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Creating line defects with a single micro-sphere width in a DNA-linked 2D colloidal crystal array using a pulsed laser

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Abstract A creation of micron-size narrow line defects in 2D colloidal crystal array was successfully accomplished using a focused pulsed laser. The 2D colloidal crystal arrays were obtained by a convective assembly of polystyrene latex micro-spheres (1.8 μm) and then immobilized onto the glass substrate by DNA hybridization process. To release a micro-sphere from the crystal, a focused laser beam was utilized to de-hybridize the DNA by raising the temperature above its melting point. From previous studies (Geiss E, Fabrication and defect design in two-dimensional colloidal photonic crystals. Ph.D dissertation thesis, 2003; Kim et al., Mater Sci Eng 26:1401–1407, 2006; Geiss et al., sudmitted), using a continuous argon ion laser, 514 nm, line-writing in colloidal crystal with 2–4 micro-sphere widths were achieved. In this study using the Nd:YAG pulsed laser's second harmonic, 532 nm, line-writing of 1 micro-sphere width was accomplished. The presence of EB (ethidium bromide) dye that intercalates into the ds-DNA strands that bind the micro-spheres to the substrate facilitated the micro-sphere releasing process by reducing the laser energy required to release a micro-sphere. Factors that influenced controlled defect insertion were the medium containing the colloidal crystal, the dyes imposed on the crystal and the self-assembled micro-sphere groups. The most effective medium condition were determined at 1 mM global EB dye in 0.1 M NaCl for removing an individual micro-sphere in single micro-spheres, groups of micro-spheres and the close-packed arrays. Single micro-sphere width simple curves and corners in the photonic crystals were then created.

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Introduction

Photonic crystals are periodic ordered arrays of two materials with large differences in their refractive indices. When the lattice distance is on the order of magnitude of the wavelength of light, the forward propagation of photons traveling into the crystal destructively interferes with the back-reflected photons [1]. This restriction of the propagation of the photons over a narrow range of wavelengths is referred to as a photonic band gap [2]. Such a unique property characteristic expects various applications such as optical computing, optical telecommunication, low-loss waveguides, optical filters and sensors [3–6]. Among a variety of methodologies to realize photonic bandgap structures, the colloidal crystallization has been widely studied due to its simple and inexpensive process [7–12]. Since the refractive index contrast of typical colloidal crystals is not enough for a full photonic band gap, the colloidal crystal as a template structure is further fabricated to form an inverse opal to realize a full photonic band gap [13–16]. In addition, many optical device applications of photonic crystal require inserting controlled defects in the photonic crystal, because defect line in the crystal can create a waveguide which can channel the prohibited wavelengths into the crystal and around tight corners or bends in the crystal [17–23]. The layer-by-layer

construction of lithographically fabricated PBG structures [24–26], multiphoton polymerization [27], focused polymerization within opaline-based photonic crystals [17, 18], and lithographic insertion of waveguides between 3D opaline arrays demonstrated the localization of controlled defects in the photonic bandgap structures [19, 21, 22].

In our previous study, we performed insertion of predetermined defects on 2D opaline colloidal crystals by utilizing an argon ion continuous laser [28–31]. The individual polystyrene micro-spheres, which were pre-bound to glass substrate by DNA hybridization, were released by a focused laser beam. The average line width of controlled defects were limited to 2–4 microspheres, due to the laser beam waist size (2.2 μm) and thermal heat transfer to neighboring particles during laser radiation (typically 1 min.). As an extension of that study, in this article, we demonstrate more precise control of microsphere release by using the Nd:YAG pulsed laser along with the spatial filter, where we could create line defects of single-microsphere width (1.8 μm) in 2D opaline photonic crystal arrays. Using the 532 nm second harmonic of the Nd:YAG pulsed laser, a single 10-ns pulse of energy was used to minimize the energy dispersion to the adjacent microspheres. This results in greater control to allow the release of single microspheres in the photonic crystal. We further studied the effects of local heat absorption in different dye-containing aqueous medium on the removal of microspheres photonic crystal. This allowed determination of the optimum conditions to achieve single-microsphere widths in line writing in the 2D photonic crystals.

Experimental

Preparation of 2D colloidal opaline arrays immobilized on the substrate by DNA hybridization

All chemical reagents were purchased from Aldrich and used without further purification. Polystyrene (PS) colloidal microspheres with 1.8 μm diameter and polydispersity of 1.04 were procured from IDC (Interfacial Dynamics Corporation) while DNA oligomers were purchased from IDT (Integrated DNA Technologies Inc.). DNA bound 2D colloidal opaline arrays were prepared as described in our previous studies [31]. Ethidium bromide dye (molecular biology grade) was procured from Sigma. The extinction coefficient of ethidium bromide at 532 nm was determined as $1.9 \times 10^3\ M^{-1}\ cm^{-1}$ by using UV-Vis spectrometer.

DNA functionalization of colloidal microspheres

About 1 mL of bifunctional (carboxyl/sulfate) PS colloids (4 wt % in water) were washed in triplicate with 10 mM MES buffer (pH 7.0), precipitated by centrifugation (5 min at 4,000 rpm) and the supernatant was removed carefully before redispersing the colloid with vortexing and/or mild sonication. The colloids were then mixed with 5 mL *ss*-DNA solution (10 μM) of sequence **B** (5′-TCT CAA CTC GTA $T_{12}(CH_2)_7NH_2$-3′) in the presence of EDC (ethyldimethyl aminocarbodiimide hydrochloride, 26 mM) and NHSS (*N*–hydroxy sulfone succinimide, 23 mM) and then gently stirred for 24 h at room temperature. Following DNA functionalization, the colloidal emulsion was dialysed against 1.0 mM MES buffer (pH 7.0) for 48 h and its concentration was adjusted to approximately 1 wt % in PS microspheres by further addition of the same buffer. Subsequently, solid SDS (sodium dodecyl sulfate) was added to the colloid to result in 3 mM SDS concentration.

