The Journal of Physical Chemistry Letters
pubs.acs.org/JPCL

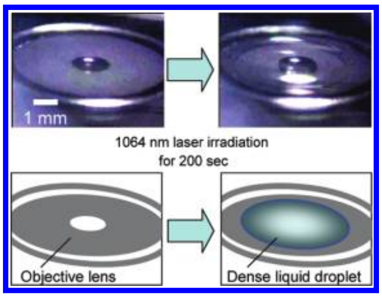
Millimeter-Scale Dense Liquid Droplet Formation and Crystallization in Glycine Solution Induced by Photon Pressure

Ken-ichi Yuyama,[†] Teruki Sugiyama,[*,†] and Hiroshi Masuhara[*,†,‡]

[†]Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan, and [‡]Department of Applied Chemistry and Institute of Molecular Science, National Chiao Tung University, Hsinchu 30010, Taiwan

ABSTRACT A millimeter-scale dense liquid droplet of glycine is prepared by focusing a CW near-infrared laser beam at the glass/solution interface of a thin film of its supersaturated heavy water solution. The formation process is investigated by direct observation with CCD and by measuring temporal change of the surface height with a displacement meter. The droplet becomes much larger than a focal spot size, a few mm width and ~150 μm height, and observable with the naked eye. Interestingly, the droplet remains for a few tens of seconds even after switching off the laser beam. Whereas the droplet is kept during laser irradiation, the crystallization is immediately attained by shifting the laser beam to the air/droplet surface. It is considered that the droplet is possibly the early stage of the multistep crystallization process and plays an important role in photon pressure-induced crystallization of glycine.

SECTION Nanoparticles and Nanostructures



Photon pressure, which is a gradient force toward a focal spot generated by a focused CW laser beam, has been widely employed as an optical tweezers technique to trap and to manipulate micrometer-sized objects in many fields of physics, optics, and biology.[1−3] Over the past decades, the study on photon pressure in solution has progressed with the size reduction of target materials from microscale to nanoscale,[4−6] and actually, we have extended a series of experiments on the dynamics of photon pressure-induced association of nanoparticles, polymers, micelles, and J-aggregates in their solutions at room temperature.[7−11] As an example, polystyrene latex nanoparticles with 24 nm diameter were trapped and gathered by photon pressure, leading to their assemblies in a focal spot.[11] In single-molecule level, Osborne et al. and Chirico et al. separately reported that the diffusion of Rhodamine 6G molecules in the focal spot were suppressed under photon pressure, although no stable trapping was achieved.[12,13] These results imply that photon pressure efficiently works even on small clusters or molecules, which have the sizes much smaller than the wavelength of the trapping laser.

In 2007, we applied photon pressure of a focused CW near-infrared laser beam to a supersaturated heavy water solution of glycine, and for the first time succeeded in inducing the crystallization, which we call "laser trapping crystallization".[14] This novel phenomenon was explained by assuming that glycine molecules form aggregates under a supersaturated condition before irradiation because the optical gradient force in our experiment is too small to trap the single molecule. Indeed, the presence of small liquid-like clusters of glycine under a supersaturated condition has been experimentally demonstrated.[15] Such clusters, which are associated with each other through weak hydrogen bonds without forming nucleus, should be gathered in the focal spot and reorganized into ordered structures by photon pressure. In 2005, Myerson et al. reported the direct confirmation on the cluster structure in water by showing small-angle X-ray scattering data, explained as follows. The glycine molecules first exist as the dimers in the supersaturated solution; then, their small liquid-like clusters are formed as the concentration increases. Finally, the clustering molecules reorganize themselves, leading to the nucleation.[15] In this letter, we demonstrate arbitrarily the formation of a single dense liquid droplet of glycine or crystallization just by controlling the focal position of a CW laser beam in a thin film of the supersaturated heavy water solution. The droplet grows up to millimeter scale, much larger than the focal spot, from which the crystallization is also attained. To our knowledge, this is the first report demonstrating such glycine droplet formation.

