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Manufacturing of Two and Three-Dimensional Micro/Nano-Structures by Integrating Optical Tweezers with Chemical Assembly

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Abstract — *Optical tweezers have been used as versatile tools for non-contact manipulation of micrometer-sized entities. This paper proposes a hybrid micro/nanoscale manufacturing system using optical tweezers and chemical linkages for fabricating 2D and 3D micro/nanostructures. A holographic multiple trap optical tweezers system is first used to trap particles in a desired pattern. The particles are then connected to form rigid units using suitable chemistry. Connection schemes based on gold seeding, complementary-DNA linkage and streptavidin-biotin chemistry are presented and possible applications of this technique are explored. This method combines the advantages of top-down and bottom-up approaches and is compatible with organic and inorganic materials.*

Keywords – *Micro/Nanorobotics, Optical Tweezers, Chemical Assembly, Micro/Nanoassembly, Rapid Prototyping, Diffractive Optics*

1. Introduction

For future nanotechnology, biotechnology and information technology products, micro/nano-manufacturing systems are indispensable. Robotics researchers have been focusing to develop such systems [1] using scanning probe microscope probes, optical tweezers, and micro/nanogrippers type of manipulation tools. The main unsolved issues in these systems are

high throughput by fast/parallel and autonomous manipulation, bonding, 3D micro/nano-assembly, and limitations to specific materials, environments, and sizes. This paper proposes a novel manufacturing method to address the issues of throughput, bonding, and assembly.

Manufacturing methods can be broadly classified as either top-down or bottom-up. The top-down approach provides for precision and can be used for controlled fabrication and assembly. However, it is not easy to parallelize it at small scales. The bottom-up approach on the other hand uses a few simple building blocks and can be massively parallel but the main problem is in process control. We propose a new hybrid approach that combines the advantages of both precision as well as chemical assembly as different from the previous works. Our method is very suited to rapid prototyping and batch fabrication and does not require masks or lithography. In addition, it is versatile and compatible with inorganic as well as organic materials. This method consists of two steps: first, a pattern of optical traps is generated so that the particles can be trapped in a desired pattern – this is the top-down part that enables spatial control. The particles are then connected chemically to form a rigid structure – this is the bottom-up part that enables parallelism. The steps involved in the process sequence are illustrated in Fig. 1 and discussed in the subsequent sections.

2. Pattern Formation Using Optical Tweezers

Optical trapping occurs due to gradient force on a particle in an optical field. Optical tweezers can be used to trap dielectric particles of sizes from about 100 nm to 20 μm. Trapping metallic or absorbing particles [2] is tougher than transparent particles but can be achieved using a donut shaped TEM_{01} Laguerre-Gaussian mode beam [3]. This mode can also be used to spin and rotate particles near the focus [4].

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Laser tweezers have been used extensively in biological systems for in-vivo manipulation of cells and single molecule studies [5, 6]. However, not much attention has been paid to using them as a tool for fabrication. Holmlin [7] used a scheme for making assemblies using coated polystyrene beads and erythrocytes. Korda [8] used a multiple-trap system to get permanent particle-pattern by gelling the surrounding fluid.

2.1. Multiple Trap Systems

However, in order to trap particles in a pattern, multiple optical traps are required. The simplest way to generate multiple beams is to use multiple light sources like VCSEL arrays [9]. Another option is to raster scan a single beam using mechanical elements [10] or acousto-optic modulators [11, 12] to create an array of traps. However, these approaches result in poor trapping and cannot be used to create a 3D array and are not easily reconfigurable. Multiple traps can also be generated dynamically using computer generated holograms and a spatial light modulator (SLM) [13, 14]. This method is attractive and best suited to our proposed fabrication technique as it can trap both dielectric and metal particles in 2D or 3D patterns and the patterns can be changed dynamically.

2.2. Holographic Optical Tweezers (HOT)

The key element in the Holographic Optical Tweezers system is a computer-generated hologram, which is created by reducing a calculated interference pattern to a series of phase or amplitude masks. The computed phase mask is then written to a spatial light modulator, which uses nematic liquid crystals to do phase-only modulation of light [15]. For a given pattern of traps, there exists a unique phase mask, which is computed using Fourier optics. The trap pattern can be changed dynamically by using a computer to continually refresh the mask on the SLM.