DNA functionalization on glass substrates

A circular type glass slides (22 mm diameter, Fisher Scientific) substrates were cleaned with piranha solution (H_2SO_4: H_2O_2 = 7:3) and then rinsed with deionized water. The substrates were then silanized with a 5% v/v VCS (vinyldimethylchlorosilane, Gelest) solution in toluene for 6 h. Following silanization, the substrates were rinsed with toluene, methanol, and dried at 110 °C for 30 min. The substrates were then immersed in a freshly-made solution of $KMnO_4$, KIO_3, and $NaHCO_3$ (with molarity of 0.5 mM, 19.5 mM, and 1.8 mM, respectively) for 48 h. Subsequently, the substrates were rinsed with an aqueous solution of $NaHSO_3$ (0.3 M), deionized water, 0.1 N HCl, deionized water and ethanol, and each substrate was reacted with 0.5 mL of *ss*-DNA solution (10 μM) of sequence **A** (5′-$NH_2(CH_2)_6T_{12}$ CGC ATT CAG GAT) in the presence of EDC (50 mM) and NHSS (50 mM) for 12 h. Following DNA functionalization, the substrates were washed with deionized water and dried in N_2.

2D opaline assembly

50 μL of **B**-*ss*-DNA-functionalized PS colloidal microspheres were mixed with 5 μL of *ss*-DNA solution (100 μM) of sequence **A′B′** (3′-GCG TAA GTC CTA AGA GTT GAG CAT-5′). The resulting colloids were deposited onto **A**-*ss*-DNA functionalized substrates and dried slowly in an enclosed environment at 25 °C for 12 h under slightly tilted substrate geometry to yield two dimensional (2D) opaline arrays.

Annealing microsphere array

Upon complete drying, the 2D opaline arrays were immersed into 5 mL of saline MES buffer solution (0.1 M

NaCl, 10 mM MES) containing 50 μL of excess **A′B′**-*ss*-DNA solution (100 μM), gently heated to 60 °C for 1 hr, and then allowed to cool to 30 °C over a period of 24 h.

Stage cell setup of 2D photonic crystal

The stage cell included the sample slide with the colloidal crystal arrays on it which was sealed in an aqueous medium by a Teflon ring (17 mm inner diameter, 22 mm outer diameter, 0.125 mm thick) and closed with a glass cover slip (Fisher Scientific 22 mm diameter, 0.225 mm thick). The focused beam was positioned directly below the microspheres on the sample slide such that a single-pulse would melt the DNA associated with that microsphere bound to the substrate. Neutral density filters (Edmund Optics) were introduced into the beam line to control the intensity of the pulsed beam. In addition to varying the beam intensity, the influence of various aqueous medium on controlled release were investigated.

Optical system design

The optical design of the system for the pulsed laser consisted of four major components as shown in Fig. 1. The Nd:YAG pulsed laser (Continuum Surelite II) emits a primary beam at 1,064 nm. By using a beam harmonic separator (CVI Lasers BSR-51-1025), the second harmonic at 532 nm can be reflected off the optic and the primary beam transmitted to a beam dump. The initial beam size of the pulsed laser is 7 mm, which is focused down to 2 mm by using a Galilean beam reducer system. For the pulsed laser system, the beam profile is non-Gaussian with multiple TEM (transverse electromagnetic) modes [32]. To obtain the central single mode in the pulsed laser system, a spatial filter with 250 μm pinhole was used in the optical design to obtain a Gaussian shaped, focused beam [33]. A 100x objective lens (Mitutoyo Plan M, working distance = 6 mm, NA = 0.70) was used to focus the beam to about 2 μm onto the sample on the stage cell. An optical microscope equipped with CCD camera (SONY XC-75) was located above the sample stage, used for aligning the beam on the microspheres and observing the microsphere release from a single 10 ns laser pulse.

Results and discussion

Study of aqueous medium conditions for 2D photonic crystal

Since the initial immobilization of the PS microspheres on the substrate was done under 0.1 M NaCl solution ($n \sim 1.34$), it was used as the control condition. As the laser beam passes through the ordered PS microspheres ($n \sim 1.57$) in aqueous medium, a significant amount of light spreads by scattering/diffraction at the interface of particle and solution, which may cause a massive release of several tens of microspheres [28, 29]. To reduce this undesirable scattering/diffraction effect, a refractive matching medium with 6 M KI, $n \sim 1.43$, was also used. Ethidium bromide (EB) dye was also used to enhance beam absorption at 532 nm. EB dye intercalates within the hybridized DNA at every fourth base pair greatly enhancing the adsorption of the 532 nm laser beam within the DNA effectively reducing the beam energy required to melt the DNA bond for microsphere release [28–30]. Two methods of introducing the EB dye into the DNA are defined as local and global EB dye. In the case of the local EB dye, the sample slide was immersed in the dye solution to allow the dye to penetrate the hybridized DNA which was then flushed with either 0.1 M NaCl or 6 M KI. Through these steps, the EB dye remained localized at the hybridized DNA such that enhanced absorption occurred only in the DNA. For global EB dye, the dye was dispersed throughout the medium (0.1 M NaCl, 6 M KI) such that enhanced absorption occurs primarily in the DNA but also everywhere in the medium.

