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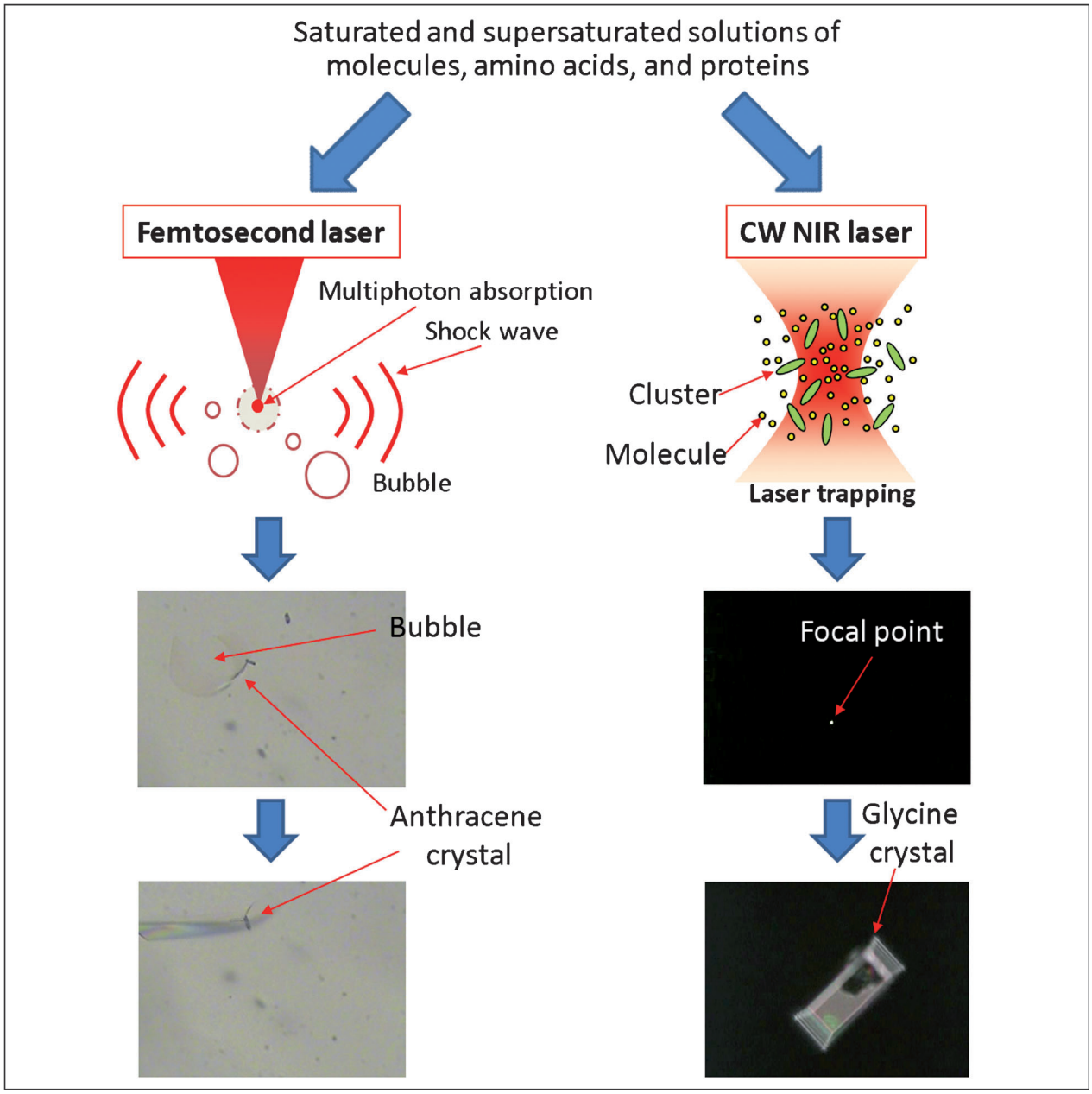
Laser-Induced Crystallization and Crystal Growth

Teruki Sugiyama*[a, b] and Hiroshi Masuhara*[c, d]



Abstract: Recent streams of laser studies on crystallization and crystal growth are summarized and reviewed. Femtosecond multiphoton excitation of solutions leads to their ablation at the focal point, inducing local bubble formation, shockwave propagation, and convection flow. This phenomenon, called "laser micro tsunami" makes it possible to trigger crystallization of molecules and proteins from their supersaturated solutions. Femtosecond laser ablation of a urea crystal in solution triggers the additional growth of a single daughter crystal. Intense continuous wave (CW) near infrared laser irradiation at the air/solution interface of heavy-water amino acid solutions results in trapping of the clusters and evolves to crystallization. A single crystal is always prepared in a spatially and temporally controlled manner, and the crystal polymorph of glycine depends on laser power, polarization, and solution concentration. Upon irradiation at the glass/solution interface, a millimeter-sized droplet is formed, and a single crystal is formed by shifting the irradiation position to the surface. Directional and selective crystal growth is also possible with laser trapping. Finally, characteristics of laser-induced crystallization and crystal growth are summarized.

Keywords: crystallization · crystal growth · nonlinear processes · polymorphism · proteins

1. Introduction

Since the laser was invented in 1960, it has been contributing to the development of modern chemistry—particularly in molecular spectroscopy and photochemistry. Early studies started on isolated molecules and clusters in the gas phase or dilute solution, then shifted to molecular complexes and polymers, and now are being extended to supramolecules, colloids, and molecular solids. The development of utilizing lasers from homogeneous to inhomogeneous systems has led us to combine lasers with optical microscopes.[1–4] Time-resolved spectroscopy has been integrated into time- and space-resolved spectroscopy, and resolutions have been improved so much that systematic studies have started in single molecular spectroscopy and are opening potential applications in the life sciences.

Spectroscopy and photochemistry under a microscope usually need high intensity excitation, which is well known in single molecule spectroscopy. In order to detect signals from small targets at a focal point, it is necessary to increase the excitation intensity, leading to various nonlinear photophysical and photochemical processes; such as multiphoton ionization, excited-state annihilation, cyclic multiphoton absorption, and so on. At higher intensities, laser ablation is induced, resulting in surface fragmentation. This is even possible for transparent samples, as multiphoton absorption is so efficient using femtosecond laser excitation. Nowadays, laser ablation is popular for use in microfabrication techniques for various materials, and is being utilized in more complex solutions and biomedical systems. One characteristic of the ablation of solution is that it induces shockwave propagation, bubbling, and local convection flow, by which generates an impulsive force which pushes the surrounding micrometer-sized targets. This force can be used to manipulate and to pattern microparticles and living cells in solution without damage. We are beginning to understand the vast potential of this manipulation and consider that this method is complementary to laser tweezers.[5]

This force is considered to generate a local area of high concentration transiently and triggers nucleation in supersaturated solutions of proteins and molecules. Indeed, we succeeded in the femtosecond laser-induced crystallization of lysozyme in 2002.[6] A supersaturated solution was repetitively irradiated by a femtosecond Titanium:Sapphire laser and left in the dark, and then within a few days, several crystals had appeared. These systematic studies have been extended from the viewpoint of the fundamental mechanism and application to complex proteins. This femtosecond approach enables us to prepare crystals more speedily and to obtain crystals with better quality compared to those obtained from the conventional methods. Even membrane proteins, which had not been crystallized previously, will give a fruitful result.

