Control of DNA Molecules on a Microscopic Bead Using Optical Techniques for Photonic DNA Memory

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Abstract. This paper focuses on a photonic DNA memory, which is a DNA memory using optical techniques. Positional information of DNA is utilized for scaling up the address space of the DNA memory. Use of the optical techniques is useful in controlling positional addresses in parallel. We performed some experiments on control of the reactions of hairpin DNA molecules on a microscopic bead. Experimental results demonstrate that operations of writing and erasing of data DNA on a bead for the photonic DNA memory can be achieved by using optical techniques.

1 Introduction

Various computations can be implemented by use of parallelism and autonomous reactions of DNA. Storing of manipulated data improves the efficiency of the computations. This means that a DNA memory, which is a memory using DNA and its reactions, is considered to be fundamental to a wide range of applications of DNA computing[1, 2, 3].

The size of DNA is a nanometer scale. This is an important characteristic for constructing a valuable memory because showing the potential of the DNA memory as a high-capacity memory. For realizing the DNA memory, the capability to store huge data using a large amount of DNA is essential. In addition to this, it is required that one can access and use arbitrary data among the stored data at his disposal.

From this viewpoint, it is important to develop a method for addressing individual data to identify them. DNA molecules are distinguished depending on their base sequences; namely, the DNA molecules have their address information inherently, and addressing with DNA base sequences is possible. However, simple use of address information that relates to base sequences requires a hard task to design the sequences for making huge address space. In addition, it is difficult to control the DNA with such a variety of base sequences accurately.

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Use of information that is independent from base sequences is another possible strategy for scaling up the address space of a DNA memory. The positional information of DNA is considered to be usable information for the purpose. It it not necessary to control the positions of DNA molecules individually at a nanometer scale. For identifying the individual DNA molecules, addressing with base sequences is suitable. The positional information at a micrometer scale is more effective to use than that at a nanometer scale. Combining addresses relating to the base sequences and to the positional information provides large address space. For realizing this idea, methods should be developed for operating the DNA memory in the individuals of an array of micrometer-scale volumes.

We have been studying methods for manipulating DNA at a micrometer scale by the basis of optical techniques[4, 5]. For example, we demonstrated that multiple microscopic beads, on which many DNA molecules were attached, were simultaneously translated by optical manipulation that uses vertical-cavity surface-emitting laser (VCSEL) array sources. We also succeeded in controlling reactions of DNA with the resolution of a few micrometer by irradiating with a laser beam. These optical techniques for manipulating DNA are promising to realize addressing of the DNA memory using the positional information of DNA molecules. The characteristics of DNA and light can be effectively utilized by combining the method for addressing the DNA memory using optical techniques at a micrometer scale and the method using molecular reactions at a nanometer scale. The DNA memory is controlled in spatially parallel owing to the parallelism of light propagation. The reaction parallelism of DNA is exploited at a micrometer scale.

It is important to develop a method for addressing the DNA memory by using optical techniques not only from the viewpoint of DNA computing but also from the viewpoint of optical computing. Optical computing is a computational technique for parallel information processing that uses inherent property of light such as fast propagation, parallelism, and a large bandwidth. Many interesting results were obtained with various demonstration systems, which were associated with, for example, optical interconnection and digital optics[6]. However, the diffraction limit determines the resolution of the light and often restricts the density and capacity of information that is dealt with in an optical system. The precise alignment of optical devices is necessary for manipulating the light in diffraction limited systems.

The DNA memory that uses optical techniques, which we refer to as a photonic DNA memory, gives a practical solution for these problems. For example, the diffraction limit is approximately 1 μm in a typical optical system. On the other hand, a volume of 1 μm^3 of a DNA solution with density of 10 μM contains 6×10^3 DNA molecules. When DNA molecules each includes information of 1 bit, the amount of information of the volume is an order of 10^3 bits. This suggests that the photonic DNA memory has potential for dealing with information more than that is dealt with in the diffraction limited optical system. The difficult alignment of the optical system is avoidable because DNA molecules float in a volume of the solution and react autonomously.

In this paper, we focus on the photonic DNA memory, which uses optical techniques for addressing with positional information. We studied a method for controlling reactions of hairpin DNA on a microscopic bead by laser irradiation. Experimental results of the operations of writing and erasing on a bead are shown.