Study of isolated microsphere groups and close-packed arrays

In an effort to establish the beam energy required to release the PS microspheres from the substrate, isolated microsphere groups of single, double, and triple were examined first. From these isolated groups, a trend in the release energy was determined to establish the required energy to release a microsphere in close-packed array. Examples of single, double, and triple microspheres are shown in Fig. 2a. An example of close-packed 2D colloidal crystal is shown in Fig. 2b.

Study of controlled versus uncontrolled release for isolated microsphere groups and close-packed arrays

Controlled microsphere release was defined as the release of the targeted microspheres without disturbing neighboring microspheres. In uncontrolled release, not only the targeted microsphere but also some neighboring microspheres will release. The following are examples of controlled vs. uncontrolled release in isolated microsphere groups and close-packed array. In Fig. 3, the pulsed laser is targeting at the circled microsphere. The boxed microspheres are surrounding microspheres disturbed after a

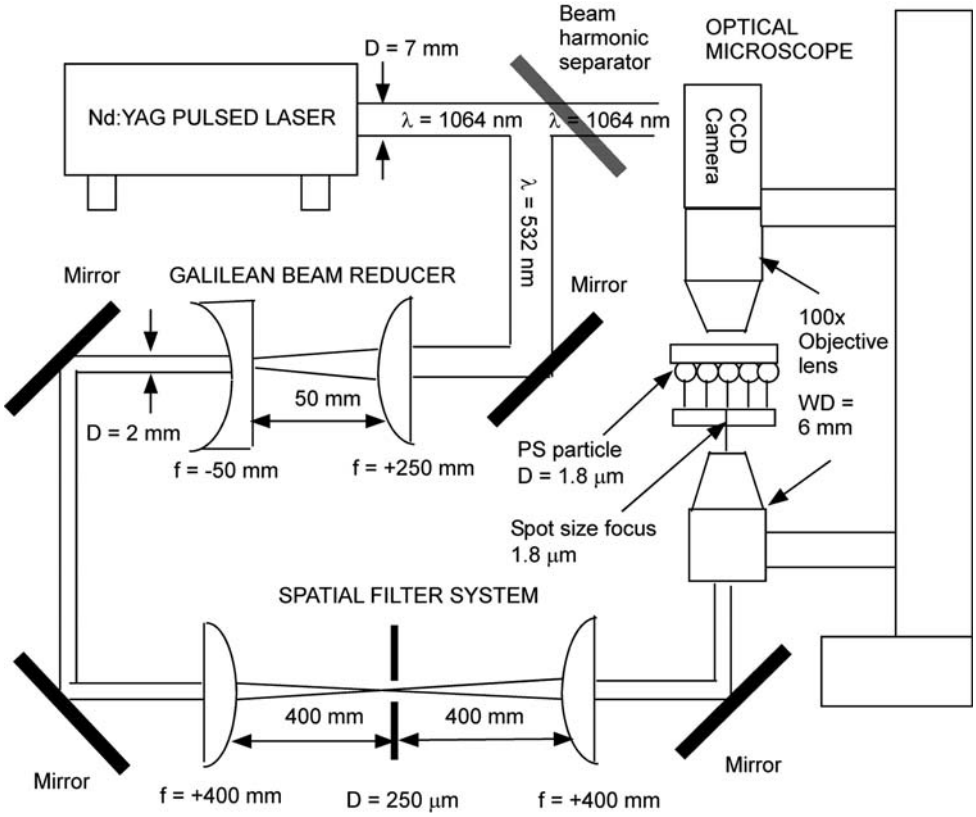

Fig. 1 Optical design setup of pulsed laser system for micro irradiation on DNA-linked PS microsphere opaline arrays

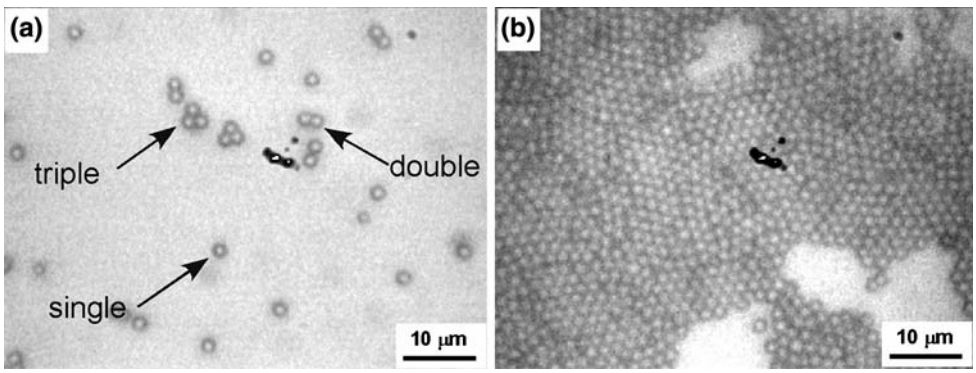

Fig. 2 Optical micrographs of polystyrene microspheres (1.8 μm diameter) linked on glass substrate by DNA in 0.1 M NaCl; (**a**) examples of isolated microsphere groups (single, double, and triple microspheres); (**b**) example of close-packed array 2D photonic crystal