In 2007, we demonstrated, for the first time, the laser trapping crystallization of glycine, which was realized by focusing an intense CW laser beam.[7] Laser trapping is one example of a laser application in science and technology that extends outside of a purely chemical application, and

[a] Prof. Dr. T. Sugiyama
Graduate School of Materials Science
Nara Institute of Science and Technology
Takayama 8916-5, Ikoma, Nara 630-0192 (Japan)
Fax: (+81) 743-72-6199
E-mail: sugiyama@ms.naist.jp

[b] Prof. Dr. T. Sugiyama
Instrument Technology Research Center
National Applied Research Laboratories
20, R&D Rd. VI, Hsinchu Science Park
Hsinchu 30076 (Taiwan)
Fax: (+886) 3-5773947
E-mail: sugiyama@itrc.narl.org.tw

[c] Prof. Dr. H. Masuhara
Graduate School of Materials Science
Nara Institute of Science and Technology
Takayama 8916-5, Ikoma, Nara 630-0192 (Japan)
Fax: (+81) 743-72-6199
E-mail: masuhara@masuhara.jp

[d] Prof. Dr. H. Masuhara
Department of Applied Chemistry and Institute of Molecular Science
National Chiao Tung University
1001 Ta Hsueh Road, Hsinchu 30010 (Taiwan)
Fax: (+886) 3-5712121 ext. 56593

has received much attention in bioscience for non-contact and non-destructive manipulation. This method does not involve photon absorption; namely, excited states of molecules are not generated. Most chemists have not paid attention to laser trapping. Therefore, we started exploratory studies on laser trapping in view of chemistry and soft materials. Initially the targets of laser trapping were polymer spheres, droplets, silica gels, glass beads, catalysts, and so forth, with sizes in the visible micrometer order.[4] Scaling down to the nanometer dimension followed in the middle of the 1990s. Polymers, micelles, J-aggregates, gold nanoparticles, and so forth, were trapped, aligned, and patterned on glass substrates in solution at room temperature.[8,9] Their assembled structures were prepared and analyzed, while trapping dynamics were followed by single particle fluorescence spectroscopy and fluorescence correlation spectroscopy. During these studies, we found that some organization is achieved in the optical trap, reflecting the properties of nanoparticles. This experience strongly stimulated the idea of forming crystals by laser trapping and was the starting point of research on laser trapping crystallization. Now, we are discovering more and more the conditions necessary for trapping crystallization and its great potential in modern crystal science.

Garetz and Myerson conducted pioneering works in laser-induced crystallization, applying a nanosecond 1064 nm laser pulse. They induced nucleation of urea,[10,11] glycine,[12–14] lysozyme,[15] and L-histidine,[16] and explained the phenomena in terms of a re-orientation of the molecules in a high-intensity laser field. This crystallization is nonphotochemical, as solvent or solute molecules are not excited by the laser. Recently, similar results have been shown with KCl,[17–19] suggesting a general applicability. On the other hand, Okutsu and co-workers presented a series of papers on the photochemical crystallization of proteins.[20,21] A tryptophan residue in one protein photochemically reacts with another one in a different protein in solution, leading to a dimer, which gives nucleus for crystallization. In the present Review, we summarize femtosecond laser-induced crystallization and laser trapping crystallization methods, and describe novel crystal growth phenomena introduced by laser irradiation. Furthermore, crystallization dynamics and mechanisms are described in view of molecular interactions and the laser effect. Finally, the future perspective and expected outcomes are considered. All our laser-induced crystallization behavior have been proposed, invented, and demonstrated on the basis of long studies in photochemistry, laser ablation, and laser trapping.

Abstract in Japanese:

レーザー照射により誘起される結晶化及び結晶成長に関する最近の新しい研究の流れをまとめて解説する。溶液のフェムト秒多光子励起は、その照射位置にアブレーションを誘起し、局所的に気泡の発生、衝撃波の伝搬、対流を引き起こす。レーザーマイクロ津波と呼ばれるこの現象により、分子やタンパク質をその過飽和溶液から結晶化させることが可能となる。尿素結晶のレーザーアブレーションにより、その結晶をもとにさらに新しい結晶の成長を引き起こすことにも成功している。強度の高いCW近赤外レーザーをアミノ酸の溶液表面で照射すると、そのクラスターの光捕捉現象が誘起され、さらに結晶化へと発展する。このレーザー捕捉結晶化では、単一の単結晶を任意の時間に任意の場所に作製することが可能であり、その結晶形態はレーザー出力、偏光、さらに溶液濃度にも依存する。異方的あるいは選択的な結晶成長も、レーザー捕捉により実現可能である。最後に、このレーザー誘起結晶化の特徴をまとめ、今後の展望について提示する。

2. Crystallization and Crystal Growth by Femtosecond Laser Irradiation

2.1. Transient Pressure Mechanism for Femtosecond Laser Ablation

It is generally considered that spectroscopic measurements and imaging are difficult during the laser ablation process, because ejected fragments may scatter monitoring and probing lights. However, we found in the laser ablation of doped poly(methyl methacrylate) and microcrystalline Cu-phthalocyanine films, the optical conditions of the irradiated sample films are initially good to absorption spectroscopy. Indeed, we succeeded in measuring the femtosecond transient absorption spectra of the latter film upon intense excitation. A decay of the Cu-phthalocyanine exciton band was observed in the time domain of 10 ps, and then was replaced by an absorption spectrum with negative and positive bands. The latter bands were confirmed to be similar to those observed

Teruki Sugiyama received his PhD from Nankai University, P.R. China in 2002. He was a Postdoctoral Fellow and appointed Assistant Professor in Department of Applied Physics at Osaka University in Japan under the supervision of Professor Hiroshi Masuhara, where he studied on preparation of organic nanoparticles utilizing laser ablation technique in solution from 2002–2006. Then, he became a Researcher at Hamano Life Science Research Foundation in 2007, and then worked at Nara Institute of Science and Technology as a Research Associate Professor from 2008–2011, where he started his current research of a new topic in laser trapping crystallization of organic compounds and proteins. Now he is extending his research in Instrument Technology Research Center, National Applied Research Laboratories in Taiwan.