Fig. 2 shows a holographic tweezers system, where an input beam is split by the SLM and the resulting beams are focused into an array of optical traps. The phase masks can be modified easily to get a 3D pattern of traps or to change the beam profile e.g. a TEM_{00} mode Gaussian beam can be converted into a Laguerre-Gaussian TEM^{*}_{01} donut shaped beam at a trap site. A single Gaussian source can yield a combination of different mode beams, which enables simultaneous trapping of metallic and dielectric particles.

Curtis et al. [16] developed a dynamic trap system, which is also commercially available [17]. They used a Hamamatsu X7550 SLM with 480x480 array of pixels, each of which is 40 μm wide with 150 distinct phase shifts from 0 to 2π. They were able to trap 1μm beads using just 1mW and also demonstrated 3D trap patterns and combinations of TEM_{00} and TEM_{01} beams.

The holographic system is best suited for making arbitrary patterns since it generates 2D and 3D trap patterns for both metallic and dielectric particles without any complex mechanical elements. The main drawback here is that the power of the input beam is distributed over all the traps but this is remedied by using a higher power laser. Also, the calculation of the phase hologram is a complicated process and limits the bandwidth of the system but the manufacturing process does not require a high refresh rate and hence the existing rates (5-10 Hz) are sufficient.

3. Pattern Linkage Using Chemical Assembly

The holographic tweezers described in the previous section can be used to trap the particles in the required pattern. Once the particles are trapped, they now need to be connected rigidly to form a microstructure. Different linkage chemistries as shown in Fig. 3 can be chosen, based on the properties of the particles and whether the particles are in contact or not. The details for each linkage method and the chemistry required are described in the following subsections.

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3.1. Direct Gold Seeding (A)

Hydroxylamine (NH_2OH) seeding of gold (Au) nanoparticles has been successfully demonstrated for increasing the size of the particles and also for forming micro/nano structures [18, 19]. NH_2OH is capable of reducing Au^{3+} ions to form bulk metal, and this reaction is strongly accelerated by the presence of Au surfaces. Consequently, no nucleation takes place in solution and all the Au goes into the production of larger Au particles. The use of gold seeding method to link the trapped particles is illustrated in Fig. 4. Once the particles are brought into contact (Fig. 4b), a solution of NH_2OH and $HAuCl_4$ is introduced. The individual particles now grow in size due to gold deposition and fuse together to form a single microstructure (Fig. 4c).

3.2. Indirect Gold Seeding (A)

For the previous direct seeding method, the particles have to be either made of gold or coated with a layer of gold. However, such particles are difficult to trap using optical tweezers. Polystyrene beads are much easier to trap but they must be coated with a gold layer for the seeding process to occur. Gold coating on polystyrene particles makes them opaque and similar to metal particles unless the coating is very thin (a few angstroms), which is very difficult to make. An alternate approach to make these particles compatible with the seeding process is to coat them with gold nano particles (Fig. 5). The density of gold nano particles and their sizes can be tailored such that they do not affect the transparency of the poly particles drastically. To make such particles, solutions containing streptavidin coated polystyrene particles and biotin coated gold nanoparticles (1-5 nm diameter) are mixed. The gold nanoparticles then assemble on the polystyrene particles as shown in Fig. 5a due to the binding between streptavidin and biotin. Biotin and streptavidin are bio-molecules that have a very strong affinity for each other and their bond strength is similar to that of a covalent bond. The nanoparticle coated polystyrene particles

are then trapped, brought into contact and the seeding agent is added. The gold nanoparticles grow in size due to the Au deposition and fuse together and thereby bonding the polystyrene particles as shown in Fig. 5b,c.

3.3. Biotin-Streptavidin Linkage

In this process, the starting material is biotin-coated particles (gold or polystyrene) and streptavidin molecules are used to link them together. Once the particles are trapped, streptavidin is introduced in solution at the trap location and bond with the biotin on the particles. This forms a linkage between the trapped particles as shown in Fig. 6.