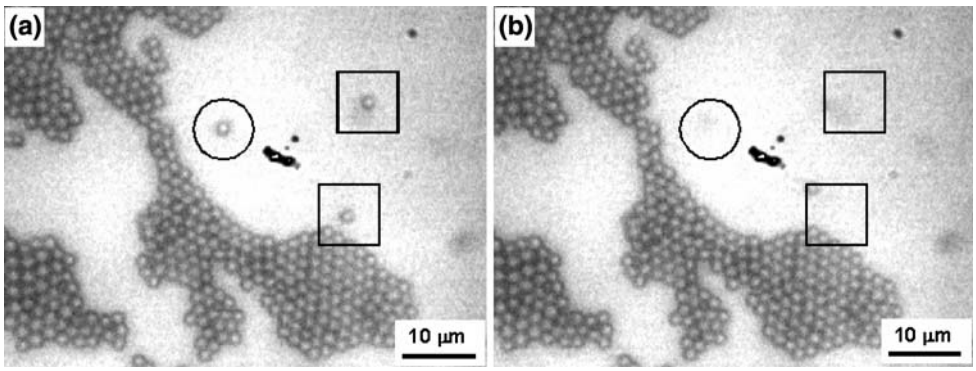

Fig. 3 Optical micrographs of uncontrolled single microsphere release in 0.1 M NaCl in 1 mM EB dye; (**a**) before single pulse, beam is targeted on circled microsphere surrounded by neighboring, boxed microspheres; (**b**) after single pulse, targeted microsphere is released and boxed microsphere is disturbed as well

single pulse. This is an example of uncontrolled release where the laser pulse releases not only the targeted microspheres but also nearby microspheres. In Fig. 4, notice that only the circled microsphere is released after a single pulse and there is no disturbance to the surrounding microspheres. For the double and triple microspheres,

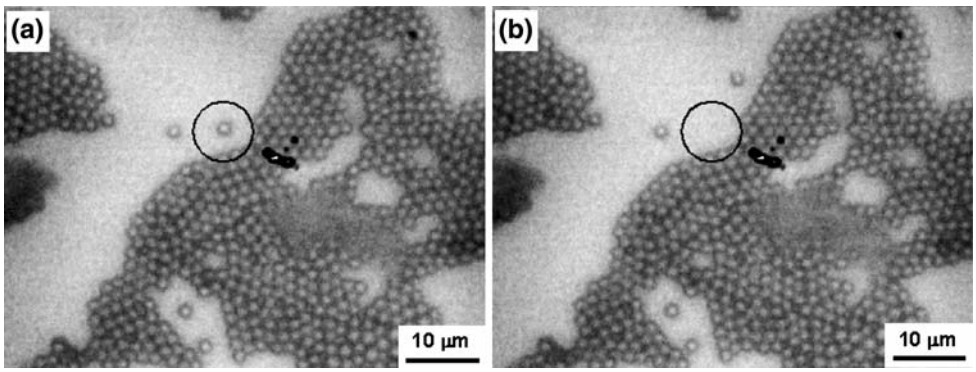

Fig. 4 Optical micrographs of controlled single microsphere release in 0.1 M NaCl in 1 mM EB dye; (**a**) before single pulse, beam is targeted on the circled microsphere; (**b**) after single pulse, targeted microsphere is released and neighboring microsphere is undisturbed

success in controlled release occurred when a target single microsphere among the group of microspheres was released without disturbing the adjacent microspheres in the isolated group (Figs. 5 and 6). Any disturbance to isolated groups and/or surrounding microspheres resulted in uncontrolled release (Figs. 7 and 8). For the case of close-packed array, controlled release was observed when only a single microsphere released in the array (Fig. 9). In uncontrolled release, massive release of microspheres resulted after a single laser pulse (Fig. 10).

For isolated microsphere groups and close-packed array, five trials at each energy level were conducted for a range of single-pulsed energies from the critical lower to the upper limit of beam energy. The critical lower limit was defined to be the single-pulsed energy at which all trials resulted in no microsphere release whereas upper limit occurred when all trials resulted in microsphere release. The medium conditions studied in the experiments were the following: (1) 0.1 M NaCl, (2) 1 mM local EB dye in 0.1 M NaCl, (3) 1 mM global EB dye in 0.1 M NaCl, (4) 0.1 mM global EB dye in 0.1 M NaCl, (5) 6 M KI, (6) 0.1 mM local EB dye in 6 M KI, and (7) 0.1 mM global EB dye in 6 M KI. In the isolated microsphere groups for a given medium, there was no trend in the release energies of the single, double, and triple. The results are presented as the fraction of microspheres release for all isolated microsphere groups averaged together and a sigmoidal curve was fit to the data. From the curve fit to the data, the release energy was defined to be the single-pulsed energy at which 50% of the trials resulted in microsphere release. For the isolated groups in 1 mM local EB dye in 0.1 M NaCl, the

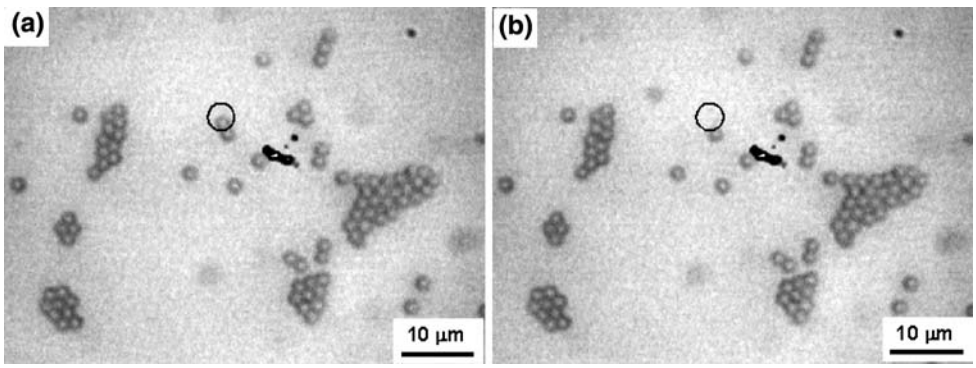

Fig. 5 Optical micrographs of controlled double microspheres release in 0.1 M NaCl in 1 mM EB dye; (**a**) before single pulse, beam is targeted on the circled microsphere; (**b**) after single pulse, targeted microsphere is released and adjacent microsphere is undisturbed

