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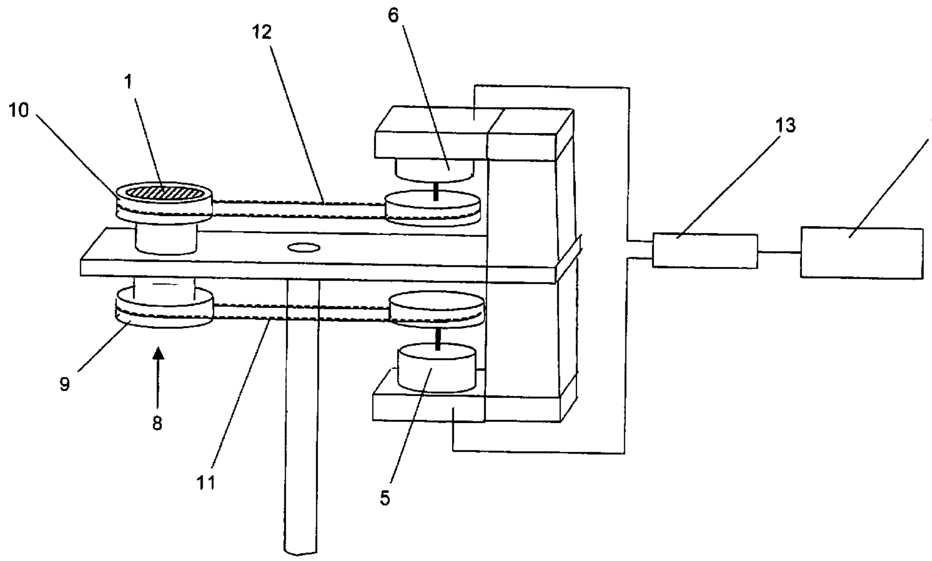
(54) **Title:** METHOD AND LASER TWEEZERS FOR ORIENTING A BODY IMMOBILIZED BY A LASER BEAM AND COMPLEMENTAL APPARATUS FOR THE LASER TWEEZERS



(57) **Abstract:** A subject of the invention is a method for orienting a body immobilized in laser tweezers. In the course of performing the method, the body is immobilized by a linearly polarized and focused light and is oriented by rotating the polarization plane of the linearly polarized light.A further subject of the invention are laser tweezers formed of linearly polarized and focused light; the laser tweezers are provided with means for rotating the polarization plane of the light beam.Another subject of the invention is a unit which complements the laser tweezers and which includes a rotatable $\lambda/2$ plate (1) and, if required, a $\lambda/4$ plate (8), both positionable in the light path of the laser light source.

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METHOD AND LASER TWEEZERS FOR ORIENTING A BODY IMMOBILIZED BY A LASER BEAM AND COMPLEMENTAL APPARATUS FOR THE LASER TWEEZERS

FIELD OF THE INVENTION

This invention relates to a method and laser tweezers for orienting a body immobilized by a laser beam and further relates to a complemental apparatus for the laser tweezers.

BACKGROUND OF THE INVENTION

It is known that light carries a momentum and refracts at the boundary of two media having different indices of refraction. As a result, the propagating direction and velocity of light (that is, its momentum) changes. Such a change of the light momentum exerts a force on the medium. The force is very small in macroscopic dimensions and in case of average light intensities. In case of a laser beam, however, such force effect may be significant on a body of microscopic dimensions. If the laser light is focused by a lens having a large numeric aperture, then, because of the above-noted refraction, the body which has a greater index of refraction than the medium, is exposed to a force which immobilizes it in the focal point. The manipulating tool based on this phenomenon are the laser tweezers (A. Ashkin, *Science*, vol.210, pages 1081-1088, 1980). Generally, laser tweezers are obtained by means of a microscope, wherein the focusing element is the microscope objective. The tool is extremely well adapted for grasping and manipulating microscopic bodies having dimensions comparable to the wavelength of light. The tool has been used particularly in the field of biology, since the size of cells and cell organisms falls into such a micron range. Further, the order of magnitude of forces appearing when using lasers of easily attainable power (a few tens of mW) is sufficient to overcome the Brownian motion of cells floating in water. In addition, the effect of the molecules exerting a biological force also falls within the order of magnitude (pN) of the forces exerted by the laser tweezers. With the use of the laser tweezers a new field of science, namely the technique of "single-particle manipulation" has developed.

A body which is significantly (generally at least ten times) smaller than the wavelength of light may also be manipulated, but in such a case the body is affixed to a larger body – generally a synthetic sphere – whose size is comparable to the wavelength of light (T.T.Perkins, D.E.Smith, R.G.Larson and S.Chu, *Science,* vol.268, pages 83-85, 1986).

In most cases, however, the laser tweezers do not orient the body they grasp; yet, in practice, a suitable control of the spatial orientation of the immobilized specimen is also of significance. For a spatial orientation an asymmetry of the trapping is necessary. Evidently, an orientation of a spherical body consisting of an isotropic material is not possible which is typical in case of synthetic spheres.

It is known that elongate bodies (such as bodies similar to a rotational ellipsoid) assume a position in the trap in which their major axis is co-directional with the light (Z.Gheng, P.M.Chapin and T.G.Mason, *Phys. Rev. Lett.* vol.89, 18303-1-3, 2002).