2 Photonic DNA Memory

In photonic DNA memory, DNA with a hairpin formation, which is referred to as hairpin DNA, is immobilized on the surface of a microscopic bead. The beads are used for translating DNA. The detail of translation is described later.

A solution of our DNA memory contains hairpin DNA, tag DNA, and anti-tag DNA. The base sequence of the tag DNA is completely or partially complement to that of the hairpin DNA. Anti-tag DNA that has a sequence complement to the tag DNA is immobilized on a substrate. The reason for using hairpin DNA is to achieve two stable states. If the temperature of the solution is decreased gradually, hairpin DNA and tag DNA molecules hybridize with each other. In contrast, if the temperature of the solution is decreased rapidly, the hairpin DNA forms the hairpin formation and does not hybridize with the tag DNA.

A tag DNA molecule includes address information of the DNA memory in its base sequence. When using tag DNA by itself as data, the state that the anti-tag DNA and the tag DNA construct dsDNA on the substrate is considered as the value "1", and the other state is considered as the value "0." One can, in contrast, append an additional DNA, genome DNA, proteins, and other molecules to the tag DNA as data. In this paper, we refer to tag DNA with or without an additional molecule as data DNA.

Let T_1 and T_1' be the melting temperature of the hairpin DNA and that of the dsDNA consisting of the tag DNA and the anti-tag DNA, respectively. Let $T_2 (> T_1, T_1')$ denotes the melting temperature of dsDNA consisting of the hairpin DNA and the tag DNA.

Figure 1 shows the molecular reaction behavior of the DNA memory considered in this paper. The procedure for writing and erasing operations is as follows. At the initial condition, the temperature is set to $T_0 (< T_1, T_1')$ and the data DNA molecules bind to the substrate by hybridization of the data DNA and anti-tag DNA. When the temperature of the solution is increased to higher than T_2, the data DNA is detached from the substrate and floats in the solution. The temperature is decreased to T_2, then the data DNA and the hairpin DNA hybridize. After the temperature is decreased to T_0, the DNA is stable. By the method, the data DNA can be read out from the substrate and written in to the bead.

When the temperature is decreased from higher than T_2 to T_0 rapidly, the hairpin DNA forms a hairpin formation, and cannot hybridize with the data DNA. As a result, the data DNA hybridizes with the anti-tag DNA, and it is immobilized to the substrate. This means that the data DNA can be read out from the bead and written in to the substrate. If hairpin DNA molecules that

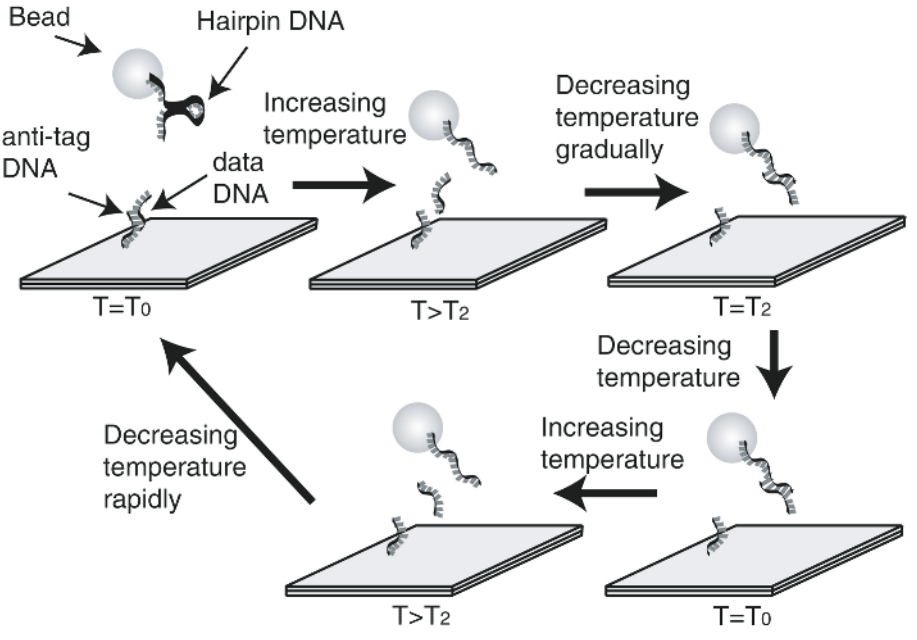

Fig. 1. The molecular reaction behavior of the photonic DNA memory

have different base sequences are immobilized to a bead, specific data DNA molecules are written in to the bead selectively due to addressing with base sequences.