Hiroshi Masuhara graduated from Tohoku University in Sendai in 1966 and received his PhD in 1971 at Osaka University, where his mentors were the late Professor Masao Koizumi and Professor Noboru Mataga, respectively. He worked in Mataga laboratory of Osaka University until 1984, and then had his own laboratory in Kyoto Institute of Technology, Osaka University, and then Hamano Life Science Research Foundation, while he was also the director of ERATO Masuhara Microphotoconversion Project, JST from 1988–1993. Now he is extending his research in Nara Institute of Science and Technology and National Chiao Tung University in Taiwan, where his groups are studying laser-induced crystallization of molecules and proteins and exploring new laser-induced phenomena in bio/nano systems by developing new laser–microscope methodologies.

in the temperature difference spectrum, so that we could estimate the local transient temperature by comparing both spectra. The temperature elevation at a fluence of 100 mJ cm^{-2} and at the ablation threshold were estimated to be 250 K and only 100 K, respectively. These temperature elevations are too small to induce melting, decomposition, and rapid vaporization, but it should be noticed that the temperature elevation rate is extremely fast; 1013 K s^{-1}. On the other hand, surface roughening, arising from the onset of fragmentation, was confirmed to occur around 10 ns, which was made clear by surface light scattering imaging.

On the basis of these femtosecond transient absorption and imaging measurements, it was concluded that the electronic excitation energy is converted to molecular and lattice vibrations in about 20 ps, whilst the attained local transient temperature is insufficient to induce morphological changes such as melting, sublimation, and decomposition. Thus, the photothermal mechanism cannot explain the present ablation, and we should focus our attention on the time lag between 20 ps and the morphological change. During this lag time, vigorous molecular motions lead to a local pressure increase and the force is directed in every direction, as the irradiated volumes are surrounded by a non-irradiated one, and thermal conduction cannot take place on the ps timescale. The mechanical stress accumulates in the film, and can be released by ejecting the upper part of the film while the lower layer remains on the substrate.

This ablation mechanism of transient pressure does not hold for nanosecond excitation, as during excitation, pulse enhanced molecular and lattice vibrations are transferred sufficiently to the surroundings. Namely, heat transfer from the irradiated area is brought about. Consequently, the photothermal heating mechanism can be applied to nanosecond ablation. It should be noted again that femtosecond laser ablation is not photothermal but photomechanical, therefore the heating effect is suppressed. This is indeed a nice advantage for molecular and protein crystallization, as thermal decomposition and denaturation can be avoided by applying femtosecond irradiation.[22]

2.2. Femtosecond Multiphoton Ablation in Solution

Multiphoton laser ablation is easily induced by focusing a femtosecond laser pulse into water, leading to bubble formation. This is brought about at the focal point, pushing the surrounding water outside, which arises from the transient pressure effect in the photomechanical mechanism as described above. Consequently, a shockwave is generated and propagates with high speed, and leads to local convection around the focal point. When the bubble shrinks, counter-convection is accompanied. As a result, the mechanical force and water flow affect small objects located near the laser focus. We call this impulsive force a laser micro tsunami, as it is induced locally and temporally in three-dimensional space in solution. These multiphoton laser excitation phenomena are well known, and its study and application have received much attention in view of medical purposes. In our case, we focused the femtosecond laser near the target not at the target, and the generated laser micro tsunami was applied in a series of experiments.[23,24] The target is not excited, no photophysical and no photochemical processes are induced, and no damage results, which is indeed an advantage of the laser micro tsunami. This approach has opened a new way to induce crystallization and to manipulate living cells.

2.3. Molecular Crystallization by "Laser Micro Tsunami"

This method was examined to prepare organic molecular crystals and proved to be useful. The 4-(dimethylamino)-*N*-methyl-4-stilbazolium tosylate crystal is an attractive material for organic nonlinear optoelectronics because of its excellent optical properties, but an appreciably large quantity cannot be obtained easily. Its crystallization based on the femtosecond tsunami was demonstrated by us in 2005.[25] We compared several crystallization experiments obtained with 30 000 shots of femtosecond laser irradiation at 1 kHz with those at 20 kHz and those without irradiation, and found that highly repetitive irradiation is most effective for nucleation. X-ray diffraction analysis revealed that the quality of the obtained crystal is the same as that using the conventional method without irradiation.

A direct confirmation of crystallization dynamics by femtosecond irradiation was successfully obtained for a simple solution system: anthracene in cyclohexane. It is well known that crystallization is very easy and the crystal growth is considerably fast, so that it is possible to monitor the crystal growth within 1 s. A single shot femtosecond laser pulse with pulse energy above 3.1 μJ pulse^{-1} led to a sufficiently supersaturated solution, when anthracene crystallization was induced at the vicinity of the laser focal point immediately after the irradiation. The threshold of crystallization was in agreement with that of the bubble formation. The evolution of the crystal is shown in Figure 1, where a bending filmlike crystal grew and changed to a normal shape. The bending form strongly suggests that the nucleation and initial growth started at the curved bubble surface, although the bubble itself was only observed at 100 ms after the irradiation.[26]

2.4. Lysozyme Crystallization by "Laser Micro Tsunami"

The laser micro tsunami is considered an effective stimulation to form a nucleus in the supersaturated solutions of molecules and proteins. Both the propagating shockwave and the local convection assisting mass transfer increase the local concentration. As a result, the system, initially prepared in the supersaturated metastable state, possibly shifts to the crystallization area in the phase diagram as a result of the laser tsunami. This concept explains the reason why we succeeded in femtosecond laser-induced crystallization for lysozyme, a standard protein. Laser tsunami crystallization has now been extended to various proteins. Lysozyme crystallization is not difficult, but a more efficient crystallization giving better quality, compared to the conventional method,

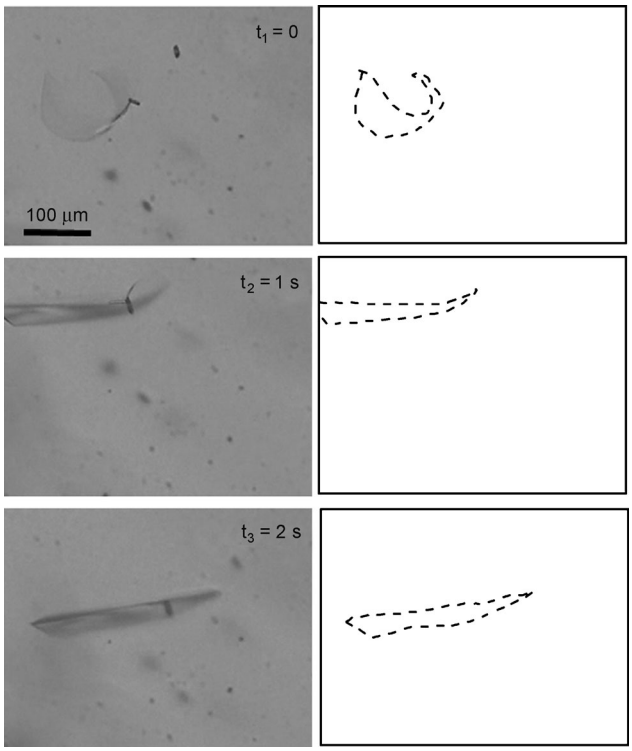


Figure 1. Microscopic images of crystallization process of anthracene upon single shot irradiation of 16.5 μJ pulse^{-1}. Right side illustrations represent the generated crystals in left side photographs. Relative times are shown in the upper right of each frame; t_1 = the 1st frame when a crystal was identified by eye after the bubble disappeared, t_2 = 1 s and t_3 = 2 s after t_1 (Ref. [26]).

