Title: METHOD FOR SENSING AN ANALYTE IN A FLUID AND SENSOR UNIT FOR SUCH METHOD

Abstract: The invention provides a method for sensing with a sensor system an analyte in an analyte fluid. The sensor system comprises a micron scale birefringent entity, a laser unit configured to generate polarized laser light, a polarization rotation device, wherein the laser unit and polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light, and a detection unit. The method comprises feeding the analyte fluid along the micron scale birefringent entity while keeping with the laser light and the polarization rotation device the micron scale birefringent entity in an optical torque trap at a polarization rotation frequency; keeping the polarization rotation frequency at a sub-critical polarization rotation frequency; and measuring with the detection unit downstream of the micron scale birefringent entity the polarization of the laser light, and sensing a perturbation on the polarization of the laser light due to the analyte.
Method for sensing an analyte in a fluid and sensor unit for such method

Field of the invention

The invention relates to a method for sensing with a sensor system an analyte in an analyte fluid. The invention further relates to a sensor system, that may be used for such sensing method, as well as to a sensor system arrangement.

Background of the invention

The last two decades have witnessed the development of novel physical techniques such as Atomic Force Microscope, Magnetic Tweezers and Optical Tweezers that allow the mechanical manipulation and measurement of microscopic actuators.

An optical tweezer is a scientific instrument that uses a focused laser beam to provide an attractive or repulsive force (typically on the order of piconewtons), depending on the refractive index mismatch to physically hold and move microscopic dielectric objects. It appears that particles can be trapped and manipulated in a single highly focused Gaussian beam. Optical tweezers are capable of manipulating nanometer and micrometer-sized dielectric particles by exerting extremely small forces via a highly focused laser beam. The beam is typically focused by sending it through a microscope objective. The narrowest point of the focused beam, known as the beam waist, contains a very strong electric field gradient. It turns out that dielectric particles are attracted along the gradient to the region of strongest electric field, which is the center of the beam. The laser light also tends to apply a force on particles in the beam along the direction of beam propagation.

Optical traps are very sensitive instruments and are capable of the manipulation and detection of sub-nanometer displacements for sub-micrometre dielectric particles. For this reason, they are often used to manipulate and study single molecules by interacting with a bead that has been attached to that molecule. DNA and the proteins and enzymes that interact with it are commonly studied in this way. When connected to individual molecules, these actuators, which control physical parameters such as the applied force at the picoNewton level and molecular extension at the nanometer scale, directly report on their physical properties, drastically changing the way biological
systems can be studied (Neuman et al., Nature Methods 5, 491 (2008) and Bustamante et al., Nature Reviews Molecular Cell Biology 1, 130 (2000)).

Optical tweezers have made critical contributions to the creation of a vibrant field of biophysical research—single molecule manipulation of nucleic acids and protein complexes. Although the possibility of manipulating biological objects was readily appreciated, the true potential of optical tweezers was realized only when microspheres were used as handles to displace attached bio molecules and measure the resulting force.

La Porta el al describe in Physical Review Letters 92 (19) 2004, 190801-1 an apparatus that can measure the instantaneous angular displacement and torque applied to a quartz particle which is angularly trapped (Optical Torque Wrench or OTW). Torque is measured by detecting the change in angular momentum of the transmitted trap beam. The rotational Brownian motion of the trapped particle and its power spectral density are used to determine the angular trap stiffness. The apparatus features a feedback control that clamps torque or other rotational quantities. The torque sensitivity demonstrated is ideal for the study of known biological molecular motors. They demonstrate angular trapping and torque detection using nominally spherical but anisotropic quartz particles. The torque acting on the particle and its deviation from the trap direction are determined by direct measurement of the change in angular momentum of the transmitted beam. The ability to measure instantaneous torque is of great importance, since it will facilitate precise measurement of the torque generated by biological structures as they rotate. The wide bandwidth and accuracy of their detection scheme allowed them to measure Brownian rotational motion of the trapped particle and to use feedback to control the applied torque or particle angle.

Studies of the effects of polarized light on such particles show that they can be set into rotation by elliptically polarized light and that both the sense and the speed of their rotation can be smoothly controlled. In this way, optical torque can be controlled by elliptical polarization (Friese et al., Nature 394, 348 (1998)).

A magnetic tweezer is a scientific instrument for exerting and measuring forces on magnetic particles using a magnetic field gradient. Typical applications are single-molecule micromanipulation, rheology of soft matter, and studies of force-regulated processes in living cells. Forces are typically on the order of pico- to nanonewtons.
Depending on the implementation, magnetic tweezers can be divided into several categories including translational or rotational, unipolar or multipolar.

The most simple setup is the unipolar translational magnetic tweezer. It consists of an electromagnet with a paramagnetic core material and a tip-shaped end. This results in a high field gradient around the tip. Any paramagnetic material within that gradient is magnetized and pulled towards the tip. The force magnitude depends on the magnitude and gradient of the magnetic field. While the magnitude can be controlled by the current that drives the electromagnet, the gradient depends on the distance to the tip of the core. Alternatively, the electromagnet can be replaced by a permanent magnetic needle.

Several electromagnets can be combined into a multipolar magnetic tweezer setup, allowing for three-dimensional translation, rotation and trapping of magnetic particles.

An example of the application of a magnetic tweezer is described in US2008/0220411. This document describes amongst others the use of remotely driven nonlinear rotation of particles (e.g. magnetic particles) for detection of cells such as microorganisms (e.g. bacteria and viruses) as well the use of remotely driven nonlinear rotation of particles for measurement of physical properties of a solution (e.g. viscosity). Especially, this document describes particles configured to bind to an analyte cell of interest. For instance, the particle may further comprise a ligand that specifically binds to a microorganism (as analyte).

**Summary of the invention**

Flow cytometry (FCM) is a technique for counting and examining microscopic particles, such as cells and chromosomes, by suspending them in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second. Flow cytometry is routinely used in the diagnosis of health disorders but has many other applications in both research and clinical practice.

A disadvantage of prior art methods, such as cytometry, to measure microscopic analytes, may be that such methods require labelling and/or may be relatively sensitive to noise. Further, such methods may not easily be tuned to detect a specific type of particles, such as for instance particles having a specific diameter or diameter range.
It is an aspect of the invention to provide an alternative sensing method and/or to provide an alternative sensor system, which can for instance be used in such method, which preferably obviate one or more of the above-mentioned drawbacks.

Now, a new sensor is proposed. The sensor may comprise a micron-scale birefringent crystal trapped in an optical-torque trap. The crystal in a liquid solution is kept in rotation, by rotating the linear polarization of the laser. The rotation frequency is set where dynamical excitability is displayed. This means that any perturbation beyond a threshold, due to the interaction with particles in solution, will trigger an identical spike in the applied torque signal, which can be measured and counted. This maximizes the signal-to-noise ratio, making the binary response of the instrument ("all or nothing") suitable for counting and sensing single particles passing in proximity of the crystal.