Note that our scheme uses the substrate as a memory device and the positional addresses are defined on the substrate. The operations of writing and reading data DNA on beads are useful in storing a cluster of data DNA temporarily and necessary for translating the data from a position on the substrate to another position to process the data.

In the photonic DNA memory, the DNA memory is operated by using optical techniques. The scheme of a method for controlling molecular reactions of the photonic DNA memory is shown in Fig. 2. A solution containing microscopic beads is put on a substrate that is coated with a sort of material for light absorption. When the substrate is irradiated with a focused laser beam, the surface of the substrate is heated up owing to light absorption, and the temperature of the solution around irradiated area increases. By the basis of the phenomenon, the temperature of the solution can be controlled by changing the power of the beam used. The positional address of the photonic DNA memory can be used by changing the irradiating position.

It is possible to generate optical field patterns at a micrometer scale. Effective use of optical devices provides a method for generating various optical field patterns, so that the operations of the photonic DNA memory can be controlled in parallel. Operating the DNA memory at a local position using light is regarded as addressing based on positional information of the DNA memory. Different

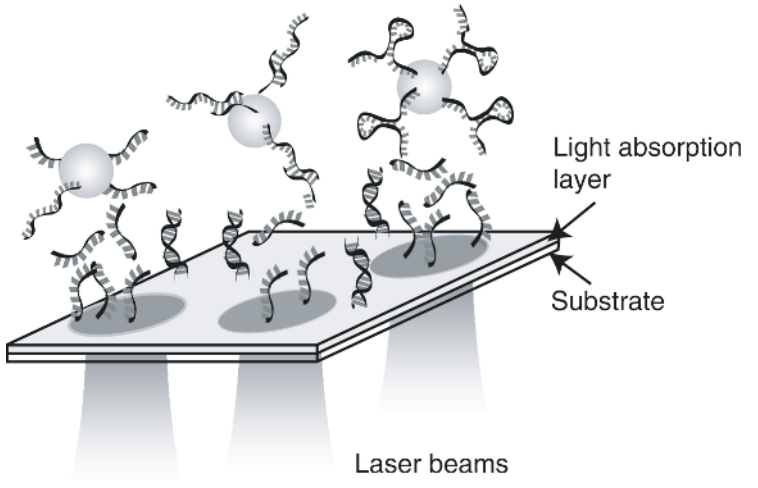

Fig. 2. The conceptual diagram of a method for controlling molecular reactions of the photonic DNA memory

positional addresses can be given at a pitch that is no more than several micrometer.

The bead with data DNA molecules can be translated by VCSEL array optical manipulation[4]. Optical manipulation is a non-contact manipulation method of an object using a radiation pressure force induced by the interaction between light and the object. A VCSEL array is high density array sources, the optical outputs of which can be controlled independently. Flexible manipulation for microscopic objects is achieved by control of spatial and temporal optical fields generated by the VCSEL array sources. The method is effective for parallel manipulation of multiple objects with compact hardware.

Use of the light in the DNA memory is effective in the following points.

1. It is possible to access to DNA memories that have different positional addresses in parallel.
2. Independent operations are executed for the DNA memories with the different positional addresses.
3. Physical interconnections are not required for flowing the data DNA.
4. Procedures of processing are programmable.

The photonic DNA memory can be applied, for example, to a programmable free-space micro-reactor array system. A variety of information is previously stored in individual reactors. Addressing with positional information of the DNA memory is performed by selecting reactors operated by optical field patterns. Operations of the DNA memory in the individual reactors are implemented by addressing with base sequences. The data DNA molecules are translated between reactors. The reactors are used as not only memories but also processing units and registers. The role of the individual reactors can be changed, so that the

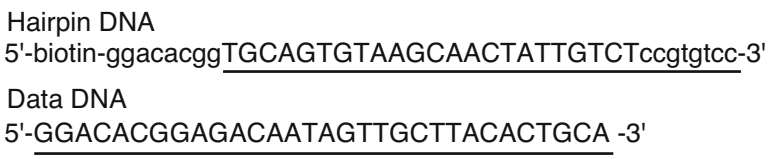

Fig. 3. The base sequences of hairpin DNA and data DNA

system is reconfigurable. Applications of the system include on-chip systems for genome analysis and DNA authentication.