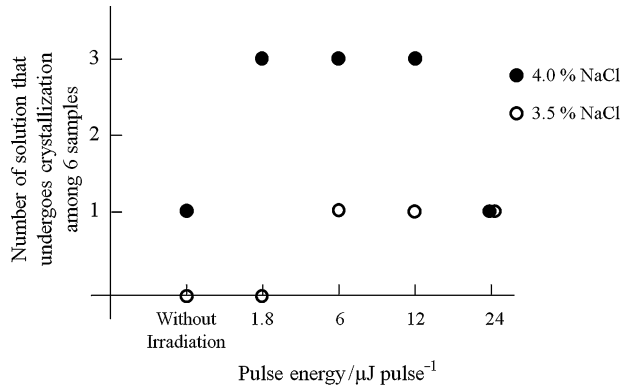


Figure 2. Pulse energy dependence of femtosecond laser-induced crystallization in lysozyme solution, indicating the number of solutions that undergoes crystallization (Ref. [23]).

has been demonstrated. The pulse energy dependence of the crystallization is shown in Figure 2, representing the statistical nature of the crystallization. Yoshikawa and co-workers reported that the effects of laser pulse energy, width, threshold values for crystallization, and bubbling were similar to each other, and that their values were lower for 200 fs pulse widths than those using 1.8 ps pulses.[27] These results indicate that the crystallization is triggered by the bubbling. Laser tsunami and the following crystallization are complex phenomena involving various processes, so the approach to clarify responsible parameters (in this case, the threshold) was crucial at the initial stage of our research.[28]

The questions we have received most frequently are whether or not multiphoton photochemistry of the protein is a key step for triggering crystallization. Namely, the photochemical reaction leading to a less soluble species possibly triggers the nucleation. By steady state illumination experiments, Okutsu and co-workers have reported that the photochemically formed tryptophan dimer is responsible for protein crystallization. To exclude such a photochemical effect, we conducted an irradiation experiment shown in Figure 3.[23] A single droplet of lysozyme aqueous solution was covered by paraffin oil and set on a microscope stage. The femtosecond laser pulse was introduced to the aqueous solution from the bottom, and, of course, the irradiation of the aqueous solution produced some bubbles and then some precipitants were observed, that is, the laser tsunami led to aggregation of proteins. In this experiment, we set the conditions to see visible μm-sized aggregates immediately after the irradiation, although the crystals came only after leaving the irradiated solution for a few hours. Next, a very intense femtosecond laser pulse was introduced to the oil phase and we confirmed that several bubbles were formed not only in the oil, but also in the aqueous solution. The bubbling in the oil phase should arise from the multiphoton excitation of oil, while the bubbles in the aqueous solution may be ascribed to migrated bubbles formed in the oil phase or/and formed by the explosion of the interface between both phases. Lysozyme is insoluble in oil, so multiphoton excitation of lysozyme is not responsible for the phenomenon. Nonetheless after several seconds, precipitants appeared in the aqueous solution, which clearly indicates that the photochemical mechanism does not contribute to the crystallization.

To confirm another relation between bubbling and crystallization, we examined a spatial correlation between bubbling and crystallization in a viscous solution of hen egg-white lysozyme.[28] By adding polyethylene glycol, the viscosity increased so that we expected that the formed bubbles would not diffuse out from the irradiated areas during the period necessary for crystal growth to form to a visible size.[28] Actually, 1 day after irradiation, lysozyme crystals were prepared and spatially associated with bubbles, with very few crystals found far from the bubbles. For example, we could observe a long-lasting bubble with an initial diameter of 120 μm. It shrunk to 80 μm in 1 hour and interestingly lysozyme crystals appeared at the surface of the bubble. This may indicate that the surface is a preferential field for the crystallization. The long-lasting bubbles are possibly composed of gas products from the photodissociation and photodegradation of solute and solvent molecules owing to multiphoton absorption at the focal point. In the present supersaturated solution, lysozyme and its clusters should be adsorbed at the surface of the formed bubble and their molecular ordering would lead to nucleation, and these nuclei undergo growth processes at the surface.

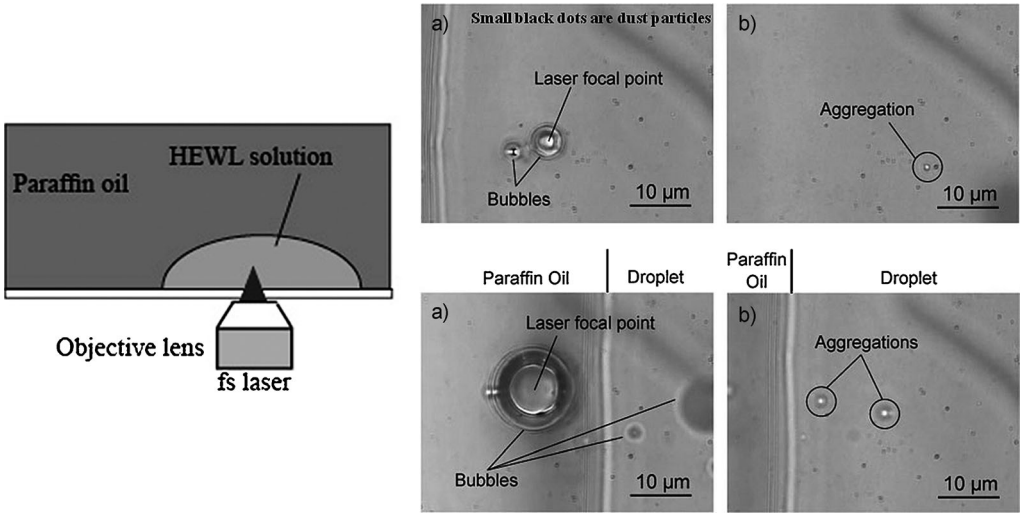


Figure 3. Femtosecond laser-induced crystallization of lysozyme upon irradiating the laser into paraffin oil surrounding the lysozyme aqueous solution (Ref. [23]).

