Integrated optical transfection system using a microlens fiber combined with microfluidic gene delivery

N. Ma,¹ P. C. Ashok,¹ D. J. Stevenson,¹ F. J. Gunn-Moore,² and K. Dholakia^{1*}

¹SUPA, School of Physics & Astronomy, University of St Andrews, North Haugh, St Andrews, KY16 9SS, Scotland, UK

²School of Biology, Medical and Biological Sciences Building, University of St Andrews, North Haugh, St Andrews, KY16 9TF, Scotland, UK

*kd1@st-andrews.ac.uk

Abstract: Optical transfection is a promising technique for the delivery of foreign genetic material into cells by transiently changing the permeability of the cell membrane. Of the different optical light sources that have been used, femtosecond laser based transfection has been one of the most effective methods for optical transfection which is generally implemented using a free space bulk optical setup. In conventional optical transfection methods the foreign genetic material to be transfected is homogeneously mixed in the medium. Here we report the first realization of an integrated optical transfection system which can achieve transfection along with localized drug delivery by combining a microlens fiber based optical transfection system with a micro-capillary based microfluidic system. A fiber based illumination system is also incorporated in the system in order to achieve visual identification of the cell boundaries during transfection. A novel fabrication method is devised to obtain easy and inexpensive fabrication of microlensed fibers, which can be used for femtosecond optical transfection. This fabrication method offers the flexibility to fabricate a microlens which can focus ultra-short laser pulses at a near infrared wavelength to a small focal spot ($\sim 3\ \mu\text{m}$) whilst keeping a relatively large working distance ($\sim 20\ \mu\text{m}$). The transfection efficiency of the integrated system with localized plasmid DNA delivery, is approximately 50%, and is therefore comparable to that of a standard free space transfection system. Also the use of integrated system for localized gene delivery resulted in a reduction of the required amount of DNA for transfection. The miniaturized, integrated design opens a range of exciting experimental possibilities, including the dosing of tissue slices, targeted drug delivery, and targeted gene therapy *in vivo*.

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1. Introduction

The introduction of therapeutic and other agents into cells which are otherwise membrane impermeable remains a key requirement in cell biology. Currently, there are a variety of transfection methods used to solve this problem, including chemical, physical, optical, electrical, and viral techniques [1]. Among these, optical transfection is a technique which offers sub-cellular selectivity, specificity, high transfection efficiency and good post-transfection cell viability. By tightly focusing a laser beam on the cell membrane, optical transfection can transiently and locally increase the permeability of the cell's plasma membrane to allow nucleic acids, for examples, to be internalized. Femtosecond (fs) laser assisted transfection has proven to be a very effective method to date amongst the use of alternative laser systems with an excellent potential for targeting single cells *in vitro* [2–6]. However in all these *in vitro* experiments, the foreign genetic materials to be transfected are homogenously mixed in the cell buffer medium. In addition, most of the optical transfection techniques that have been used employ free space (bulky) optical setups for targeting light to the transfection site. This approach limits the potential application of the technology for *in vivo* experiments.

Development of an integrated system for localized gene delivery through a microfluidic system is necessary in order to move this technology towards *in vivo* applications. However, combining a micro-capillary based gene delivery system to a bulk optics based system is not easily achievable, since the beam is delivered from outside the Petri dish through an objective in bulk optics based system and the drug has to be delivered into the medium. In addition, the transfection efficiency achieved by a bulk optic setup is highly dependent on the quality of the optical transfection beam and accurate targeting of beam focus on cell membrane, so an expertise in optical alignment is necessary to achieve efficient transfection. Our solution is to use a fiber based light delivery system and combine it with a micro-capillary based gene

delivery system to achieve spatial localization of both optical transfection beam and gene delivery.

A fiber based system offers not only compactness but also the advantage that once the laser has been fiber coupled, no specialist optical alignment is required to perform optical transfection. To the best of our knowledge, the only reported fiber based femtosecond optical transfection technique to date uses an axicon tipped optical fiber for light delivery [7]. However the hydrofluoric acid (HF) based etching makes the fabrication procedure of axicon tips hazardous and also the transfection efficiency is very sensitive to the quality of the axicon tip. In addition, the short working distance produced by the axicon makes the targeting of the beam focus at the cell membrane very difficult: particular care has to be taken to make sure both fiber tip and cells are not damaged. These limitations make axicon tipped fiber based optical transfection a non-viable technique for use in wider applications. A different solution is to fabricate a microlens at the tip of the fiber, whose properties can be tailored in order to obtain the optimum output beam characteristics for optical transfection and cell transfection.

