(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 March 2006 (30.03.2006)

PCT

(10) International Publication Number
WO 2006/032844 A2

(51) International Patent Classification:
G21K 1/00 (2006.01) *B01J 19/00* (2006.01)
B01L 3/00 (2006.01) *G01N 30/00* (2006.01)

(21) International Application Number:
PCT/GB2005/003476

(22) International Filing Date:
9 September 2005 (09.09.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0421166.0 23 September 2004 (23.09.2004) GB

(71) Applicant *(for all designated States except US)*: **THE UNIVERSITY COURT OF THE UNIVERSITY OF ST ANDREWS** [GB/GB]; College Gate, North Street, St Andrews KY16 9AJ (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **DHOLAKIA, Kishan** [GB/GB]; School of Physics and Astronomy, University of St Andrews, North Haugh, Fife KY16 9SS (GB). **McGLOIN, David** [GB/GB]; School of Physics and Astronomy, University of St Andrews, North Haugh, Fife KY16 9SS (GB). **PATERSON, Lynn** [GB/GB]; School of Physics and Astronomy, University of St Andrews, North Haugh, Fife KY16 9SS (GB).

(74) Agents: **SZCZUKA, Jan, Tymoteusz** et al.; Marks & Clerk, 19 Royal Exchange Square, Glasgow G1 3AE (GB).

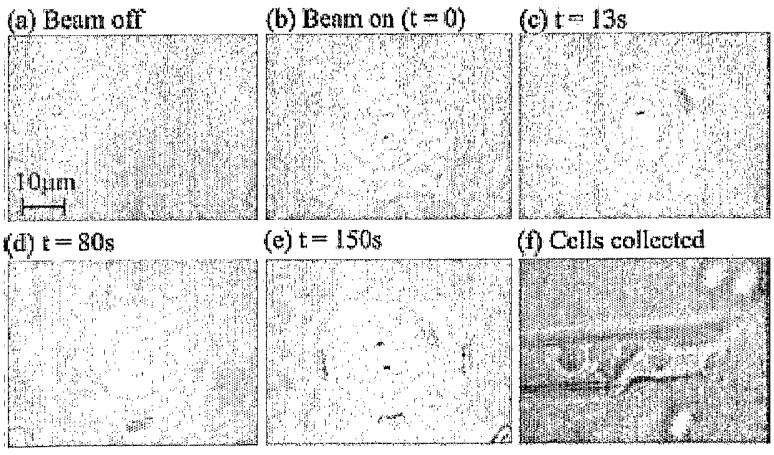
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PARTICLE SORTING IN A TAILORED LANDSCAPE



(57) Abstract: A method for sorting/separating at least two different particles in a fluid, the method comprising defining within the fluid a static optical landscape/pattern having one or more optical wells or troughs that are substantially the same size or slightly larger than at least one of the particles. By exploiting differing particle responses to the same light pattern, separation/sorting can be done. This type of sorting may potentially be performed to separate particles that are of different sizes, shapes or refractive indices.

WO 2006/032844 A2

Particle Sorting in a Tailored Landscape

The present invention relates to a system and method for sorting particles and in particular for sorting cells in the absence of a fluid flow.

A variety of particle sorting or separation schemes exist, ranging from gel-electrophoresis, capillary electrophoresis, and analytical centrifuging to novel, entropic barriers. Examples of these are described by J. Han, H. G. Craighead, Science 288, 1026-1029 (May 12, 2000) and D. Nykypanchuk, H. H. Strey, D. A. Hoagland, Science 297, 987-990 (Aug 9, 2002). The majority of these known techniques separate a polydisperse mixture in a flowing fluid into bands containing particles that travel at different velocities along the direction of flow. This typically leads to batch processing. In electrophoresis a gel may be used to obtain a size-dependent mobility. Recovery of fractions is achieved through post-processing of the gel. However, despite its widespread use and effectiveness this methodology is slow and importantly, due to limited pore sizes, has difficulty in separating objects at the microscopic size level, for example cells, chromosomes, and colloidal matter.

