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Dielectrophoretic Field Cages Made from Indium Tin Oxide: Trapping of Submicron Particles within Transparent Electrode Structures

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Indium tin oxide (ITO) layers on glass have been structured by KrF excimer laser ablation into planar electrode arrays. These have been assembled to form three-dimensional hybrid structures and energized by high-frequency (1–50 MHz) a.c. Particulate materials of different origin, suspended in aqueous solutions, can be manipulated using negative dielectrophoresis. Polystyrene latexes of submicrometer diameter and viable mammalian cells have been trapped in dielectrophoretic field cages made from ITO, as previously shown for noble metal electrodes. Optical observation of the trapped objects is facilitated by the transparency of the electrode material. Fine-structured ITO electrodes can be used for contactless particle handling in complex microsystems.

1. Introduction

A dielectric body in an a.c. electric field polarizes. Depending on the relaxation characteristics of particle and medium and on the field frequency, the induced polarity can be parallel or anti-parallel to the external field. In spatially inhomogeneous fields, the particle will be moved towards the electrodes in the first case (positive dielectrophoresis) or away from them (negative dielectrophoresis) in the second case.(1)

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Negative or positive dielectrophoresis occurs according to the shape and the effective complex permittivity of the particle and the surrounding liquid ($\tilde{\varepsilon} = \sigma + i\omega\varepsilon\varepsilon_0$, where ε is the relative permittivity, σ is the conductivity and ω is the radian frequency of the field). If both the conductivity and the permittivity of a given particle are lower than those of the solution, repulsion from the electrodes occurs. In most artificial polymer particles, such as latex or chromatographic carrier material, negative dielectrophoresis has been found at high frequencies (MHz range) and in conductive electrolyte solutions (> 1 mS/m).(2) Many viable cells show negative dielectrophoresis at all frequencies when the conductivity of the medium is higher than 0.5 S/m. In previous work with such conductive solutions, ultramicroelectrodes allowed the application of electric fields sufficently strong for particle and cell manipulation (kV/m to MV/m).(3)

Since the electric field distribution between electrodes depends on their shape and configuration, field minima can be produced in the bulk solution by appropriate design and drive. Over the central region of a crosslike electrode quadrupole, a field force funnel can be formed. Such a funnel can be closed at the top by a barrier such as a cover slip. Alternatively, three-dimensional octopole electrodes will produce a closed trap or a dielectrophoretic field cage. Spatial deformations of funnel/cage shape are possible by altering the driving regime and therefore very complex field formed cages with permanent or temporary openings can be created (For review see refs. 4 and 5). With traps and cages generated by micrometer and submicrometer diameter metal electrodes, solids such as latex spheres or liquid droplets, as well as pollen, plant protoplasts, yeast cells, trypsinized mammalian cells, mammalian ova or spermatozoa, viruses or vesicles suspended in electrolyte solutions can be (individually) moved, confined or arranged into structured microaggregates.(5−14) Traps generated in microelectrode arrays have been shown to be promising components for complex miniaturized systems for particle handling and measurement.(2,7)

In addition to facilitating the collection and confinement of different particles from suspensions without the need for physical contact, data which would otherwise be unobtainable from individual particles can be measured as a function of field frequency (rotation spectroscopy) using propagating a.c. fields.(15,16) The importance of these data and of such miniaturized systems for applications in life sciences, medicine and biotechnology is obvious.

The conventional metal electrodes used so far to generate dielectrophoretic microtools cause problems during the optical observation of objects under investigation. Particularly in complex arrays such as three-dimensional field cages, observation of electrode surfaces is obscured. Similar problems in liquid crystal display panel fabrication or in voltammetry of color-changing films(17) have been solved using (finely structured) ITO layers, deposited on glass.

Some electrical and optical properties of ITO films have been measured; in particular, the high-frequency dielectric constant was found to be 3.4–4.0.(18) Such films have been used in cell growth investigation.(19) Here, we report their application to complex field cages and traps.

