IR Laser Evaporation of Phenol Solvated with Water Molecules from Liquid Beam of Aqueous Phenol Solution

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A continuous liquid flow in a vacuum (liquid beam) of an aqueous solution of phenol was irradiated with an IR laser at 2.92 μm, and species from the liquid beam surface were ionized by a UV laser at 266 nm and mass-analyzed by a time-of-flight mass spectrometer. The mass spectra showed that the product ions were solvated phenol cluster ions, Phenol$^+$ $(H_2O)_m$ ($m = 0-4$). The mechanism of the cluster ion formation was investigated by measuring the abundances of the product ions as a function of the IR laser power, the UV laser power, and the delay time between an IR laser pulse and the subsequent UV laser pulse. It was concluded that phenol and its hydrated clusters are isolated within several microseconds from the liquid beam due to selective excitation of the solvent water molecules under irradiation of an IR laser having a fluence of less than 1100 mJ pulse^{-1} cm^{-2}.

1. Introduction

Molecules exhibit a variety of functions in solutions where the molecules cooperate with the solvent molecules in the manifestation of these functions. It is evident that any protein and DNA molecules could not function properly in aqueous solutions if the solvent water molecules do not interact with the protein and the DNA molecules. In fact, the water molecules are actively involved in forming specific structures of protein and DNA molecules, in promoting the catalytic activity of enzymes, in helping proton transfer in biological systems, and so forth. Such molecular functions in solution can be elucidated through investigating the function of each component molecule and its ensemble of solvent molecules, which can be isolated in the gas phase. To achieve this goal, we have to develop a technique to isolate a component molecule from solution into the gas phase in the first place. Thermal evaporation is one of the most popular techniques, but is not always applicable to molecules that decompose readily by heating. To overcome this difficulty, several alternative techniques have been reported for isolation of such molecules in liquid media. Electrospray is one of the most promising techniques, by which molecules are introduced into vacuum with a lower probability of decomposition, but a sizable portion of the molecules thus isolated are multiply charged so that the intrinsic properties of the molecules could not be elucidated.[1−5] Under these circumstances, various laser techniques are available to overcome this difficulty. For example, a matrix containing molecules of interest can be irradiated by a laser for isolation in the gas phase (matrix assisted laser desorption/ionization or MALDI).[6−9] Molecules in a liquid beam (continuous liquid stream in the vacuum) can also be isolated by IR-laser desorption and subsequent IR-multiphoton ionization as demonstrated by Brutschy et al. (laser-induced liquid beam ionization/desorption or LILBID).[10−15] These methods are found to be particularly suitable for isolation of biomolecules in the gas phase because of their sensitivity and of the fact that they provide species identification.

Recently, we have explored a technique for isolation of solute molecules from a liquid beam, by using resonant excitation of the solvent molecules by IR laser irradiation.[16,17] The molecules thus isolated are ionized by delayed irradiation of a UV laser. Taking advantage of this technique, we have isolated bare neutral resorcinol molecules as well as those solvated with water molecules. In this study, we have employed a 1.9-μm laser, which is resonant to the combination band of the OH stretch and the bending vibration of a water molecule. The 1.9-μm laser excitation is not efficient because of the low absorption cross section at this wavelength. We could perform more effective isolation if the fundamental vibrational mode of the solvent water molecules is excited at 2.92 μm, because the absorption cross section of the fundamental mode is much higher than that of the combination mode.

In the present study, a liquid beam of an aqueous solution of phenol was irradiated with a 2.92-μm IR laser. Species ejected into the gas phase were ionized by a UV laser at 266 nm, and then mass-analyzed by a time-of-flight mass spectrometer. Hydrated phenol clusters were found to be isolated from the liquid beam. The dynamics of the cluster isolation (ejection) was investigated by changing several important parameters, which control the ejection dynamics.