A further degree of freedom is the control of the direction of light propagation by a rotation about an axis. In this connection it is known that an orientation may be feasible if the grasped body is of an optically birefringent material, and the light of the laser tweezers is polarized. If the light is linearly polarized, the specimen orients itself, and the optical axis of the birefringent material will lie in the plane of polarization. A restoring torque is exerted on the grasped body in case it deviates from its position of equilibrium. In case the light is circularly polarized, the body is exposed to a continuous torque which causes the body to rotate. The direction of rotation depends on the direction of the circular polarization, that is, the direction of rotation may be reversed at will (M.E.J.Friese, T.A.Nieminen, N.R.Heckenberg and H.Rubinsztein-Dunlop, *Nature*, vol.394, pages 348-350, 1998).

In case the light of the laser tweezers is not circularly polarized, but has phase singularity, it is capable of generating a torque and thus can rotate the body which the laser tweezers grasped (H.He, M.E.J.Friese, N.R.Heckenberg and H.Rubinsztein-Dunlop, *Phys. Rev. Lett.*, vol.75, pages 826-829, 1995).

A further mechanism can cause rotation in case the light itself does not carry a pulse torque, but the grasped body is helical, and the light, as it scatters on the body, acquires a pulse torque. This effect causes the body to rotate. This phenomenon dynamically corresponds to a wind-driven propeller (R.C.Gauthier, *Appl. Phys. Lett.*, vol.67, pages 2269-2271, 1995; E.Higurashi, R.Sawada and T.Ito, *Appl. Phys. Lett.*, vol.72, pages 2951-2953, 1995; and P.Galajda and P.Ormos, *Appl. Phys. Lett.*, vol.78, pages 249-251, 2001).

An asymmetric intensity distribution of the trapping light bundle may also effect an orientation of the trapped body (A.T.O'Neil and M.J.Padgett, *Opt. Lett.*, vol.27, pages 743-745, 2002). Such an asymmetry may be achieved in a simplest manner by using an aperture having the shape of an oblong rectangle.

The force of all orienting mechanisms have the same order of magnitude: in case of typical parameters (a laser intensity of 10 mW, the birefringence of calcite, a size in the micron range, etc.) the order of magnitude of the torque is 10^{-19} - 10^{-17} Nm, dependent upon the various parameters.

SUMMARY OF THE INVENTION

It is an object of the invention to provide an improved, simple and in practice well-usable method and improved laser tweezers as well as a complemental apparatus for orienting a body immobilized in the laser tweezers.

This object is accomplished by the inventive method, according to which the body is immobilized by a linearly polarized, focused light and is oriented by rotating the polarization plane of the linearly polarized light.

The rotation may be expediently effected by a $\lambda/2$ plate positioned in the light path.

If required, the body to be oriented is converted into a flat body before immobilizing it in the laser tweezers. The conversion into a flat body is achieved by affixing the body, which by itself has no asymmetry or is significantly smaller than the wavelength of the linearly polarized light, to an asymmetric carrier whose size is comparable to the wavelength of light.

According to an expedient method of realization, the carrier is obtained by polymerizing a resin placed into the sample liquid.

The essential aspect of the laser tweezers according to the invention resides in that they are provided with means for rotating the polarization plane of the light beam. According to a preferred embodiment such a means is a rotatable $\lambda/2$ plate placed in the light path. Expediently, upstream of the $\lambda/2$ plate (as viewed in the direction of light propagation) a rotatable $\lambda/4$ plate is positioned in the light path.

The units which rotate the $\lambda/2$ and $\lambda/4$ plates are computer-controlled.

The apparatus serving for complementing the laser tweezers includes a rotatable $\lambda/2$ plate, and if required, a rotatable $\lambda/4$ plate, both positioned into the light path of the light source.

The invention is based on the observation that if the laser tweezers are formed by a linearly polarized light, flat bodies orient themselves therein. The orientation is such that the large body side is aligned with the polarization direction of the light. The force of the orientation has a magnitude with which, in case of an intensity of 10 mW, it can easily overcome the Brownian motion of a body having the size of a few microns.

The orienting effect resulting from the solution according to the invention extends the possibilities of utilization of laser tweezers, namely, a simple orientation and spatial manipulation of a body grasped by the laser tweezers. The orienting effect can be directly utilized for orienting and manipulating elongate, non-spherical bodies, such as most living cells. In case the body to be oriented does not have the shape necessary to be oriented by the above-outlined mechanism (either because it is spherically symmetrical, or because its size is much smaller than the wavelength of light), the body to be oriented has to be affixed to a suitable auxiliary body permitting an orientation.

In the laser tweezers according to the invention the direction of orientation may be altered by changing the polarization plane of the light constituting the laser tweezers, and the force of the orienting polarization effect may be altered without changing the trapping force of the laser tweezers. If a $\lambda/4$ plate is placed in the path of the polarized light, the plate, dependent upon its orientation with respect to the orientation of the polarization plane of the light, renders the light linearly polarized or circularly polarized. In the circularly polarized light the flat body is not oriented, but the force of the trapping does not significantly change. Thus, the force of the trapping may be controlled between limit values, that is, between a maximum and a zero value measured in the linearly polarized light.

In the apparatus complementing the laser tweezers the components which hold the λ/2 and λ/4 plates are so dimensioned that both plates may be placed in the light path of the laser tweezers regardless of the tweezers' design. Both plates may be rotated and placed into a desired position independently from one another by computer-controlled motors. The apparatus thus offers the possibility to orient, manipulate and rotate bodies which meet the above-described criteria, independently from the trapping force of the laser tweezers.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an image of a flat body oriented in laser tweezers.

Figure 2 is a diagram illustrating the difference (shift) between the polarization plane and the direction of orientation as a function of the rotational velocity as the body grasped by the laser tweezers is rotated by the polarization plane of the light.

Figure 3 is a diagram explaining the Fresnel equations characterizing the refraction and reflection of polarized light.