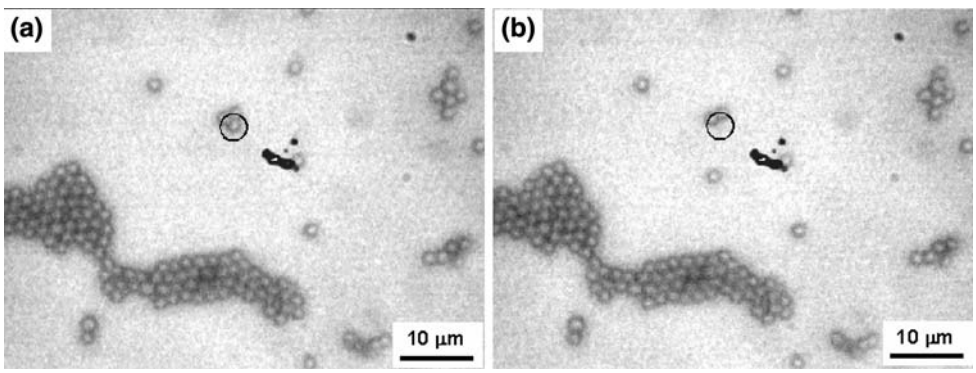

Fig. 6 Optical micrograph of controlled triple microspheres release in 0.1 M NaCl in 1 mM EB dye; (**a**) before single pulse, beam is targeted on the circled microsphere; (**b**) after single pulse, targeted microsphere is released and adjacent microspheres are undisturbed

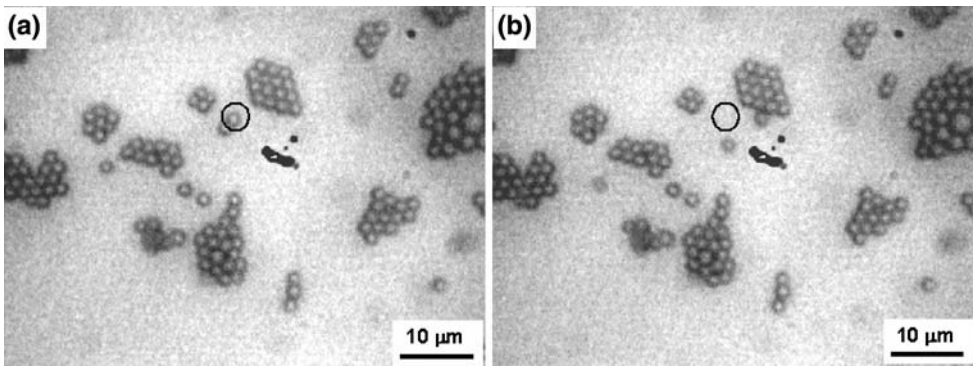

Fig. 7 Optical micrographs of uncontrolled double microspheres release in 0.1 M NaCl in 1 mM EB dye; (**a**) before single pulse, beam is targeted on the circled microsphere; (**b**) after single pulse, targeted microsphere is released and adjacent microsphere is also disturbed

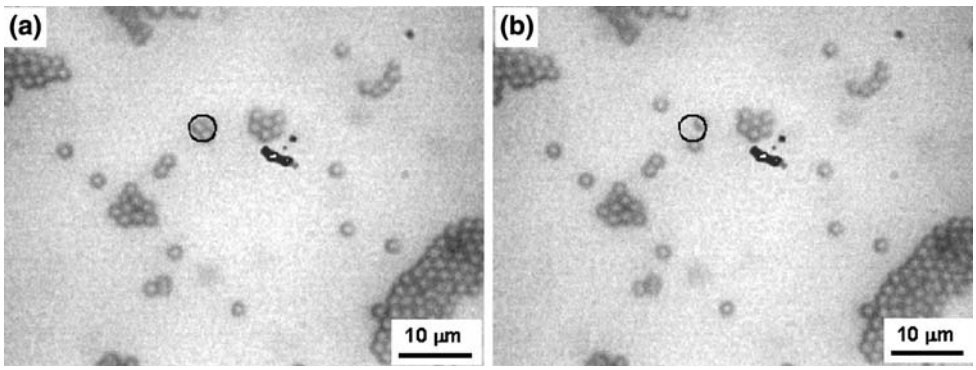

Fig. 8 Optical micrographs of uncontrolled triple microspheres release in 0.1 M NaCl in 1 mM EB dye; (**a**) before single pulse, beam is targeted on the circled microsphere; (**b**) after single pulse, targeted microsphere is released and adjacent microspheres are disturbed

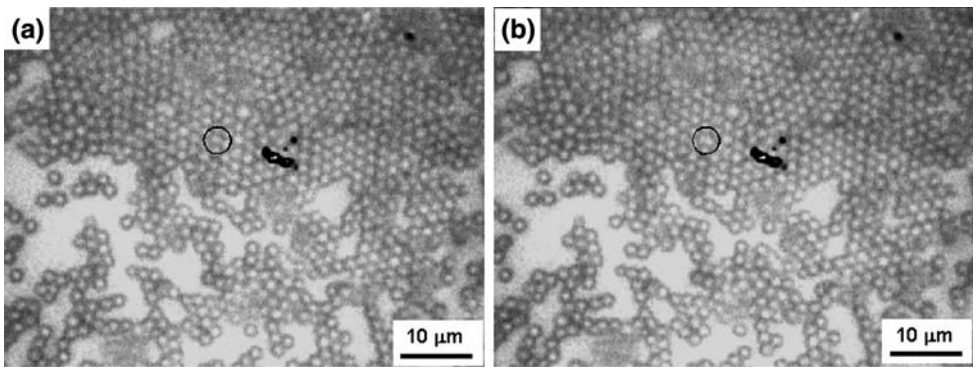

Fig. 9 Optical micrograph of controlled released in close-packed cluster in 0.1 M NaCl in 1 mM EB dye; (**a**) before single pulse, beam is targeted on circled microsphere; (**b**) after single pulse, only circled microsphere is release without massive release in close-packed cluster