Hence, a small particle in a flow can be detected passing in proximity of the rotating crystal, as its collision with the crystal (or equivalently an increase of its rotational drag coefficient due to proximity effects) will give rise to a phase slip of the rotating crystal with respect to the driving force of the laser polarization. This event is detectable as a deterministic pulse in the torque signal. In this way particles driven by a flow and entering in contact with the crystal may easily be monitored.

With respect to cytometry, the present method may be non-linear, may maximize the signal-to-noise ratio, and may not require labelling. Further, detecting the shift in the critical frequency of rotating magnetic particles, and not making use of the excitable regime, as demonstrated in the prior art, makes such technique slow and not suitable for counting fast single events, as a scan of the frequency is needed. In the present method however, the frequency is kept fixed and any event may be immediately visible. Using the excitable behaviour of the non linear system may allow counting and sensing proximity events due to sub-micron particles with constant maximum signal-to-noise ratio in the milliseconds regime. Further, existing methods are linear in their response, which limits their signal-to-noise ratio at low signal. Also (in the case of fluorescence based measurements) they require previous manipulation and labelling of the particles. In contrast, the present method may be used to exploit a nonlinear response which may give it very high signal-to-noise. Hence, the present method may be faster and more accurate than magnetic alternatives. With respect to
commercially available cytometry, the non linear response and use of label-free particles are advantageous.

Hence, in a first aspect, the invention provides a method for sensing with a sensor system an analyte in an analyte fluid, wherein the sensor system comprises (a) a micron scale birefringent entity, (b) a laser unit configured to generate polarized laser light, (c) preferably a polarization rotation device, wherein the laser unit and (optional) polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light, and (d) a detection unit, wherein the method comprises:

a. feeding the analyte fluid along the micron scale birefringent entity while keeping with the laser light and the (optional) polarization rotation device the micron scale birefringent entity in an optical torque trap at a polarization rotation frequency;

b. keeping the polarization rotation frequency at a sub-critical polarization rotation frequency, where the critical polarization rotation frequency is defined as the maximum frequency of the linear response regime between input polarization rotation frequency and micron scale birefringent entity rotation frequency; and

c. measuring with the detection unit the polarization of the laser light downstream of the micron scale birefringent entity, and sensing a perturbation on the polarization of the laser light due to the analyte.

Hence, a micron scale birefringent entity, such as a birefringent microscopic particle (micron scale birefringent entity), held in an Optical Torque Wrench (OTW), and rotationally forced by the rotating linear laser polarization, is an excitable system. The micron scale birefringent entity then can be used as a local controllable non-linear sensor for single perturbation events, such as a passing analyte. The micron scale birefringent entity may rotate at a defined frequency due to the polarization of the laser unit and polarization rotation device. The response of the system is set in the non-linear excitable regime, and the detection unit measures the polarization of the laser light downstream of the micron scale birefringent entity, in the absence or presence of the analyte. Were an analyte to approach the micron scale birefringent entity, a non-linear response is given, with high signal to noise ratio.

Hence, this method may be used to analyse fluids on the presence of analytes. A high signal to noise ratio may be achieved. The method may further be used to determine the concentration of such analyte in the fluid.
Torque and polarization are related. Here, polarization variations or perturbations are measured to retrieve the torque perturbation due to the analyte observation by the micron scale birefringent entity. The response seems to be non-linear and single perturbations can be detected. Hence, with the method of the invention, it is possible to sense with the detection device single perturbations occurring at the micron scale birefringent entity via the non-linear variations of the output polarization, i.e. the polarization of the laser light downstream from the micron scale birefringent entity.

General

The analyte may be any particle, but may especially be selected from the group consisting of a virus, a bacterium, and a cell. The analyte will in general have dimensions in the range of about at least 0.02 μm, likely at least about 0.1 μm. The term “analyte” may also relate to a plurality of analytes. It may also refer to a plurality of different analytes. For instance, the analytes may vary in dimensions and/or in chemical nature. With the method of the invention, by choosing the desired rotation frequency of the micron scale birefringent entity, one may select a desired particle size range for detection.

The analyte fluid (“fluid”) may be any fluid in which the analyte may be present. Especially, it may be a fluid wherein the particle may be dispersed or float, like dust particles in air. In a specific embodiment, the analyte fluid is a liquid, such as water. In yet another embodiment, the analyte fluid is a gas. A too high concentration of the analyte may not be desired. Preferably, the concentration of the analyte in the analyte fluid is selected to provide mean distances between adjacent analytes of at least about the diameter, more preferably at least about 2* the diameter of the micron scale birefringent entity. For instance, the concentration of the analyte in the analyte fluid is selected to provide mean distances between adjacent analytes in the range of about 0.5-500 μm, especially in the range of about 10-200 μm.

Herein, phrases like “a method for sensing with a sensor system an analyte in an analyte fluid” and “measuring with the detection unit downstream of the micron scale birefringent entity, the polarization of the laser light, and sensing a perturbation on the polarization of the laser light due to the analyte” relate to a method and to measuring of the analyte fluid per se. The outcome of the method and sensing may be that there are
no analytes in the fluid, or at least in an amount below the detection limit within the
time frame measured.

*Micron scale birefringent entity*

The micron scale birefringent entity preferably has a cylindrical shape. For
instance, it may have the shape of a cylinder, an elliptical cylinder, a (truncated) cone,
or a sphere. However, in another embodiment, the geometry of the micron scale
birefringent entity can be even irregular. As will be clear to the person skilled in the art,
the presence of an ordinary and an extraordinary axis (birefringence) is preferred. A
cylinder has a diameter; in case the micron scale birefringent entity is no “perfect”
cylinder, as diameter a mean diameter is meant (averaged over the length of the
cylinder). The length of the micron scale birefringent entity is preferably in the range of
0.01-10 μm; the diameter of the micron scale birefringent entity is preferably in the
range of 0.1-5 μm, such as 0.5-2 μm.

The entity is a birefringent material, i.e. the material is anisotropic. Birefringence,
or double refraction, is the decomposition of a ray of light into two rays (the ordinary
ray and the extraordinary ray) when it passes through certain types of material, such as
calcite crystals or boron nitride, depending on the polarization of the light. In a specific
embodiment, the micron scale birefringent entity comprises quartz. In a further specific
embodiment, the micron scale birefringent entity comprises a cylindrically shaped
quartz crystal. However, as mentioned above, also other materials may be applied.

The micron scale birefringent entity may in an embodiment be arranged in a
second fluid. This second fluid may be the same fluid as the analyte fluid or another
fluid. In a specific embodiment, the micron scale birefringent entity is arranged in the
analyte fluid. In principle, any fluid may be chosen to arrange the micron scale
birefringent entity in. Preferably, the index of refraction of the micron scale
birefringent entity is larger than the surrounding fluid. This allows trapping the micron
scale birefringent entity by the laser light.