Microlensed fibers are widely used in the field of communication for increasing coupling efficiency between terminals and interconnect [8]. These fibers are also used in the field of biomedical optics as endoscopic probe heads for Optical Coherence Tomography (OCT) [9,10], two-photon microscopy [11], near field microscopy [12] and spectroscopy [13]. There are various fabrication procedures reported for the fabrication of microlensed fibers. Melting the fiber tip by an electric arc discharge [10] or heating [14] to form a lens are the most widely used methods to fabricate a communication standard microlensed fiber. However, these methods cannot provide high reproducibility and only lenses with a comparatively large radius of curvatures can be fabricated. As already alluded to, selective chemical etching [7] can only create short working distance axicon tipped fibers and the fabrication process is hazardous due to the involvement of HF. Polishing techniques [15,16] can make axicon lenses of different angles; however, the technique is complex, time consuming and expensive. Another reported method is based on photoresist reflow [17], whose fabrication procedure is relatively simpler. However the flexibility of this fabrication method is highly limited and in addition these lenses may not be appropriate for high peak power pulsed lasers. Femtosecond two-photon lithography [18] is a highly flexible technique, micro-structures are directly inscribed on surfaces point by point, but this technology is in its infancy and the manufacturing cost is unacceptably high for practical applications. There are also other indirect methods using coreless silica fiber [8], micro-silica spheres [13] or a combination of these techniques [14]. All these procedures have disadvantages such as complexity, high cost or lack of flexibility.

Here, we report a simple, inexpensive method for fabricating a polymer microlens at the tip of a standard optical fiber using commercially available ultraviolet (UV) curable adhesive. A Scanning Electron Microscope (SEM) image of the fabricated microlens is shown in Fig. 1. This fabrication procedure affords the flexibility to tailor the lens characteristics by changing the parameters of curing the adhesive. A fabrication procedure was developed to manufacture microlenses which yield a very small focal spot (2~3 μm) at a relatively large working distance (~20 μm). The efficiency of femtosecond transfection with this novel microlens is studied with Chinese Hamster Ovary (CHO-K1) cells. We also carried out optical transfection experiments with an axicon tipped fiber, as described in [7], and compared its efficiency with that of our novel microlensed fiber based transfection.

Further, using the microlensed fiber mentioned above, an integrated system is engineered and this new system achieves highly localized delivery of DNA-containing fluid during transfection which is the first of its kind, to the best of our knowledge. In order to observe the cell boundaries during transfection, a multimode fiber based illumination system is also embedded into the integrated system. This new integrated system was then used for the successful optical transfection of CHO-K1 cells and Human Embryonic Kidney (HEK-293) cells with plasmids encoding for the mitochondrial targeted DS-Red protein (BD Biosciences, Oxford, UK). Also the amount of DNA required for transfection could be reduced as the DNA was delivered locally, close to the cell to be transfected. We suggest that this miniaturized

optical transfection system could readily be adapted for a myriad of *in vivo* applications, including the optical injection of membrane impermeable drugs.

Fig. 1. SEM image of the polymer microlens fabricated at the facet of an optical fiber. (inset): shows the apex of the microlens.

2. Experimental

2.1 Fabrication of microlens tipped fiber

The polymer microlens was fabricated onto the tip of a standard fiber by using a UV curable adhesive, cured with a focused UV beam. The commercially available single mode fiber had a mode field diameter of 5.6 μm , cladding diameter 125 μm , and an operating wavelength of 830 ± 100 nm (Thorlabs, SM800-5.6-125). A UV curable adhesive (Norland, NOA 65) with optimum sensitivity for curing in the 350 – 380 nm range was used as the photopolymer for fabrication of the microlens due to its good adhesion to glass, fast curing time, easy processing, suitable refractive index (1.524 for polymerized resin) and high transmission efficiency ($\sim 98\%$) at 800 nm. These characteristics make the polymer lens ideal for the delivery of high peak power pulsed laser light without damaging the structure.