2. Experimental Section

The apparatus employed in the experiment, a liquid beam and a reflectron time-of-flight (TOF) mass spectrometer, has been described in detail previously.[18] Therefore, an overview of the apparatus is summarized here together with details relevant to the present experiment. A continuous laminar liquid flow of a 0.7-M aqueous solution of phenol was introduced into a vacuum chamber through a nozzle having an aperture with 20 μm in diameter. A constant liquid flow was supplied by a Shimadzu LC-6A pump designed for a liquid chromatograph. The flow rate was maintained at 0.2 mL/min with a pressure of typically 20 atm inside the nozzle. The source chamber was evacuated by a 1200 L s^{-1} diffusion pump and a liquid N_2 trap to achieve pressures down to $10^{-5}-10^{-6}$ Torr during injection

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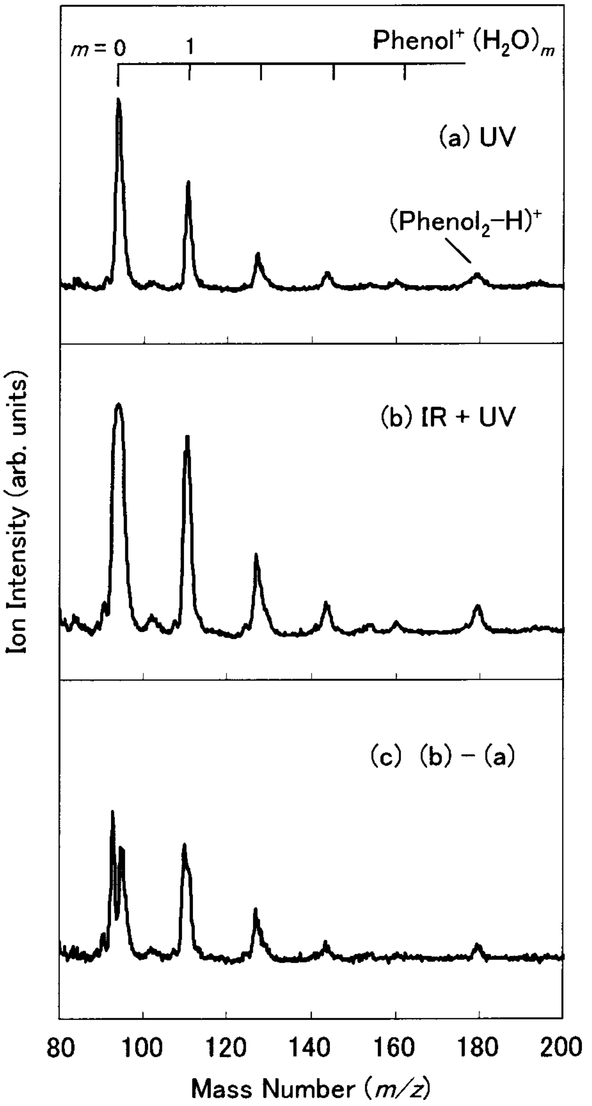


Figure 1. A mass spectrum of ions produced by irradiation of a 266 nm laser on a liquid beam of a 0.7-M aqueous solution of phenol (a), and that of ions produced by irradiation of an IR laser and subsequent irradiation of a UV laser with the delay time of 1 μs; both the lasers illuminate the same position of the liquid beam (b). A difference spectrum ((b) − (a)) is also shown (c). All the mass spectra are represented in the same intensity scale.

of the liquid beam. Commercially available phenol and water (deionized and distilled) were used without further purification as a sample solution.

After traveling a distance of 2 mm from the nozzle, the liquid beam was crossed with an IR laser (2.92 μm, 3430 cm^{-1}) and a UV laser (266 nm) in the first acceleration region of the TOF mass spectrometer. The wavelength of the IR laser is resonant to the OH stretch vibrational mode of a water molecule. The output of the IR laser was obtained by difference frequency generation between the fundamental output of a Quanta-ray GCR-3 Nd:YAG laser and the output of a Quanta-ray PDL-3 dye laser (779.5 nm pumped by the second harmonic of the Nd:YAG laser) by use of an INRAD Autotracker III with a $LiNbO_3$ crystal. The UV laser beam was the fourth harmonic from a Quanta-ray GCR-12 Nd:YAG laser. The laser power (typically ~150 and ~300 μJ/pulse for the IR and the UV lasers, respectively) was monitored by a LAS PM-200 energy meter. The IR and UV lasers were independently focused onto the liquid beam by lenses with focal lengths of 775 and 750 mm, respectively. The fluences of the IR and UV lasers were ~1100 (spot diameter of ~0.13 mm) and ~600 (spot diameter of ~0.25 mm) mJ $pulse^{-1}$ cm^{-2}, respectively.

