Optical Manipulation of Symbiotic Chlorella in Paramecium Bursaria Using a Fiber Axicon Microlens

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Abstract. In this paper, chemically etched axicon fiber was proposed for laser trapping of symbiotic chlorella from paramecium bursaria. We fabricated axicon micro lenses on a single-mode bare optical fiber by selective chemical etching technique. The laser beam from fiber axicon microlens was strongly focused and optical forces were sufficient to move a symbiotic chlorella. From experimental results, it was found that our proposed fiber axicon microlens was a promising tool for cell trapping without physical contact.

1. Introduction
The handling of individual cells is a key technique in cell engineering such as gene introduction, drug injection and clone technology. However, a wide variety of naturally occurring bacteria cannot be isolated by classical microbiological methods. More sophisticated electronic enumeration and sampling systems such as flow cytometry cannot prevent the formation of cell aggregates.

Ashkin et al. proposed the optical trapping of dielectric particles by a single-beam gradient force trap for the first time[1]. This laser beam trapping method is considered to be useful for manipulation of biological object because of noninvasive technology. Since then, this method was developed as an optical tweezers technology for various biological objects, such as viruses, bacteria and yeast cells, as well as for various dielectric particles. Its operation principle is based upon the electromagnetic momentum preservation. In recent years, a great deal of experimental and theoretical work has been done on the various types of laser beam trapping to manipulate microorganisms. We have already developed an optical trapping system with a single-mode lensed fiber probe [2]-[5]. This system is simpler and more flexible than conventional optical tweezers. Microsphere dispersed in liquid could be trapped by a single laser beam from an lensed optical fiber end inserted at an angle, and could be freely manipulated in 2-D plane synchronized to the lensed fiber probe.

In this paper, we investigated the manipulation of cells using fiber axicon microlens[6][7]. We fabricated axicon micro lenses on a single-mode bare optical fiber by selective chemical etching technique using an etching solution of buffered hydrofluoric acid(BHF), a mixture of 50% weight hydrofluoric acid (HF) and 40% aqueous solution of ammonium fluoride (NH4F). In our experiments, a semiconductor laser module at 980nm was used as a light source. From our experiment, we could verify that the laser beam from fiber axicon microlens was strongly focused and optical forces were sufficient to move a microorganisms and biological cells without physical contact. Furthermore we tried to trap and manipulate the symbiotic Chlorella in paramecium bursaria using a fiber axicon microlens. In our experiments, we used surfactant to flow out many symbiotic algae from paramecium bursaria. From these experimental results, it was found that our proposed fiber axicon microlens was a...
promising tool for the isolation of microorganisms which cannot be obtained in pure culture by conventional methods.

2. Chemically etched axicon fiber
We fabricated axicon microlenses on a single-mode bare optical fiber by using selective chemical etching technique. We also investigated the optical properties by measuring the beam profile of light emanating from the axicon microlens by objective lens imaging. A fiber can be sharpened by utilizing the difference in etching rate between its core and cladding when immersed in an etching solution of buffered hydrofluoric acid (BHF), a mixture of 50% weight hydrofluoric acid (HF) and 40% aqueous solution of ammonium fluoride (NH₄F).

Fig. 1. SEM images of optical fiber axicon microlens. The volume ratio of NH₄F:HF is 2:1. (a) The apex angle is 114deg. (b) Tilted SEM image of (a).

Fig. 2. Laser beam profile and CCD camera images of field distribution at the beam waist in liquid for 114deg axicon.

The Teflon beaker filled with BHF was warmed in a double boiler to a temperature of 35 degree Celsius. A cleaved fiber was immersed in the 2:1 etching solution for 35min. Then an axicon microlens was formed at the cleaved fiber. Fabricated axicons had apex angles of 114deg for 2:1 solutions as shown in Fig. 1. We also studied the laser beam profiles emanating from the axicon lens by objective-lens imaging experiments.
The smallest central spot size in liquid is $\sim 4\mu m$ for 114deg axicon with 980 nm wavelength light-source. On the other hand, the fiber used for fabrication was a standard single-mode fiber with an MFD of 5.8μm at $\lambda=980$nm. From these results, we can say that fiber axicon microlens is useful tool for trapping and manipulation of an object.