First, we describe the formation of a single millimeter-scale dense liquid droplet that is induced by focusing the laser beam of 1064 nm at the glass/solution interface of a glycine solution. To evaluate the large droplet formation quantitatively, the

Received Date: February 25, 2010
Accepted Date: April 1, 2010
Published on Web Date: April 06, 2010

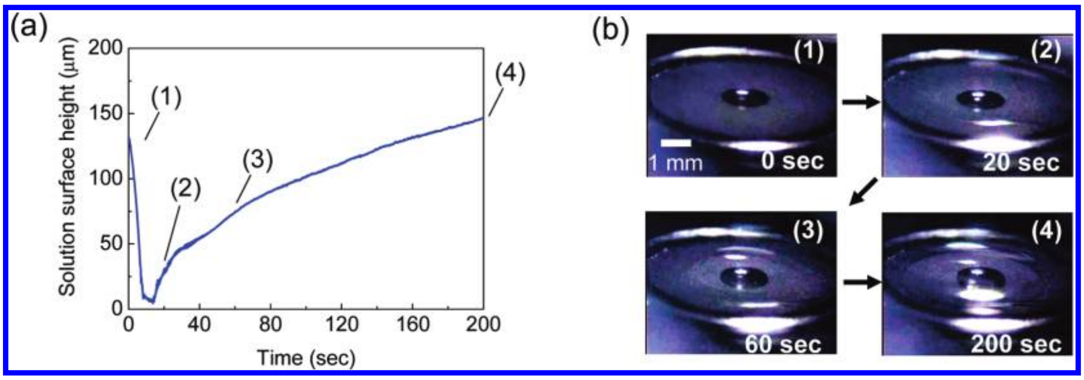


Figure 1. (a) Temporal change of the solution surface height upon focusing the laser beam at a glass/solution interface. (b) CCD images around a focal spot simultaneously measured with part a. (1)−(4) in part b correspond to (1)−(4) in part a.

temporal change of solution surface height at the focal axis was measured using a laser displacement meter, and the surrounding area at the focal spot was simultaneously and directly observed with a CCD camera during laser irradiation. Figure 1a,b shows the surface height change as a function of irradiation time and a series of associated CCD images during laser irradiation, respectively. Before irradiation, the surface height was estimated to be 130 μm, which corresponds to the thickness of the solution layer, and only the top of the objective lens was observed as a dark disk through the solution, as shown in (1) of Figure 1a,b, respectively. Just after starting irradiation, the surface height monotonously decreased to ~5 μm, whereas no apparent change was identified by direct CCD observation. The behavior of such surface depression is generally known for a thin film-shaped solution and is explained from the viewpoint of inhomogeneous surface temperature distribution.[16−18] Actually, we have checked that the surface depression also occurred upon using a liquid film of pure D_2O as a sample due to the temperature elevation of a few degrees.

Note that further irradiation induced the surface height elevation, although no apparent change was observed through the CCD camera in (2) of Figure 1a,b. Then, the surface continued to elevate, and simultaneously, a circular droplet became clearly visible around the focal spot, as shown in (3) of Figure1a,b. As a matter of course, both phenomena are never seen in neat D_2O. The droplet gradually grew to a larger size of 5 mm in diameter in 200 s, which could be seen even by the naked eye as in Figure 1b (4). The millimeter-scale droplet formation was reproducibly observed for all examined samples. The solution height was then estimated to ~145 μm in Figure 1a (4). Therefore, the droplet formation and growth behaviors are well followed by surface height by using a displacement meter.

We consider that the droplet formation is due to an increase in the solution concentration upon laser trapping and examined temporal change of the refractive index of the solution by monitoring the backscattering with a He−Ne laser.[4,5] The detailed results are presented as Supporting Information, and they indicate that the refractive index of the solution at the focal spot became higher with laser irradiation. Because the refractive index of a glycine solution becomes higher in the visible region as the concentration increases,[19] we conclude that the formation of the dense liquid droplet in glycine solution is due to the high local concentration realized by photon pressure. The trapping force acting on one glycine molecule is too small to overcome the micro Brownian motion in the solution. Actually, the laser intensity in this work is estimated to be ~0.4 GW/cm^2 at the focal point, and the size of the object we can trap is approximately calculated to be >17 nm even by estimating the refractive index of the cluster to 1.4.[4] Therefore, we imply that the large liquid-like clusters are gathered in the focal spot by photon pressure, leading to the formation of the dense liquid droplet.