In order to fabricate the microlens the cleaved tip of the fiber was dipped into uncured UV curable adhesive so as to form a hemisphere of adhesive at the tip. A violet diode laser (Toptica Photonics CVLS-LH050-2V1, $\lambda = 405$ nm, maximum output power = 40 mW) beam was shaped to TEM_{00} mode by coupling it to a single mode fiber. The output from the fiber was collimated using a lens and focused using an objective, so as to produce a proper beam profile to cure the UV adhesive. By adjusting the focusing optics and with correct positioning of the tip of the fiber with respect to the apex of the objective, the profile of the curing beam was tailored so as to obtain a microlens with desirable output characteristics for optical transfection as shown in Fig. 1. The microlens fiber which was fabricated using this procedure could focus ultra short laser pulses at a near infrared wavelength, to a small focal spot (~ 3 μm), whilst keeping a relatively large working distance (~ 20 μm). A detailed description of this novel fabrication method for polymer microlens tipped fiber and its characterization is given as an Appendix. This fabrication technique offers an inexpensive alternative to fabricate microlenses at the tip of the fiber. Further, by tailoring the beam profile of the curing beam, it is possible to fabricate a customized microlens tipped fiber with a desirable output beam profile for various different applications.

2.2 Cell transfection using the microlens fiber

CHO-K1 and HEK-293 cells were routinely cultured in modified eagles medium (MEM) containing 10% fetal calf serum (FCS), 18 IU/ml of penicillin, 18 $\mu\text{g}/\text{ml}$ of streptomycin, 1.8 mM of L-Glutamine (“complete medium”) in a humidified atmosphere of 5% CO_2 / 95% air at 37 $^\circ\text{C}$. Cells were grown to sub-confluence in 30 mm diameter glass-bottomed Petri dishes (World Precision Instruments, Stevenage, UK) in 2 ml of culturing medium. Prior to experimentation, the cell monolayer was washed twice with OptiMEM (Invitrogen) and for all

experiments (except the integrated system where the solution was delivered microfluidically), the sample was bathed in 1 ml solution of OptiMEM containing 9 $\mu\text{g/ml}$ mitoDsRED plasmid, encoding a mitochondrially targeted *Discoideum* Red Fluorescent protein (BD Biosciences, Oxford, UK).

Cell transfection was instigated by a femtosecond Ti: Sapphire laser emitting at 800 nm, with output pulse duration of ~ 100 fs and a pulse repetition frequency of 80 MHz (Coherent, MIRA). At the output end of both microlens and axicon tipped fibers, the pulses undergo stretching due to non-linear phenomena occurring inside the fiber - self-phase modulation (SPM) and group velocity dispersion (GVD) - giving an overall pulse duration of approximately 800 fs, as measured using a home built autocorrelator [19]. A home built optical transfection setup was used for the optical transfection experiment as shown in Fig. 2.

Fig. 2. Schematic of the experimental setup for fiber based optical transfection. A collimated laser beam generated by Ti: Sapphire laser is directed onto a half wave plate and an optical isolator. Lens L1 and L2 expands the incoming laser beam by 1.6 times and subsequently couples the beam into the 35 cm long optical fiber, through a fiber collimator. For axicon and microlens tipped fiber transfection, an LED light source is used for illumination. For integrated system, the illumination fiber is connected to a home built fiber illuminator and a micropipette is connected to liquid delivery tube. (a) SEM image of an axicon of 110.9° fabricated at the tip of an optical fiber (b) Side view of the tip of the integrated system.