3.4. DNA-DNA linkage

The affinity of single strand DNA molecules to their complementary strands can also be exploited to link particles. Complementary single strands of DNA hybridize to form a double stranded molecule in the presence of ions like Na^+ or Mg^{2+}. The connection process differs a bit for polystyrene and gold particles. One set of particles are coated with single stranded DNA molecules of Type A and another set is coated with molecules of type B, which is complementary to type A. The DNA molecules need to be modified at one end to attach them to the particles. If gold particles are used, thiol (-SH) chemistry is used to attach the DNA molecules to the surface. If polystyrene beads are used, then biotin-streptavidin chemistry is used to attach the DNA molecules to the surface.

The pattern in this case is made in two steps. First, the type A particles are trapped and the type B particles are trapped such that they form an alternating pattern. Once the particles are brought into contact, Mg^{2+} ions are added to aid hybridization, thus linking up the particles (Fig. 7a). The Mg^{2+} ions are added last to prevent random hybridization during the trapping process. The linkage between the DNA strands as a result of hybridization is rather weak and can be

easily broken by changes in pH or by shear forces. However, this bond can be strengthened using the silver (Ag) staining process [20].

The silver staining process involves three main steps. The first step is the exchange of ions in the vicinity of the DNA with Ag^+ ions and the formation of DNA-Ag^+ complex. This is followed by the deposition of silver on the DNA strands to form nanometer-sized aggregates. The final step is the development of the silver, much as in the standard photographic process, using an acidic solution of hydroquinone and silver ions under low light conditions. The deposition is limited only to the silver formed over the DNA resulting in a rigid connector between the particles. The linkage chemistry with complementary DNA is complicated, as it requires trapping different types of particles when compared to the gold seeding and biotin-streptavidin linkage method.

3.5. Connection of Separated Particles (DNA Bridge)

This method is used when the particles cannot be brought into physical contact with each other. This may be due to the limitations of the optical trap array, the optics system used, or the particles themselves. In such a case a DNA bridge can be used to link up the particles. After trapping the particles in the desired pattern, a solution with long DNA molecules like □ DNA with linker molecules attached to both ends is introduced and these assemble on the surface of the particles as in Fig. 8a. The linker is biotin when using streptavidin coated polystyrene particles and thiol when using gold particles.

To get directional assembly, DNA are stretched out in a particular direction using either flow [20] or a high frequency electric field [21, 22]. Drag forces due to the flow can cause particles to escape the trap but the electric field doesn't affect the particles and is hence preferred. The field can be applied using an external source or by micro-fabricated electrodes. The electric field

causes the DNA to stretch in one direction and the other end of the biotinylated DNA can attach to the neighboring particles as seen in Fig 8b. If the pattern needs to be connected in two directions, electric field can be applied sequentially in both directions. The process using the gold microspheres is similar to the one described above, except that the linkage chemistry is different. The DNA molecules are modified so that they have thiol groups at both ends and they attach to the gold particles by the thiol-gold bond. Finally, since the DNA bridge between the particles is compliant, it can be made rigid by using the same silver staining process described in previous section.

4. Discussion

The simplest methods for joining particles in contact are the direct seeding and biotin-streptavidin binding since they do not need elaborate preparation of the beads or DNA. The approach with complementary DNA is complicated since it requires trapping different types of particles. Connecting separated particles by stretching DNA is an exciting prospect since networks of nanowires can be obtained using this approach.

The main advantage of this method over conventional lithographic techniques is that it does not require the use of masks. The tweezers array is dynamically configurable and can be used to make different shapes just by using software to define a new pattern. The critical dimensions of the pattern are influenced mainly by the size of the particles, which in turn is limited by the capabilities of the tweezers array like laser power, grating resolution, etc. Structures made using this technique are free floating and this could pose some handling problems once the structure is released. Finally, the usable starting materials are limited by requirements of refractive index though other beam modes could be used but with some loss in stability.