Figure 4 is a diagram showing the computation results concerning the magnitude of the torque exerted on the flat body by the polarized light as a function of the relative orientation between the polarization plane and the body.

Figure 5 is a perspective view of an apparatus according to the invention.

Figure 6 is a schematic side elevational view illustrating the adaptation of the apparatus of Figure 5 to a microscope.

Figure 7 is a perspective view illustrating the adaptation of the apparatus of Figure 5 to a microscope.

DETAILED DESCRIPTION

The invention and the underlying phenomena will be described in two Examples.

Example 1.

Flat bodies were obtained, having defined geometrical parameters and oriented by linearly polarized light. The arrow N in Figure 1 indicates the direction of polarization. Further, the orienting torques were determined for different, relative positions between the body and the polarization plane of the light. Measuring was based on the following principle: As a starting point, the orienting torque is zero in the position of equilibrium, and, deviating from such a position, a restoring torque is generated which increases as the deviation increases. Thus, the restoring torque increases, as the angle formed between the direction of polarization and the longitudinal direction of the body increases. The polarization plane of the laser light may be arbitrarily set by a $\lambda/2$ plate 1 (shown in Figures 5 and 6) placed in the light path. A rotation of the $\lambda/2$ plate 1 evidently entrains the body into rotation too, since its orientation follows the direction of polarization. A perfect, ideal following by the body is impeded by the friction of the liquid: the direction of polarization set by the $\lambda/2$ plate 1 does not agree entirely with the orientation of the body. The friction generated during rotation may be overcome by the body by the difference which develops between the orientation of the body and the direction of polarization. Thus, the body lags behind the polarization, that is, it has a phase lag. The phase lag may be determined for any revolution by determining the rotational phase of the $\lambda/2$ plate 1 and the body. The rotational resistance affecting the body may be computed based on the known laws of hydrodynamics. Since different phase shifts (that is, polarization orientations relative to the body) and thus different torque values of hydrodynamic resistance are associated with different rates of rotation, as a result of this measuring process the magnitude of the torque exerted by the light on the body is obtained as a function of the angle formed between the polarization plane of the light and the body. The measuring results are illustrated by the graph of Figure 2.

The phenomenon is based on that the refraction and reflection depend on the polarization of the light. An exact, detailed description thereof would be very complex, because in the instant case the dimension of the grasped body falls into the wavelength range of the laser tweezers 2. Under these circumstances an exact computation of the light scattering is not solved for bodies of complex shape. As an approximation it is assumed that the wavelength of the light is smaller than the characteristic dimensions of the body. In the present instance a discussion of known radiation optics is in order. In creating a computer-generated model of the phenomenon, the laser tweezers 2 are produced by a great number (100,000) of light rays. The distribution and direction of the light rays correspond to the real intensity and direction distribution of the laser tweezers. For each individual light ray it is examined how the light ray enters into a mutual effect with the grasped body, that is, whether the ray impinges on the surface of the body. If such an impingement does take place, then one part of the light is reflected from the body surface, while the other part continues to propagate in the medium after refraction. The extent of both the reflection and the refraction depend on the polarization of the light ray or, stated differently, on the relative orientation between the polarization and the refracting surface. The relationship is given by the Fresnel equations as follows:

$$r_{\perp} = -\frac{\sin(\alpha - \beta)}{\sin(\alpha + \beta)} \qquad t_{\perp} = \frac{2\sin\beta\cos\alpha}{\sin(\alpha + \beta)}$$

$$r_{\parallel} = \frac{tg(\alpha - \beta)}{tg(\alpha + \beta)} \qquad t_{\parallel} = \frac{2\sin\beta\cos\alpha}{\sin(\alpha + \beta)\cos(\alpha - \beta)}$$

The explanation of the quantities are given in Figure 3.

The equations yield the vector-related reflection coefficients and transmission coefficients of the electric field intensity in case of a polarization direction which is perpendicular or parallel to the plane of incidence. Based on the above equations the course of the post-refraction ray may be computed for each polarized ray, that is, the momentum change may be determined. The momentum changes to which the individual rays undergo exert a force on the body, and by adding up the momentum changes, the entire force (torque) acting on the body may be determined.

With the aid of the above procedure the magnitude of the torque exerted on an oblong rectangular body was determined in the possible relative positions between the body and the polarization plane of the light of the laser tweezers 2. The diagram of Figure 4 shows the computed magnitude of the torque applied to the body for different orientations. The results have qualitatively well agreed with the measured results shown in Figure 2. The quantitative agreement was also satisfactory: the model calculation yields the order of magnitude of the torque.

The practical significance of the above phenomenon is very substantial, since its use offers the possibility of orienting originally flat bodies grasped in the laser tweezers 2. In case of bodies which are not asymmetrical by themselves, suitable auxiliary bodies (carriers) ensure that any object may be oriented. Carriers may be made in numerous ways; the Example which follows describes the making of such bodies from a material that polymerizes when exposed to light.

<u>Example 2.</u>

The bodies were made by introducing the light

(λ=514 nm) of an Ar-ion laser into a microscope, focusing the light by the objective of the microscope and then directing the focused light into the specimen compartment where a resin polymerizing to the effect of light was positioned. The resin was a Norland NOA 63 substance which polymerizes when excited by a light having a wavelength of less than 340 nm. The light of 514 nm wavelength is, in focus, of sufficient intensity to cause a two-photon excitation; thus the resin is polymerized in a very small volume. A three-dimensional body of arbitrary shape (in this instance the shape of a flat cross) may be drawn by moving the focus. The specimen to be oriented may be affixed to the body and then may be manipulated at will by the laser tweezers 2 (which may be a conventional, home-made arrangement or may be mounted on the microscope 3) complemented by a simple apparatus 4 according to the invention. It is the essence of the complemental apparatus 4 that it holds a unit, such as a $\lambda/2$ plate 1, in the light path of the laser tweezers 2 and rotates the polarization plane of the light beam.