As notable features of the formed dense liquid droplet with the size of a few millimeters, much larger than the focal spot, it does not disappear soon and remains for a few tens of seconds even after switching off the laser beam. Furthermore, although the droplet has a higher concentration compared with the initial supersaturated solution, it keeps the liquid-like phase prior to the nucleation. Taking these features into account, we suspect that the formation of the dense liquid droplet of glycine is ascribed to liquid−liquid phase separation via binodal decomposition.[20,21] He et al. reported the attempt of the phase separation in glycine aqueous solution by means of super cooling, whereas the separation has not been successful so far.[22] In the present work, the formation of the millimeter-scale dense liquid droplet was observed only after the initial surface depression to a few micrometers, and there was no droplet formation on the way of the depression. As a prospective mechanism of this phenomenon, we should consider the notable theoretical results reported by Louchev et al.[23] They presented that dissolved molecules in the solution are efficiently supplied into the surface depression area because of the mass transfer by convection, and the optical gradient force is enhanced by a magnitude of one to two orders when photon pressure works just on the depression area. This theoretical approach was carried out based on our previous experimental result that polymer molecules in the solution were efficiently trapped by photon pressure accompanied by surface depression, forming the deposition on the cover glass.[24] Therefore, we consider that the droplet formation is triggered by the high condensation of liquid-like clusters induced by photon pressure, and the droplet having large association energy of the clusters is energetically

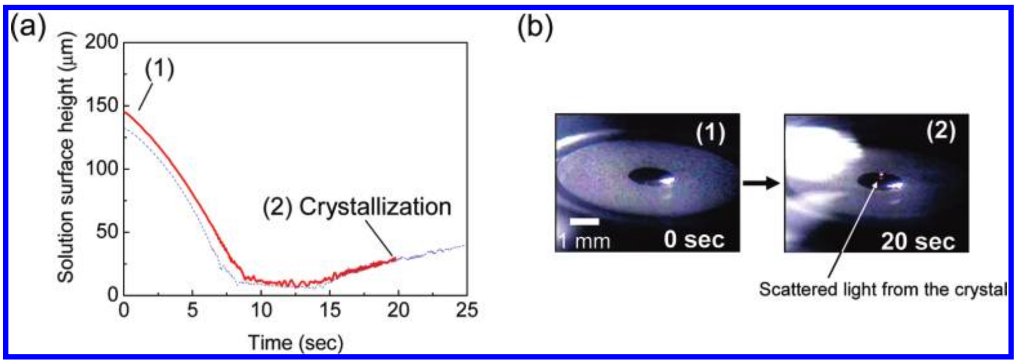


Figure 2. (a) Red line shows the temporal change of the solution surface height upon focusing the laser beam at an air/solution interface. The temporal change of Figure 1a is represented as a blue dotted line for comparison. (b) CCD images around a focal spot simultaneously measured with part a. (1)−(2) in part b corresponds to (1)−(2) in part a.

favorable compared with molecular reorientation leading to crystallization.[22]

Next, we describe the crystallization of glycine, which is induced by focusing the laser beam at the air/solution interface. Because the crystallization is realized just upon irradiation but attained only by shifting the focal spot from time to time to the moving surface, the precise examination of temporal surface height change during laser irradiation is indispensable. Although we have already reported the crystallization itself,[14] the dynamics of the solution deformation accompanying crystallization is directly measured for the first time in the present work. The height change measurement is now made possible here by a displacement meter with keeping the focal position of trapping laser always at the solution surface, and the results are shown in Figure 2a,b. Initially, the surface height decreased to a few micrometers and then elevated to ~30 μm in ~20 s, as shown in Figure 2a. This surface elevation after the depression was reproducibly observed for all examined samples. Around 20 s, the crystallization was induced at the focal spot and was clearly confirmed by the scattering light of He−Ne laser adjusted coaxially with the trapping laser, as shown in Figure 2b (2). The crystal formation that occurred at the surface height ranged from 20 to 70 μm, depending on samples. Further laser irradiation kept the crystal trapped two-dimensionally at the surface, where the crystal grew rapidly with rotating, and the measurement of the surface height was not available anymore. The crystallization was observed only after the initial surface depression and the following elevation. The temporal change of surface height before the crystallization is quite similar to that due to the droplet formation of Figure 1a when they are compared with each other on the same time scale as that shown in Figure 2a. Hence, the surface elevation before the crystallization possibly corresponds to the droplet formation. We suppose that the dense droplet was initially produced at the solution surface; then, the crystallization was induced at the surface on the way of droplet growth. To demonstrate this assumption, we did the following experiment. First, the focal spot was kept at the glass/solution interface until the millimeter-scale dense liquid droplet was formed, and then it was shifted to the droplet surface. Within a few seconds, the crystallization was immediately induced at the droplet interface; the image of direct observation was quite similar to that of Figure 2b (2). The molecular arrangement may be triggered by 2D ordering at the air/droplet surface, leading to the crystallization.