2. Materials and Methods

ITO layers, 40 nm thick, sputtered onto AF 45 glass chips, 9 × 9 mm [DESAG, Grünenplan, Germany] were structured using 248 nm light from a KrF excimer laser system [Exitech EX-PS-750, Long Hanborough, Oxford, UK]. The precise ablation in the central parts of multipole arrays was accomplished using specially designed masks. These masks were made from platinum/titanium film deposited on thin quartz plates. The metal layer was ablated to form the required pattern at 15 × final size. The masks were then used to shape the laser beam prior to its passage through the objective (with 15-fold reduction) onto the workpiece. Peripheral parts of the ITO structures were freed from the conducting material by direct writing with the laser beam.

The optical transparency of an unstructured ITO layer for 190 – 900 nm light was quantified spectrophotometrically [UVIKON 930, Kontron Instruments GmbH, Neufahrn, Germany]. As reference an AF45 chip previously treated with hydrochloric acid to remove the ITO layers was used.

Electrical connection of the chip to a carrier was made using silver-filled epoxy and gold wire. These bonding spots were additionally fixed with conventional epoxy. Two chips, carrying identically shaped quadrupoles, were mounted face to face at a distance of 20 – 80 μm. The mounted hybrid chip functioned as a three-dimensional octopole dielectrophoretic field cage. The ohmic resistance of an ITO slab (1 × 10 × 0.04 mm) on the glass was estimated with a conventional multimeter.

Latex particles, 9.9 μm, 3.4 μm and 0.46 μm in diameter [Polysciences, Inc., Warrington, PA, U.S.A.] were used after washing and equilibration in the measuring electrolyte solutions. Mouse L 929 cells, trypsinized from confluent culture in HEPES buffered Dulbecco's Modified Eagle Medium (DMEM), were resuspended in culture medium. A single-cell suspension was achieved by diluting an aliquot with 300 mM aqueous inositol solution (1 + 3).

The electrodes on the chip carrier were connected to a HP 8116A pulse/function generator or a laboratory-made rectangular four-phase generator.(6) Experiments were set up in the manner shown in Fig. 1. Recording was carried out using a microscope camera and video equipment in combination with an epifluorescence microscope (Axioscop, Zeiss, Oberkochen, Germany).

3. Results

The ITO electrode structures generated by laser ablation are comparable in accuracy and working properties to gold electrodes fabricated by microlithography on planar substrates. Corrosion, which can occur with gold layers on different metal plating bases, is unlikely with the homogeneous conductor and was not observed. Gaps as fine as 2 μm could be made easily with the ablation/direct writing technique. The transparency of the electrodes allowed good differential interference contrast microscopic observation even of faint structures such as cells (Fig. 2(d)). ITO has an absorption peak at 260 nm but optical transmission of the layer reaches 50% at 320 nm, 75% at 400 nm, and 98% at 550 nm