Lithographically fabricated two-dimensional, asymmetric artificial gels are also used. Examples of these are described in the articles by D. Ertas, Physical Review Letters 80, 1548-1551 (Feb 16, 1998); T. A. J. Duke, R. H. Austin, Physical Review Letters 80, 1552-1555 (Feb 16, 1998) and C. F. Chou et al., Biophysical Journal 83, 2170-2179 (Oct, 2002). These gels yield separation transverse to the direction of flow. Because of this, they can be operated in a continuous fashion, with various fractions taken up by separate collection channels. However, sorting based on diffusion becomes impractically slow at the microscopic scale and above.

Another sorting or fractionation scheme that has been proposed is described in the article "Kinetically Locked-in Colloidal Transport in an Array of Optical Tweezers" by P. T. Korda et al, Physical Review Letters 89, Number 12, Art. No. 128301 (16 Sep, 2002). In this case, a monolayer of colloidal spheres is allowed to flow through an array of discrete optical traps. By varying the orientation of the trap lattice it was shown that the direction of flow of the spheres could be varied. Because of this, it was suggested that the lattice could be used to continuously fractionate mesoscopic

particles. However, because of the use of a lattice of localized discrete traps, the observed kinetically locked-in channelling along low-index lattice vectors was intrinsically limited to small-angle deflections. In practice, this limits the practicality of the lattice for use in fractionation.

PCT/GB2004/001993 describes yet another optical fractionation scheme. In this, three-dimensional optical lattices are used for sorting and fractionation of biological and colloidal material in a microfluidic flow, see also M. MacDonald, G. Spalding and K. Dholakia, Nature 426, 421 (2003). However, a problem with both this arrangement and that proposed by Korda et al is that it requires the presence of a fluid flow. In some circumstances, this can be a significant disadvantage.

According to the present invention, there is provided a method for sorting/separating at least two different particles in a fluid, the method comprising defining within the fluid an optical landscape/pattern having one or more optical wells or troughs that are substantially the same size or slightly larger than at least one of the particles, the optical landscape/pattern being arranged to cause sorting/separation of the particles in the absence of fluid flow.

Different particles are affected by the optical field in different ways. By exploiting differing particle responses to the same light pattern, separation/sorting can be done. This type of sorting may potentially be performed to separate particles that are of different sizes, shapes or refractive indices. Thermal activation leads to a differing residence time within the optical wells for the particles, so that for example smaller particles hop across the landscape at different rates than relatively larger particles. In contrast, the larger particles may be of such size that they straddle two or more wells in the patterns and thus rather than respond to each individual well are likely to respond to the overlying envelope of the pattern. This allows the particles to be sorted and separated without the need to implement flows and/or microfluidic systems. This is a significant advantage and offers compatibility with standard microscopes for example where a beam pattern may be projected directly onto the sample in the microscope, thereby to initiate sorting.

Adjacent wells may be concentric. The adjacent wells may be concentric rings. The adjacent well may be symmetric.

The optical landscape may be provided by a Bessel beam that defines a plurality of concentric rings or wells. An advantage of this is that particles within the rings of the Bessel landscape gradually hop or migrate towards the centre. Once particles are gathered in the centre area, increasing the optical power of the beam raises the central field potential causing physical movement of the captured particles upwards. Elevating the centre particles in this way makes it easier to remove them from the main body of the fluid.

The optical landscape may be tilted, thereby creating an asymmetry in the barrier heights of the optical potential in a given well direction, which encourages movement of the particles in a pre-determined direction.

According to another aspect of the present invention, there is provided a method for sorting/separating at least two different particles in a fluid, the method comprising defining within the fluid an optical landscape/pattern having one or more optical wells or troughs and varying the optical landscape to identify parameters that cause sorting/separation of one of the particles from the main body of the fluid.

According to yet another aspect of the invention, there is provided a system for sorting/separating at least two different particles in a fluid, the system comprising means for defining within the fluid a static optical landscape/pattern having one or more optical wells having widths that are substantially the same size or slightly larger than at least one of the particles, the landscape defining a potential gradient for causing selected particles to accumulate within a pre-determined region of the landscape. This system could be incorporated in a microscope.