In summary, we have succeeded in forming a single dense liquid droplet of glycine under photon pressure caused by focusing the laser beam at the glass/solution interface. The droplet was on the millimeter scale, much larger than a focal spot, and remained even after cutting off photon pressure. These phenomena are well explained in terms of liquid−liquid phase separation. Upon shifting the laser beam to the droplet surface, the crystallization was immediately induced. In general, it is much more difficult to predict where or when crystallization and nucleation accurately occur. However, this difficulty could be overcome in principle by this laser trapping crystallization, and the formation processes of dense liquid droplet and crystal could be easily investigated spectroscopically as they occur spatio-temporarily. Therefore, the present understanding of the droplet formation will lead us to elucidate the early stages of the nucleation and hopefully will open a new stage of research on crystallization and crystal growth. For example, we have recently reported the polymorph control of glycine under photon pressure, and the formation of γ-glycine, which cannot be prepared with a conventional crystallization method, is achieved.[25] The γ-glycine formation was explained on the basis of the local molecular concentration depending on laser power and may be triggered by the molecular arrangement reflecting the electromagnetic field. We consider that the molecular dynamics leading to crystallization via the droplet formed by changing laser polarization is critical, and the result will be reported in the near future.

EXPERIMENTAL SECTION

An experimental setup for optical trapping system used in this work is shown in Figure 3. A near-infrared laser beam of 1064 nm from a CW Nd^{3+}/YVO_4 laser (Spectra Physics, J20-BL-106C) was used as an optical trapping light source, which was focused at a glass/solution interface or an air/solution surface via an objective lens (60× magnification, NA = 0.90). A He−Ne laser (Coherent, 6069DU) was adjusted to be coaxial with a trapping laser path by using some optical elements to confirm a focal position before the trapping laser

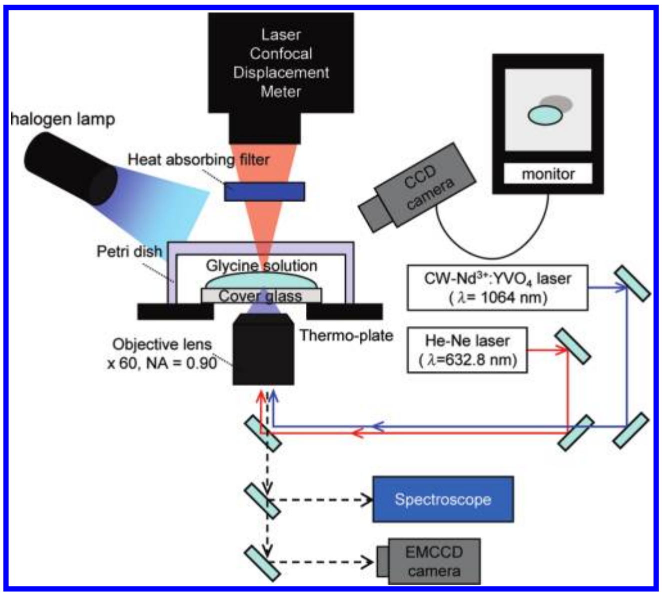


Figure 3. Experimental setup for optical trapping system coupled to backscattering and surface height measurement apparatuses and a CCD camera.

was turned on. Reflected beam patterns of the He−Ne laser were monitored using an EMCCD camera (Flovel, ADT-40C). For the backscattering measurement, the reflected He−Ne laser was measured using a spectrograph (Oriel, MS127i) with a charge-coupled device (DU420-OE). The surrounding area at the focal spot was directly observed under halogen light irradiation, and the images were captured from the upper side of a sample using another CCD camera (Flovel, HCC-600) combined with a zoom lens. For the measurement of the solution surface height during laser irradiation, a laser confocal displacement meter (Keyence, LT-9030M) was outfitted with an inverted microscope. This device has great advantages in terms of noncontact and real-time measurement of surface height, of which principle is described in ref 26. The laser beam of 670 nm from the inside of this device was adjusted to be coaxial with the trapping laser path, and the voltage data were collected by a data logger (KEYENCE, NR-500) every 50 ms. To prevent the trapping laser beam from penetrating into a light-receiving element of the device, a heat-absorbing filter equipped with a cooling system was set between the sample and the device. We confirmed experimentally that the device and He−Ne laser had no effect on the surface height change.