3 Experiments

We performed some experiments on writing and erasing of data DNA on a microscopic bead by using optical techniques. The operations on the bead are required to take data DAN selectively from the substate (DNA memory) and to use it in processing.

The base sequences of hairpin DNA and data DNA are shown in Fig. 3. In the hairpin DNA, the part of the sequence indicated with small letters is the part of a stem, and the part with capital letters forms a loop. The underlined letters of the hairpin DNA and the data DNA indicate a complementary part of the sequences. The detail of molecular reactions of the hairpin DNA and the data DNA is described in [7].

The hairpin DNA molecules which were modified with biotin at the 5'-end were mixed in a solution that contains polystyrene beads of 6 μm diameter coated with streptavidin. The hairpin DNA molecules were immobilized to the surface of the bead by biotin-streptavidin binding. The beads were extracted and put into a TE buffer solution. Fluorescence molecules (Molecular Probes, Alexa Fluor 546) were attached to the data DNA. The absorption and fluorescence emission maxima of the florescence molecules are 555 nm and 570 nm, respectively. The solution of the data DNA was mixed with the solution that contained beads with the hairpin DNA.

The substrate used in the experiments was a glass substrate that was coated with titanylphthalocyanine of $0.15-\mu$m thickness as a layer of light absorption. The sample was irradiated from below with a beam that was generated from a semiconductor laser of a wavelength of 854 nm and focused with an objective lens (Olympus Corp., LUMPlan Fl 60×). A fluorescence microscope with a cooled CCD was used for observation.

We measured the optical power required for the operation of erasing on a bead. The data DNA and the hairpin DNA that was immobilized on the bead were annealed previously in a tube. The sample was put on the substrate and irradiated with the laser beam. An irradiation cycle consisted of the first phase of irradiating for 10 seconds and the second phase of capturing a fluorescence image for 2 seconds. This irradiation cycle was repeated. The power of the irradiation beam used was 1, 2, 3, 4, or 5 mW.

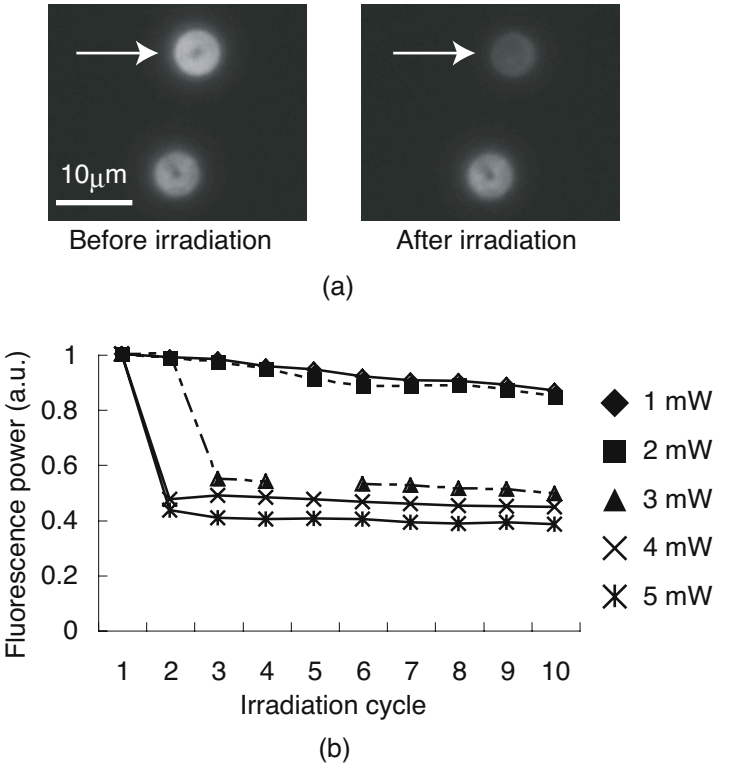

Fig. 4. (a) Fluorescence images captured before (left) and after irradiation (right). (b) The relationship between the number of irradiation cycles and fluorescence power.