Similar approaches were adopted for clarifying crystallization processes of other proteins. Yoshikawa and co-workers studied femtosecond laser-induced crystallization of thaumatin by adding agarose gel, and monitored the process over a time period ranging from μs to days.[27] Crystals and bubbles were observed in the same area, which is ascribed to the suppressed diffusion of nuclei and bubbles. They also examined fluorescence molecule-labeled lysozyme and reported that the shrinking of the bubble causes a local increase in the protein concentration.

This laser tsunami crystallization is useful to induce crystallization in solutions that have a low supersaturation degree. Murai and co-workers demonstrated that the use of a gel solution with agarose enhanced nucleation of egg white lysozyme crystals, and the crystallization was realized at a saturation degree that is 3 to 5 times lower than that required without agarose and for spontaneous crystallization (without laser irradiation) with agarose.[29] Additionally, fluorescence imaging of the labeled lysozyme revealed that the cavitation bubbles generate in the high concentration region around the focal point, which may trigger the nucleation. Furthermore, the high concentration region remains for longer in the agarose system, so that the nucleation probability should be increased. The present laser tsunami crystallization method is improved by combining it with the solution-stirring technique,[30] which has received much attention as a successful crystallization method for proteins.[31]

2.5. Directional Crystal Growth by Laser Ablation

When nanoparticles are ejected from a molecular crystal in its supersaturated solution by femtosecond laser ablation, the nanoparticles and/or remaining holes in the crystal may become a seed for nucleation. Indeed, we found a quite novel phenomenon of laser-induced crystal growth in urea solution. One representative example is shown in Figure 4,

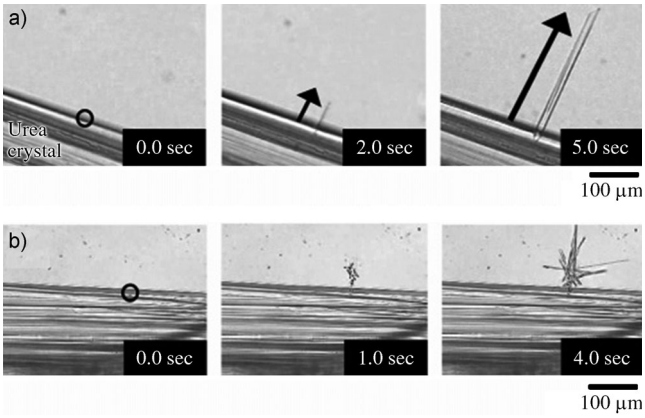


Figure 4. Subsequent crystal growth of urea from the irradiated area marked with an open circle by a single shot of an 800 nm pulse at (a) 0.12 and (b) 0.21 μJ pulse^{-1} (Ref. [32]).

where a urea crystal is irradiated with a single 800 nm femtosecond pulse.[32] It is interesting to see that the daughter crystal grew from the irradiated area, and the crystal shape is similar to a needle, which is characteristic of urea crystals (Figure 4b). The daughter crystal tended to grow perpendicularly to the mother crystal, and the orientation of needle axis to the daughter crystal was independent of polarization of the laser pulse. At the higher energy irradiation (Figure 4b), several crystals were prepared near the irradiation area. The number of the daughters increased with both the laser energy and the number of pulses.

This growth was considered to be induced by etching and fragmentation, which were directly confirmed by AFM observation. The ablation was done in air on a glass substrate and the obtained results are shown in Figure 5, where a hole etched in the urea crystal and fragments ejected on to the glass substrate are clearly identified. At higher laser fluence (Figure 5b), many fragments, with sizes ranging from tens of

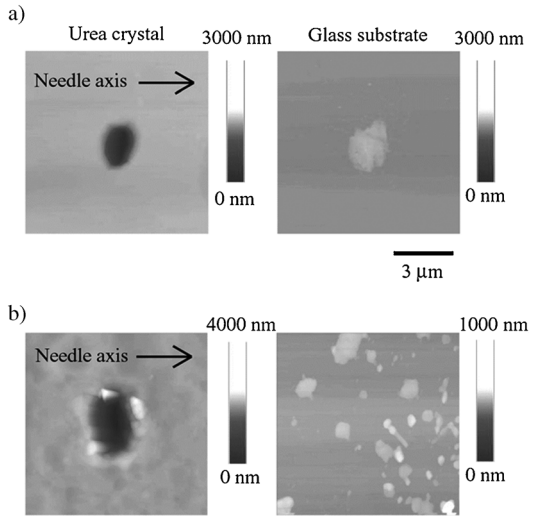


Figure 5. AFM images of the ablated fragments on a cover glass substrate (right) and the corresponding etched area of a urea crystal (left) by one shot irradiation of an 800 nm laser pulse with (a) 0.031 μJ pulse^{-1} and (b) 0.056 μJ pulse^{-1} through a 20× objective lens. Reproduced with permission from Ref. [32].

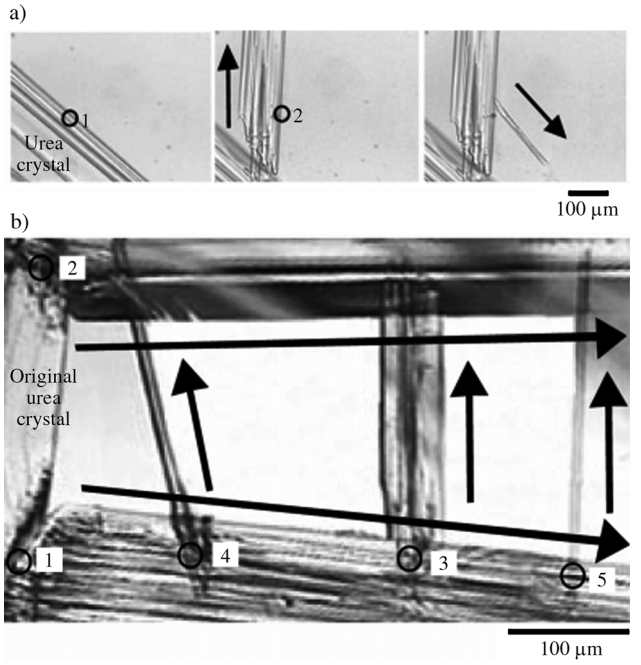


Figure 6. a) Crystal patterning procedure for urea. The first pulse was irradiated to the open circle area numbered 1, the second pulse was to the circle 2, and so on. A single shot of the 800 nm femtosecond laser pulse with 0.12 μJ pulse^{-1} was irradiated through a 10× objective lens. b) The ladderlike spatial pattern of urea crystals obtained by successive single shot laser irradiation of the five open circle areas each under the same irradiation conditions. Reproduced with permission from Ref. [32].

nm to a few μm, were observed and they spatially dispersed on the substrate. These fragmentations are considered to be caused by the transient pressure effect as explained above, thus the destructive thermal effect should be suppressed in this femtosecond ablation.