For sample preparation, the glycine supersaturated heavy water solution (23 wt %) was prepared similarly as in the previous report.[14] We prepared a cover glass of 22 mm square with a highly hydrophilic surface by removing some contaminations on a glass surface using plasma treatment instrument (SAKIGAKE-Semiconductor Co., A1000), and the cover glass was dipped in an alkaline detergent for 1 h. It was rewashed with distilled water just before the experiment and set on the stage of the microscope. A 40 μL portion of the glycine solution was put on the cover glass, which was immediately capped with a Petri dish to suppress solvent evaporation. The trapping laser power used in all experiments, after passing through an objective lens, was fixed at 1.1 W.

SUPPORTING INFORMATION AVAILABLE Temporal change of the reflected light intensity during the droplet formation is included. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author:

*To whom correspondence should be addressed. E-mail: sugiyama@ms.naist.jp; masuhara@masuhara.jp.

ACKNOWLEDGMENT The present work is partially supported by a KAKENHI grant (a Grant-in-Aid for Scientific Research) in the Priority Area "Strong Photon-Molecule Coupling Fields" from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) (no. 21020022) to T.S., a KAKENHI (C) grant (no. 20550136) to T.S., and a KAKENHI (S) grant (no. 18106002) to H.M. from the Japan Society for the Promotion of Science (JSPS), the MOE-ATU Project (National Chiao Tung University) of the Ministry of Education, Taiwan to H.M., and the National Science Council of Taiwan (0970027441) to H.M.

REFERENCES

(1) Ashkin, A.; Dziedzic, J. M.; Bjorkholm, J. E.; Chu, S. Observation of a Single-Beam Gradient Force Optical Trap for Dielectric Particles. *Opt. Lett.* **1986,** *11*, 288–290.

(2) Sasaki, K.; Koshioka, M.; Misawa, M.; Kitamura, N.; Masuhara, H. Laser-Scanning Micromanipulation and Spatial Patterning of Fine Particles. *Jpn. J. Appl. Phys.* **1991,** *30*, L907–L909.

(3) Ashkin, A.; Dziedzic, J. M.; Yamane, T. Optical Trapping and Manipulation of Single Cells Using Infrared Laser Beams. *Nature* **1987,** *330*, 769–771.

(4) Tsuboi, Y.; Shoji, T.; Kitamura, N. Optical Trapping of Amino Acids in Aqueous Solutions. *J. Phys. Chem. B* **2010,** *114*, 5589–5593.

(5) Singer, W.; Nieminen, T. A.; Heckenberg, N. R.; Rubinsztein-Dunlop, H. Collecting Single Molecules with Conventional Optical Tweezers. *Phys. Rev. E* **2007,** *75*, 011916-1–011916-5.

(6) Ito, S.; Yoshikawa, H.; Masuhara, H. Laser Manipulation and Fixation of Single Gold Nanoparticles in Solution at Room Temperature. *Appl. Phys. Lett.* **2002,** *80*, 482–484.

(7) Yoshikawa, H.; Matsui, T.; Masuhara, H. Reversible Assembly of Gold Nanoparticles Confined in an Optical Microcage. *Phys. Rev. E* **2004,** *70*, 061406 1–6.

(8) Borowicz, P.; Hotta, J.; Sasaki, K.; Masuhara, H. Laser-Controlled Association of Poly(*N*-vinylcarbazole) in Organic Solvents: Radiation Pressure Effect of a Focused Near-Infrared Laser Beam. *J. Phys. Chem. B* **1997,** *101*, 5900–5904.

(9) Hotta, J.; Sasaki, K.; Masuhara, H. A Single Droplet Formation from Swelled Micelles by Radiation Pressure of a Focused Infrared Laser Beam. *J. Am. Chem. Soc.* **1996,** *118*, 11968–11969.

(10) Tanaka, Y.; Yoshikawa, H.; Masuhara, H. Two-Photon Fluorescence Spectroscopy of Individually Trapped Pseudoisocyanine J-Aggregates in Aqueous Solution. *J. Phys. Chem. B* **2006,** *110*, 17906–17911.