In a specific aspect, the micron scale birefringent entity may be arranged in
vacuum. In principle, the method of the invention may also be applied in vacuum to
sense analytes. Hence, according to a further aspect, the invention also provides a
method for sensing with a sensor system an analyte in vacuum, wherein the sensor
system comprises (a) a micron scale birefringent entity, (b) a laser unit configured to
generate polarized laser light, (c) preferably a polarization rotation device, wherein the laser unit and polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light, and (d) a detection unit, wherein the method comprises:

a. keeping with the laser light and the polarization rotation device the micron scale birefringent entity in an optical torque trap at a polarization rotation frequency;

b. keeping the polarization rotation frequency at a sub-critical polarization rotation frequency, where the critical polarization rotation frequency is defined as the maximum frequency of the linear response regime between input polarization rotation frequency and micron scale birefringent entity rotation frequency; and

c. measuring with the detection unit the polarization of the laser light downstream of the micron scale birefringent entity, and sensing a perturbation on the polarization of the laser light due to the analyte.

**Laser unit and polarization rotation device**

The laser unit may for instance comprise a continuous laser, such as a continuous Nd-YAG laser. Hence, in an embodiment, the laser unit comprises a Nd-YAG laser. In an embodiment, the laser unit is configured to generate IR light, i.e. light having a wavelength in the range of about 800 nm and up, such as in the range of 800-2000 nm, such as at about 1.3 or about 1.5 micron. Especially, the laser light of the laser unit is chosen to fit with the dimensions of the micron scale birefringent entity, although other wavelengths may also be possible, like in the visible.

In an embodiment, the laser unit may be configured to generate linearly polarized laser light. In such instance, a polarization rotation device is necessary to obtain the desired rotation of the polarization of the laser light, see below. In another embodiment, the laser unit may be configured to generate elliptically polarized laser light. The polarization of the laser light can be rotated with the polarization rotation device (see below).

The laser unit and polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light. The presence of the polarization rotation device is preferred.

In an embodiment, the polarization rotation device comprises a rotating wave plate device. The rotating wave plate device is a device comprising a rotatable wave
plate, especially rotatable by a rotor. The wave plate is preferably a half wave plate (½ λ plate). The rotation of the rotating wave plate may impose the rotation of the polarization on the laser light, and thereby imposes the rotation on the micron scale birefringent entity in the tweezer. The speed of rotation of the rotating wave plate device may especially be tuneable i.e. is configured to be able to vary the polarization rotation frequency of the polarization of the laser light. The combination of the linearly polarized light and the rotating wave plate leads to the rotation of the linear polarization of the laser light.

In yet another embodiment, the polarization rotation device comprises a quarter wave plate, preferably the polarization rotation device comprises an electro-optical modulator (EOM) in combination with a quarter-wave plate (¼ λ plate). This combination may impose the rotation of the polarization on the laser light, and thereby imposes the rotation on the micron scale birefringent entity in the tweezer. The electro-optical modulator may especially be tuneable, i.e. is configured to be able to vary the polarization rotation frequency of the polarization of the laser light. The combination of the linearly polarized light, the EOM and the quarter wave plate leads to the rotation of the linear polarization of the laser light.

In yet another embodiment, the laser unit is arranged to generate elliptically polarized laser light. Depending on the extend of ellipticality, one of the above mentioned options (EOM + ¼ λ plate, rotating ½ λ plate) may be applied to control the frequency of the polarization rotation of the laser light. The ellipticality is preferably not larger than 60%, preferably not larger than 40% (defined as the ratio between minor and major axis of the elliptical electric field). As will be clear to a person in the art, an ellipticality of 0% refers to linear polarization, and is herein thus indicated as linear polarization.

Hence, downstream of the polarization rotation device, the polarisation of the laser light rotates with a polarization rotation frequency (upstream of the polarization rotation device, the polarization of the laser light may not rotate).

In a preferred embodiment, the laser unit and the polarization rotation are configured to be able to vary the polarization rotation frequency at least in a range larger than 0 Hz and equal to or smaller than 500 Hz, especially in the range of about 0.5-200 Hz, even more especially in the range of about 0.5-50 Hz. In general, within this range the method of the invention may be performed. In general, the critical
polarization frequency will also be found within the range of larger than 0 Hz and equal to or smaller than 500 Hz. Usually, the critical polarization frequency will be in the range of about 10-100 Hz, dependent upon the type (i.e. especially geometry) and size of the micron scale birefringent entity.

The critical polarization rotation frequency is, as mentioned above, defined as the maximum frequency of the linear response regime between input polarization rotation frequency and micron scale birefringent entity rotation frequency. The input polarization rotation frequency is the rotational frequency of the polarization of the laser light (thus upstream of the micron scale birefringent entity). The micron scale birefringent entity rotation frequency is the rotational frequency of the micron scale birefringent entity. Below (i.e. “subcritical”) the critical polarization frequency, an increase of the rotation frequency of the laser light is linearly followed by the micron scale birefringent entity. Hence, in such instance, the input polarization rotation frequency and micron scale birefringent entity rotation frequency are equal. Beyond the critical polarization frequency, the micron scale birefringent entity cannot follow the rotation any more (for instance due to drag). In such instance, the input polarization rotation frequency and micron scale birefringent entity rotation frequency are not equal (especially, the former being larger than the latter). The fact that the micron scale birefringent entity cannot follow the rotational frequency of the laser light can be perceived as perturbations on the polarisation of the laser light downstream of the micron scale birefringent entity. The torque on the micron scale birefringent entity temporarily varies (torque spike), and this can be derived from the downstream laser light polarization. The micron scale birefringent entity is maintained with the laser light and the polarisation rotation device at a rotational frequency lower than the critical (polarization) rotational frequency. The input polarization rotation frequency is the rotation frequency from the polarization of the laser light (i.e. the polarization rotation frequency of the laser light).

It further appears that the sensor system and sensing method is most sensitive close or at the critical polarization rotation frequency, but also most sensitive to noise, such as thermal noise. This may lead to the creation of undesired spikes in the signal. It also appears that the lower the polarization rotation frequency, the less the system and method suffer from noise, such as thermal noise. Therefore, the polarization rotation frequency is chosen below the critical polarization rotation frequency (i.e. sub critical).
In a preferred embodiment, the sub-critical polarization rotation frequency is selected from the range of 50-99%, such as 70-99%, especially 70-95% of the critical polarization frequency. An option to reduce/suppress thermal noise may be to use a plurality of micron scale birefringent entities (which can be measured coincidentally, see also below).

The method of the invention may in an embodiment further involve a calibration procedure for determining the critical polarization rotation frequency of the micron scale birefringent entity. As indicated above, the critical polarization rotation frequency may depend upon the type of micron scale birefringent entity, on the dimensions thereof and on the type of fluid in which the micron scale birefringent entity is arranged and should be tweezed & wrenched by the laser light.

