MICROVISON-ACTIVATED AUTOMATIC OPTICAL MANIPULATOR FOR MICROSCOPIC PARTICLES

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ABSTRACT

We have demonstrated an automatic optical manipulator that integrates microvision-based pattern recognition and optoelectronic tweezers (OET) for processing microscopic particles. This system automatically recognizes the positions and sizes of randomly distributed particles and creates direct image patterns to trap and transport the selected particles to form a predetermined pattern. By integrating the OET with a programmable digital micromirror device display (DMD), we are able to generate 0.8 million pixels of virtual electrodes over an effective area of 1.3 mm × 1 mm. Each virtual electrode is individually controllable for parallel manipulation of a large number of microscopic particles. Combining the automatic microvision analysis technology with the powerful optical manipulator, this system greatly increases the functionality and reduces the processing time for microparticle manipulation.

1. INTRODUCTION

Tools for manipulating microscopic particles are very important in the fields of cell biology and colloidal science. Optical tweezers and dielectrophoresis are two of the most widely used mechanisms for manipulating microparticles. Optical tweezers use direct optical forces to deflect the motion of microscopic particles [1]. They are noninvasive and have very high positioning accuracy. Holographic optical tweezers further extend the benefits to manipulating multiple particles [2]. However, they require very high optical power, and have limited working area (< 100 μm × 100 μm) due to the need of tight focusing with high numerical aperture (N.A.) lenses. These limit their use in large-scale parallel manipulation applications. On the other hand, dielectrophoresis (DEP) control the particle motion by non-uniform electric field [3,4]. It has high throughput and large working area, but requires a fixed electrode pattern for a given function. Programmable DEP cage array consisting of two-dimensional electrodes with integrated driving circuits has been demonstrated on a CMOS (complementary metal-oxide-semiconductor) chip [5]. However, the resolution is limited by the pitch of the electrode and the driving circuits of the unit cell (~ 20 μm in [5]), and the cost may prohibit its use as disposable devices.

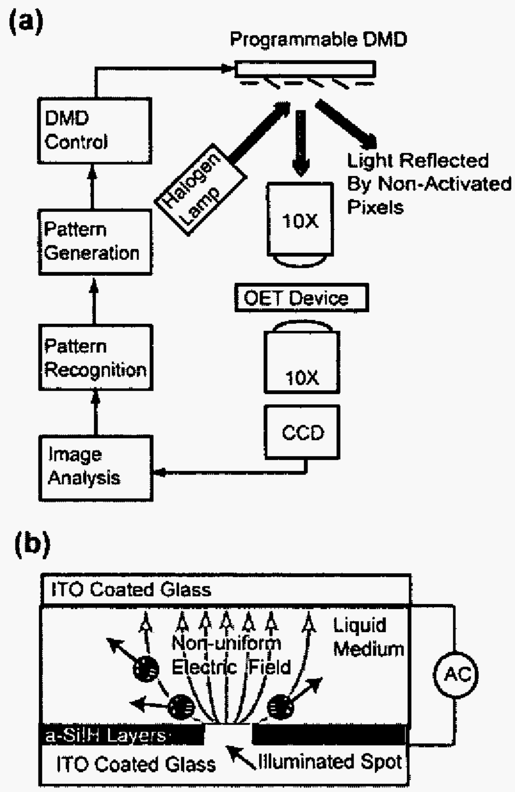


Fig. 1 (a) Schematic diagram of the microvision-based automatic optical manipulation system. (b)The structure of the OET device

Our group has developed a novel optoelectronic tweezers (OET) to address DEP forces on a photoconductive surface using optical beams [6,7]. OET enables us to pattern virtual electrodes optically. The electrode size can be varied continuously by the optical spot size down to the diffraction limit of the objective lens. Because of the optoelectronic gain in the photoconductor, the required optical power density is five orders of magnitude lower than that of optical tweezers. This enables us to use a digital optical project with incoherent light source to manipulate microparticles. We have reported the use of "light walls" to confine microparticles in virtual microfluidic channels and switch them by light pistons [8]. Interactive manipulation of virtual DEP cage array has also been demonstrated by manually changing the optical patterns [9].