The present laser-induced crystal growth is unconventional and considered useful in forming complex structures in supersaturated solutions, and the fragmentation results in nucleation and needle crystals growing perpendicular to the mother crystal. The subsequent processing gives a set of blocks as shown in Figure 6. Another idea is to use this phenomenon to generate a new single crystal when multicrystalline materials or amorphous precipitates are formed unintentionally. Sometimes proteins and newly synthesized compounds are very expensive and/or difficult to obtain, and thus our approach will be used in relevant fields in near future.

3. Crystallization and Crystal Growth by Laser Trapping

3.1. Laser Trapping Crystallization of Glycine

Laser trapping is a well-known phenomenon of gathering macromolecules, micelles, nanoparticles, and molecular clusters in solution at room temperature.[33–36] The trapping arises from the photon pressure of a focused laser beam, and the force increases along with the size and polarizability of the target object. Once the assembling is started at the focal spot, the polarization increases with the effective volume. Consequently, the formed assembly experiences a large trapping force, developing further trapping. Similarly, when laser trapping is applied to a supersaturated solution of an organic compound, it is expected that the solute clusters formed in the solution are gathered to the focal spot, increasing the local solute concentration nonlinearly. Consequently crystallization is expected to be induced. For the first demonstration, we chose glycine as a solute for laser trapping induced crystallization. It is known for glycine that the dimer formation becomes prominent as the solution concentration increases, and consequently the liquid-like solute clusters are formed by the link of the dimers through intermolecular interactions.[37] D_2O was used as a solvent to suppress the heating in this experiment, since H_2O has a non-negligible absorption coefficient owing to the overtones of the OH vibration at 1064 nm.[38] In this glycine/D_2O solution, we succeeded in the first demonstration of laser trapping crystallization, as we named it, which is described in this paper.[7]

Figure 7 shows a series of crossed-Nicol images around the focal point during laser irradiation. Immediately after irradiation with a linear-polarized CW NIR laser beam with 1064 nm wavelength at the air/solution interface of the su-

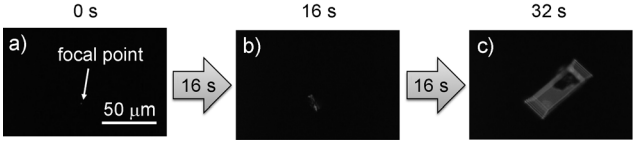


Figure 7. Crossed-Nicol images of glycine crystallization during laser irradiation (Ref. [7]).

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persaturated solution thin film, only the laser reflection from the surface was observed as a small spot (Figure 7a). After 16 s irradiation, one glycine crystal with a size of 10–30 μm was clearly identified at the focal point by an EMCCD camera (Figure 7b). Although we do not know how small a size we can detect with our set-up, the nucleation was possibly induced at around 16 s. The formed crystal grew larger upon further irradiation while being trapped at the surface, and finally it measured about 50 μm (Figure 7c).

The laser power of 0.4 GW cm^{-2} used in this experiment is too small for one glycine molecule to be trapped stably at the focal spot.[39] Therefore, we suggest that the laser trapping of the large liquid-like clusters, as described above, provides the increase in local concentration followed by the nucleation. It is noteworthy that this laser trapping crystallization occurs only at the air/solution interface. This result indicates that both efficient trapping of the clusters at the surface and the re-orientation of glycine molecules contribute to the crystallization mechanism.

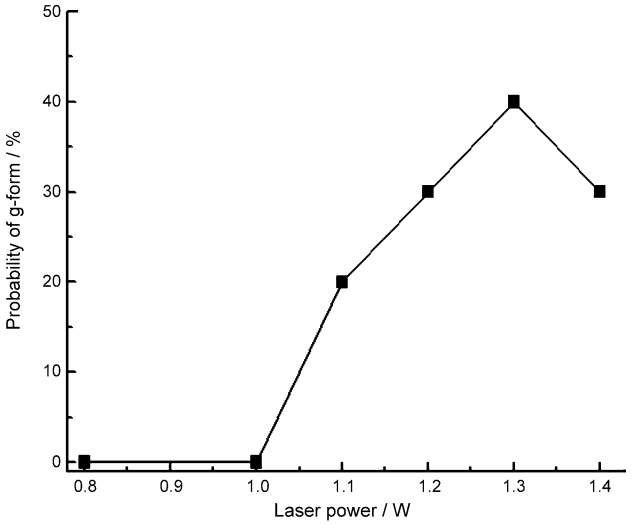

Figure 8. Laser power dependence of probability of a γ-glycine crystal prepared by laser trapping. Reproduced with permission from Ref. [40].

3.2. Control of Crystal Polymorph of Glycine

We have also succeeded in controlling the crystal polymorph of glycine by tuning the laser power using laser trapping crystallization.[40] Glycine is one of the most representative compounds to be studied for the polymorphic crystallization mechanism and process,[41–44] and it is known to have three kinds of polymorph, namely of α-, β-, and γ-form. The α-form is always produced by conventional crystallization methods,[45,46] β-form is obtained by cooling a saturated solution with acetic acid,[47] and the γ-form is prepared through some experimental procedures of adding some additive salts or highly acidic/basic compounds.[41,48,49] Furthermore, glycine requires a much higher supersaturation degree to crystallize into the γ-form in solution.[50] However, such a higher supersaturated solution is not easily prepared since concentration fluctuation in solution leading to the α-form is generally unavoidable. The difficulty of γ-form preparation is expected to be overcome by utilizing this laser trapping technique.

We investigated the polymorph of crystals prepared under various laser powers between 0.8 and 1.4 W. The experiment was done for 10 samples at each condition. Note that only a single crystal was always formed at the focal point. FTIR measurement was carried out for all prepared crystals, and two kinds of spectra were obtained. All their vibrational absorption peaks agree well with those of the α- or γ-forms,[51,52] which was also supported by single X-ray crystallographic analysis. Thus, we successfully prepared γ-glycine by this method, and found that the formation probability strongly depends on laser power as shown in Figure 8. The α-form was always obtained at a laser power less than 1.0 W, while the γ-form became prominent with an increase in the power and a maximum probability of 40% was achieved at 1.3 W. However, when the power was set to 1.4 W, which is the maximum laser power in this experiment, the probability decreased to 20%.