Various aspects of the invention will now be described by way of example only and with reference to the accompanying drawings, of which:

Figure 1 is a photograph of a Bessel beam;

Figure 2 is a plot of potential well depth versus distance from the centre for a Bessel beam;

Figure 3 is an illustration of a particle flow process;

Figure 4 illustrates a sorting process in which particles gradually hop towards the centre of the beam and are subsequently directed upwards into a capture chamber;

Figure 5 is a block diagram of an arrangement for generating a Bessel beam;

Figure 6 is a series of images showing one-micron spheres gradually accumulating in the centre of a Bessel beam;

Figure 7 is a series of images showing how five-micron spheres and one-micron spheres respond differently to the optical potential, with the larger spheres accumulating in the middle;

Figure 8 is a series of images showing the separation of red and white blood cells in a Bessel beam as a function of time and the collection of white blood cells in a microcapillary reservoir;

Figure 9 is a series of images showing a chromosome attached to a sphere being transported across the optical landscape of a Bessel beam into the central maximum where the chromosome-sphere complex is vertically guided;

Figure 10 is a series of images showing the separation of silica-sphere-labeled T-lymphocytes from other unlabeled lymphocytes in the Bessel beam as a function of time, and

Figure 11 is a schematic view of a linear optical landscape for sorting particles.

Particles of differing shape, refractive index and size respond differently in the presence of a tailored, static optical landscape. By exploiting the differing Brownian dynamics in such a tailored optical landscape, particle sorting can be effectively done without the need for causing a fluid flow. Any suitable optical landscape can be used, provided the features of the light patterns, such as modulation/periodicity, are comparable in size to at least one of the particles. The field must also have some form of periodicity and some way of enticing the particles of choice to accumulate somewhere in the pattern. This can be done by adding a tilt or asymmetry to the light field. Alternatively, an intensity gradient is needed. In a preferred example, the optical landscape may be that defined by a Bessel beam. In this case, the field intensity gradient encourages the smaller particles to move towards the centre beam.

Figure 1 shows an example of a Bessel beam. This has a set of concentric rings and a centre beam. Each ring of the Bessel beam forms a 2D annular potential well within which particles can reside and undergo restricted Brownian motion. Random hopping between wells occurs according to Kramer's theory (L. I. McCann et al, Nature 402 (Issue 6763) 785-787 (16 Dec, 1999); Landa, 1998; Lindner et al., 1999). As shown in Figure 2, the rings define a series of potential wells that increase in depth towards the centre. The center of the Bessel beam is like a rod of light that propagates without spreading.

During sorting, particles are dispersed in a fluid sample and the circularly symmetric Bessel beam pattern is projected upon them. Typically, this will be done with the beam being projected onto the sample in a substantially vertical direction. Large particles may respond only to the overall envelope of the light pattern if the size of the particle is well in excess of the width of ring. The smaller particles stay in each ring and may "hop out" and migrate very slowly towards the centre. The Kramer's residence time is key here. If a radial tilt is imposed on the pattern, as shown in Figure 3, then particles may move faster. When the particles are accumulated in the middle of the beam, increasing the power guides them upwards for example to a second chamber where they can be transported away as shown in Figure 4.

Figure 5 shows an arrangement for testing the method in which the invention is embodied. This has a neodymium YAG laser with a beam expander at its output. Light from the beam expander is input to an axicon, thereby to produce a Bessel beam. The Bessel beam is directed onto a dielectric mirror and focused using a suitable lens onto a sample stage. The sample stage is back-lit using white light so that the Bessel beam and movement of particles can be viewed by a suitable camera arrangement. The choice of various lenses dictates the size of the central maximum and also spatial extent and periodicity.

To test the invention, samples of spheres or cells were placed on the sample stage in the beam path in a chamber approximately of 1cm diameter and of 100 microns depth. For the samples of spheres (colloids) either water or a mixture of heavy water D2O and water was used. The heavy water shows reduced heating. The beam may be

generated at any wavelength but for the purposes of experiment, 1064nm and 1070nm were used. These were selected because heating of the water in the sample is not a significant issue at these wavelengths. Figure 6 shows the accumulation of 1 micron spheres in the centre of an untilted Bessel beam. Figure 7 shows how 5 micron spheres and 1 micron spheres respond differently to the optical potential, with the larger spheres accumulating in the middle.