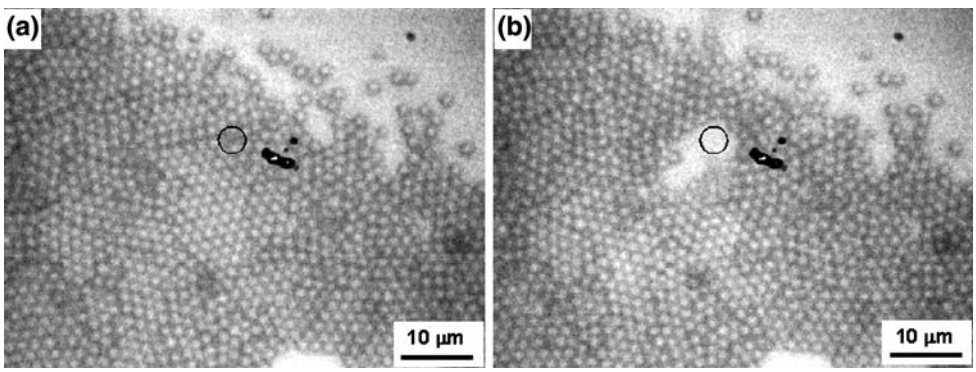

Fig. 10 Optical micrograph of uncontrolled release in close-packed cluster in 0.1 M NaCl in 1 mM EB dye; (**a**) before single pulse, beam is targeted on the circled microsphere; (**b**) after single pulse, massive release of microspheres from the large void of array

release energy is about 0.25 μJ (Fig. 11a). For the close-packed array in same medium condition, the release energy is almost 0.35 μJ (Fig. 11b).

The presence of the EB dye lowered the beam release energy by almost an order of magnitude for both 0.1 M NaCl and 6 M KI environments as shown in Table 1. There

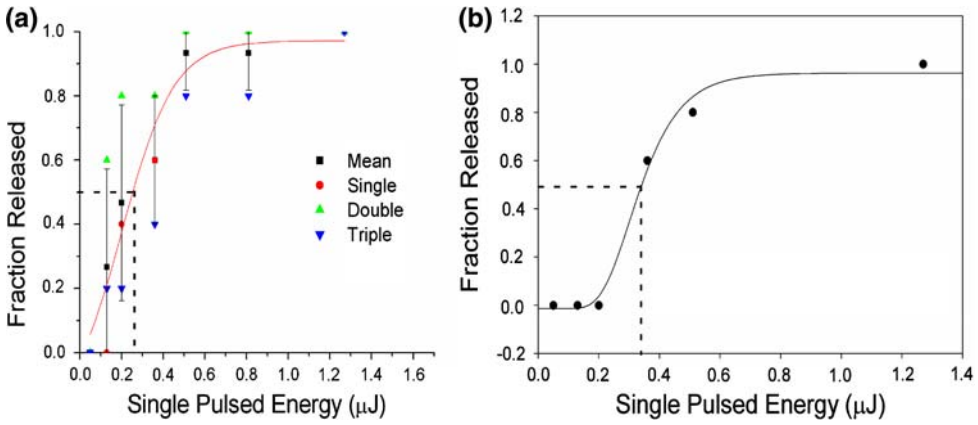

Fig. 11 Sigmoidal fit to fraction released versus single pulsed energy for 12-bp DNA-PS Sample in 1 mM local EB in 0.1 M NaCl; (**a**) single microsphere ●, double microspheres ▲, triple microspheres ▼, mean of single, double, and triple microspheres ■; (**b**) close-packed array ●

Table 1 Release energy for average isolated microspheres and close-packed microspheres at 0.5 fraction released for 5 trial run

	0.1 M NaCl (μJ)	1 mM global EB in 0.1 M NaCl (μJ)	1 mM local EB in 0.1 M NaCl (μJ)	0.1 mM global EB in 0.1 M NaCl (μJ)	6 M KI (μJ)	0.1 mM global EB in 6 M KI (μJ)	0.1 mM local EB in 6 M KI (μJ)
Average isolated microspheres	2.1	0.2	0.25	0.5	1.6	0.5	0.4
Close-packed microspheres	1.1	0.2	0.35	0.3	1.1	0.4	0.4

Table 2 Fraction of controlled release from all trials resulting in microsphere release for isolated microsphere groups and close-packed array

	0.1 M NaCl	1 mM global EB in 0.1 M NaCl	1 mM local EB in 0.1 M NaCl	0.1 mM global EB in 0.1 M NaCl	6 M KI	0.1 mM global EB in 6 M KI	0.1 mM local EB in 6 M KI
Single microsphere	0.07	1.00	0.53	0.67	0.08	1.00	0.09
Double microspheres	0.07	0.82	0.54	0.44	0.00	0.67	0.10
Triple microspheres	0.00	0.60	0.56	0.39	0.00	0.32	0.28
Close-packed microspheres	0.00	0.67	0.25	0.68	0.00	0.25	0.17

are small differences in beam release energy between the global and local EB dye in either the 0.1 M NaCl and 6 M KI aqueous solutions. For the 0.1 mM global EB dye in 0.1 M NaCl, the release energies for both average isolated microspheres and close-packed microspheres are comparable to the energies measured for the 0.1 mM global EB in 6 M KI. The laser beam release energy for 0.1 mM global EB dye in 0.1 M NaCl is greater than 1 mM EB global dye in 0.1 M NaCl.