A collimated laser beam was directed into a combination of a half-wave plate and an optical isolator (Laser2000, UK, I-80-2), which were used to eliminate the back reflection from the beam path. A magnifying telescope of magnification 1.6X was used to expand the incoming laser beam which was subsequently coupled to the 35 cm long optical fiber, through a fiber collimator (Thorlabs, F810FC-780). The fiber output power was adjusted using a variable neutral density (ND) filter wheel appropriately placed in the beam path. During optical transfection the average power of the beam was kept at 20 mW, with a peak power per pulse of 0.24 kW. The fiber was mounted on a xyz translation stage and was carefully inserted into the medium. A mechanical shutter (Newport, UK, model 845HP-02) controlled the time duration of the laser dosage on the cell membrane. Each cell was irradiated with 3 - 5 laser doses. The duration of each dose was experimentally optimized to be 100 ms. The peak intensity of the pulse was estimated to be $4.4 \times 10^9 \text{ W/cm}^2$ and the total energy per dose was estimated to be 2 mJ.

To compare the performance characteristics of the novel microlens fiber with the previously reported axicon tipped fiber, optical transfection was also performed with an axicon tipped fiber as described previously [7]. For microlens tipped fiber transfection, due to the restrictions imposed by the geometry of the fiber and the imaging path, the fiber was tilted

at $\sim 5 - 10^\circ$ with respect to the vertical axis. With a white LED light source on top and an imaging system below the sample, the sample cells were observed during the transfection procedure as shown in Fig. 3(b). During laser irradiation no visual response was observed. After the laser treatment, the cell monolayer was washed three times by and thereafter bathed in 2 ml complete medium and returned to the incubator. The sample was viewed 48 hours later under a fluorescent microscope, where successfully transfected cells expressed the red fluorescent protein as shown in Fig. 6.

The microlens tipped fiber has a long working distance ($\sim 20 \mu\text{m}$) which makes it easy to be positioned and focused on the cell membrane. In contrast to the axicon tipped fiber based transfection, transfection with a microlens tipped fiber does not need focusing and re-focusing for transfection of each cell. During the transfection, the beam focus was fixed at $5 \mu\text{m}$ above bottom of the Petri dish, which is the average height of these cells adhered to the bottom of the dish. Without any further axial positioning, the tip of the fiber could be laterally scanned in order to transfect different cells within one Petri dish.

Fig. 3. Illumination at the cell sample during optical transfection using (a) axicon tipped fiber; (b) microlens tipped fiber and (c) integrated system. It can be seen that, with the fiber based illumination, the cell boundaries are clearly visible during the transfection procedure, when transfected with the integrated system (c).

2.3 Design of a wholly integrated system for localized drug delivery

In order to achieve localized drug delivery during optical transfection, the microlens fiber mentioned in the previous section was integrated with a micro-capillary tube (Harvard Apparatus, Inner Diameter (I.D.) = 0.58 mm , Outer Diameter (O.D.) = 1 mm). The implementation of this integrated drug delivery system in the optical transfection device is shown in Fig. 2. A barbed T junction (Harvard Apparatus, 72-1487) with three ports was used to build this integrated system as shown in Fig. 4(a). The micro-capillary was attached to port 1 of the T junction using a flexible plastic tubing (Tygon T3601, I.D. = 0.8 mm , O.D. = 2.4 mm) as a connector. The microlens fiber was inserted through port 2, into the micro-capillary and the tip of the fiber was positioned close to the tip of the micro-capillary, as shown in Fig. 4(a). A cleaved multimode fiber of core diameter $200 \mu\text{m}$ (Thorlabs, BFL37-200) was also inserted similarly into the micro-capillary in order to achieve uniform illumination in a liquid environment. The optimum distance of the tip of the multimode fiber from the apex of the microlens was experimentally estimated to be $\sim 1 \text{ cm}$ so as to achieve the best contrast for the sample. A slide clamp (WPI, Luer Valve Assortment, 14042) was used to seal the flexible tubing, attached to the fiber inlet port, in order to ensure that the system was air-tight during sample injection. The flexible tubing, attached to port 3 was used for DNA delivery, whilst the other end was connected to a micropipette. A photograph of the integrated system is shown in Fig. 4(b), where a gene delivery system and a fiber based illumination system are combined with the microlens tipped fiber.