The method of the invention was also tested to determine whether it could be used to separate cells. To do this, a Bessel beam was imposed on a fluid sample that included red blood cells (erythrocytes) and white blood cells (lymphocytes). Figure 8 shows the gradual separation of red and white blood cells in this Bessel beam as a function of time (in minutes). From this is can be seen that the lymphocytes are transported to the center and erythrocytes align in the outer rings and are 'locked' into these rings. Hence, by appropriate selection of beam parameters, the lymphocytes can be drawn towards and accumulated in the middle of the pattern, whereas the erythrocytes re-orient due to their bi-concave shape and stay "locked" in the rings. Lymphocytes can be readily extracted with a capillary from this system.

Sorting particles is of particular interest in the biological field. Whilst the invention can be successfully applied to the sorting of erythrocytes and lymphocytes, the main barrier to optical trapping and accumulating of many biological macro-molecules like chromosomes is their low scattering and the fact their refractive index is almost identical to that of immersing solution. This limits the trapping force created by photons, which are scattered and refracted by the trapping object. For guiding, sufficient scattering is generated by increasing the laser power. The small relative refractive index results in little exchange of optical momentum and thus little effect on their mobility. Additionally the macromolecule is hugely exposed to the intense laser radiation causing denaturation and damage due to absorption. An alternative way of handling of macromolecules is to attach them to colloidal particles of higher refractive index, which receive most of scattering and refraction from laser field. This particle can act as a cargo carrier in this instance. Biological molecules (e.g. DNA, cells) attached to such a bead can be readily manipulated by using the bead as an anchor.

The attachment of spheres has not previously been applied to chromosomes. To test chromosome sorting, streptavidin coated microspheres were attached non-covalently to biotin-labelled Chinese hamster ovarian (CHO) chromosomes. Non-covalent binding creates elastic linkage between a chromosome and a bead. The microspheres and attached beads were introduced into a fluid and the beam was projected onto it. While a bead diffuses rapidly, following the form of the potential and overcoming potential barriers, it stretches the linkage, which after relaxation provides sufficient force to transport the chromosome across the potential barriers. The shape of chromosome is far from spherical and their linear contour size varies between 1 to 10 μm. They do not lend themselves to rapid and controlled transit across the potential landscape. However, Figure 9 shows that by attaching beads, chromosomes can accumulate and be guided in an optical landscape. The behaviour is dictated by the power level and periodicity of the beam. Hence, by attaching beads to chromosomes, chromosome sorting is provided.

In further tests equal volumes of erythrocytes and lymphocytes were mixed together in a sample chamber and exposed to a Bessel beam of 5.0 μm core size, 3.2 μm ring size, and propagation distance of approximately 3 mm. In a first set of experiments, a qualitative study of the cell sorting was performed. Samples of cells were exposed to the Bessel beam for total beam powers of 100 mW to 800 mW in increments of 100 mW, after which the movement of the cells was observed. At low powers (up to 300 mW) all cells (lymphocytes and erythrocytes) were transported slowly towards the central core of the Bessel beam, where they were finally trapped by forming a vertical stack at the top of the sample chamber. The biconcave-shaped erythrocytes, aligned on their sides with the longest axis of the cell in the direction of beam propagation. Theoretical studies confirm this alignment behaviour for erythrocytes. At higher powers (above 400mW) the erythrocytes move towards the central core but before reaching the center, align vertically in the outer rings of the Bessel beam. Once reoriented, the erythrocytes are "locked-in" to the specific ring and are guided upwards within that ring. In contrast, the spherically shaped lymphocytes move directly towards the central Bessel core, where they form a vertical stack along the central maximum as described earlier, responding rather to the overlying potential and not being "locked" within any ring of the Bessel beam.

Measuring the time it takes from one cell to be transported from the fifth ring of the Bessel beam into the central core for different powers gives an idea of the throughput of this system. A single erythrocyte or lymphocyte was placed at the same position on the fifth ring and allowed to travel towards the center of the beam at three power regimes; low denotes a power across the whole Bessel beam of 150mW, medium denotes 350mW, and high denotes a laser power of 550mW. This process was repeated with 70 cells for each power. All lymphocytes were transported towards the beam core at all power regimes, where they were guided vertically upwards through the sample. The velocity of each cell increased as it travelled from ring to ring, closer to the central maximum. As beam power is increased the velocity of the cells traveling from ring five to the central core increases.