It is expedient in general to adapt existing laser tweezers 2 to the process according to the invention.

Turning to Figures 5, 6 and 7, the complemental apparatus 4 according to the invention may be adapted practically to any laser tweezers 2. It is a condition for its use that the light of the laser tweezers 2 be polarized. The light bundle producing the laser tweezers 2 is typically a single-mode laser light, that is, it is linearly polarized. Should this not be the case, the light should be polarized by suitable methods.

With particular reference to Figure 5, the essential components of the apparatus 4 are two optical elements which are placed in the light path of the laser tweezers 2 and whose orientation may be varied by electric motors 5 and 6, controlled by a computer 7.

The first element, as viewed in the direction of light propagation, is a $\lambda/4$ plate 8 which, dependent upon the angle it forms with the polarization direction of the linearly polarized light, either transforms the linearly polarized light into a circularly polarized light, or leaves it linearly polarized. Thus, the role of the element 8 is to switch the orienting effect on or off. As discussed earlier, the linearly polarized light orients the flat body. In contrast, a circularly polarized light does not have such an effect; in case of a circularly polarized light the plane of polarization rotates at the frequency of light. Evidently, a body of micron size cannot follow such a rotation. Thus, by means of rotating the $\lambda/4$ plate 8 the orienting effect may be switched without appreciably affecting the trapping force of the laser tweezers 2. This represents a very significant and advantageous property.

The second element is a $\lambda/2$ plate 1 which rotates the direction of polarization of the linearly polarized light passing therethrough. Thus, by means of the second element the orientation of the grasped body may be set.

The $\lambda/4$ plate 8 and the $\lambda/2$ plate 1 are mounted on respective, bearing-supported holders 9 and 10. The holders 9 and 10 are rotated by respective motors 5 and 6 via respective toothed belts 11 and 12. The motors 5 and 6 are, with the intermediary of a control 13, connected to a computer 7 for controlling the rotation of both optical elements 8 and 1. The jig in which the optical elements and their drive are mounted may be installed in the light path of the laser tweezers 2. In case of home-made laser tweezers 2 such a solution is evident. If the laser tweezers 2 are mounted on the microscope 3, the apparatus 4 is installed in the microscope 3, whereby the $\lambda/4$ plate 8 and the $\lambda/2$ plate 1 take the place of the fluorescence filters, as illustrated in Figures 6 and 7. Figure 6 clearly shows the path of the light emanating from a laser light source 14. After being deflected by mirrors 15, the light first passes through the $\lambda/4$ plate 8 and then through the $\lambda/2$ plate 1.

The apparatus 4 significantly widens the possibilities of application of the laser tweezers 2.

CLAIMS

1. A method of orienting a body immobilized in laser tweezers, including the steps of immobilizing the body by a linearly polarized light and orienting the body, characterized in that the orienting step is effected by rotating the polarization plane of the linearly polarized light.

2. The method as defined in claim 1, characterized in that the rotation is effected by a $\lambda/2$ plate (1) placed in the light path.

3. The method as defined in claim 1 or 2, characterized in that the body, before being immobilized in the laser tweezers, is transformed into a flat body.

4. The method as defined in claim 3, characterized in that the transformation of the body, which is lacking an asymmetry by itself or which is substantially smaller than the wavelength of the linearly polarized light, is effected by affixing the body to an asymmetrical carrier having a size comparable to the wavelength of light.

5. The method as defined in claim 4, characterized in that the carrier is made by polymerizing a resin placed in a specimen liquid.

6. Laser tweezers produced from linearly polarized and focused light, characterized in that the laser tweezers are provided with means for rotating the polarization plane of the light beam.

7. Laser tweezers as defined in claim 6, characterized in that the means for rotating the polarization plane of the light beam is a rotatable $\lambda/2$ plate (1) positioned in the light path.

8. Laser tweezers as defined in claim 7, characterized in that a rotatable $\lambda/4$ plate (8) is positioned ahead of the $\lambda/2$ plate (1).

9. Laser tweezers as defined in claim 8, characterized in that the units rotating the $\lambda/2$ plate (1) and the $\lambda/4$ plate (8) are provided with computer control.

10. A complemental unit for laser tweezers, characterized in that the unit includes a rotatable $\lambda/2$ plate (1) and, if required, a $\lambda/4$ plate (8), both positionable in the light path of a laser light source (14).