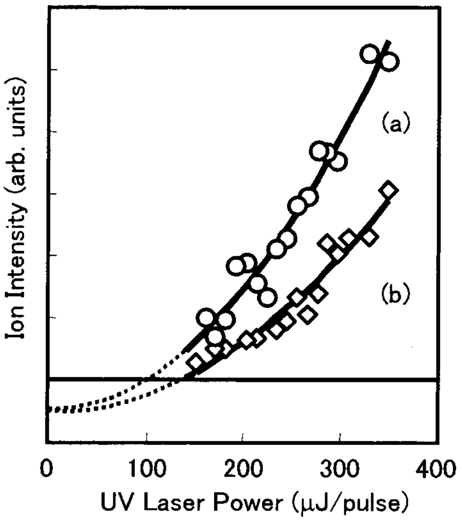


Figure 2. Dependences of the $\sum_{m=1}^{3}$ $Phenol^+ (H_2O)_m$ intensity on the power of the UV laser with (○) and without (◇) IR-laser irradiation.

The mass-to-charge ratios, m/z, of ions produced in the gas phase from the liquid beam were analyzed by the reflectron TOF mass spectrometer. Ions ejected from the liquid beam were accelerated by a pulsed electric field in the direction perpendicular to both the liquid and the laser beams after a time delay (~0.5 μs) from the photoexcitation. The ions were then steered and focused by a set of vertical and horizontal deflectors and an einzel lens. After traveling a 1.5-m field-free flight tube, the ions were reversed by the reflectron which provides a reversing field tilted by 2° off the beam axis. Trains of spatially mass-selected ions were detected after flying another 0.5-m field-free region by a Murata EMS-6081B Ceratron electron multiplier. Signals from the multiplier were amplified and processed by a Yokogawa DL 1200E digital oscilloscope based on an NEC 9801 microcomputer. The mass resolution, defined as $m/\Delta m$, was typically 200 at usual experimental conditions.

3. Results

Figure 1a shows a typical mass spectrum of ions produced by irradiation of a 266-nm laser on a liquid beam of a 0.7-M aqueous solution of phenol. Peaks in the mass spectrum are assigned to hydrated phenol cluster ions, $Phenol^+ (H_2O)_m$ ($m = 0-4$) and a dehydrogenated phenol dimer ion, $(Phenol_2-H)^+$.[18] Our previous studies demonstrated that the cluster ions are ejected from the liquid beam by Coulomb repulsion exerted from neighboring ions (Coulomb ejection model).[19-21] Figure 1b shows a typical mass spectrum of ions produced by irradiation of an IR laser (2.92 μm) and subsequent irradiation of a UV laser (266 nm) with a delay time of 1 μs; both the lasers illuminate the same position of the liquid beam. Peaks in the mass spectrum are assignable to $Phenol^+ (H_2O)_m$ ($m = 0-4$) and $(Phenol_2-H)^+$.[22] Figure 1c shows a difference spectrum (Figure 1b − Figure1a). Figures 1a−1c are represented in the same intensity scale. The intensities of the ions increase substantially by irradiation of the IR laser before the UV laser irradiation.

Figure 2 shows the dependence of the sum of the intensities of all the cluster ions containing one phenol ion, ($Phenol^+ (H_2O)_m$ ($m = 1-3$)) on the UV laser power, with (curve a) and without (curve b) irradiation of the IR laser. The dependence is

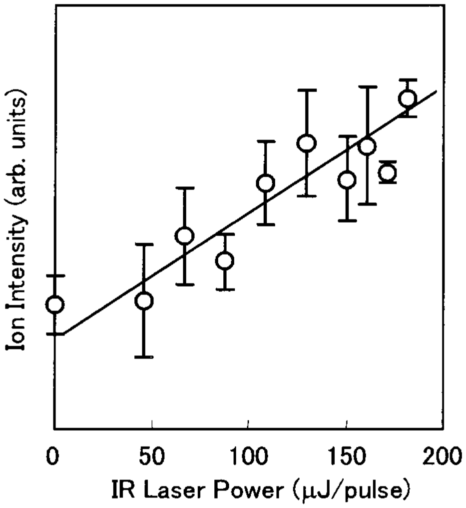


Figure 3. Intensity of $\sum_{m=1}^{3}$ Phenol$^+$ $(H_2O)_m$ produced by irradiation of IR and UV lasers as a function of the power of the IR laser.