If the data DNA molecules are immobilized to a bead, the fluorescence power observed around the bead is high because fluorescence molecules is concentrated on the bead. After denaturing, the data DNA dispersed in the solution, and the intensity around the bead decreases.

As an example, the fluorescence images captured before and after irradiating a bead with 5 mW for 10 seconds is shown in Fig. 4(a). Decrease of fluorescence intensity means that the data DNA was denatured by laser irradiation. Figure 4(b) shows the relationship between the number of irradiation cycles and fluorescence power. The fluorescence power was averaged values of 5 measurements. It can be seen from Fig. 4(b) that, with the irradiation power of 1 or 2 mW, the fluorescence power changes little. This result suggests that the temperature did not increase to the temperature required for denaturing. When the irradiation power was no less than 3 mW, in contrast, the fluorescence power decreased. We can conclude that the power of no less than 3 mW is required for erasing operation on the bead by denaturing the hairpin DNA and the tag DNA.

We investigated a suitable irradiating condition for writing the data DNA on a bead. The sample solution was prepared by mixing the solution of the beads including the hairpin DNA with the solution of the data DNA. The mixed

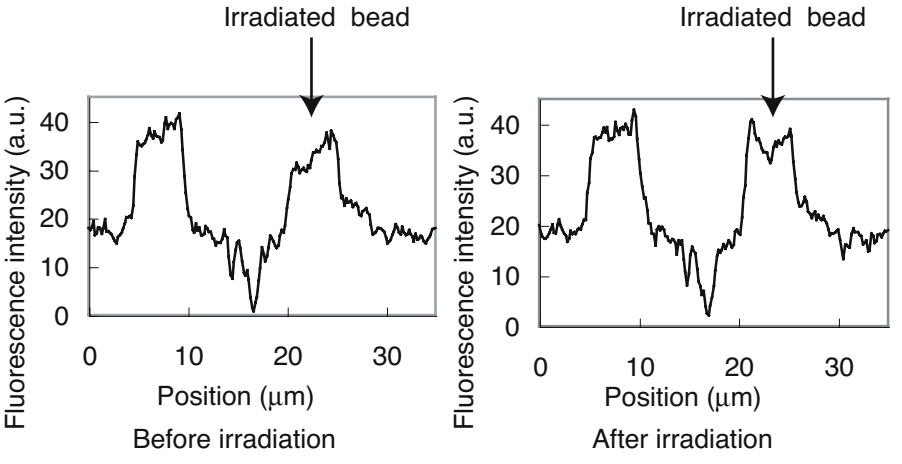

Fig. 5. Cross sections of a target bead of fluorescence images captured before (left) and after irradiating the bead with 2 mW for 30 seconds (right)

solution contained the enough data DNA for reaction. A suitable irradiation condition for hybridization was found by changing laser power, irradiation time, and other parameters.

As an example, we irradiated a target bead with a laser beam of 2 mW for 30 seconds and stopped irradiating. Cross sections of the bead of fluorescence images captured during this trial are shown in Fig. 5. The fluorescence intensity of the bead did not change, which means failure in writing.

In contrast, when a target bead was irradiated with the irradiation schedule shown in Fig. 6 (a), the obtained fluorescence images are shown in Fig. 6 (b). Figure 6 (c) shows cross sections of fluorescence images along the line indicated in Fig 6 (b). The background fluorescence intensity is removed to show these figures.

The fluorescence intensity of the irradiated bead increases obviously. This result indicates that the tag DNA hybridizes with the hairpin DNA on the bead. We succeeded in writing the data DNA on the bead using the optical technique. The fluorescence intensities of another beads around the target bead did not change, and a hybridization reaction can be controlled with the resolution of no more than 10μm. If the reaction area is divided to many small areas of 10μm square, different positional addresses can be given to the individual small areas.

In the next experiment, we repeated writing and erasing operations on a bead. At the beginning, the data DNA molecules were not attached to a bead with the hairpin DNA. The following steps were repeated 3 times. Step 1: writing with the irradiation schedule shown in Fig. 6 (a), step 2: erasing by irradiating with the laser beam of 5 mW for 10 seconds.