At low power, many erythrocytes behave similarly to lymphocytes in that they are transported to the center of the Bessel beam, although at a lower velocity. Upon arrival at the central core, the erythrocytes align and are guided upwards. Many erythrocytes are also aligned and guided upwards in the first and second rings of the Bessel beam. The average time for an erythrocyte to be transported towards the beam center, reorient, and become locked-in to a ring or the central core, starting from ring five, in a low-power Bessel beam, is 84s. As higher powers are used, we see the erythrocytes locking into rings further from the Bessel beam center, as the potential wells are becoming more difficult for the cells to traverse. For example, at 350mW this lock-in time is 28s and the reoriented erythrocytes are found in the third, fourth, and fifth rings. For higher power of 550mW, the erythrocytes also align in the third, fourth, and fifth rings after an average of 23s, with a larger proportion in the outer rings. This demonstrates that, at a laser power of 550 mW, erythrocytes will reorient in the outer rings of the Bessel beam, lock in to those rings, and be guided upwards, while lymphocytes will rapidly move directly to the beam center, where they will also be guided upwards, but separated from the erythrocytes. Frames (a)-(e) of Figure 8 show this behaviour. Two lymphocytes are transported to the beam center then guided upwards to the top of the sample chamber, and erythrocytes align, lock in, and are guided upwards in the third, fourth, and fifth rings of the Bessel beam. Frame (f) of Figure 8 shows how the guided lymphocytes may be collected in a separate reservoir using a microcapillary. Similar experiments have been performed with

Gaussian beams, but the behaviour described earlier has not been seen, verifying the need for a periodic landscape.

To enhance sorting, beads can be attached to one or more populations of the cells that are to be separated. Streptavidin-coated, silica microspheres (5.17 μm diameter, from Bangs Laboratories) were attached to the T-cell subpopulation via a mouse CD2 primary antibody and a secondary, biotinylated, antimouse antibody attachment (Vector Laboratories). Attaching silica microspheres, targeted to a specific subpopulation of cells via antibody-antigen binding, enhances separation, as the microspheres react to the optical landscape more strongly than cells. The silica spheres, because of their enhanced refractive index, exhibit more scattering and are therefore simultaneously guided vertically and transported laterally more readily than any unattached cells. Thus, separation of the T-cells from the ensemble of unlabelled cells was achieved, as shown in Figure 10. This purified subpopulation of cells may be collected and used for downstream analysis, such as biochemical or functional studies. This demonstrates passive sorting of cell populations in the optical potential landscape of a Bessel beam. The likely differences in various cell types and their deformability imply that this method may have widespread applicability. In addition, no microfluidic flow is required for this technique and thus it is readily compatible with standard microscope technology.

By taking advantage of the differing Kramer's residence time for polydisperse mixtures of objects leading to differing rates of motion across optical landscapes, there is provided a new method for optical fractionation or sorting in the absence of any flow, purely initiated by projecting a light pattern onto a sample area. This is a simple and effective means for separating particles of different size and/or shape and/or refractive indexes within the landscape.

A skilled person will appreciate that variations of the disclosed arrangements are possible without departing from the invention. For example, the light induced sorting is not restricted to the use of a Bessel beam and other forms of two and three-dimensional light patterns may be used. In particular, linear interference fringes could be used. In this case, due to optics in the system the fringes in the beam centre might

be brighter than those at the edges, as illustrated in Figure 11. If particles of different size are placed in this pattern the large ones will move towards the centre (thicker lines are brighter /more powerful fringes), whereas the small particles may get locked into one line and only hop infrequently towards the middle. Using a spatial light modulator (SLM) is a good way of generating such patterns, especially if a controlled tilt on the light pattern is to be used.

Whilst all of the embodiments described above use light to produce an optical landscape directly within the fluid, light could instead be used to generate a light patterned electric field, which is then used as the sorting mechanism. This can be done, for example, by using dielectrophoresis techniques, such as those described by Chiou et al in "Massively Parallel Manipulation of Single Cells and Microparticles Using Optical Images", Nature 426, (21 Jul, 2005). Also, whilst the sorting arrangement illustrated is a standalone system, it will be appreciated that it could readily be incorporated in another system or arrangement, such as a standard microscope. In this case, the beam pattern would be projected directly onto the sample in the microscope, thereby to initiate sorting. Accordingly, the above description of a specific embodiment is made by way of example only and not for the purposes of limitation. It will be clear to the skilled person that minor modifications may be made without significant changes to the operation described.