Here we discuss the preparation probability of γ-glycine depending on laser power in terms of two effects: photon pressure and local temperature elevation at the focal spot. As illustrated in Figure 9a, photon pressure should be induced by the interaction of a focused laser beam with glycine clusters, and the force is proportional to the laser power. Simultaneously, laser heating at the focal spot is induced, which mainly arises from the absorption of 1064 nm-photons by the glycine molecule itself. Since laser trapping

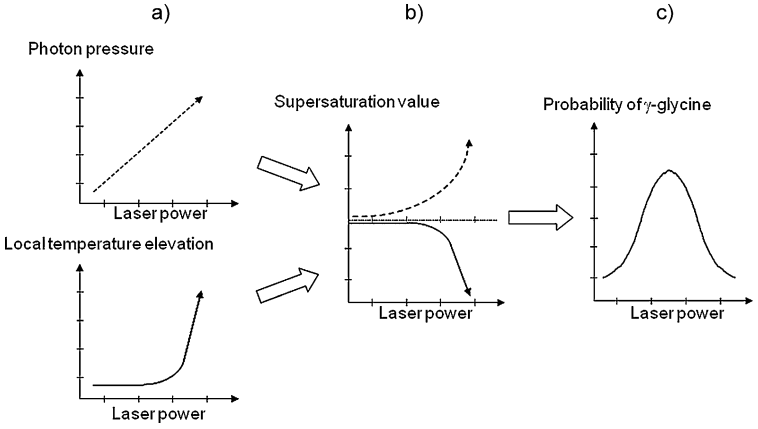

Figure 9. A bell-shaped curve of the multiplication of two effects; photon pressure and local temperature elevation. Reproduced with permission from Ref. [40].

of the clusters at the high power causes the increase in molecular concentration at the focal spot, the absorption of NIR photons by the molecules becomes larger and larger, leading to further temperature elevation. As heating is always compensated by thermal dissipation to the surroundings, the temperature elevation becomes more prominent at a higher power. Namely, the temperature elevation is nonlinearly increased with the laser power. Next, we consider the changes in the supersaturation value at the focal spot, which depends on both photon pressure and local temperature elevation, as illustrated in Figure 9b. Once the cluster is trapped, further trapping should be induced, and as a result, the supersaturation value at the focal spot nonlinearly increases with the laser power. Conversely, the local temperature elevation, illustrated in Figure 9a, nonlinearly decreases the supersaturation value. Consequently, the probability can be represented by the multiplication of these two compensating factors, giving a bell-shaped curve, as illustrated in Figure 9c. Thus, a high degree of supersaturation is realized within a few minutes by this method, giving a single γ-glycine crystal. We believe that this technique will become a standard crystallization method for crystal polymorph control in the near future.

3.3. Dense-Liquid-Droplet Formation of Glycine

Laser trapping crystallization of glycine is induced by focusing a laser beam at the air/solution interface. Thus, focal position is one of the key factors for the crystallization. Indeed, when the focused laser beam is used to irradiate the solution, which is common in laser trapping experiments, a particle-like assembly of glycine with almost the same dimensions as the spot size is confirmed although no crystallization is realized.[33] During the investigation of photon pressure-induced phenomena depending on focal positions, recently, we successfully demonstrated the formation of a single millimeter-sized dense liquid droplet of glycine.[39] This phenomenon is the first demonstration of a laser trapping behavior, which we summarize and describe here.

For all laser trapping experiments, laser heating and the accompanying phenomena should be always considered. Since the glycine molecule itself has a noteworthy absorption coefficient at 1064 nm of the trapping laser, irradiation causes the elevation in local temperature at the focal spot. When the laser beam is used to irradiate at the solution/glass interface of the solution thin film, the generated heat is mainly transferred to the solution surface. Consequently the temperature distribution at the solution surface becomes non-uniform and results in local surface depression.[53,54] Actually, on measuring the surface deformation with a surface displacement meter, the surface depression started immediately after laser irradiation. The temporal change of the local surface height and the corresponding CCD images are shown in Figure 10a and 10b, respectively. Immediately after the irradiation, the surface linearly depressed, and finally the solution thickness reached about 5 μm at around 18 s. It is interesting to see that the height suddenly recovered by further irradiation. This amazing phenomenon is observed only for the glycine solution, never for a neat D_2O liquid film, which shows only the initial depression. After the surface height was elevated up to about 70 μm after 60 s irradiation, and spherical droplet formation gradually ensued around the focal spot. Eventually, at 200 s, the droplet grew large up to a lateral diameter of 5 mm and height of about 150 μm, which was clearly identifiable with the naked eye.

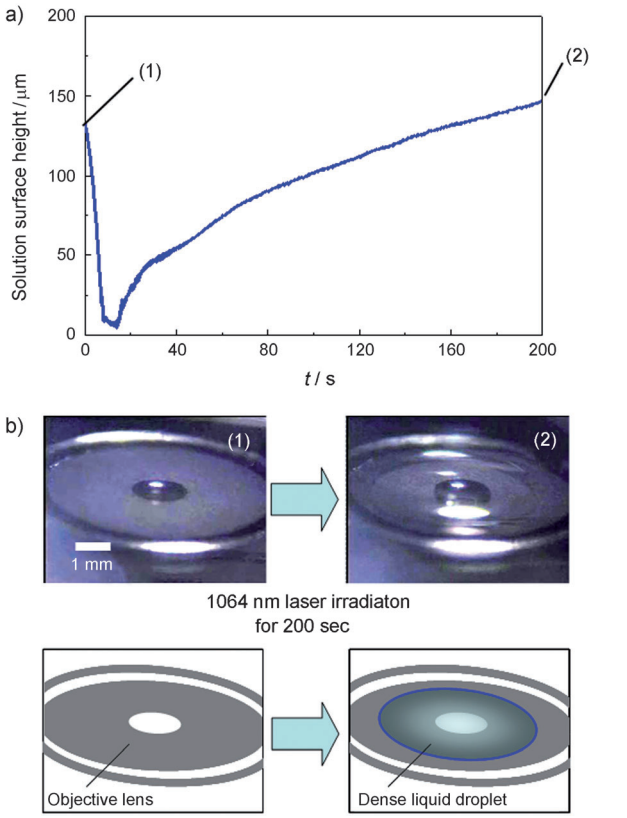

Figure 10. a) Temporal change of the solution height upon focusing a laser beam at the glass/solution interface. b) CCD images around a focal spot simultaneously captured with (a). (1) and (2) in (b) correspond to those in (a). Reproduced with permission from Ref. [39].