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5. Applications

The simplest application is in making motifs and patterns. These small building blocks could then be assembled into larger assemblies since they are large enough to be individually manipulated. Since the trap system is dynamic and easily reconfigurable, this method is well suited for rapid prototyping, where flexibility and speed are desired and the whole process can be done in solution without a substrate. Another application would be to make novel materials by using particles with different properties e.g. composite nanowires can be made using different particles in the gold seeding process; separated particles can be connected using DNA to get meshes of nanowires. Finally, this method is compatible with both organic and inorganic materials. Hence it is possible to selectively trap cells and integrate them into a microstructure consisting of a cage around the cell. This cage can then be tracked or manipulated further downstream in the analysis using an electric field.

6. Conclusion

This novel method for manufacturing 2D and 3D microstructures using optical traps and chemical assembly combines the advantages of the top-down (tweezers) and bottom-up (chemistry) approaches. The tweezers give good control and can be rapidly reconfigured, while the different chemistry schemes offer a fast and reliable connection method. This method does not require large capital investments unlike traditional lithography. The 3D structures in this case do not require any substrate and can be made directly in fluid suspensions. The assembly process is applicable to a large variety of materials and is compatible with both living and non-living components. This could have potential applications in cell marking and tracking and for rapid prototyping of structures involving biological entities.

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List of Figure Captions:

Fig. 1. (a) An array of optical traps, (b) pattern for type I particle, (c) type I particles trapped, (d) pattern for type II particle, (e) type II particles trapped, (f) particles linked to form structure.

Fig. 2. Holographic optical tweezers system [13]

Fig. 3. Classification of the different linkage chemistry

Fig. 4. Micro/nanostructure formed using direct seeding.

Fig. 5. Microstructure formed using direct seeding: (a) gold nanoparticle coated polystyrene beads (b) bring into contact (c) gold seeding to yield final structure

Fig. 6. Microstructure using biotin-streptavidin linkage: (a) A single biotin coated polystyrene particle, (b) two particles in contact, (c) streptavidin linkage formed between particles.

Fig. 7. DNA-DNA linkage with silver staining: (a) Polystyrene particles coated with type A (red) and type B (blue) DNA, linked by hybridization. (b) Silver stained DNA connector after development with hydroquinone solution.

Fig. 8. Stretching DNA molecules using an electric field, to link the particles together, (a) DNA molecule coiled, (b) Applied electric field cause the DNA to stretch and link to next particle.

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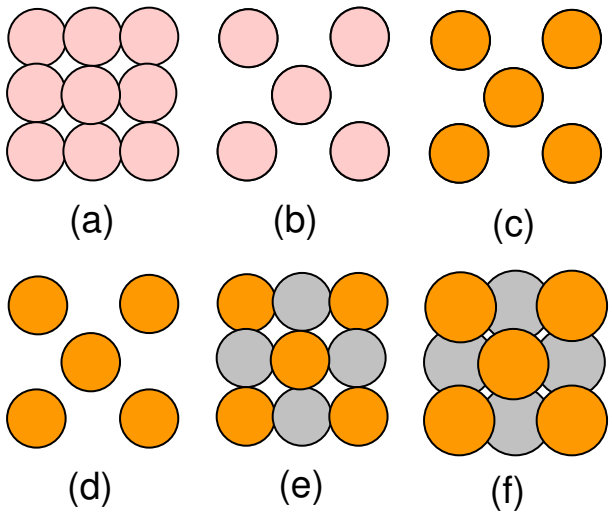