Claims

1. A method for sorting/separating at least two different particles in a fluid, the method comprising using light to define within the fluid a potential landscape/pattern having one or more potential wells having widths that are substantially the same size or slightly larger than at least one of the particles, the landscape/pattern being arranged to cause sorting/separation of the particles in the absence of fluid flow.

2. A method as claimed in claim 1 wherein using light to define a potential landscape/pattern involves using light to generate an optical potential landscape/pattern for causing the sorting/separation of the particles.

3. A method as claimed in claim 1 wherein using light to define a potential landscape/pattern involves using light to generate an electric potential landscape/pattern for causing sorting/separation of the particles.

4. A method as claimed in claim 3 involving exposing a dielectrophoresis material with light, thereby to produce a light induced electric potential landscape/pattern.

5. A method as claimed in any of the preceding claims, wherein the landscape defines a potential gradient for causing selected particles to accumulate within a pre-determined region of the landscape.

6. A method as claimed in any of the preceding claims, wherein adjacent wells are concentric.

7. A method as claimed in any of the preceding claims, wherein the adjacent wells are concentric rings.

8. A method as claimed in any of the preceding claims, wherein adjacent wells are symmetric.

9. A method as claimed in any of the preceding claims comprising allowing one or more particles of a given type to collect in a pre-determined region and manipulating the optical landscape in that region to move the collected particles away from the main body of the fluid.

10. A method as claimed in any of the preceding claims wherein the optical landscape is tilted.

11. A method as claimed in any of the preceding claims wherein more that three optical wells are provided and the potential of the optical wells increases gradually in a pre-determined direction.

12. A method as claimed in any of the preceding claims wherein the potential landscape is generated using a Bessel beam that defines a plurality of concentric rings or wells.

13. A method as claimed in any of the preceding claims wherein the particles are of different sizes and the larger of the particles straddles two or more optical wells.

14. A system for sorting/separating at least two different particles in a fluid, the system comprising a light source that is operable to define within the fluid a potential landscape/pattern having one or more potential wells having widths that are substantially the same size or slightly larger than at least one of the particles, the landscape/pattern being arranged to cause sorting/separation of the particles in the absence of a substantial fluid flow.