**Detection unit**

The detection unit may comprise any system that allows detection of the polarization of the laser light. For instance, a detector as described by La Porta et al. (Physical Review Letters 92 (19) 2004, 190801-1) or Friese et al. (Nature 394, 348 (1998), ISSN 0028-0836) may be applied.

The detection unit is arranged to measure the polarization of the laser light downstream of the micron scale birefringent entity, and sense a perturbation thereon, if any. By comparing the upstream polarization and the downstream polarization, perturbations on the downstream polarization in time may be measured, due to the presence of analytes flowing along the micron scale birefringent entity. When an analyte is “perceived” by the torque wrenched micron scale birefringent entity, the downstream polarization changes temporarily. The polarization measurement may be used to determine the torque. The torque on the micron scale birefringent entity changes due to an analyte.

The terms “upstream” and “downstream” relate to an arrangement of items or features relative to the propagation of the light from a light generating means (here the laser unit), wherein relative to a first position within a beam of light from the light generating means, a second position in the beam of light closer to the light generating means is “upstream”, and a third position within the beam of light further away from the light generating means is “downstream”. For instance, the micron scale birefringent entity is downstream of the laser unit and the laser unit is upstream of the micron scale
birefringent entity; the detection unit is downstream of the micron scale birefringent entity and downstream of the laser unit, but the micron scale birefringent entity is upstream of the detection unit.

Sensor system and analyte fluid feeding channel

The sensor system comprises (a) a micron scale birefringent entity, (b) a laser unit configured to generate polarized laser light, (c) preferably a polarization rotation device, and (d) a detection unit. Those items are discussed above.

Preferably, the sensor system further comprises an analyte fluid feeding channel (further also indicated as "channel"), wherein the channel comprises a channel cavity (herein further also indicated as "cavity") in fluid connection with the fluid feeding channel, wherein the channel cavity is configured to host the micron scale birefringent entity and to allow free rotation of the micron scale birefringent entity. Such system may especially allow sensing of an analyte in an analyte fluid by the micron scale birefringent entity (in the channel cavity).

Hence, in a further aspect, the invention also provides a sensor system for sensing an analyte in an analyte fluid, wherein the sensor system comprises:

a. an analyte fluid feeding channel, wherein the channel comprises a channel cavity in fluid connection with the fluid feeding channel, wherein the channel cavity is configured to host a micron scale birefringent entity and to allow free rotation of the micron scale birefringent entity in a fluid in the channel cavity;

b. a laser unit configured to generate polarized laser light and preferably a polarization rotation device, wherein the laser unit and optional polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light;

c. a detection unit, configured to measure the polarization of the laser light downstream of the micron scale birefringent entity and to sense a perturbation on the polarization of the laser light due to the analyte.

As indicated above, the sensor system is configured to keep, during use of the system, with the laser light and the optional polarization rotation device the micron scale birefringent entity (in the fluid) in an optical torque trap at the polarization rotation frequency.
In an embodiment, the channel cavity hosts the micron scale birefringent entity. Further, in an embodiment, the laser unit and the optional polarization rotation device are configured to keep with the laser light the micron scale birefringent entity in an optical torque trap at said polarization rotation frequency. Especially, the sensor system comprises the polarization rotation device. Hence, in such embodiment the laser unit and polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light, and the laser unit and the polarization rotation device are configured to keep with the laser light the micron scale birefringent entity in an optical torque trap at said polarization rotation frequency.

Preferably, the fluid connection is configured to prevent migration of the micron scale birefringent entity from the channel cavity into the analyte fluid feeding channel. This can for instance be achieved by a fluid connection having a smallest cross-section smaller the micron scale birefringent entity, i.e. the “mesh size” is too small to allow the micron scale birefringent entity pass the fluid connection. Hence, this may on the one hand allow a free rotation of the micron scale birefringent entity and a good sensing by the micron scale birefringent entity and on the other hand allow a free flow of the analyte fluid. Drag by the flow of the analyte fluid on the micron scale birefringent entity may in this way also be reduced. Further, the fluid connection may allow migration of fluid from the channel into the cavity and vice versa. As mentioned above, the fluid may for instance be a gas or a liquid, especially a liquid, such as water.

In a specific embodiment, the sensor system may comprise a plurality of micron scale birefringent entities. This may for instance advantageous to track the movement of an analyte. A plurality of micron scale birefringent entities may also be applied to discriminate between noise spikes and analyte spikes. In a specific embodiment, the channel cavity may host a plurality of micron scale birefringent entities.

When more than one micron scale birefringent entity is applied (at the same time), it is desirable that (a) one or more laser units configured to generate polarized laser light (beams) and optionally one or more polarization rotation devices, wherein the one or more laser units and the one or more polarization rotation devices are configured to rotate at one or more polarization rotation frequencies the polarization of the laser light (beams), and (b) one or more detection units, arranged, relative to the propagation of the laser light (beams), downstream of the micron scale birefringent entities, respectively, and configured to measure the polarization of the laser light
(beams) and to sense one or more perturbations on the polarization of the laser light (beams), respectively, due to the analyte. Here, the term “beams” is added to indicate that each micron scale birefringent entity is tweezed by its own beam. Downstream of the micron scale birefringent entity, in principle each beam is (individually) detected by the detection unit.

To create a plurality of beams, one or more laser units may be applied, but alternatively or additionally, also beam splitters may be applied. A commonly-used method to generate a plurality of laser traps, which may also be applied in an embodiment, is to use time-sharing. For instance, a single laser beam may be generated, but it is rapidly shifted in position (on a faster timescale than the micron scale birefringent entity can respond), which means that a trap can effectively be present at several locations simultaneously. This shifting may typically be accomplished by an acousto-optical deflector (AOD). Further, optionally, a plurality of detection units may be applied, especially arranged, relative to the propagation of the laser light beams, downstream of the micron scale birefringent entities, and configured to measure the polarizations of the laser light beams and to sense the perturbations on the polarizations of the laser light beams due to the analyte, respectively.

However, the method and sensor system of the invention may also comprise a plurality of sensor systems. Hence, in a specific embodiment of the method of the invention, the method further comprises comparing the measurements of the sensor systems and filtering out noise spikes.

Whatever the arrangement may be, the distance between the micron scale birefringent entities is preferably large enough that they do not interact. Hence, adjacent micron scale birefringent entities preferably have a distance from each other of at least 1/2 the diameter of the micron scale birefringent entities (“shortest distance”), preferably a distance from each other of at least the diameter of micron scale birefringent entities. The term diameter for each micron scale birefringent entity is used as defined above. For determining the shortest distance of adjacent micron scale birefringent entity having different dimensions, the largest diameter is chosen to determine this shortest distance.

The term “along” especially indicates that during application of the method and/or during use of the sensor system, the micron scale birefringent entity is arranged substantially at one position (while rotation), and the fluid passes along the liquid.
Passing along the micron scale birefringent entity may also including flowing/streaming along the micron scale birefringent entity.