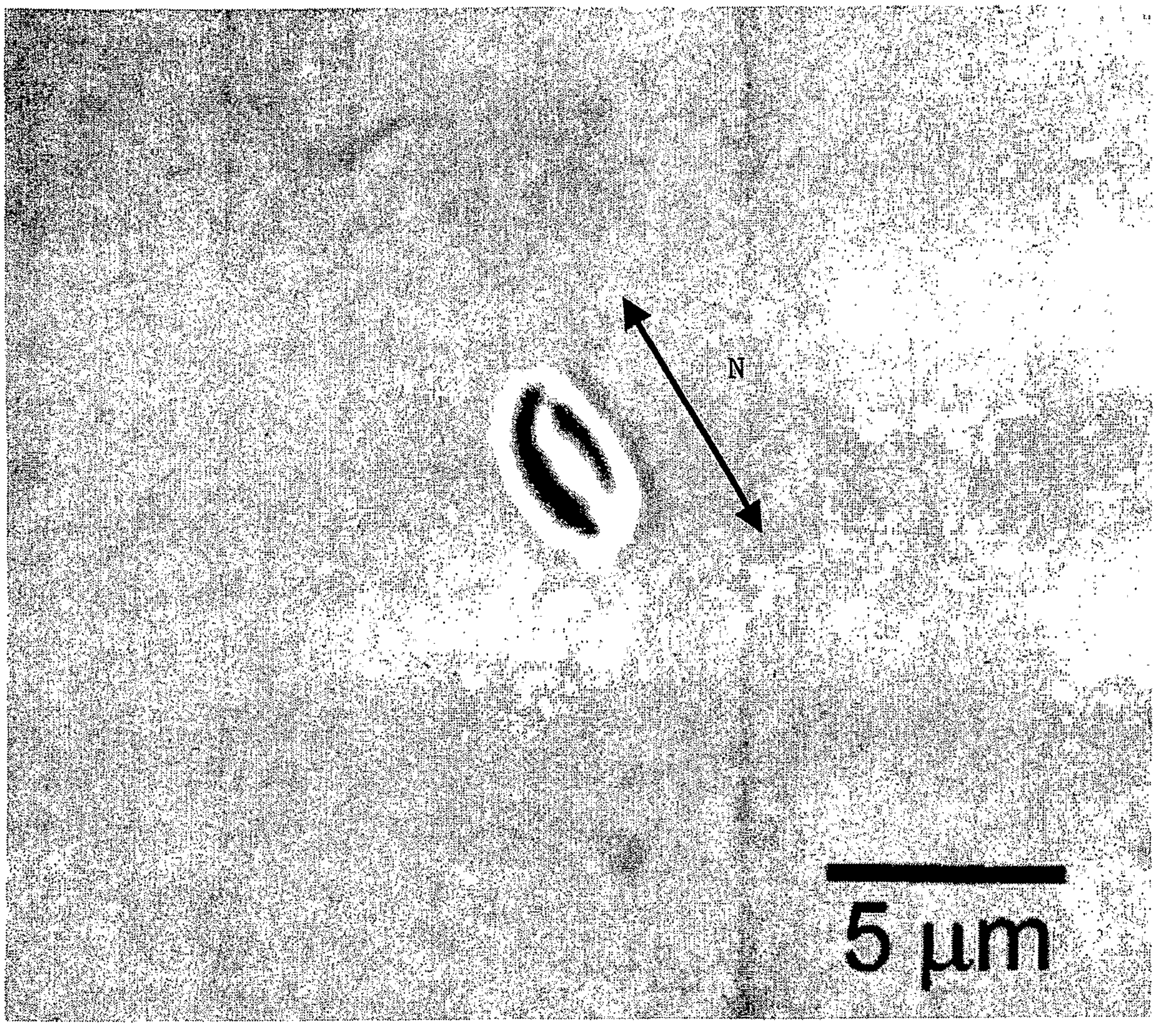
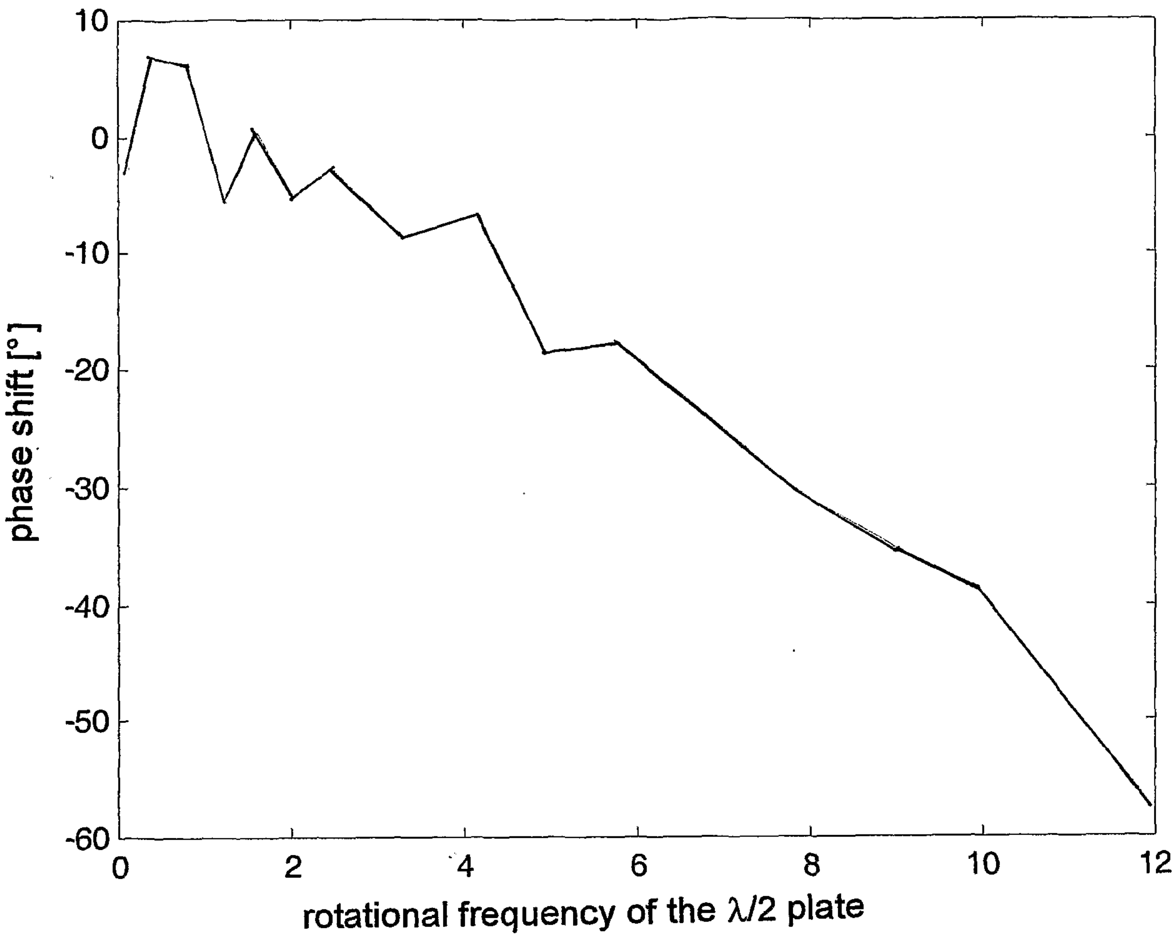