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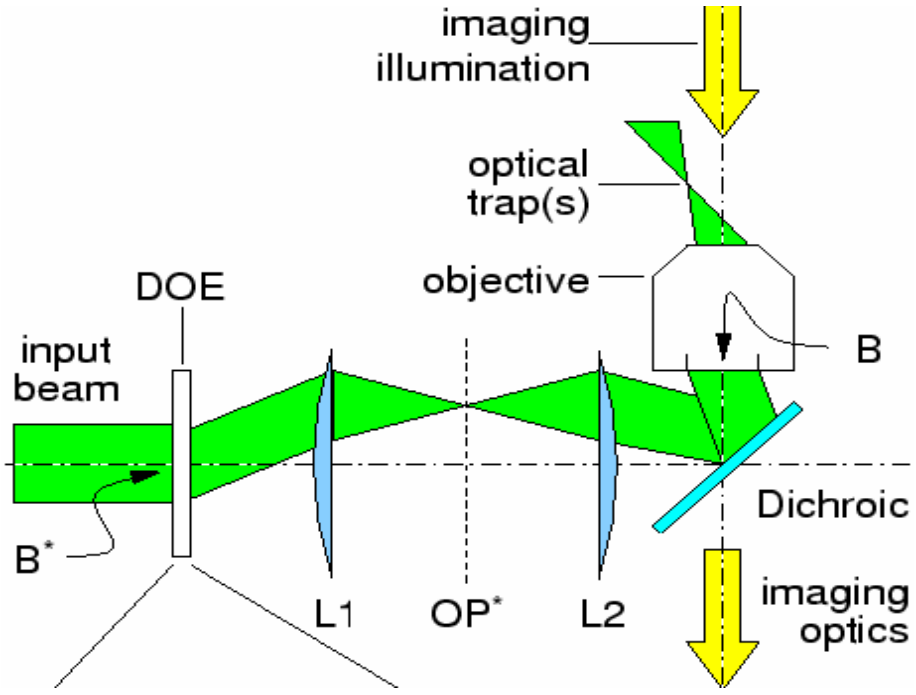

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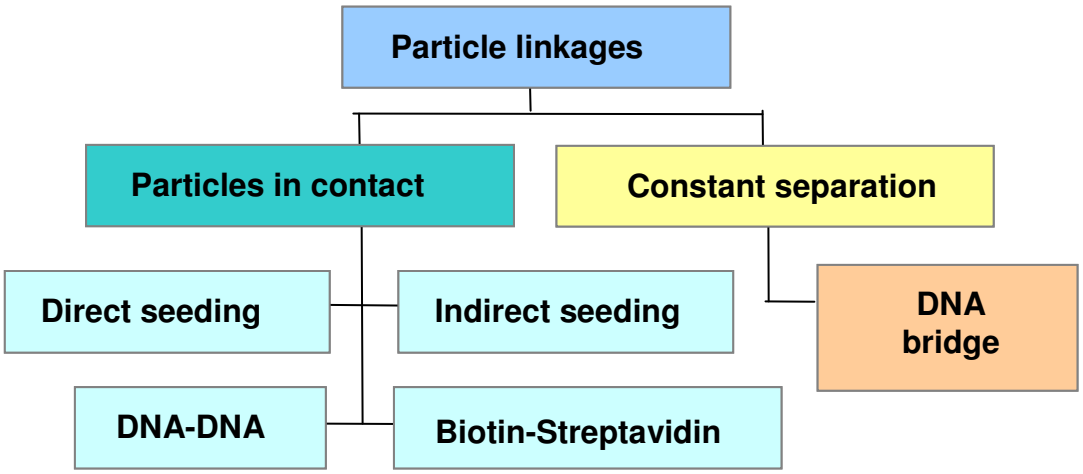
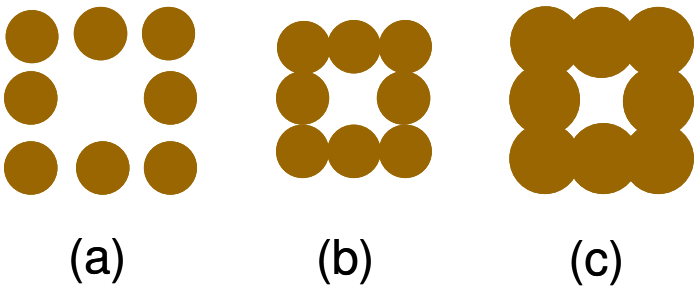


Fig. 3.