Sensor system arrangement

As mentioned above, also a plurality of sensor systems may be applied. Hence, in a further aspect, the invention provides a sensor system arrangement comprising a plurality of sensor systems as defined herein. Such sensor system arrangement thus comprises a plurality of micron scale birefringent entities per se. Preferably, the plurality of sensor systems have a mutual analyte fluid feeding channel. This may again for instance be advantageous to track the movement of an analyte. A plurality of micron scale birefringent entities may also be applied to discriminate between noise spikes and analyte spikes.

In a specific embodiment, the plurality of sensor systems comprise a mutual channel cavity hosting the plurality of micron scale birefringent entities. The cavity is preferable large enough that during performing the method for sensing, the micron scale birefringent entity can be tweezed at sufficient distance(s) of each other (see also above with respect to the shortest distance between adjacent micron scale birefringent entity). Alternatively, or additionally, the channel may comprise a plurality of channel cavities, wherein the plurality of channel cavities each host one or more micron scale birefringent entity. Hence, in a further embodiment, the invention provides a sensor system arrangement as defined herein, wherein the mutual analyte fluid feeding channel comprises a plurality of channel cavities hosting the plurality of micron scale birefringent entities, respectively.

Control unit

As will be clear to the person skilled in the art, the sensor system and sensor system arrangement may further comprise a control unit, configured to control at least one or more of the devices of the sensor system and sensor system arrangement, respectively. The control unit may be arranged to control one or more of the laser unit, the polarization rotation device, the detection unit, and other apparatus, such as for instance a flow controller. The flow controller may be configured to control the flow of the analyte fluid in the analyte fluid feeding channel. For instance, the control unit may
be arranged to control the flow of the analyte fluid and/or the rotational frequency $\omega$ of the micron scale birefringent entity.

Specific embodiments

Specific embodiments are described below, wherein the number are only used for reference purposes:

1. A method for sensing with a sensor system an analyte in an analyte fluid, wherein the sensor system comprises (a) a micron scale birefringent entity, (b) a laser unit configured to generate polarized laser light, (c) optionally a polarization rotation device, wherein the laser unit and optional polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light, and (d) a detection unit, wherein the method comprises:
   a. feeding the analyte fluid along the micron scale birefringent entity while keeping with the laser light and the optional polarization rotation device the micron scale birefringent entity in an optical torque trap at a polarization rotation frequency;
   b. keeping the polarization rotation frequency at a sub-critical polarization rotation frequency, where the critical polarization rotation frequency is defined as the maximum frequency of the linear response regime between input polarization rotation frequency and micron scale birefringent entity rotation frequency; and
   c. measuring with the detection unit downstream of the micron scale birefringent entity the polarization of the laser light, and sensing a perturbation on the polarization of the laser light due to the analyte.

2. The method according to method 1, wherein the analyte fluid is a liquid.
3. The method according to method 1, wherein the analyte fluid is a gas.
4. The method according to any one of methods 1-3, wherein the laser unit is arranged to generate linearly polarized laser light.
5. The method according to any one of methods 1-3, wherein the laser unit is arranged to generate elliptically polarized laser light.
6. The method according to any one of the preceding methods, wherein the analyte is selected from the group consisting of a virus, a bacterium, and a cell.
7. The method according to any one of the preceding methods, wherein micron scale birefringent entity comprises quartz.
8. The method according to any one of the preceding methods, wherein micron scale birefringent entity comprises a cylindrically shaped quartz crystal.

9. The method according to any one of the preceding methods, wherein the laser unit comprises an IR laser.

10. The method according to any one of the preceding methods, wherein the polarization rotation device comprises a rotating wave plate device.

11. The method according to any one of the preceding methods, wherein the polarization rotation device comprises an electro-optical modulator in combination with a quarter-wave plate.

12. The method according to any one of the preceding methods, wherein laser unit and the optional polarization rotation device are configured to be able to vary the polarization rotation frequency at least in a range larger than 0 Hz and equal to or smaller than 500 Hz.

13. The method according to any one of the preceding methods, wherein the sub-critical polarization rotation frequency is selected from the range of 70-99%, especially 70-95% of the critical polarization frequency.

14. The method according to any one of the preceding methods, wherein the micron scale birefringent entity is arranged in a second fluid.

15. The method according to method 14, wherein the micron scale birefringent entity is arranged in the analyte fluid.

16. The method according to any one of the preceding methods, wherein the sensor system comprises an analyte fluid feeding channel, wherein the channel comprises a channel cavity in fluid connection with the fluid feeding channel, wherein the channel cavity is configured to host the micron scale birefringent entity and to allow free rotation of the micron scale birefringent entity, and wherein the fluid connection is configured to prevent migration of the micron scale birefringent entity from the channel cavity into the analyte fluid feeding channel.

17. The method according to any one of the preceding methods, wherein the method further involves a calibration procedure for determining the critical polarization rotation frequency of the micron scale birefringent entity.

18. The method according to any one of the preceding methods, wherein a plurality of sensor systems is applied, and wherein the method further comprises comparing the measurements of the sensor systems and filtering out noise spikes.
19. The method according to method 18, wherein adjacent micron scale birefringent entities have a distance from each other of at least 1/2 the diameter of the micron scale birefringent entities, preferably a distance from each other of at least the diameter of micron scale birefringent entities.

20. A sensor system for sensing an analyte in an analyte fluid, wherein the sensor system comprises:
   a. an analyte fluid feeding channel, wherein the channel comprises a channel cavity in fluid connection with the fluid feeding channel, wherein the channel cavity is configured to host a micron scale birefringent entity and to allow free rotation of the micron scale birefringent entity in a fluid in the channel cavity;
   b. a laser unit configured to generate polarized laser light and optionally a polarization rotation device, wherein the laser unit and optional polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light;
   c. a detection unit configured to measure the polarization of the laser light downstream of the micron scale birefringent entity and to sense a perturbation on the polarization of the laser light due to the analyte.

21. The sensor system according to claimsystem 20, wherein the fluid connection is configured to prevent migration of the micron scale birefringent entity from the channel cavity into the analyte fluid feeding channel.

22. The sensor system according to any one of sensor systems 20-21, wherein the channel cavity hosts a plurality of micron scale birefringent entities.

23. A sensor system arrangement embodiment, comprising a plurality of sensor systems according to any one of sensor systems 20-22, and having a mutual analyte fluid feeding channel.

24. The sensor system arrangement embodiment according to claim arrangement embodiment 23, wherein the plurality of sensor systems comprise a mutual channel cavity hosting the plurality of micron scale birefringent entities.