The features of this amazing droplet are summarized as follows. First, the size is much larger than the focal spot of 1 μm, where the trapping force works. Since the trapping force is generated through the interaction of the laser beam with glycine clusters, the clusters outside the focal spot do not experience this force. Thus, we consider that the growth to mm-order for the droplet arises from the spontaneous molecular assembly, and is possibly assisted by molecular transfer toward the depression area, owing to convection. Second, the droplet surface height becomes higher than the initial thickness of the thin solution. Backscattering measurements with a He–Ne laser showed that the reflected light intensity increased nonlinearly with irradiation.[39] This result indicates that the droplet has a higher molecular concentration compared with the initial solution since the refractive index of glycine solution increases with concentra-

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tion.[55] Thus, the dense droplet prefers to conglobulate because of the high surface Gibbs energy, resulting in the higher surface height. Third, the droplet remains for several seconds, even after turning off the laser beam, and keeps the liquid-like phase prior to nucleation in spite of the high concentration. These characteristic features can be well explained on the basis of liquid–liquid phase separation (LLPS). In 2007, He and co-workers attempted to induce LLPS of glycine in the aqueous solution by the rapid cooling method, however, a successful separation has never been realized so far.[56] Louchev and co-workers recently reported a worthwhile theoretical result that stated mass transfer is enhanced by a thermocapillary effect and as a result, effectively supplies solute molecules to the surface depression area, where the trapping force works.[57] These results support that photon pressure is spatially integrated with the thermocapillary effect, which induces a highly concentrated area that is sufficient to lead to LLPS. The droplet is also regarded as some kind of crystal precursor, since the nucleation is easily triggered within a few seconds by shifting the laser focus to the droplet surface. This success will give us valuable information to elucidate the early stages of the crystallization process.

3.4. Control of Glycine Crystal Growth by Laser Trapping

We have described above three notable phenomena of laser trapping of glycine clusters in solution: crystallization, polymorph control, and dense liquid droplet formation. Next, we describe another interesting result of conducting the crystal growth by focusing the laser beam close to the spontaneously generated crystal.[58] In order to explain the phenomenon of this crystal growth, we give here Figures 11 and 12, where two samples A and B with one and three crystals around a focal spot are prepared, respectively. Figure 11 shows a series of crossed-Nicol images on irradiation for sample A—the white arrow tip denotes the focal spot. Note that the laser beam was irradiated at the solution/glass interface. Immediately after focusing the laser beam at a point 18 μm away from the crystal edge, crystal growth toward the focal spot was observed and the growth rate increased with irradiation time. When the edge of the growing crystal reached the focal spot and overlapped with it, the growth soon stopped. Then, by shifting the focal spot away from the edge again, it started re-growing. This is the first demonstration of crystal growth induced by laser trapping. For sample B, three spontaneously generated crystals could be observed around the focal spot (labeled I–III in Figure 12a). When the focused laser beam was irradiated at the point between crystals I and II, as indicated by the white arrow, crystal I immediately started growing two-dimensionally. This growth behavior seemed to be quite different from that in sample A, which showed one-dimensional growth. This may arise from an anisotropic property of the crystal growth, and crystal I in sample B probably has a two-dimensional crystal growth face. Simultaneously, crystal II conversely started shrinking from its upper side, which is located far from the

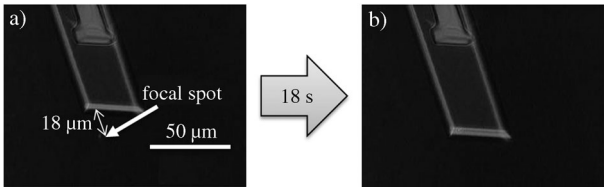

Figure 11. Crossed-Nicol images of laser trapping induced crystal growth of spontaneously generated glycine crystal. Reproduced with permission from Ref. [59].

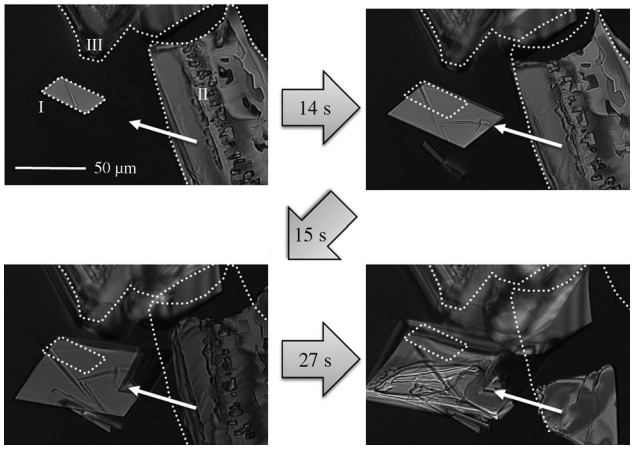

Figure 12. Crossed-Nicol images of laser trapping induced crystal growth and dissolution of spontaneously generated glycine crystal. Reproduced with permission from Ref. [59].

focal spot. Upon further laser irradiation, crystals I and II gradually became larger and smaller, respectively, and finally the latter was completely dissolved. Incidentally, crystal III also grew slightly toward the focal spot by laser irradiation.

It is important to discuss the long range effect of the trapping force on crystal growth. Since the focal spot size is estimated to be only 1 μm, it is far too small to allow the force to reach the crystal. The key to explain this unique phenomenon is to remember the result of the formation of the giant dense droplet, as described above. The formed droplet has a millimeter size, which means that the high concentration area is spread out over quite a large area compared to the focal spot. Therefore, the crystal can grow larger by use of the solutes in the high concentration area. On the other hand, the solution before irradiation should be under saturation since no spontaneous growth proceeded. The solutes used for the droplet formation should be compensated by the dissolution of other crystals in order to keep the chemical equilibrium. These phenomena are much like Ostwald ripening, which arises from the difference of surface free energy. This successful demonstration of crystal growth will give us new trials of selective growth to a desired direction and crystal shape control by optimizing experimental conditions.

4. Conclusions and Perspective

Femtosecond laser irradiation realizes new crystallization of molecules and proteins which are not available by conventional methods, offering fast and efficient crystallization that produces high quality crystals. It is considered that nucleation takes place at the surface of laser-induced bubbles, which is followed by its growth. Laser trapping crystallization is made possible upon irradiation at an air/solution interface, and replaced by single droplet formation when irradiated at a glass/solution interface. Crystal polymorph control is achieved by tuning laser power, polarization, and concentration, while crystallization has recently been made possible even from unsaturated solutions.[59]

Both laser-induced crystallization methods are being developed further to crystallize molecules, amino acids, and proteins, which are not obtainable using conventional techniques, and to control the crystal polymorph. In addition to the conventional conditions of concentration, solvent, and temperature, laser parameters, such as pulse width (or CW), wavelength, power, polarization, repetition rate, irradiation time, and so on, can be tuned for getting successful results. Practical preparation of new crystals is very much expected in many research fields, for which femtosecond crystallization will be fruitful. Elucidation of dynamics and mechanism of nucleation and crystal growth is important and will receive much attention. In the case of laser trapping crystallization, one single crystal is always fabricated in a focused position at an arbitrary time, which can be probed by spectroscopic and imaging methods, so that the studies into crystallization dynamics and mechanism are very promising. Femtosecond laser crystallization and laser trapping crystallization are complementary to each other, and their concerted studies will open new horizons of crystal chemistry.

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