Fig. 1


10
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-10
-20
-30
-40
-50
-60
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6
8
10
12
rotational frequency of the λ/2 plate

Fig. 2

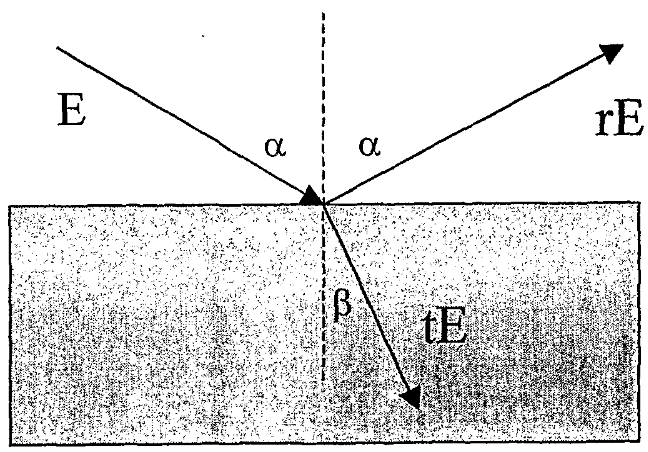
E
α
α
rE
β
tE

Fig. 3

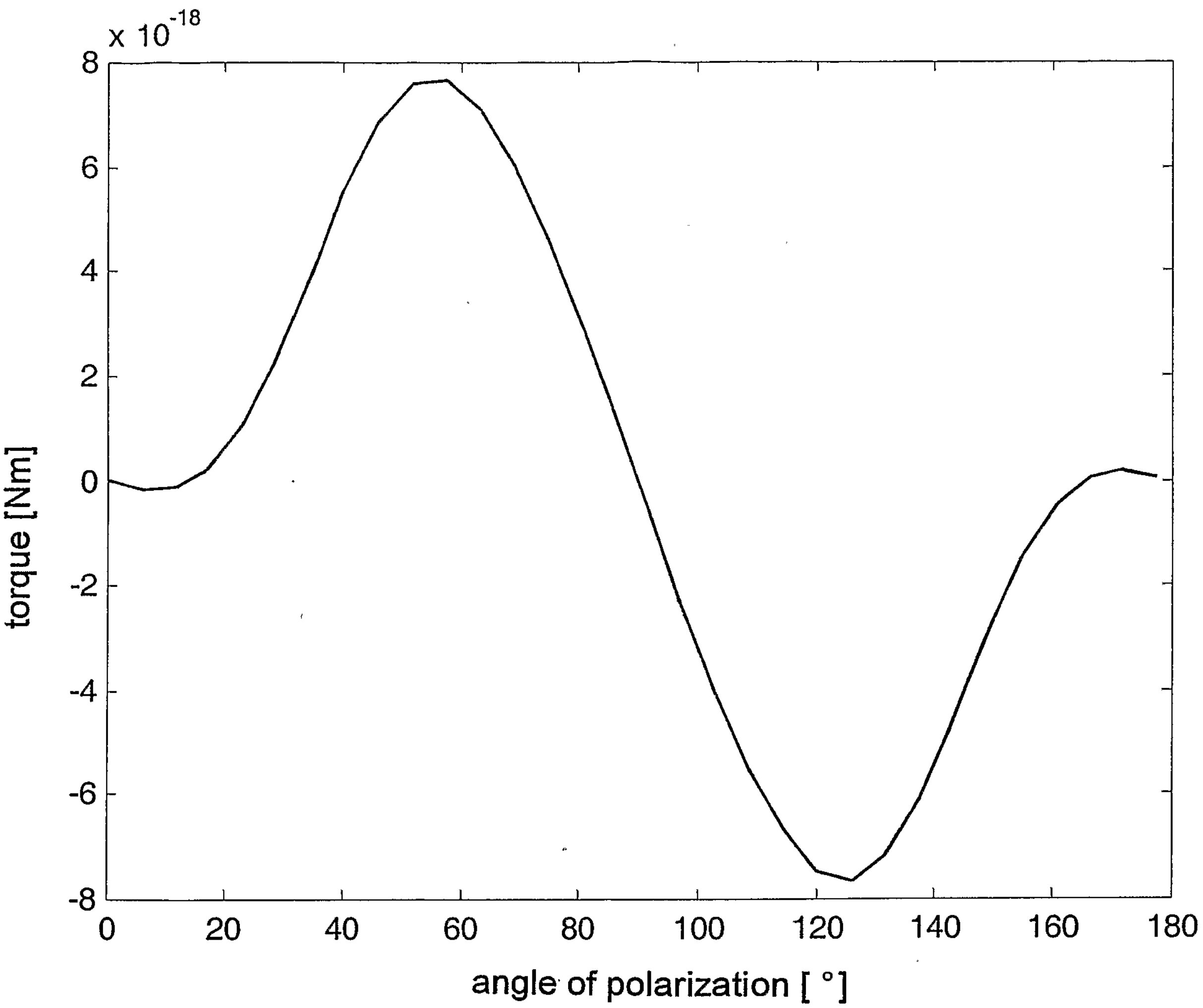
x 10^-18
8
6
4
2
0
-2
-4
-6
-8
torque [Nm]
0
20
40
60
80
100
120
140
160
180
angle of polarization [°]