25. The sensor system arrangement embodiment according to claim arrangement embodiment 23, wherein the mutual analyte fluid feeding channel comprises a plurality of channel cavities hosting the plurality of micron scale birefringent entities, respectively.
The invention also provides a system comprising a central processing unit arranged to perform the method as described herein. In a further aspect the invention also provides a computer program product comprising data and instructions, said computer program being arranged to be loaded by such a system, and after being loaded providing said central processing unit with the capacity to perform the method. In yet another aspect, the invention provides a data carrier comprising such a computer program product. The term “substantially” herein, such as in “substantially consists”, will be understood by the person skilled in the art. The term “substantially” may also include embodiments with “entirely”, “completely”, “all”, etc. Hence, in embodiments the adjective substantially may also be removed. Where applicable, the term “substantially” may also relate to 90% or higher, such as 95% or higher, especially 99% or higher, even more especially 99.5% or higher, including 100%. The term “comprise” includes also embodiments wherein the term “comprises” means “consists of”.

Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

The devices herein are amongst others described during operation. As will be clear to the person skilled in the art, the invention is not limited to methods of operation or devices in operation.

It should be noted that the above-mentioned embodiments illustrate rather than limit the invention, and that those skilled in the art will be able to design many alternative embodiments without departing from the scope of the appended claims. In the claims, any reference signs placed between parentheses shall not be construed as limiting the claim. Use of the verb "to comprise" and its conjugations does not exclude the presence of elements or steps other than those stated in a claim. The article "a" or "an" preceding an element does not exclude the presence of a plurality of such elements. The invention may be implemented by means of hardware comprising several distinct elements, and by means of a suitably programmed computer. In the device claim enumerating several means, several of these means may be embodied by
one and the same item of hardware. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage.

5 Brief description of the drawings

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying schematic drawings in which corresponding reference symbols indicate corresponding parts, and in which:

Figure 1:

1a: scheme of a birefringent cylinder forced by a rotating linear polarization in the optical trap; EA indicates the extraordinary axis.

1b: mean value of the measured torque (pN nm) as a function of the polarization rotation frequency \( \omega \) (Hz).

1c: torque time traces (TTT) of the points A,B and C indicated in figure 1b.

The relative polarization angle is shown in with reference PA; torque (pN nm) as function of time (ms) is displayed.

Figure 2: Potential V \((x)\), and corresponding phase space, for different values of \( \omega \). In the noise-free system, the bifurcation at \( \omega = \omega_c \) (dashed line) separates the excitable region \( \omega < \omega_c \), curves E1 and E2) from the periodically modulated one \( \omega > \omega_c \), curves blue M1 and M2). Here \( \tau_1/\gamma = 1 \), and \( \omega = [0, 0.5, 1, 2, 6] \omega_c \) for E1, E2, \( \omega = \omega_c \), M1 and M2, respectively;

Figure 3:

3a: Demonstration of excitability. A,B,C (left): keeping \( \omega = 31.25 \text{Hz} \), an increasing perturbation \( \varphi_P \) is inserted at times indicated by the dots P. Torque (pN nm) as function of time (ms) is displayed. A,B,C (right): the part of the trace (P1) following each perturbation, set at \( t = 0 \) (P2), is shown. Polarization angle (rad) as function of time (ms) is displayed;

3b (D): the probability of successful excitation of one spike as a function of the perturbation amplitude \( \varphi_P \). The line F is the fit with Eq.3. Spike probability as function of perturbation amplitude (rad) is displayed;

3c (E): the superposition (dots) of the events (triggered in B) is fitted by eq.2 (line F, \( \tau_0 = 1200 \text{pN nm}, \omega_c = 38 \text{Hz}, \tau_0 = 6.3 \text{ms})\); the spike probability as function of time (ms) is displayed;
Figure 4: Probability distribution for the interspike-time of events triggered by thermal noise. The value of $\omega$ (36 Hz) is such to have the excitability threshold overcome by thermal events (inset). The line F fits the data using eq. 4 with $s = 42$ ms, $\tau_0 = 13$ ms, $\lambda = 0.06$, $K = (35$ ms)$^{-1}$.

Figure 5:

5a: a bead stuck on the surface (left) is moved at constant speed along the white line, while the excitable cylinder is kept rotating at the center;

5b: The torque traces along the path are shown for different values of the distance $d$. Torque (pN nm) as function of time (ms) is displayed;

Figure 6 schematically depicts an embodiment of the sensor system of the invention;

Figure 7 schematically depicts an embodiment of the analyte fluid channel; and

Figures 8a and 8b schematically depict various embodiments of the sensor system and sensor system arrangement;

Figure 9 schematically depicts embodiments of the micron scale birefringent entity.

Description of embodiments

Torque is an additional physical parameter of biological relevance, as witnessed by its role in diverse cellular processes such as DNA replication, transcription and packaging, ATP synthesis and bacterial propulsion. For this reason, there is growing interest in the development of single-molecule techniques which allow modification and simultaneous measurement of force and torque. In particular, the high spatio-temporal resolution of optical tweezers can be combined with the angular control of microscopic anisotropic particles, mediated by spin moment transfer, in the so called Optical Torque Wrench (OTW) (see for instance Forth et al., Phys. Rev. Lett. 100, 148301, (2008), La Porta et al, Phys. Rev. Lett. 92, 190801 (2004), and Deufel et al., Nature Methods 4, 223 (2007)). Despite the large amount of knowledge built on standard linear optical tweezers, much less information is available for its angular counterpart, due to its relatively recent development and remaining technical challenges. Expanding the fast dynamical capabilities of optical tweezers to include both the detection and application of torque will add a key component at present missing in the existing single-molecule techniques.
Here, we measure the torque applied to a single nano-fabricated birefringent particle (micron scale birefringent entity) by the linear polarization of the trapping laser, and we show that the torque dynamics of the system casts the Optical Torque Wrench within the general class of excitable dynamical systems.

The distinctive feature of excitability is the existence of a stable (or 'rest') state separated by a threshold from a 'firing' state and a 'refractory' state. Perturbations smaller than the threshold produce small linear response around the stable state, while perturbations larger than the threshold force the system to undergo a large deterministic excursion in the phase space (measured as a spike) before coming back to the stable state again. The refractory time is the minimum time between two successive spikes, corresponding to the time needed to the recovery of the initial state after one deterministic excursion. Based on this non-linear behaviour, we propose a conceptually new sensing technique capable to detect single perturbation events, triggered by proximity effects, with controllable sensitivity, high signal-to-noise ratio and spatial localization typical of optical tweezers.

To investigate the angular dynamics of a birefringent particle (micron scale birefringent entity), we rotationally force it by applying a linear laser polarization rotating at constant frequency $\omega$ in an OTW. To constitute the optical trap, a 4W laser emitting at 1064 nm is focused in a flow cell by a 1.49 NA objective. We use a fast (~MHz) electro-optical modulator (EOM) in combination with a quarter-wave plate as a polarization control system (Arthur). As result, the angle of the linear polarization of the trapping laser field (ellipticity smaller than 5%) is proportional to the voltage applied to the EOM in the angular range $0 - \pi$. To continuously rotate the polarization at a constant rate, we use a saw tooth voltage signal of controlled frequency $\omega$. The torque transferred to the trapped particle (micron scale birefringent entity) is optically measured by fast intensity detectors from the imbalance of the two circular components of the polarization at the output of the trap (Friese et al., Nature 394, 348 (1998), ISSN 0028-0836). The total bandwidth of the detection system is 200 kHz. An input polarization reference and a calibration procedure (similar to the one performed for linear optical tweezers (La Porta et al, Phys. Rev. Lett. 92, 190801 (2004), Tolic-Norrelykke, et al., Review of Scientific Instruments 77, 103101 (pages 11) (2006)) are necessary to obtain the absolute value of the applied torque in physical units. The
birefringent particles employed are cylinders of slightly conical shape (1.7\(\mu\)m height, 0.9\(\mu\)m large diameter, 0.6\(\mu\)m small diameter).