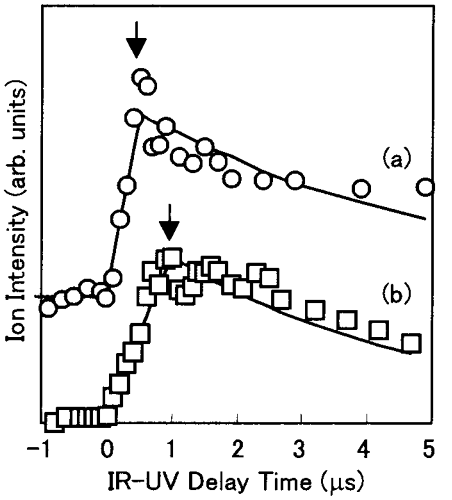


Figure 4. Dependence of intensity of $\sum_{m=1}^{3}$ Phenol$^+$ $(H_2O)_m$ on the delay time from irradiation of the IR laser to that of the UV laser.

reproduced by the following formula:

$$N = \alpha I^2 - \beta \tag{1}$$

where N and I represent the intensity of $\sum_{m=1}^{3}$ Phenol$^+$ $(H_2O)_m$ and the power of the UV laser, respectively, and α and β are constants. Note that β derived from curve a is found to be approximately equal to that from curve b.

Figure 3 shows N as a function of the power of the IR laser. The intensity increases almost linearly with the increase in the IR laser power. Figure 4 shows the dependence of N on the delay time from irradiation of the IR laser to that of the UV laser. The spot diameters of the UV laser for obtaining curves a and b are ~250 and ~500 μm, respectively. The intensity increases, reaches a maximum, and then gradually decreases with increasing delay time. The peak maximum of curve a appears at the delay time of ~0.5 μs, while that of curve b appears at ~1 μs. Throughout the measurements, the UV laser was less tightly focused than the IR laser.

4. Discussion

4.1. Cluster Ejection from Liquid Beam Surface. As shown in Figure 1, IR-laser irradiation onto the liquid beam prior to UV-laser ionization causes the intensities of Phenol$^+$ $(H_2O)_m$ ($m = 1-3$) to increase. The intensity increment is attributed to resonant vibrational excitation (OH stretch vibrations) of the solvent water molecules. Clusters are ionized in the gas phase by multiphoton excitation under irradiation of the UV laser, as shown by the hyperlinear dependence of the ion intensity on the UV-laser power (see Figure 2).

Mere UV-laser irradiation produces cluster ions in the gas phase (Coulomb ejection model).[19−21] Briefly, the mechanism involves UV laser ionization of chromophore molecules in the liquid beam, producing ions and electrons. As electrons in the vicinity of the liquid beam surface (<2 nm) escape into the vacuum, an electron-depleted region is formed in the vicinity of the liquid beam surface. Then, an ion in the surface region is ejected into the vacuum, with several accompanying solvent molecules (provided that the Coulomb repulsion energy built by its neighboring ions exceeds the solvation energy of the ion). This model predicts the presence of a threshold power of the UV laser, below which no ions are ejected because of insufficient Coulomb repulsion energy. If this model is true, only a part of the ions produced in the liquid beam ought to be ejected into the vacuum. Actually, we have observed that a sizable fraction of the ions remains inside the liquid beam.[21] The model shows that the number (defined as "abundance") of ions ejected into the vacuum, N_{UV}, is given by

$$N_{UV} = aI^n - b \tag{2}$$

where I is a power of the UV laser and n is the number of photons involved in the multiphoton ionization of the molecule (we found $n = 2$), and a and b are parameters. The term, aI^n, represents the number of ions produced by the multiphoton ionization in the liquid beam, and the parameter, b, represents the number of ions remaining in the liquid beam after ejection of ions from the liquid beam surface into the vacuum. Under irradiation of a weak laser ($aI^n < b$), no ions are ejected from the liquid beam because sufficient Coulomb repulsion energy is not provided by the neighboring ions.[21]

On the other hand, neutral clusters and molecules are ejected in the vacuum by irradiation of the IR laser, and are subjected to photoionization by the UV laser. Therefore, the abundance, N_{IR}, of ions which are produced by photoionization of the neutral clusters is given by

$$N_{IR} = cI^n \tag{3}$$

where c is a parameter. Under irradiation of the IR and UV lasers at the same spot, the abundance of the ions, N_{IR+UV}, produced in the gas phase turns out to be

$$N_{IR+UV} = N_{UV} + N_{IR} = (a + c)I^n - b \tag{4}$$

Figure 2 shows the least-squares fitting of the observed laser-power dependences by using eqs 2 and 4 (see solid curves); the calculation reproduces the observed dependences well. Note that both the solid curves give approximately the same intercept with the ordinate. This finding leads us to conclude that hydrated phenol clusters are ejected by irradiation of the IR laser and then ionized by the UV laser.