15. A microscope including a system as claimed in claim 14.

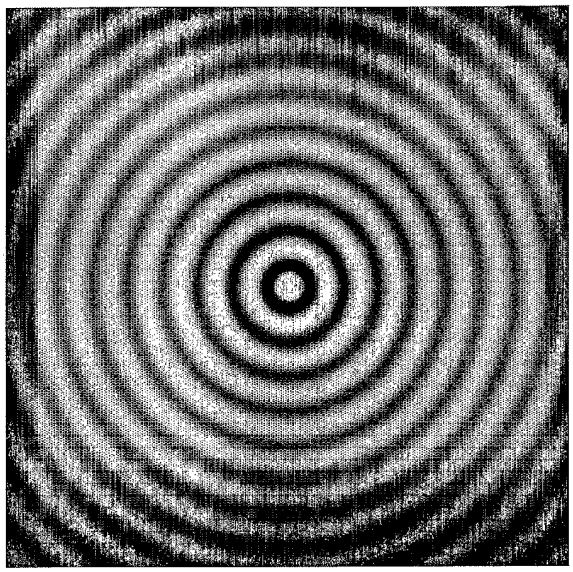


Figure 1

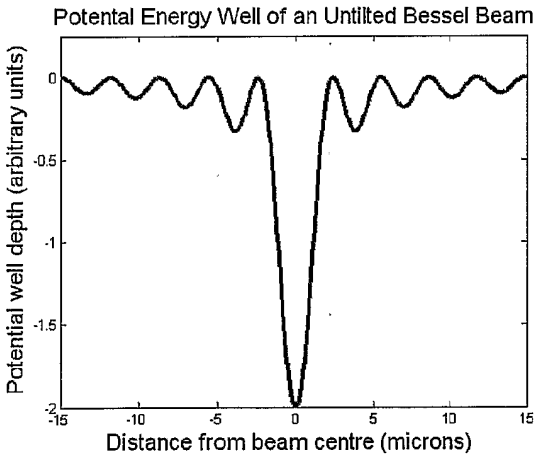


Figure 2

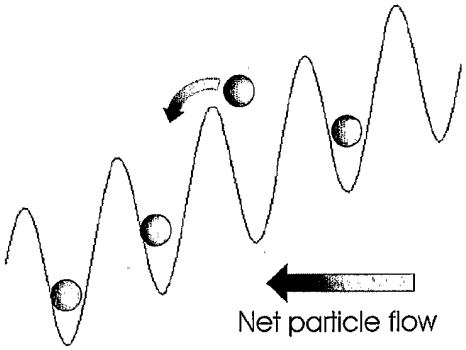


Figure 3

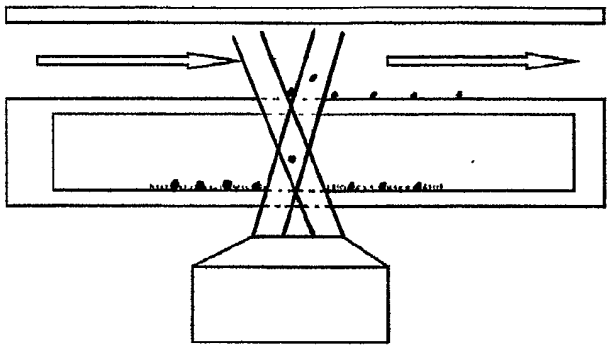


Figure 4

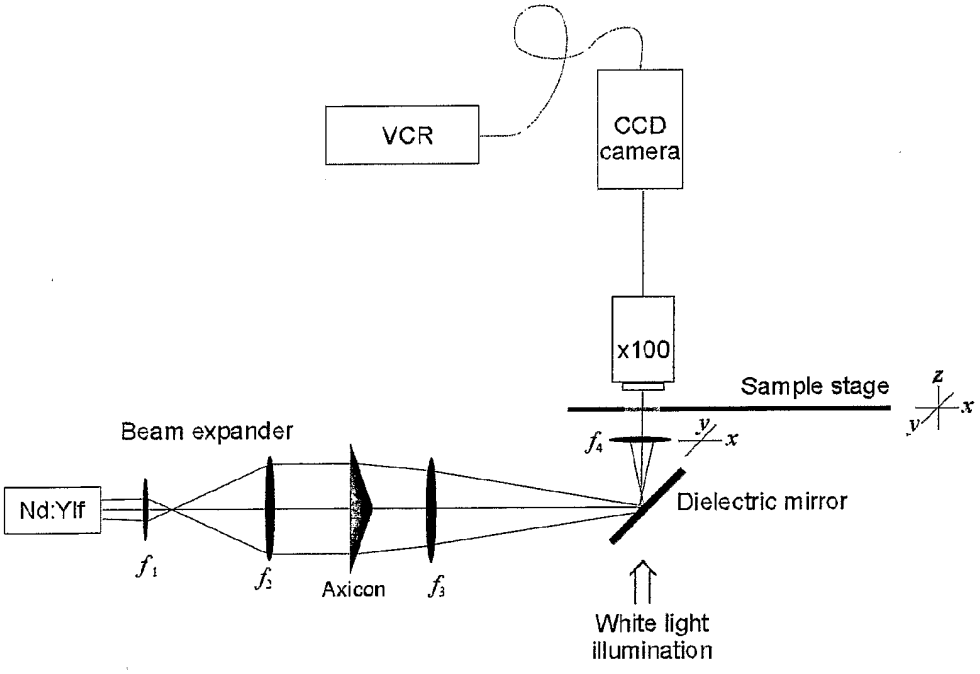


Figure 5