Measurements of the torque exerted on the rotating birefringent particle (i.e. the micron scale birefringent entity, indicated with reference 140) are shown in Fig.1A/1B, with reference 111 indicating the polarized laser beam light. The response of the mean value of the applied torque as a function of the polarization rotation frequency \(\omega\) (top panel) clearly indicates the presence of two distinctive regions. For \(|\omega| < \omega_{\text{exp}} = 35.5\) (±0.5) Hz the absolute value of the mean torque increases linearly with the imposed frequency, while increasing \(|\omega|\) beyond \(\omega_{\text{exp}}\) the absolute value of the mean torque decays to zero (negative frequencies indicate opposite sense of rotation). This behaviour is typical of rotationally forced systems, with optical, magnetic or magneto-mechanic forcing. The physical reason for the decreasing of the average torque beyond the critical point \(\omega_c\) becomes clear looking at its temporally resolved signal: the drag torque acting on the rotating cylinder overcomes the maximum torque which can be transferred by the laser polarization, inducing a phase slip between the micron scale birefringent entities extraordinary axis and the polarization. This event appears as a spike in the torque signal (fig.1B). At higher forcing frequencies the polarization scans the quasi-static particle (micron scale birefringent entity), which cannot follow the fast polarization rotation, giving rise to a sinusoidal torque signal.

These observations can be understood starting from the analytical expression for the torque (per unit area) optically transferred to the birefringent micron scale birefringent entity, which can be written [3] as \(\tau_{\text{opt}} = -\tau_o \sin(2(\theta - \theta_o))\), with \(\tau_o = \varepsilon/(2\Omega)E_o^2 \sin(kL\Delta n)\). Here \(\varepsilon\) is the permittivity, \(\Omega\) is the laser optical frequency, \(\theta - \theta_o\) is the angle between the extraordinary axis of the particle (micron scale birefringent entity) (\(\theta\)) and the polarization (\(\theta_o\)), \(k\) is the wave vector, \(L\) is the length of the cylindrical particle (micron scale birefringent entity), \(\Delta n\) is the quartz birefringence (\(\Delta n = +0.009\)) and a linear input polarization is assumed. Setting \(\theta_o = \omega t\), the equation of motion can be written in the rotating reference as:

\[
\dot{x} = -\tau_o/\gamma \sin(2x) - \omega
\] (1)
where \( x = \theta - \omega t \), \( \gamma \) is the rotational drag coefficient and inertial effects are neglected. Equation 1 has become a classic example of how complex behaviour can arise from a relative simple law.

In fact the system, which is periodic in \( x \) and therefore can be described simply by the motion of a particle (micron scale birefringent entity) on a circle (see fig.2), is excitable for \( \omega < \omega_c = \tau_o / \gamma \). One stable and one unstable solution coexist there, while the distance between them defines the excitability threshold.

Perturbations overcoming the threshold will produce a large deterministic trajectory (the "firing state") back to the stable point (the "resting state", where the rotation of the particle (micron scale birefringent entity) is in phase with that of the polarization).

Increasing further the value of the parameter \( \omega \), the two stationary solutions merge through saddle-node bifurcation at \( |\omega| = \omega_c \), giving rise to a limit cycle, i.e. to periodic spiking in time which tends to become sinusoidal at high \( \omega \). This is visible in Fig.2 where the potential \( V(\dot{x}) \) (defined as \( \dot{x} = -V'(x) \)) is shown for different values of \( \omega \): at the critical frequency the potential barrier defining the excitability threshold disappears, leaving the system in a periodically modulated state.

With the deterministic frame given by Eq.1 in mind, we sought to experimentally demonstrate the excitabile character of the rotating birefringent cylinder in our OTW (Fig.3). The system is prepared below the critical point (\( \omega \) about equal to or smaller than \( \omega_c^{exp} \)) where spikes are not present. A perturbation is then inserted periodically in the EOM voltage, at the times indicated by dots P in the Fig. 3a time traces. As result of the perturbation, the polarization phase \( \theta_o \) suffers a sudden jump (in less than 10 \( \mu s \)) of controlled amplitude \( \varphi_P \) (\( \theta_o = \omega t + \varphi P \) with \( \varphi P = [0, 1.08] \) rad). Small perturbation amplitudes (\( \varphi P < 0.2 \)rad, Fig.3A) do not trigger torque spikes, while the probability of triggering spikes increases with higher perturbation amplitudes (Fig.3B,C). In the right panels of Fig.3A,B,C we show the excitable spikes triggered by the imposed perturbations, artificially set at \( t = 0 \). This shows that the delay between the triggering perturbation and triggered spike is a statistical variable, whose mean value and spread decrease for higher perturbation amplitude.

In Fig.3E we superpose the spikes triggered by the perturbation of Fig.3B, showing that the pulse shape is conserved. This shows that the path followed by the system during an event is deterministic as expected for an excitable response. Directly
integrating Eq.1, we can write the analytical expression of the torque which fits well the measured signal during one 'firing' event, as shown by the fit line F, as

$$\frac{\tau}{\tau_0} = \frac{\cot^2 \left( x' - \frac{\pi}{4} \right) - 1}{\cot^2 \left( x' - \frac{\pi}{4} \right) + 1}$$

where \( \cot \left( x' - \frac{\pi}{4} \right) = \sqrt{\frac{\omega_c + \omega}{\omega_c - \omega}} \left[ \frac{1 - \exp \left( 2 \sqrt{\omega_c^2 - \omega^2} (t - t_{0i}) \right)}{1 + \exp \left( 2 \sqrt{\omega_c^2 - \omega^2} (t - t_{0i}) \right)} \right] \) \( \approx \)

\(- (\omega_c + \omega) (t - t_0)\), \( t_0 \) is the time at which the peak torque is reached, and the approximation is valid when \( \omega \) about equal to or smaller than \( \omega_c \). These measurements clearly show the excitable behaviour of the OTW, as predicted by Eq.1.