The fractions of controlled release shown in Table 2 is the ratio of the number of trials with controlled single microsphere release over the total number of trials that resulted in either single microsphere or multiple microsphere release. EB dye provided not only reduction of laser energy, but also an increased probability for controlled single microsphere release in both the 0.1 M NaCl and 6 M KI aqueous medium. The global EB dye was more effective for controlled single microsphere release than local EB dye in both cases as well. Comparing 1 mM global EB dye in 0.1 M NaCl with 0.1 mM global EB dye in 0.1 M NaCl, the higher concentration of EB dye significantly improved the probability for controlled single microsphere release as well as minimizing the energy required. Based on this, it was used in all our defect writing experiments described below.

The best medium condition observed for controlled microsphere release in both isolated microsphere groups and close-packed microspheres was 1 mM global EB dye in 0.1 M NaCl. The EB dye was intercalated into the DNA concentrating the energy adsorption in the DNA directly on the microsphere exposed to the focused laser beam reducing the energy required for release. The higher concentration 1 mM global EB dye in 0.1 M NaCl was expected to be more effective than the 0.1 mM global EB dye in 0.1 M NaCl since the concentration of the EB dye in the DNA was expected to be higher. The primary reason for the global EB dye condition being more effective is due to the reduction of concentration of the EB dye in the DNA by the reverse

diffusion of EB dye out of the DNA when the global EB solution was replaced by the solution without the EB dye.

For laser writing of curves and corners, the pulsed laser beam was first positioned at the circled microsphere to release the targeted microsphere and then repositioned to the adjacent microsphere to create the waveguide of the desired pattern. Primarily single microsphere line width was achieved as shown in Figs. 12 and 13. This was much more effective than the 2–4 microsphere line width previously studied using the continuous 514 nm argon-ion laser [28–30].

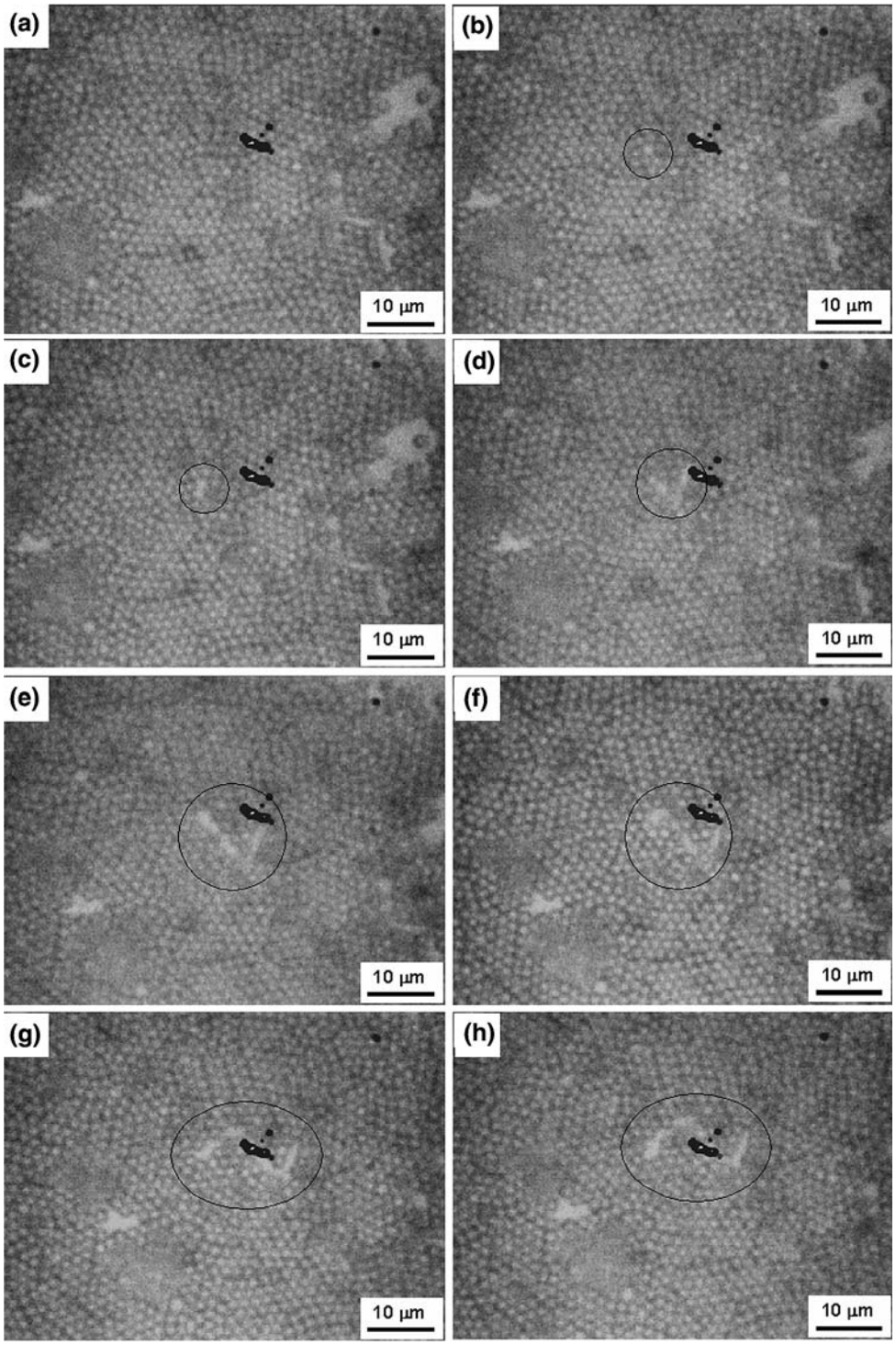

Fig. 12 Curved line writing (12-bp DNA-PS sample in 0.1 M NaCl in 1 mM global EB dye, 1.27 μJ incident energy). (**a**) before single pulse; (**b**) after single pulse; (**c**) after 3rd pulse; (**d**) after 10th pulse; (**e**) after 20th pulse; (**f**) after 22nd pulse; (**g**) after 24th pulse; (**h**) after 26th pulse