In this paper, we report on an *automatic* optical manipulator by integrating the OET with a microvision-based analysis system. The microvision system automatically recognizes the particle positions and sizes, generates the desired trapping patterns, and calculates the moving paths of the particles. It enables close-loop control of trapping, transporting, and assembling a large number of particles in parallel.

2. INTEGRATION OF MICROVISON ANALYSIS WITH OET

Fig. 1(a) shows the schematic diagram of the microvision-based optical manipulation system. It is constructed on a Nikon inverted microscope. A 150W halogen lamp illuminates on a programmable digital micromirror device (DMD) microdisplay. The DMD pattern is imaged onto the OET device through a 10× objective lens.

The structure of the OET device is shown in Fig. 1(b). It consists of a top indium-tin-oxide (ITO) glass and a bottom photosensitive amorphous silicon surfaces. The liquid medium containing the particles are sandwiched between these two surfaces. The OET is biased by a single ac voltage source. Without light illumination, most of the voltage drops across the amorphous silicon layer because its impedance is much higher than the liquid layer. Under optical illumination, the conductivity of the amorphous Si increases by several orders of magnitude, shifting the voltage drop to the liquid layer. This light-induced virtual electrode creates a non-uniform electric field, and the resulting DEP forces drive the particles of interest. The light-induced DEP force can be positive or negative, controlled by the frequency of the applied ac signal. Negative DEP force repels particles away from the high field region, and is preferable for single particle cage, which can be easily formed by a light wall around the particle. Positive DEP tends to attracts multiple particles. We have employed negative DEP force in our automatic optical manipulator experiments.

The image on the OET device is captured by a CCD camera through the inverted microscope and sent to a computer for image processing. The software "Processing" [http://processing.org/] analyzes the real time video frames and generates the corresponding optical patterns for trapping and moving the particles. These patterns are then transferred to the DMD. We used TI's DMD Discovery Kit [10], which allows direct control of individual pixels. The resolution of the projected optical image on the OET device is 1.3 μm, defined by the pixel size of the mirror (13 μm). The effective optical manipulation area on the OET is 1.3 mm × 1 mm. By combining the DMD mirrors with OET device, the silicon-coated glass is turned into a million-pixel optical manipulator.

3. EXPERIMENT

Fig. 2 illustrates process of automatically recognizing and arranging randomly distributed particles into a predetermined pattern. First, the images of the particles are captured and analyzed by the microvision system (Step 1), which identifies the positions and the sizes of all particles (Step 2). The software then generates a ring trap around each particle (Step 3). It also calculates the trajectories of the particles to reach their final positions (Step 4).

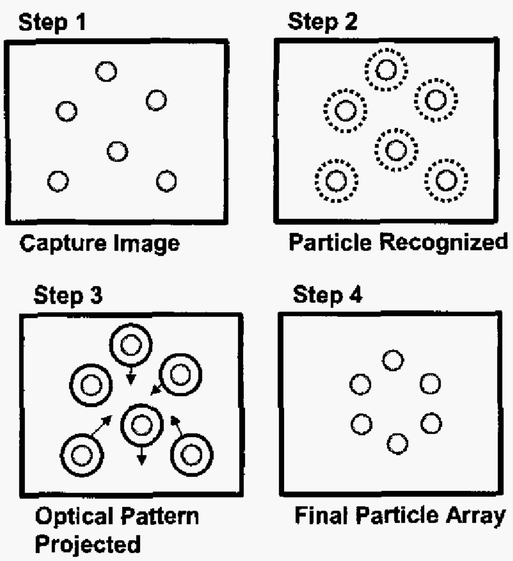
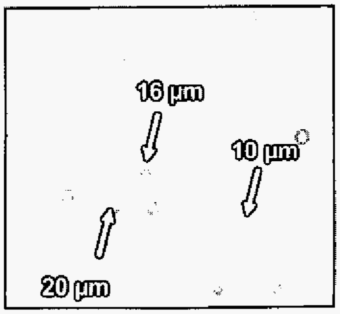


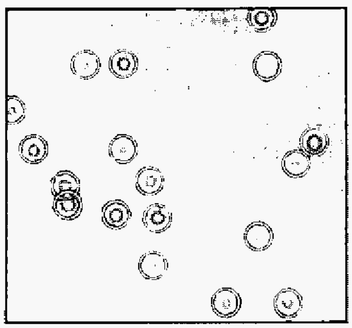
Fig. 2 Steps to arrange randomly distributed particles into a specific pattern.