(11) Hosokawa, C.; Yoshikawa, H.; Masuhara, H. Cluster Formation of Nanoparticles in an Optical Trap Studied by Fluorescence Correlation Spectroscopy. *Phys. Rev. E* **2005,** *72*, 021408 1–7.

(12) Osborne, M. A.; Balasubramanian, S.; Furey, W. S.; Klenerman, D. Optically Biased Diffusion of Single Molecules Studied by Confocal Fluorescence Microscopy. *J. Phys. Chem. B* **1998,** *102*, 3160–3167.

(13) Chirico, G.; Fumagalli, G.; Baldini, G. Trapped Brownian Motion in Single- and Two-Photon Excitation Fluorescence Correlation Experiments. *J. Phys. Chem. B* **2002,** *106*, 2508–2519.

(14) Sugiyama, T.; Adachi, T.; Masuhara, H. Crystallization of Glycine by Photon Pressure of a Focused CW Laser Beam. *Chem. Lett.* **2007,** *36*, 1480–1481.

(15) Chattopadhyay, S.; Erdermir, D.; Evans, J. M. B.; Ilavsky, J.; Amentisch, H.; Segre, C. U.; Myerson., A. S. SAXS Study of the Nucleation of Glycine Crystals from a Supersaturated Solution. *Cryst. Growth Des.* **2005,** *5*, 523–527.

(16) Costa, G. D.; Calatroni, J. Transient Deformation of Liquid Surfaces by Laser-Induced Thermocapillarity. *Appl. Opt.* **1979,** *18*, 233–235.

(17) Costa, G. D. Optical Visualization of the Velocity Distribution in a Laser-Induced Thermocapillary Liquid Flow. *Appl. Opt.* **1993,** *32*, 2143–2151.

(18) Bezuglyi, B. A.; Ivanova, N. A.; Zueva, A. Y. Laser-Induced Thermocapillary Deformation of a Thin Liquid Layer. *J. Appl. Mech. Tech. Phys.* **2001,** *42*, 493–496.

(19) Soto, A.; Arce, A.; Khoshkbarchi, M. K. Experimental Data and Modelling of Apparent Molar Volumes, Isentropic Compressibilities and Refractive Indices in Aqueous Solutions of Glycine + NaCl. *Biophys. Chem.* **1998,** *74*, 165–173.

(20) Vekilov, P. G. Dense Liquid Precursor for the Nucleation of Ordered Solid Phases from Solution. *Cryst. Growth Des.* **2004,** *4*, 671–685.

(21) Bonnett, P. E.; Carpenter, K. J.; Dawson, S.; Davey, R. J. Solution Crystallization via a Submerged Liquid−Liquid Phase Boundary: Oiling Out. *Chem. Commun.* **2003,** 698–699.

(22) He, G.; Tan, R. B. H.; Kenis, P. J. A.; Zukoski, C. F. Metastable States of Small-Molecule Solutions. *J. Phys. Chem. B* **2007,** *111*, 14121–14129.

(23) Louchev, O. A.; Juodkazis, S.; Murazawa, N.; Wada, S.; Misawa, H. Coupled Laser Molecular Trapping, Cluster Assembly, and Deposition Fed by Laser-Induced Marangoni Convection. *Opt. Exp.* **2008,** *16*, 5673–5680.

(24) Nabetani, Y.; Yoshikawa, H.; Crimsdale, A. C.; Müllen, K.; Masuhara, H. Effects of Optical Trapping and Liquid Surface Deformation on the Laser Microdepostion of a Polymer Assembly in Solution. *Langmuir* **2007,** *23*, 6725–6729.

(25) Rungsimanon, T.; Yuyama, K.; Sugiyama, T.; Masuhara, H.; Tohnai, N.; Miyata, M. Control of Crystal Polymorph of Glycine by Photon Pressure of a Focused Continuous Wave Near-Infrared Laser Beam. *J. Phys. Chem. Lett.* **2010,** *1*, 599–603.

(26) Takamasa, T.; Hazuku, T. Measuring Interfacial Waves on Film Flowing Down a Vertical Plate Wall in the Entry Region Using Laser Focus Displacement Meters. *Int. J. Heat Mass Transfer* **2000,** *43*, 2807–2819.