their dynamic behavior. Hence, we adopted fluorescent-labeled protein and protein crystals as a model system. We have used hen egg-white lysozyme (HEWL) crystals and fluorescent-labeled HEWL (F-HEWL) [1], and reported the intrinsic picture of diffusion at a solution-crystal interface [2]. In this study, we demonstrate intrinsic pictures of adsorption. First we observed F-HEWL molecules adsorbed on a crystal surface by SMV, and also observed elementary steps in the same field of view by laser confocal microscopy. We found that F-HEWL adsorbed preferentially on steps, showing that F-HEWL molecules behave like solute HEWL molecules, because of very small size of the fluorescent label compared to that of HEWL. Next we tracked the adsorption kinetics, and found that the amount of adsorbed F-HEWL increased after a certain "induction period". This phenomenon clearly indicates that the adsorption proceeds through successive multiple elementary processes. In addition, we also found that F-HEWL molecules that stayed on a crystal surface for longer period adsorbed faster. This result supports the successive adsorption that proceeds gradually on a crystal surface.

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Keywords: single-molecule visualization, adsorption kinetics, protein

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Wavelength dependence of the crystallization by the laser irradiation

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Introduction : Light-induced crystallization have attracted attention as an application of temporal and spatial control of crystallization. We have proposed a new nucleation technique using a femtosecond laser at a wavelength of 780 nm and succeeded in producing high-quality protein crystals [1]. In order to optimize a laser condition, we investigated wavelength dependence of the crystallization by the laser irradiation about nucleation probability. Experiment : We compared the probability of nucleation, when focused femtosecond laser beams were irradiated in protein solutions, such as Lysozyme and Glucose Isomerase, with various laser conditions (wavelength:260 nm, 390 nm, 780 nm, energy: 13.5-94 μJ/pulse). Trials were carried out using a batch method at 23 °C. At the same time we measured the strength of the impulse wave with shock wave sensor, and estimated deformations of solution. Result: In each wavelength, nucleation was promoted by femtosecond laser irradiation with certain energy level. However, nucleation probabilities were almost same in all wavelength of laser irradiated. These energy levels were comparable with threshold values of deformation of solution. Accordingly, nucleation was not dependent on wavelength of laser, but deformations of solution by the laser irradiation. From these results, we conclude that 780 nm laser is suitable for nucleation, because there is little denaturation of the protein by the laser irradiation at a fundamental wavelength of commercial femtosecond laser and there is no absorption to a plastic crystallization plate and a tape for sealing. [1] H. Adachi, et al., Jpn. J. Appl. Phys. 42 (2003) L798.

Keywords: wavelength, laser radiation, nucleation

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Growth of large protein crystals for neutron crystallography by hanging a seed crystal

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