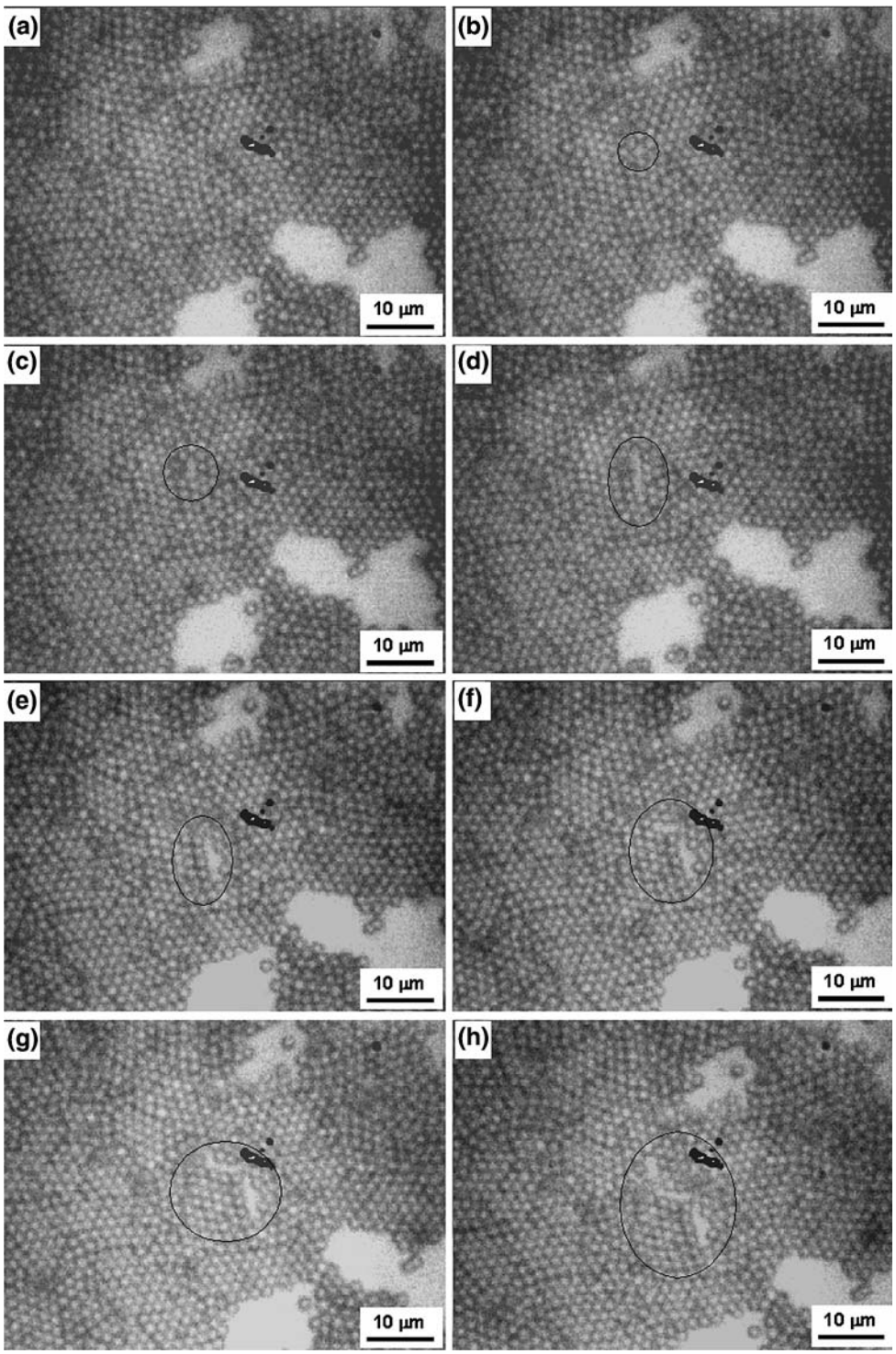

Fig. 13 Corner line writing (12-bp DNA-PS sample in 0.1 M NaCl in 1 mM global EB dye, 1.27 μJ incident energy). (**a**) before single pulse; (**b**) after single pulse; (**c**) after 2nd pulse; (**d**) after 4th pulse; (**e**) after 6th pulse; (**f**) after 8th pulse; (**g**) after 10th pulse; (**h**) after 12th pulse

Conclusion

From these studies, it has been demonstrated that line writing in a 2D photonic crystal with line width of 1 microsphere (1.8 μm) can be done with sequenced single 10 ns pulse of the 532 nm focused beam from the second harmonic of the Nd:YAG pulsed laser. The presence of EB dye in both the 0.1 M NaCl and 6 M KI lowered the laser release energy almost by an order in magnitude. This reduction in energy dispersion in surrounding microspheres

resulted in greater control to release single microspheres in the close-packed array. The global EB dye was more effective than local EB dye for greater fraction of controlled microsphere release. The most effective aqueous medium condition for single microsphere removal was determined to be 1 mM global EB dye in 0.1 M NaCl. This was used for the effective one microsphere line width writing for both straight and curved lines.

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