Figure 7 shows the fluorescence power measured after the individual steps. The fluorescence power increases after step 1 and decreases after step 2. This is an expected result. Note that efficiencies of writing and erasing indicate almost the same values during 3 repetitions.

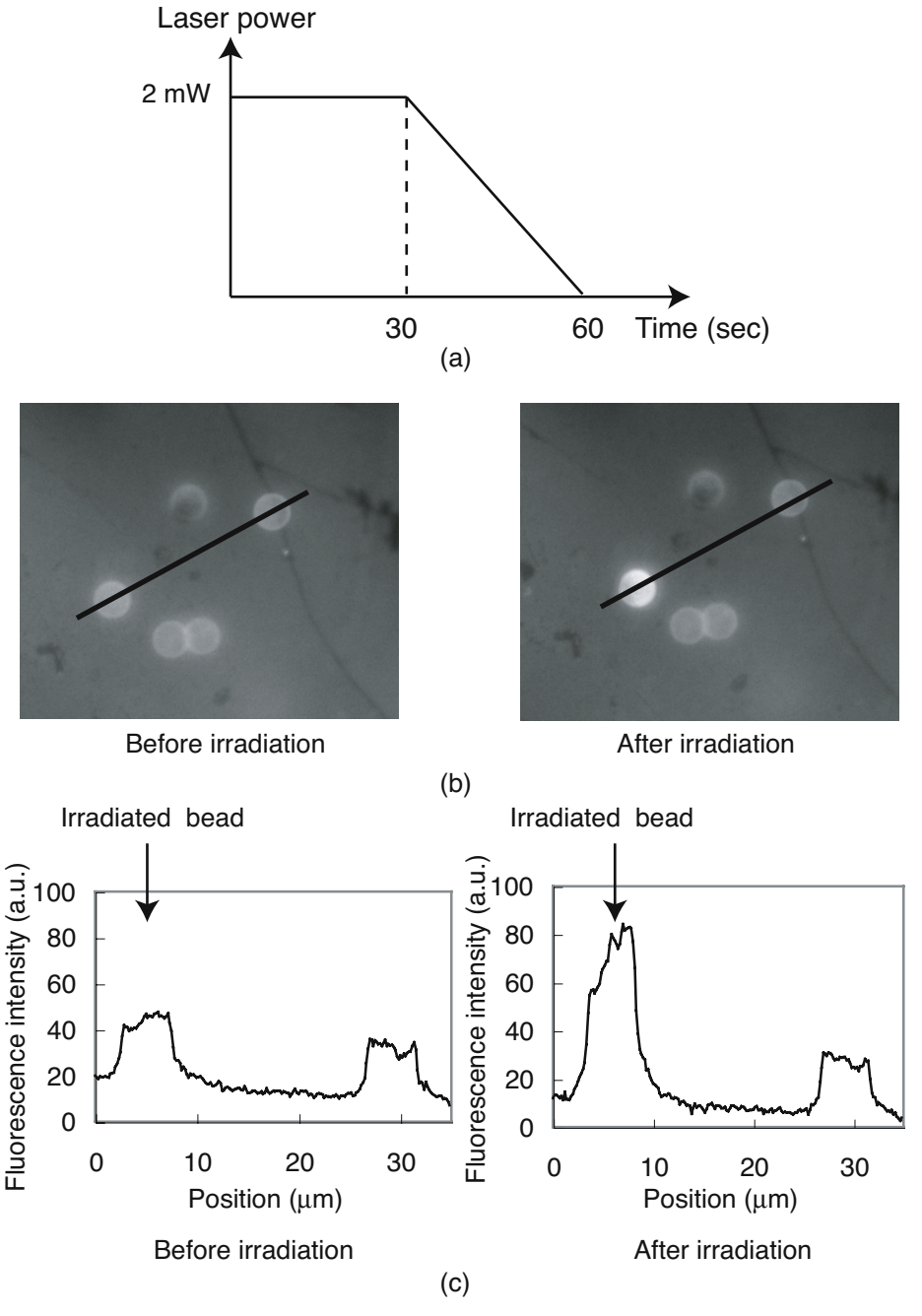

Fig. 6. Experimental results on writing data DNA on a bead. (a) Irradiation schedule, (b) fluorescence images, and (c) cross sections along the line shown in (b).