4.2. Mechanism of Cluster Ejection. As described in the Section 4.1, clusters are ejected from the liquid beam surface by irradiation of the IR laser. As the clusters travel in the gas phase, they evaporate their solvent molecules so as to release their internal energy gained from the incident IR laser. Let us consider how much energy the clusters gain. Assuming that the velocity of a nascent cluster, v, is given as the root-mean-square value, one obtains

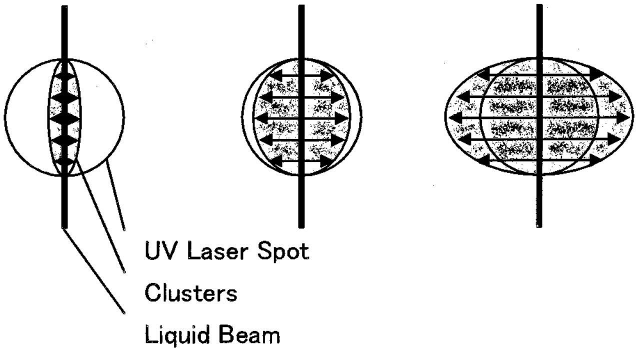
$$v = \left(\frac{3k_BT}{m}\right)^{1/2} \tag{5}$$

where m is the mass of the nascent cluster, k_B is the Boltzmann constant, and T is the internal temperature of the cluster. The nascent cluster is likely to contain one phenol molecule solvated with 4 water molecules, i.e., Phenol $(H_2O)_4$, because the largest cluster observed in the mass spectrum is this cluster. The velocity, v, is measured as ~250 m/s as mentioned in Section 4.3. If this is the case, the internal temperature of the nascent cluster turns out to be 419 K by using eq 5. The liquid beam surface is cooled to ~250 K because of evaporation,[23,24] at the spot where the laser is irradiated. It follows that the temperature rise of the solution amounts to ~169 K and the spot diameter should be ~0.13 mm if the energy of the irradiated IR laser is fully converted into the internal energy of the solution.[25] The spot diameter thus estimated is a likely value in the present experimental condition. Although the average temperature amounts to 419 K (250 + 169 K), the cluster ejection is likely not to be simply a thermal phenomenon, because the IR laser excites the liquid beam locally and instantaneously. Local solvent sites in the liquid beam are superheated by selective excitation of internal modes related to the OH stretching vibration of solvent water molecules by the IR laser irradiation. The superheated small local sites thus produced gasify rapidly, and as a result hydrated clusters remain in the vacuum. In this dynamic picture, the abundance of the clusters left in the vacuum increases proportionally with the increase in the IR laser power, since the number of the local sites increases proportionally to the IR laser fluence introduced if the superheated local sites possess always the same diameter irrespective of the laser fluence. Actually, the abundance of the clusters increases linearly with the IR laser fluence (see Figure 3). The clusters are neither produced by laser ablation nor thermal evaporation, because there is no threshold value in the laser fluence for production of clusters (laser ablation) and the abundance does not increase super-linearly with the laser fluence (thermal evaporation),[26] as shown in Figure 3. Note that a process, by which cold and fast products are generated,[16,17] is not observed probably because of a relatively low fluence of the laser employed.

4.3. Time Evolution of Ejected Clusters. As shown in Figure 4, the intensity of the cluster ions increases, reaches a maximum, and then gradually decreases, as the IR−UV delay time increases. The maxima of curves a and b appear at the delay time of ~0.5 μs and ~1 μs, respectively; curve b is obtained with the UV laser less tightly focused. The difference in the delay time arises from the difference in the time needed for the clusters produced by the IR laser to reach the edges of the UV-laser spots. The spot diameter of the UV laser (~250 or ~500 μm) is larger than that of the liquid beam (20 μm). The geometries of the liquid and the laser beams are shown in Scheme 1.