3. Fiber trapping system

Figure 3 shows the experimental set up for laser trapping with a fiber axicon microlens inserted at an angle. The purpose of this paper is to show that it is possible to make efficient optical tweezers using a properly modified single mode fiber. The fiber was mounted on a linear actuator stage and immersed in water drop. In our previous work, it was found that the fiber insertion angle into a water drop was important parameter for fiber trapping and manipulation. A single-beam fiber optic trap was not created at all when the fiber insertion angle was less than 20 degrees. Therefore, in our experiment, a fiber probe was inserted at an angle of 50 degrees. In our experiments, a semiconductor laser module at 980nm was used. The output of laser light was coupled into an optical fiber which had an optical connector at the fiber end. The trapping fiber was attached to an xyz manipulator and the fiber was introduced into a water drop at an angle. The trapping fiber could be freely moved in the water drop by controlling the xyz manipulator. A microscope was used to observe the trapped objects and the trapping behavior was recorded on a VTR with a CCD camera.

This optical fiber trapping method has following merits: (i) optical trapping systems using optical fibers are simple and inexpensive. (ii) trapped objects can be moved easily and freely, synchronized to the trapping fiber. (iii) optical sources can be changed easily using optical connectors. (iv) the trapping point is easily noticeable, because the fiber axicon microlens points out the focal point.

![Experimental setup for laser trapping](image-url)
From our experiment, we could verify that the laser beam from fiber axicon microlens was strongly focused and optical forces were sufficient to move a microsphere and biological cells without physical contact as shown in Fig. 4. In Fig. 4, a 2μm-diameter microsphere diapered in water solution was trapped near the fiber end. Under the trapped status, we could freely move the trapped microsphere to the forward and backward or right and left directions synchronized to the trapping fiber.

Next, we tried to trap and manipulate the symbiotic Chlorella in paramecium bursaria using a fiber axicon microlens. Many researchers study with a chlorella of the second and third-generation. But the chlorella of second and third-generations are not known an exact species of chlorella. So, the study become instability and unreliable. In our experiments, we used surface-active agents to flow out many symbiotic algae from paramecium bursaria as shown in Fig. 5.
According to the results in Fig. 5, after 5min we could see the symbiotic Chlorella, and we could trap the symbiotic Chlorella by the laser beam from axicon fiber as shown in Fig. 6(a). Furthermore, we could isolate the symbiotic Chlorella from paramecium bursaria as shown in Fig. 6(b).

![Trapped Symbiotic Chlorella](image1) ![Isolated symbiotic Chlorella](image2)

Fig. 6. (a)Symbiotic Chlorella trapped using axicon fiber. (b) Isolated symbiotic Chlorella.

Furthermore, we tried to investigate the cell tapping performance of this special designed fiber probe. A 980 nm laser diode with a power of 10mW and 20mW was used for the optical tweezers. Constant moving speed was achieved with a motorized stage (KT-LS13-M, Zaber Technologies Inc.). Maximum forward and backward (see Fig. 3.) trapping forces for the symbiotic Chlorella were investigated. When the moving speed increased, the drag forces increased proportionally and were equal to the optical force. When the Chlorella escaped from the trap, the current drag force was equal to the maximal trapping force. Experimental results are listed in Table 1. According to results, the forward trapping force was always larger than that obtained from backward trapping. This is because the scattering force coming from reflection always pushes the micro object to move in the direction of light propagation. Optical forces were sufficient to move the symbiotic Chlorella without physical contact.

| laser power from fiber end | maximum forward speed | maximum backward speed |
|----------------------------|-----------------------|------------------------|
| 10mW                       | ~ 60μm/s              | ~ 18μm/s               |
| 20mW                       | ~110μm/s              | ~ 34μm/s               |

### 4. Conclusions

In this paper, chemically etched axicon fiber was proposed for trapping. We fabricated axicon micro lenses on a single-mode bare optical fiber by selective chemical etching technique using an etching solution of buffered hydrofluoric acid(BHF), a mixture of 50% weight hydrofluoric acid (HF) and 40% aqueous solution of ammonium fluoride (NH₄F). From these experimental results, it was found that our proposed fiber axicon microlens was a promising tool for the isolation of microorganisms which cannot be obtained in pure culture by conventional methods.
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