Fig. 4

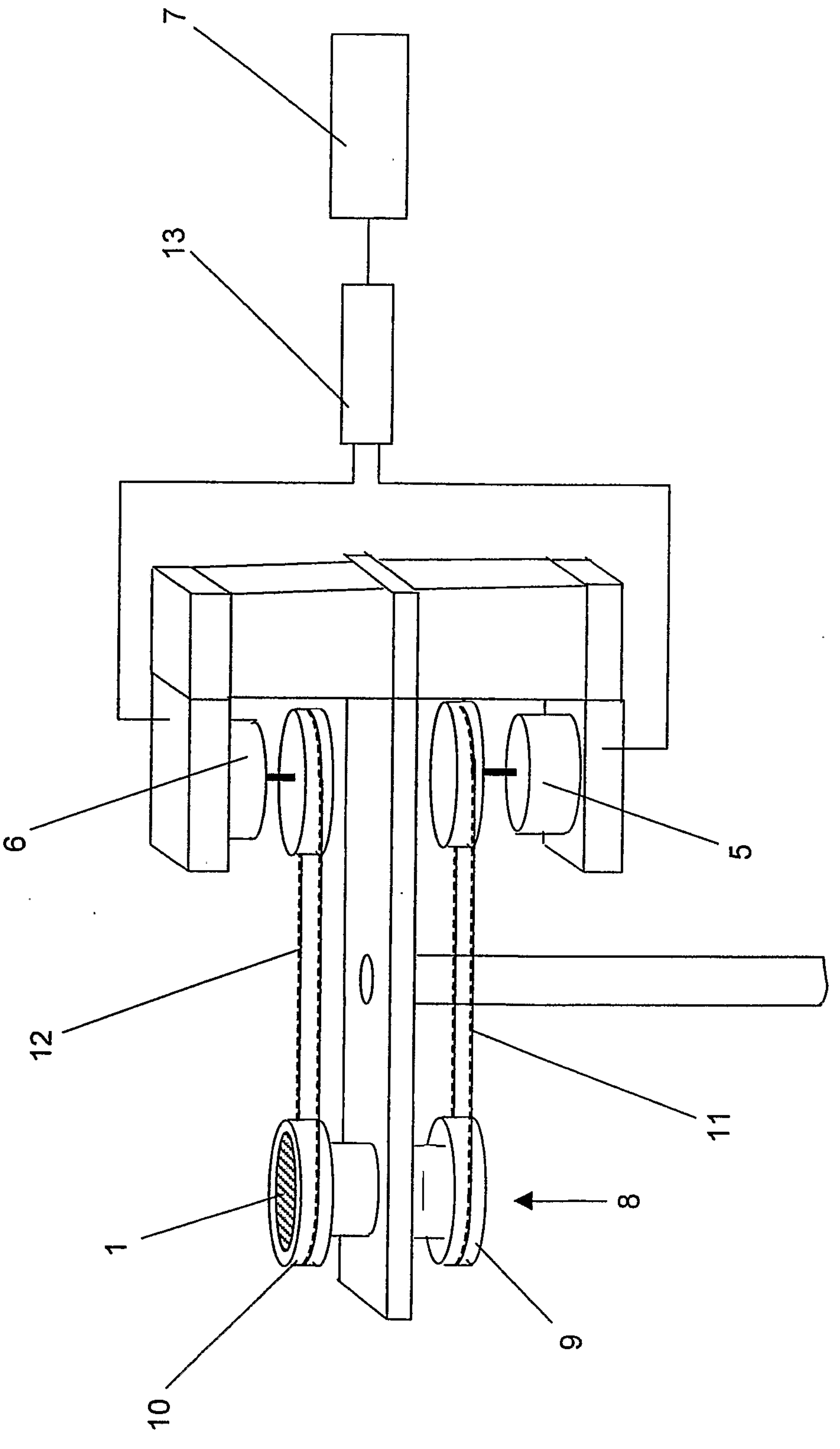

Fig. 5

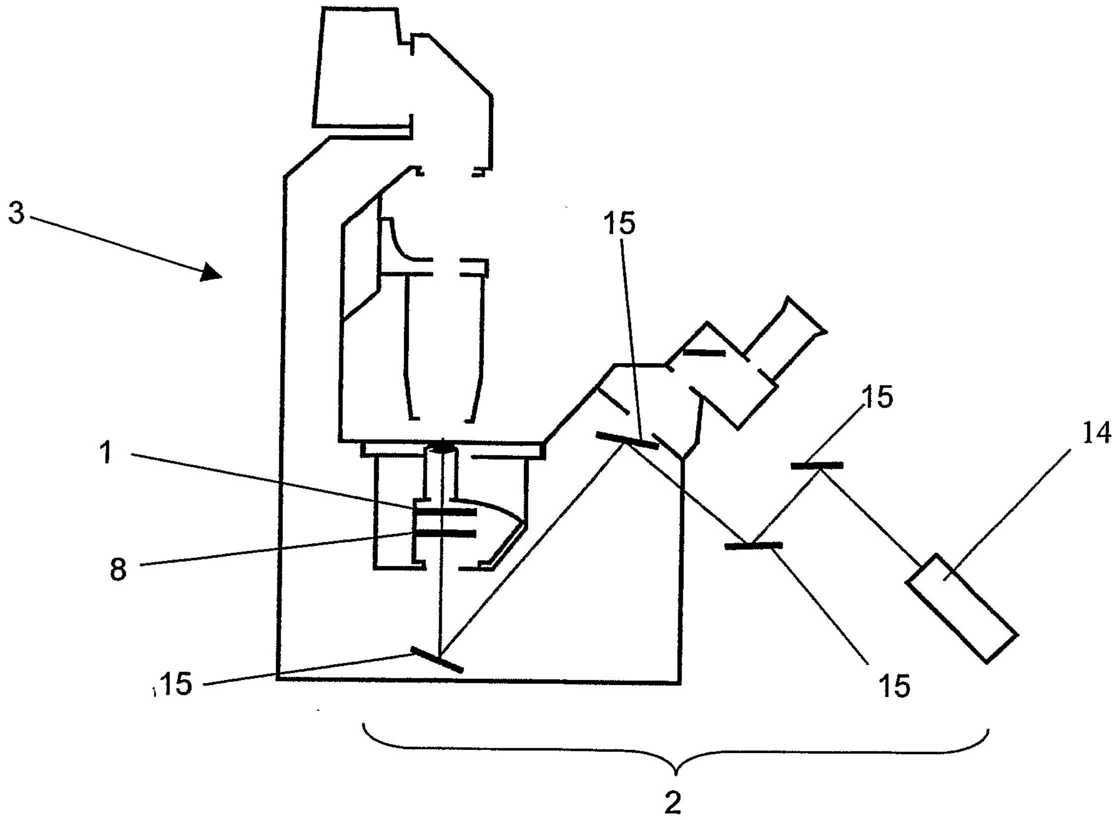

Fig. 6

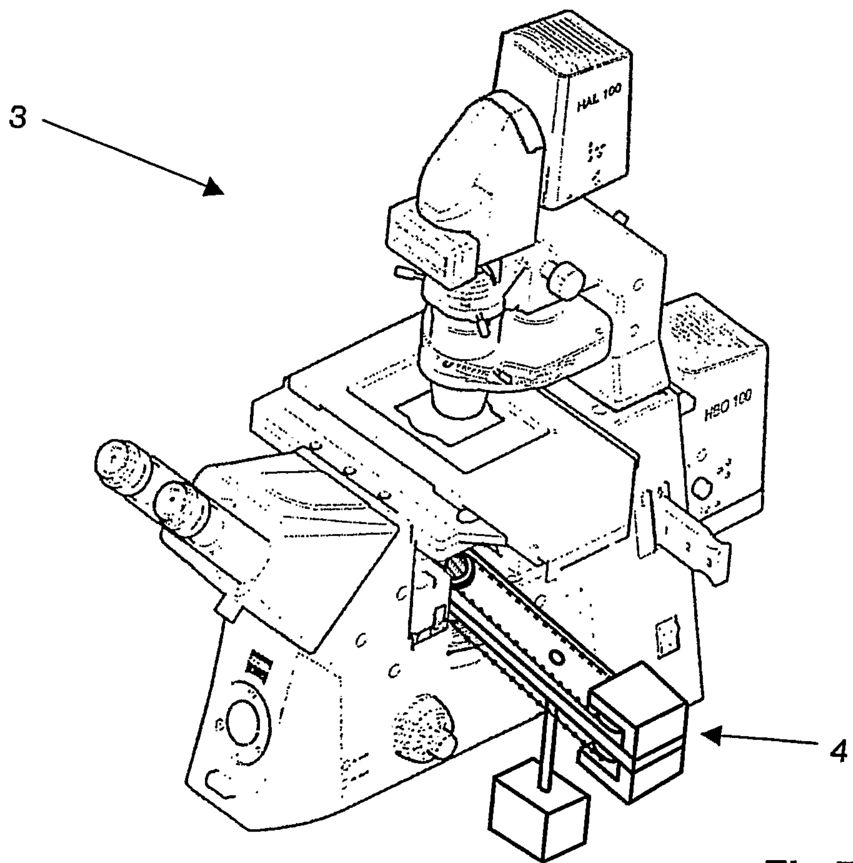

Fig.7

INTERNATIONAL SEARCH REPORT

International Application No
PCT/HU 03/00073

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G21K1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G21K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, INSPEC, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HIGURASHI E ET AL: "Optically driven angular alignment of microcomponents made of in-plane birefringent polyimide film based on optical angular momentum transfer" J. MICROMECH. MICROENG. (UK), JOURNAL OF MICROMECHANICS AND MICROENGINEERING, MARCH 2001, IOP PUBLISHING, UK, vol. 11, no. 2, March 2001 (2001-03), pages 140-145, XP0002266764 ISSN: 0960-1317	1,2,6,7
A	the whole document	4,5,9,10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 January 2004	04/02/2004

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/HU 03/00073

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	FRIESE M E J ET AL: "Optical alignment and spinning of laser-trapped microscopic particles" NATURE (UK), NATURE, 23 JULY 1998, MACMILLAN MAGAZINES, UK, vol. 394, no. 6691, 23 July 1998 (1998-07-23), pages 348-350, XP002267270 ISSN: 0028-0836 cited in the application page 348, right-hand column, paragraph 2 -----	1,2,6,7
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