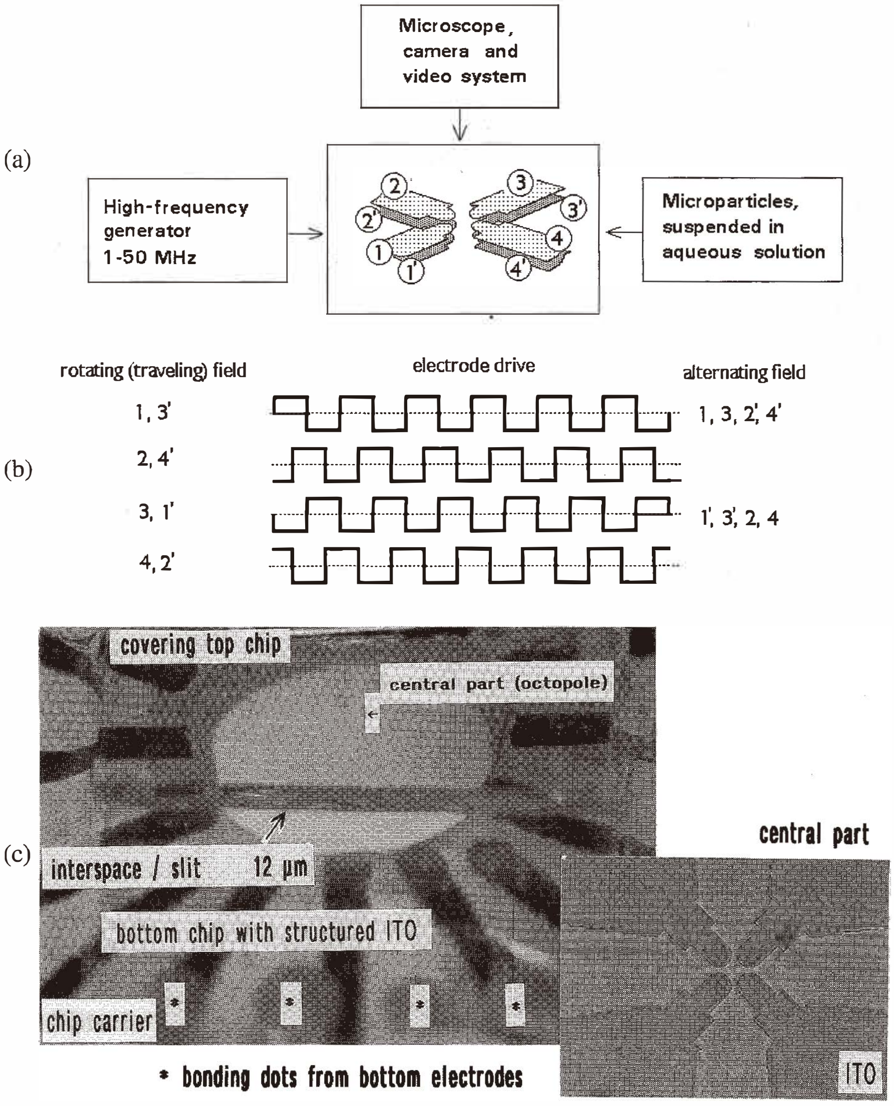

Fig. 1. Experimental setup. (a) Schematic view of apparatus used. (b) Electrode connections of the octopole to a four-phase generator as given for rotating and alternating fields. (c) Two glass chips with ITO electrodes were mounted face-to-face on a carrier for individual electrode connections. The slit between the chips is filled with an aqueous particle suspension. The inset shows the enlarged central ITO structure. The precisely arranged and driven electrodes formed an octopole dielectrophoretic field cage allowing particle confinement in the central region between top and bottom planes. Note the mask-ablated center and the serrated edges, ablated by direct writing with the laser beam.