For optical transfection, the sample was bathed in 1 ml OptiMEM, whilst 1 ml of OptiMEM containing $20 \mu\text{g/ml}$ mitoDsRED plasmid was loaded into the pipette for delivery. The pipette, loaded with sample was connected to the capillary tube of the integrated system

as shown in Fig. 2. Controlled injection of DNA locally into the medium, in the vicinity of the cell to be transfected, was achieved using the pipette during optical transfection. The flow rate of the buffer containing DNA, inside the micro capillary was estimated to be 630 $\mu\text{m/s}$. Each cell was exposed to this flow for ~ 5 s, which results in a delivery of 0.02 μg of DNA per cell.

In order to demonstrate the ability of the system to optically transfect cell lines, CHO-K1 and HEK-293 cells were transfected using this integrated system. An image of cells recorded during optical transfection with the integrated illumination system, is shown in Fig. 3(c). Despite the poor image contrast due to a shadow cast by the optical transfection fiber, when targeted cells were imaged, the cell boundaries were clearly visible, which permitted them to be transfected.

In order to ensure the sterility of the drug delivery system, before each transfection experiment 2 ml of 70% ethanol was run through to sterilize the whole system and was subsequently dried using filtered air. The same capillary tube was used for multiple transfection experiments and the subsequent experiments showed that the system remains sterile with the above mentioned sterilization procedure.

Fig. 4. [a] Design of the integrated system. A glass capillary tube with 580 μm inner diameter is connected to port 1 of a barbed T junction through a piece of plastic tube. One optical fiber for laser delivery and another multimode fiber for illumination are inserted into the glass capillary through port 2. A piece of plastic tube and a slide clamp are used to hold two fibers and seal the fiber inlet at the same time. Another piece of plastic tube for liquid delivery is connected to port 3 of T junction. [b] Photograph of the integrated system.

3. Results and discussions

Figure 5 show the comparison of the transfection efficiency of CHO-K1 cells achieved using an axicon tipped fiber, microlens tipped fiber and our integrated system respectively, and in addition the transfection efficiency of HEK-293, transfected with the integrated system. The transfection efficiency is calculated in a similar way to those presented in previously reported studies. It is defined as the number of cells correctly expressing the targeted red fluorescent protein after 48 hours of the laser treatment, divided by the total number of cells that were laser treated in a particular area of interest [1,3]. An example of successfully transfected fluorescent cells is shown in Fig. 6. In order to monitor for potentially spontaneous transfected cells, each photoporated sample dish was accompanied by a control sample dish in which cells were cultured, bathed in plasmid DNA solution and then experienced the fiber presence in the absence of laser radiation. The number of treated cells and the results are

summarized in Table 1. The number of spontaneously transfected cells varied between 0 – 2 cells for each sample dish.

Fig. 5. The transfection results of CHO-K1 and HEK-293 cells using 3 different methods. The error bar shown is standard error of the mean.

Table 1. Transfection results

	Cell type	No of Dish treated	Total No. of treated cells	Transfection Efficiency ($\pm$ SEM) (%)
Axicon tipped fiber	CHO-K1	15	450	30.22 $\pm$ 5.36
Microlens tipped fiber	CHO-K1	20	800	40.25 $\pm$ 3.39
Integrated system	CHO-K1	15	525	45.71 $\pm$ 4.84
	HEK-293	5	175	64.00 $\pm$ 4.10

Our results show that the efficiency of the fiber based optical transfection technique is comparable to that of the free space transfection [3]. Also as shown in these results, the microlens tipped fiber provides significantly higher transfection efficiency with a smaller error than the axicon tipped fiber method. This reflects the fact that due to the longer working distance, the manipulation of a microlens tipped fiber is easier and more stable when compared to an axicon tipped fiber. During the transfection procedure, the axial focal position needed to be found only at the beginning of the procedure and then multiple cells in the same sample dish could be photoporated by solely moving the fiber mount laterally. This results in reduced damage to cells and the fiber tip, high transfection efficiency and more consistency.