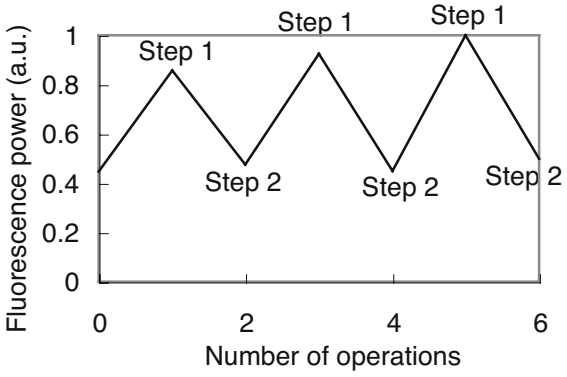

Fig. 7. The experimental result of repetitions of writing and erasing operation on a bead

4 Conclusions

We demonstrated that the operations of writing and erasing of data DNA on beads with hairpin DNA can be controlled by laser irradiation. The method is a fundamental technique for realizing the photonic DNA memory, which is a memory based on the nature of DNA and optical techniques. The use of optical techniques is effective to scale up the address space of a DNA memory because it provides a method for addressing based on positional information of DNA.

For practical use, lots of beams are required for parallel operation. VCSEL array sources are usable because, with the device, one can generate multiple laser beams simultaneously and modulate them independently. Fortunately, much effort is being made to increase the pixel number of VCSEL arrays, and the VCSEL arrays are expected to be applied to the photonic DNA memory. Future issues include optimization of writing conditions, transfer of data DNA molecules between a substrate and a bead, and demonstration of DNA memory using multiple kinds of data DNA.

Acknowledgments

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References

1. Chen, J., Deaton, R., Wang, Y.: A DNA-based memory with *in vitro* Learning and associative recall. In: Chen, J., Reif, J. (eds.): DNA computing: 9th International Workshop on DNA Based Computers, DNA 9. Lecture Notes in Computer Science, Vol. 2943. Springer-Verlag, Berlin Heidelberg New York (2004) 145-156

2. Kameda, A., Yamamoto, M., Uejima, H., Hagiya, M., Sakamoto, K., Ohuchi, A.: Conformational addressing using the hairpin structure of single-strand DNA. In: Chen, J., Reif, J. (eds.): DNA computing: 9th International Workshop on DNA Based Computers, DNA 9. Lecture Notes in Computer Science, Vol. 2943. Springer-Verlag, Berlin Heidelberg New York (2004) 219-224
3. Takahashi, N., Kameda, A., Yamamoto, M., Ohuchi, A.: Aqueous computing with DNA hairpin-based RAM. In: Ferretti, C., Mauri, G., Zandron, C. (eds.): DNA computing: 10th International Workshop on DNA Computing, DNA 10. Lecture Notes in Computer Science, Vol. 3384. Springer-Verlag, Berlin Heidelberg New York (2005) 355-364
4. Ogura, Y., Kawakami, T., Sumiyama, F., Suyama, A., Tanida, J.: Parallel translation of DNA clusters by VCSEL array trapping and temperature control with laser illumination. In: Chen, J., Reif, J. (eds.): DNA computing: 9th International Workshop on DNA Based Computers, DNA 9. Lecture Notes in Computer Science, Vol. 2943. Springer-Verlag, Berlin Heidelberg New York (2004) 10-18
5. Ogura, Y., Sumiyama, F., Kawakami, T., Tanida, J.: Manipulation of DNA molecules using optical techniques for optically assisted DNA computing. In Dobisz, E.A., Eldada, L.A. (eds.): Nanoengineering: Fabrication, Properties, Optics, and Devices. Proceedings of SPIE, Vol. 5515. SPIE, Bellingham, WA (2004) 100-108
6. Tanida, J., Ichioka, Y.: Optical computing. In Brown, T.G., Creath, K., Kogelnik, H. (eds.): The Optics Encyclopedia, Vol. 3, Wiley-VCH, Berlin (2003) 1883-1902
7. Takinoue, M., Suyama, A.: Molecular reactions for a molecular memory based on hairpin DNA. Chem-Bio Infomatics Journal, **4** (2004) 93-100