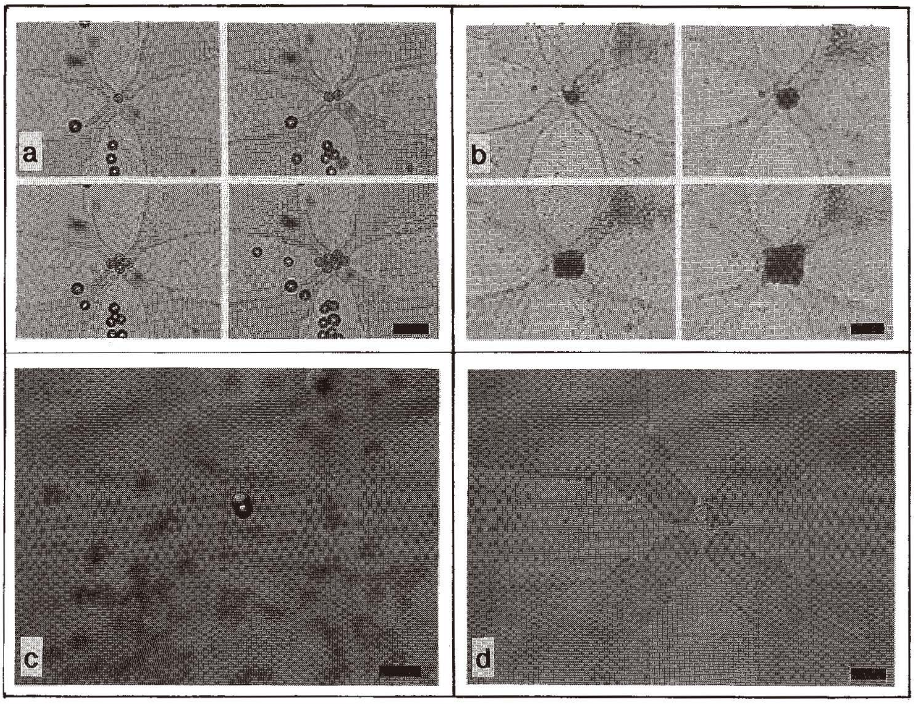

Fig. 2. Microparticles confined in the central region of dielectrophoretic field cages. In all cases, the electrodes are the curved-tip structures running diagonally into the center of the photographs. (a) Collection of 3.4 μm latex particles from a streaming suspension in an octopole field cage. Planar tip-to-tip distance: 5 μm, small gaps between neighboring electrodes: 2 μm. Cage height: 11μm, drive: alternating field, 1 MHz, 5 V_{ptp}. Consecutive photographs of particle aggregation are shown. Bar: 10 μm. (b) Latex particles (0.46 μm diameter) forming a particle cushion. Octopole cage as in (a), alternating field: 2 MHz, 5 V_{ptp}. Consecutive frames from a video sequence showing different stages of particle aggregation from a suspension. The final shape and size of the particle aggregate depend on dielectric properties and driving conditions. Typically, a stationary stage is reached after a few seconds. Note the good visibility of the particle cushion outside the center. Bar: 10 μm. (c) A stack of three 9.9 μm latex spheres in a 76-μm-high octopole cage. Planar tip-to-tip distance is 25 μm, and small gap is 5 μm. Electrode drive: 1 MHz, 10 V_{ptp} alternating field. Particles outside the dielectrophoretic cage settle by gravity, and are therefore out of focus and only faintly visible. Bar: 20 μm. (d) Trapping of a single mouse L 929 cell in cell culture medium DMEM, diluted 1 : 4 with 300 mM inositol. Octopole cage: height 35 μm, four-phase rotating a.c. driven at 1 MHz, 20 V_{ptp}. Bar: 20 μm.

wavelength light. The material would, therefore, be compatible with most microscopic techniques. The conductivity of the material used was measured to be 1.67 × 10^5 S/m (resistance between the sides of any square of the material was 150 Ohm, measured with d.c.).

Latex particles from 10 μm to as small as 460 nm could be confined inside three-dimensional field cages (Figs. 2(a) – 2(c)) as shown before for noble metal electrode structures. They could also be held inside planar dielectrophoretic field traps (not shown).

4. Discussion

The ITO was structured to yield wide connections to the central quadrupole in order to prevent losses due to low conductivity in comparison with metal electrodes. At high conductivities in cell culture medium (1.5 S/m) the large active electrode area caused significant losses. For the design used, osmotically balanced dilutions had to be substituted for the original medium (Fig. 2(d)). For other designs, metallic connections could be used leaving free/transparent only the central dielectrophoretical/optical working window of the electrode array.

ITO has been proven to be useful for electrode manufacture in micrometer and submicrometer field cage development. Good optical transparency in the visible light region allows observation of cells or other dispersed solid particles or liquid droplets down to submicrometer size in aqueous solutions during manipulation by complex miniaturized electrode systems. Fluorescent indicators from the xanthene, acridine and cyanine series all have absorption peaks above 400 nm and are therefore usable in ITO structures. This opens up new possibilities for combination with optical techniques such as correlation spectroscopic methods(20–22) and laser tweezers(23) for small particles such as cells or even macromolecules.(24)

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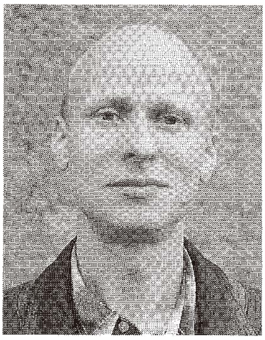

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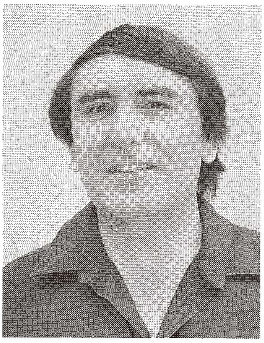

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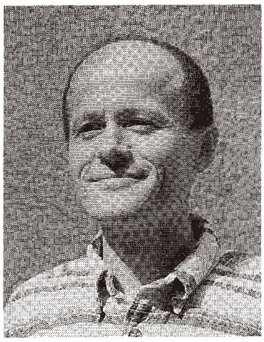

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