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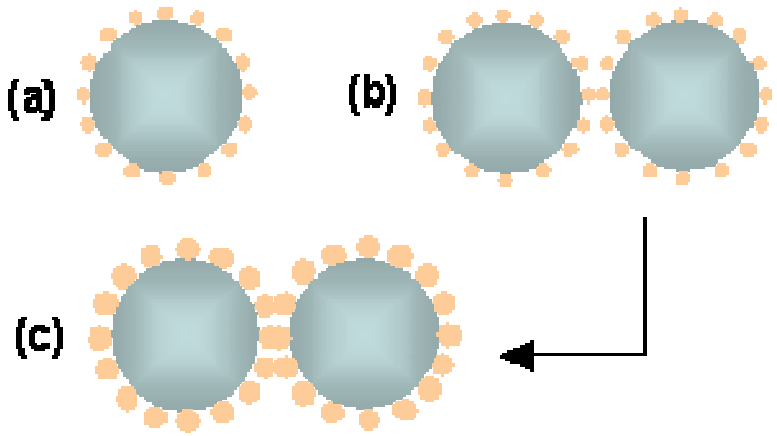
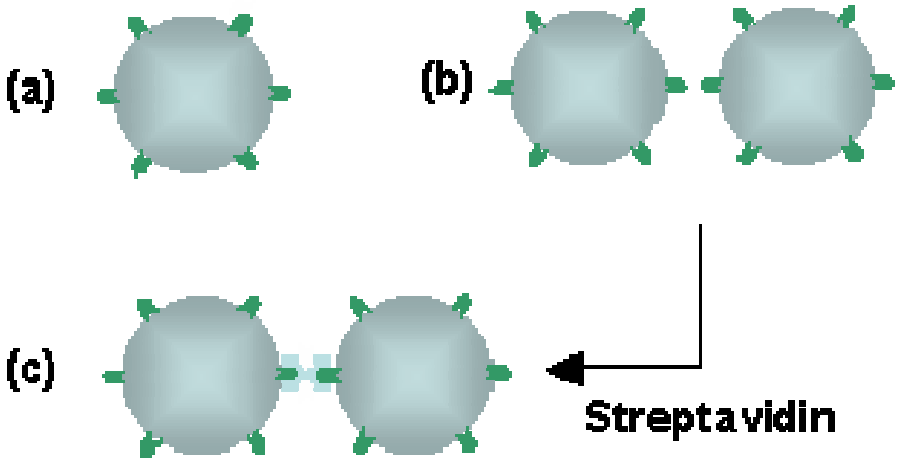


Fig. 5.



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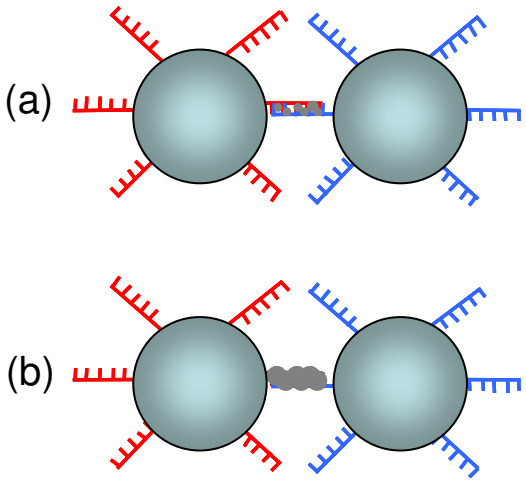

Fig. 7.

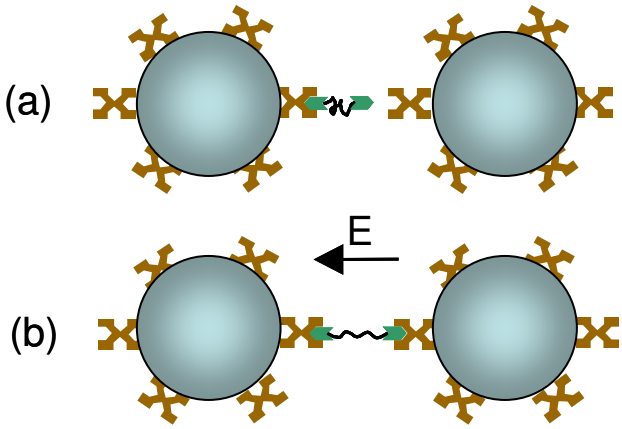

Fig. 8.