The transfection efficiency for the integrated system with localized gene delivery is comparable to that of non-localized gene delivery systems. An integrated system providing high transfection efficiency proves that it did not have any detrimental effects to cells. The slightly higher transfection efficiency might be attributed to the higher local concentration of DNA near the transfected cells. The average concentration of the DNA in the cell medium after treating 35 cells in a dish is estimated to be 1.3 $\mu\text{g/ml}$, in contrast to the average DNA concentration of 9 $\mu\text{g/ml}$ used for the non-integrated system. It was shown in previously reported studies that the transfection efficiency reduces dramatically below 9 $\mu\text{g/ml}$ of DNA concentration [20,21]. The resultant transfection efficiency obtained shows that our localized gene delivery is successful in creating a localized high concentration of plasmid in the vicinity of the cell to be transfected. This means that the total required amount of DNA for performing transfection procedure is significantly low for integrated system compared to that of non-integrated systems.

Fig. 6. Fluorescent microscope image of optically transfected CHO-K1 cells, incubated for 48 hours after transfection. The bright cells are transfected successfully resulting in the uptake of the plasmid and thereby expressing the mitochondrially targeted red fluorescent protein.

4. Conclusion

We report the first realization of an optical transfection system which can deliver genes locally, near the vicinity of the cell to be transfected. In order to achieve this, a fiber based optical transfection system is developed which is viable and easy to implement compared to its previously reported counterpart. A novel fabrication method is proposed to fabricate a polymer microlens at the tip of optical fiber. This technique is cheap, less complex and has the flexibility to fabricate customized microlenses for specific applications compared to other existing microlens fabrication techniques. This fabrication technique is used to fabricate a ‘micro-stick’ which can produce a tightly focused beam ($\sim 3\ \mu\text{m}$ focal diameter) with a comparatively large working distance ($\sim 20\ \mu\text{m}$), which is ideal for fiber based femtosecond optical transfection of cells. This microlens tipped fiber was used for optical transfection of CHO-K1 cells and the transfection efficiency achieved is comparable to that of conventional free-space optical transfection setups and significantly better than the previously reported axicon tipped fiber based optical transfection. By combining the microlens tipped fiber with a micro-capillary system an integrated system that achieves localized drug (gene) delivery during transfection is realized. A multimode fiber based illumination system was also combined with this integrated system to allow the efficient visual identification of the cell boundaries during optical transfection. This integrated system was used for optical transfection of CHO-K1 and HEK-293 cells and the obtained transfection efficiency was better than that of the non-integrated system. Also with the integrated system, the total amount of DNA required for transfection could be lowered significantly. This new technique opens up prospects for a portable “hand-held” system that can locally deliver therapeutic agents and transfect cells within a fiber geometry placing minimal requirements upon any microscope system. We are working towards a wholly fiber based optical transfection system on a microscope free platform which would be compatible with an endoscopic system to achieve *in vivo* optical transfection.

Appendix

Fabrication of microlens

The custom made setup used for fabrication is shown in Fig. 7. Laser light was coupled through an objective ($\times 10$, Newport, UK) into a single mode optical fiber (Thorlabs, S405-HP) with a coupling efficiency of $\sim 45\%$, in order to improve the lateral profile of the laser beam. The optical fiber was then carefully cleaved and the facet was examined using SEM. The lateral beam profile of the output beam was measured using a long working distance

(WD) objective (Mitutoyo x100 infinity-corrected, WD = 6 mm) to confirm a high quality TEM_{00} Gaussian beam profile. A very high quality beam profile and optical alignment are essential for this lens fabrication method. The laser beam was then directed to an objective (x60 Nikon) and focused near the cleaved tip of an optical fiber, which was vertically mounted on an xyz translation stage with 1 μm resolution. The cleaved tip of the fiber was imaged onto a CCD camera (WAT-250D) to position the curing beam at the core of the fiber. The exposure of the curing time was controlled by a shutter (Newport, UK, model 845HP-02).

Fig. 7. Microlens tipped fiber fabrication setup. A violet diode laser (405 nm) is coupled into a piece of single mode fiber (fiber 1) through a 10X objective lens to obtain an output beam with perfect Gaussian lateral distribution. The beam, directed by a 5 cm tube lens and a beam splitting cube is focused by a 60X objective at the cleaved facet of a fiber. The lens is placed at 6 cm away from the fiber to make the beam converging. The fiber on which microlens is to be fabricated (fiber 2) is mounted in the setup using an xyz stage with 1 μm resolution, after dipping it into the uncured UV curing adhesive which forms a drop of the adhesive at fiber tip. The adhesive at the tip of the fiber 2 is cured for 5 s with the laser power of 0.5 mW.