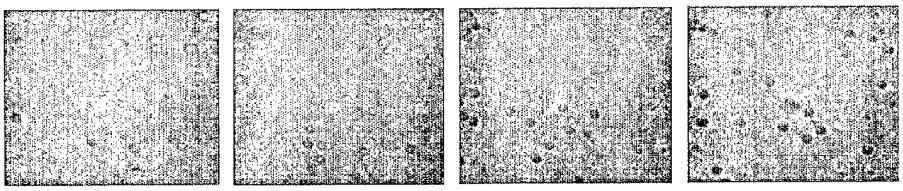


Figure 6

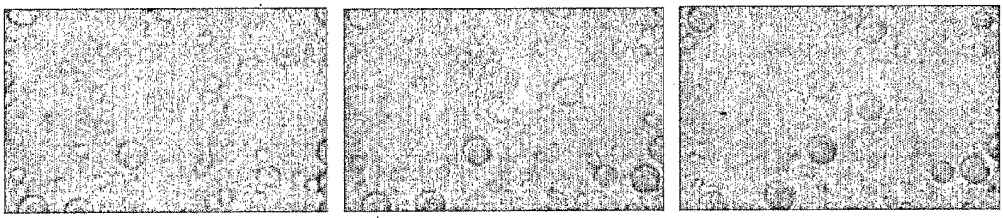


Figure 7

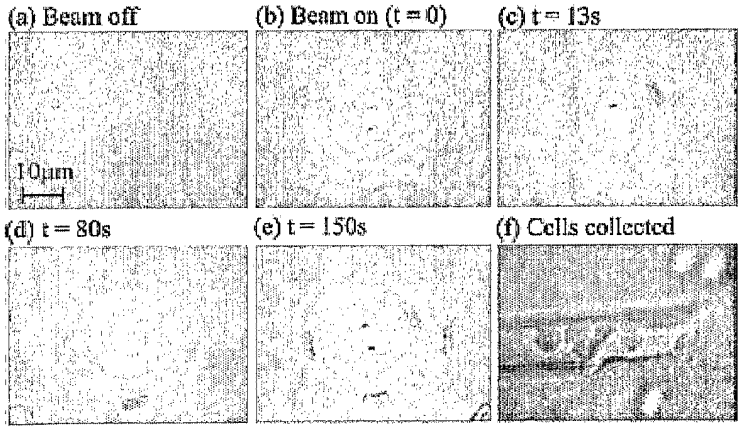


Figure 8

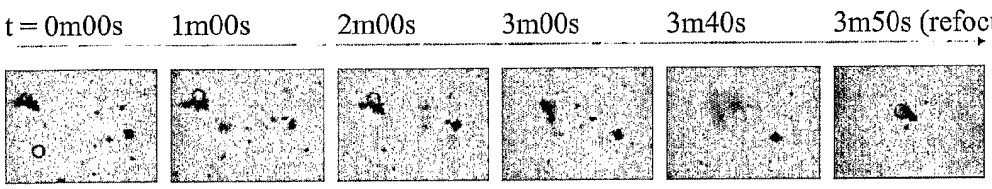


Figure 9

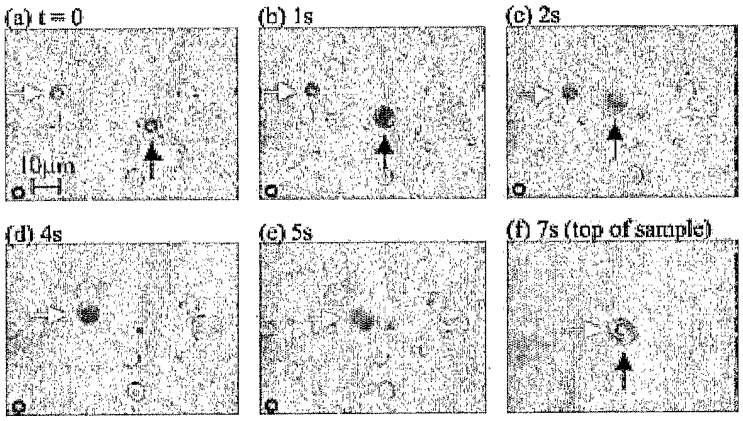


Figure 10

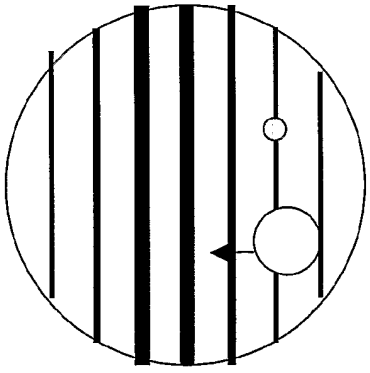


Figure 11