Let us consider more quantitatively a time evolution of the cluster ejected by irradiation of a pulse IR laser. According to the cluster ejection model discussed in Section 4.2., the abundance of the clusters increases rapidly and reaches a stationary value, but decreases slowly after the laser pulse is off, because the superheated region is being depleted with time by diffusion and is cooled by evaporation, as well. In practice, the abundance is measured by the ionization with the UV laser at a Gaussian spot (radius R) whose center coincides with the liquid beam center. The rate of the cluster ejection, r, is assumed to decrease exponentially with the IR − UV delay time, t, as

SCHEME 1



$$r = r_0 e^{-\lambda t} \tag{6}$$

where r_0 represents the initial rate of the cluster ejection at $t = 0$ and λ is the decay rate of the cluster ejection rate with time. The parameter λ is related to the evaporative cooling. Assuming that the clusters travel at a velocity, v, toward the edge of the ionization spot, they reach the edge at $t = R/v$ when the ion abundance reaches its maximum (see Figure 4). The abundance of the ejected clusters, $A(t)$, at time t is expressed as

$$A(t) = \int_0^t r_0 e^{-\lambda\tau}\, d\tau = \frac{r_0}{\lambda}(1 - e^{-\lambda t}) \tag{7}$$

in the time range of $0 < t < (R/v)$, in which the clusters are within the UV laser spot. When the clusters expand outside the spot of the UV laser ($t < (R/v)$), the abundance of the clusters in the UV laser spot turns out to be

$$A(t) = \int_{t-(R/v)}^{t} r_0 e^{-\lambda\tau}\, d\tau = \frac{r_0}{\lambda}(e^{-\lambda(t-(R/v))} - e^{-\lambda t}) \tag{8}$$

in the time range of $t > (R/v)$. The intensity of a given cluster ion is proportional to the abundance of the corresponding neutral cluster on the assumption that detection efficiency of every cluster ion is equal. The time dependences of the intensities of the cluster ions (see solid lines in Figure 4) are predicted by eqs 7 and 8 by setting $t_{max} = (R/v)$ with leaving r_0 and λ as the fitting parameters, where t_{max} represents the IR−UV delay time giving the maximum ion intensity. The radius of the ionization region by the UV laser, R, is estimated as follows: At first, the diameter of the UV-irradiation region where Coulomb ejection occurs on the liquid beam surface, was determined by measuring the intensity of a cluster ion with changing the spatial position of the laser spot. The diameter was determined to be ~0.05 mm (fwhm). This region must be smaller than the region in which UV-laser ionization actually occurs, because an ion produced in the liquid beam must be concentrated sufficiently enough to supersede the solvation energy of the ion. In our previous study, the region that ionization actually occurs was measured by observing the abundances of all the ions ejected outside and left inside the liquid beam together. The diameter was found to be ~0.25 mm, in comparison with the diameter of ~0.05 mm (fwhm), which was measured simultaneously by observing the abundance of the ions ejected outside. We conclude, therefore, that the diameter of the ionization region or the spot diameter of the UV laser is ~0.25 mm (= $2R$). The velocity of the clusters, v, is then calculated to be ~250 m/s by use of the measured value of $R/v = 0.5$ μs.

5. Conclusion

We have demonstrated isolation of solute molecules from a liquid beam of a solution by resonant vibrational excitation of the solvent molecules. The clusters thus isolated are ionized by a UV laser, and the cluster ions produced are identified mass-spectroscopically. In the present study, we applied this technique to isolation of phenol molecules from an aqueous solution of phenol by using an IR laser (2.92 μm) and a UV laser (266 nm), and showed that phenol and its hydrated clusters are isolated from the aqueous solution of phenol. This technique is particularly suitable for isolation of biological molecules from their aqueous solutions.

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$$V = 2\pi l^2 R \tag{a}$$

where l is the radius of the liquid beam (10 μm) and R is the radius of the IR laser spot. As the cross section of the IR laser is circular, only a part of the incident IR laser is absorbed to the liquid beam as

$$I_{\text{eff}} = \frac{4lR}{\pi R^2} I \tag{b}$$

where I and I_{eff} are the power of the incident IR laser and that actually absorbed to the liquid beam, respectively. The temperature rise, ΔT, is then calculated as

$$\Delta T = \frac{I_{\text{eff}}}{CV} \tag{c}$$

where C is the specific heat of water. Equations a−c give $R = 0.065$ mm when $\Delta T = 169$ K and $I = 150$ μJ.

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