Particle Recognition

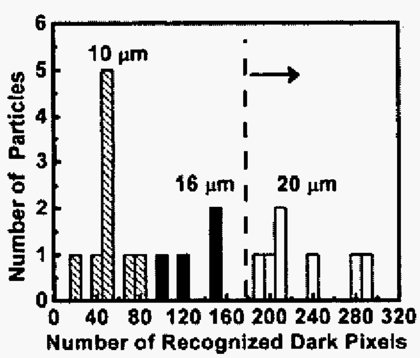
Particle recognition is achieved by using a dark-pixel recognition algorithm to scan through each pixel of the captured image. The brightness value and the position of each pixel are then recorded and calculated to determine the size of each particle and its center position. Fig. 3(a) shows an image of randomly distributed particles with three different sizes, 10 μm, 16 μm, and 20μm. The brightness value of the pixels at the particle edge is smaller than that of the background and the color is darker too. By setting a threshold brightness value between the background and the particle edge, we can recognize the edge pixels of each particle. Averaging the x and y position data of the edge pixels of each particle, we can determine its position. Fig. 3(b) shows the recognized particles marked by a white ring pattern generated by the microvision analysis system. The same algorithm also determines the size of each particle by counting the number of the recognized dark pixels. Fig. 3(c) is the histogram data showing the number of particles and the number of the dark pixels recognized for each particle on this image. Bigger particles have more dark pixels than smaller ones. By setting a threshold number for the recognized pixels, as indicated by the dash line in the histogram figure, the system can selectively pick up particles with certain sizes. For example, in Fig. 3(d), the seven largest beads (20 μm) are selected by setting a threshold number equal to 180. This recognition algorithm is used specifically for determining spherical particles with different sizes. Other algorithms can be developed to recognize particles with different colors, shapes, or textures.



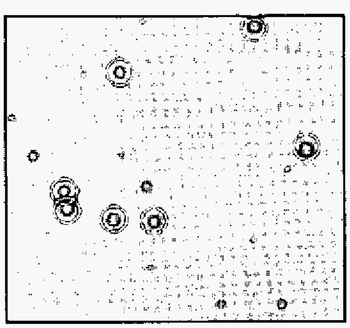
(a)



(b)



(c)

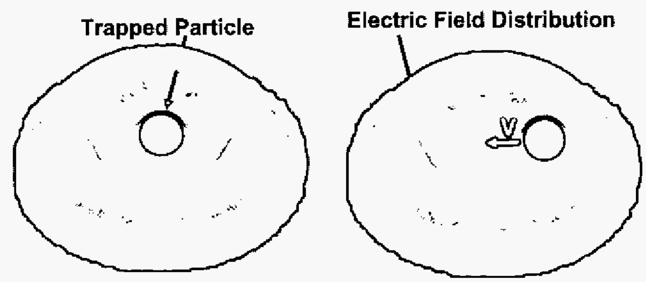


(d)

Fig. 3(a) Test image for particle recognition system. Polystyrene particles with three different sizes, 10 μm, 16 μm, and 20 μm, are mixed and randomly distributed in the liquid medium. (b) The microvision system recognizes the position of each particle and projects a ring mark on each particle. (c) The histogram showing the number of particles versus the number of recognized dark pixels in this test image. (d) By setting a threshold for the dark pixels, the largest particles are selectively picked up.

Particle Trapped by an Optical Ring Pattern

Trapping of a single particle is achieved by operating OET in the negative DEP regime. We create an optical ring pattern to form a virtual DEP cage that allows only one single particle to be trapped inside the ring, as shown in Fig. 4. In static state, the trapped particle will be focused at the center or the ring pattern where the minimum electric field strength occurs. When the optical ring moves, the trapped particle also move in the same direction but with a position deviated from the ring center so that the DEP force pushes the particle in the direction toward the center. This deviation distance depends on how fast the particle moves. When the optical ring moves too fast, the particle will escape the optical ring because the DEP force is not strong enough to hold it. The escaping speed of a 20 μm particle is 40 μm/sec in our current system. To trap a particle with a smaller size, a smaller optical ring would be required to ensure a single particle in the ring.