The steps involved in microlens fabrication are schematically shown in Fig. 8. A well-cleaved optical fiber was vertically dipped and raised from a drop of UV curable adhesive such that a hemisphere of adhesive on the fiber tip would be formed. This fiber was then mounted to the curing setup as shown in Fig. 7. Keeping the power of the laser below the threshold (<0.1 mW), where the curing process would not be initiated, the laser beam was positioned at the centre of fiber tip facet and defocused by 20 μm from the tip of the fiber in order to get correct beam shape which could produce the desired lens structure. The power of the laser is increased to 0.5 mW to start the curing process. At the beginning of the polymerization process, UV adhesive would partially cure around the centre of fiber facet followed by a growth towards the direction of the laser. After an exposure for 5 s, the unpolymerized adhesive was removed using acetone and a 'micro-stick' (an extended microlens as shown in Fig. 1) was created. At the apex of 'micro-stick' a curved facet is formed which acts as a focusing surface for the output beam from the fiber.

Fig. 8. Different steps involved in the fabrication of lens at the tip of the fiber.

The fabrication procedure is highly flexible, and by changing the parameters such as light distribution near to the focus of the curing beam, intensity of the curing beam or curing time, it is possible to fabricate different structures at the tip of the fiber as shown in Fig. 9 which shows that, the structures created are strongly related to the beam distribution.

Fig. 9. Different structures can be made by changing the curing beam distribution. Using curing beam (a), (b) and (c) with appropriate laser power and curing time structures like (d), (e) and (f) respectively were fabricated.

Characterization and modeling of the microlens

The fabricated lens was characterized using SEM and beam profiling. The SEM images of the microlens at the tip of the fiber are shown in Fig. 1. The physical parameters of the lens were estimated from the SEM images. The height of the lens was estimated as $11 \pm 1 \mu\text{m}$, base diameter $7 \pm 1 \mu\text{m}$ and top diameter $5 \pm 1 \mu\text{m}$. An 800 nm laser from a Ti-Sapphire laser (Coherent, MIRA) was coupled to the microlens tipped fiber and the output beam from microlens was profiled in water from a series of lateral cross-sections with a $5 \mu\text{m}$ step change using a water immersion objective (x60 Olympus UPlanSApo) in conjunction with a CCD camera (WAT-250D). The working distance of the lens (distance of the focal plane from the apex of the lens) and the diameter of the focal spot were estimated from the beam. The estimated working distance of the beam was $20 \pm 5 \mu\text{m}$, focal spot diameter was $3 \pm 0.05 \mu\text{m}$ and the beam divergence was 15° .

A ray tracing model of the lens, fabricated at the tip of the fiber was built using optical design software (Zemax Development Corporation) from the parameters estimated from the SEM images and beam profiling. In the Zemax model, a radial source with a Gaussian profile was defined at a wavelength of 800 nm, which propagates from a cylinder of refractive index similar to that of the core of the fiber used. The output beam from the cylinder had same

numerical aperture ($NA = 0.12$) and mode field diameter ($MFD = 5.6 \mu\text{m}$), as was defined by the specifications of the single mode fiber used for the experiments. A microlens was defined at the surface of the cylinder with a material of refractive index same as that of cured UV adhesive (Norland 65 – refractive index 1.524). The lens was designed with physical dimensions estimated from the SEM image, keeping the radius of curvature of the surface close to the surface of the cylinder as 0 and the radius of curvature of the second surface (apex of the microlens) as a variable. The whole system was immersed in water and the radius of curvature of the lens was estimated which provided the experimentally measured working distance.

Figure 10 shows the ray diagram of the model used for estimating the lens parameters. The radius of curvature was estimated to be $7 \mu\text{m} \pm 0.5 \mu\text{m}$. With all the estimated parameters, beam profiling was performed on the model at a step size change of $5 \mu\text{m}$ and the diameter of the focal spot ($2.9 \mu\text{m}$) and divergence (16°) was calculated which was comparable to that of the experimentally obtained values.

Fig. 10. Ray tracing model used to estimate the parameters of the fabricated microlens.

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