(a) Static state *(b) Moving State*

Fig.4(a) Electric field distribution induced by a single optical ring pattern. In static state, the particle is trapped in the electric field minimum in the center. (b) During moving, the particle is displaced from the center as a result of the balance between the DEP and the viscous forces .

Parallel Manipulation of Multiple Particles

Once the particle positions are recognized, the software generates the corresponding ring-shaped traps and calculates the transport trace for each particle. These optical patterns are stored as image files and are batch loaded to the DMD control software to create dynamic optical patterns to trap and transport particles. These processes are shown in Fig. 5(a). The image of the randomly distributed particles was scanned vertically from left to right. The first six particles were identified and trapped by the OET (0 sec). The trapped particles were transported by moving the ring traps, and reached the hexagonal configuration in 12 seconds. Fig. 5(b) and (c) shows the video sequences of rearranging the particles into linear and triangular shapes and the unwanted particles were swept away by a scanning line pattern.

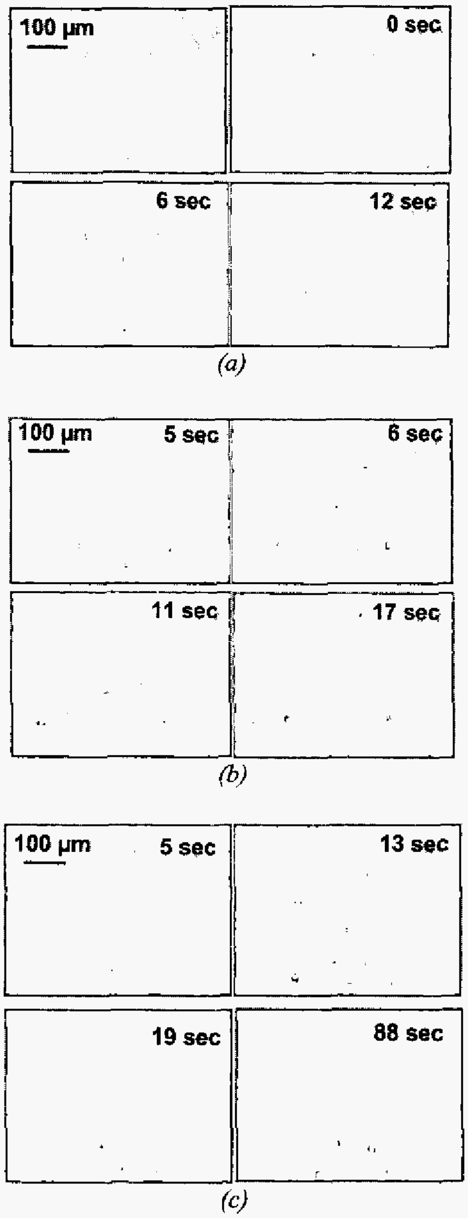


Fig.5 Examples of microvision-based automatic optical manipulation of microscopic particles. (a) Randomly distributed particles arranged into a hexagonal shape. (b) The hexagonal pattern is transform into a line. (c) The line pattern is transform into a triangle shape. The unwanted particles are swept away by a scanning line.

CONCLUSION

We have demonstrated an automatic optical manipulator that allows a feedback control through a microvision analysis system. This system can automatically recognize particles with specific size from a mixture of particles with different sizes and generate optical manipulating patterns to trap and move these selected particles to form a predetermined pattern. The large optical manipulation area (> 1 mm × 1mm) of our OET device permits parallel manipulation of a large number of microscopic particles. The automatic parallel optical manipulation system greatly reduces the time for sorting and patterning microscopic particles. With further optimization, the system will be able to sort particles with different colors, shapes, or textures. More sophisticated optical manipulation functions can also be performed. The automatic optical manipulator has many potential applications in biological cell analysis and colloid science fields.

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