We can now take advantage of the vast literature existing on excitable systems to understand several nontrivial features in the behaviour of the OTW. It is possible to write the probability to excite one spike as a function of the amplitude \( \phi_P \) of the imposed perturbation as

$$P(\phi_P) = \frac{1}{2} \left[ -\text{erf}(\sqrt{2K_BT} \left[ 1 - \left( \frac{\omega}{\omega_c} \right)^2 \right]^{1/4}) \right]$$

where \( A = \sqrt{\frac{\tau_0}{2K_BT}} \left[ 1 - \left( \frac{\omega}{\omega_c} \right)^2 \right]^{1/4} \), \( B = \arccos \left( \frac{\omega}{\omega_c} \right) \),

and \( K_BT = 4.1 \) pN nm is the thermal energy. Fitting the data in Fig.3D with Eq.3, we find that \( \omega = 0.90 \omega_c \) and \( \tau_0 = 1150 \) pN nm, well in agreement with the value of maximum measured torque (see Fig.1).

Thermal noise clearly acts as a perturbation on the systems. Therefore the appearance of spikes in the torque signal is anticipated before the bifurcation, for \( \omega < \omega_c \), when the threshold separating the saddle and the node is comparable with the thermal noise amplitude, resulting in \( \omega_{c,\text{exp}} < \omega_c \). The noise has also a perturbing effect
on the period of the deterministic solution for values of \( \omega \) greater than \( \omega_c \). As a result, the periodicity of the deterministic solution beyond the critical point cannot be observed experimentally except for frequencies much higher than \( \omega_c \), where the error relative to the period decreases (Fig.1C). Events excited by thermal noise in the vicinity of the critical point are visible in Fig.1B. Taking the inter-spike time as observable, we show in Fig.4 its probability distribution. The analytical expression for this probability can be written as:

\[
P(t) = \frac{\exp \left( \frac{-e^{2\lambda(t-t_o)}}{e^{2\lambda(t-t_o)} - 1} \ln 2 \right)}{\sqrt{1 - e^{2\lambda(t-t_o)}}} e^{-Kt} \tag{4}
\]

and fits well the experimental data. Here \( s \) is the refractory time, \( t_o \) is the deterministic time of escape from the saddle point, \( \lambda = \sqrt{(1 - (\omega/\omega_c)^2)} \) and \( K \) is the Kramer decaying rate.

Having clarified the behaviour of the OTW and its dynamical origin, we show in Fig.5 that the excitable nature of this opto-mechanical system can be used as a novel sensing technique, inspired by the functioning of an actual neuron. The sensing principle relies on the following elements: the rotating cylinder is sensitive to any type of perturbation that overcomes the barrier between the saddle and the node, and any such perturbation produces an identical excitable spike in the torque signal which spans from its minimum to its maximum value, i.e. with the highest possible signal-to-noise ratio. Moreover, as the threshold is continuously controllable by the parameter \( \omega \), the sensitivity to perturbations is continuously adjustable, even to the point where the technique becomes sensitive to thermal fluctuations. Also, spatial localization of the sensor is assured with great accuracy by the three-dimensional optical trap. Therefore, if the perturbation originates from a particular object which comes in proximity to the cylinder (for example if forced by a flow in a designed micro-channel) its presence can be recognized simply from the spiking torque signal.

In Fig.5, we test this idea in simple and controlled conditions, using a polystyrene bead (3\( \mu \)m diameter) stuck on the glass surface as source of the perturbation. Imposing
a constant velocity to the microscope piezo-stage (on a total distance of 14μm along x, white line in the image), a flow driving the bead is simulated. We also control the lateral distance $d$ (along $y$) between the surfaces of the cylinder and the bead, while the trap was kept about 3μm from the glass surface (contact between the two was possible for small $d$). We set $\omega < \omega_c$ in order for the excitable cylinder not to be sensitive to thermal fluctuations, and we record the torque signal during the movement of the stuck bead, for different values of $d$.

As shown in the temporal traces, spikes are excited in a region where the distance between the two objects is minimum, starting at a lateral distance $d$ comparable with the diameter of the bead, indicating that the possible mechanism of perturbation is a change in the rotational drag coefficient and/or optical diffraction from the bead. Regardless from the actual origin of the perturbation, the proximity of the object can be easily recognized from the presence of excited spikes in the torque signal, while their density encodes distance information.

In conclusion, we have shown both experimentally and theoretically that a birefringent microscopic particle (micron scale birefringent entity) held in a Optical Torque Wrench, and rotationally forced by the rotating linear laser polarization, is an excitable system. The particle (micron scale birefringent entity) then can be used as a local controllable non-linear sensor for single perturbation events.

Figure 6 very schematically depicts an embodiment of a sensor system 100. The sensor system 100 comprises a micron scale birefringent entity 140 which is configured in such a way, the “free” rotation or movement is allowed, i.e. there is enough room that the micron scale birefringent entity 140 can be rotated by the laser.

The sensor system 100 comprises a laser unit 110, especially configured to generate polarized laser light 111 and (optionally) also a polarization rotation device 120. Upstream of the polarization rotation device 120, the laser light 111 may for instance have a linear or elliptical polarization, which polarization does not rotate; downstream of the polarization rotation device 120, the laser light 111 rotates with the polarization rotation frequency. This laser light provides the “input” polarization rotation frequency provided to the micron scale birefringent entity 140. Further, the sensor system 100 may comprise optics, indicated with reference 130, to focus the laser light 111 to allow optical tweezing of the micron scale birefringent entity 140. The laser unit 110 and polarization rotation device 120 are configured to rotate at a
polarization rotation frequency the polarization of the laser light 111. As a subcritical polarization rotation frequency, the micron scale birefringent entity 140 will rotate with a micron scale birefringent entity rotation frequency equal to the polarization rotation frequency (input polarization rotation frequency).

5 Downstream of the micron scale birefringent entity 140 the laser light is measured, especially its polarization. To this end, the sensor system 100 further comprises a detection unit 150 configured to measure the polarization of the laser light downstream of the micron scale birefringent entity 140 and to sense a perturbation on the polarization of the laser light due to the analyte. The analyte is present/carried in a analyte fluid. The flow of the analyte fluid is schematically depicted with reference 181. The sensor system 100 may further comprise a controller 160, configured to control for instance the rotation frequency \( \omega \) of the micron scale birefringent entity 140 and the flow 181 of the analyte fluid.

10 With the sensor system 100, more precisely with the detection unit 150, a nonlinear perturbation on the micron scale birefringent entity rotation frequency due to the presence of analyte in the proximity of the micron scale birefringent 140 entity may be sensed.

15 Figure 7 schematically depicts an embodiment of part of an analyte fluid feeding channel 170 that may be comprises by the sensor system 100. Here, in this embodiment, channel 170 comprises a channel cavity 171 in fluid connection 172 with the fluid feeding channel 170. The channel cavity 171 is configured to host the micron scale birefringent entity 140 and to allow free rotation of the micron scale birefringent entity 140. The fluid connection 172 is configured to prevent migration of the micron scale birefringent entity 140 from the channel cavity 171 into the analyte fluid feeding channel 170. For instance, assuming a circular fluid connection 172, the diameter thereof may be smaller than the smallest diameter of the micron scale birefringent entity 140. Within the channel 170, the analyte fluid, indicated with reference 180, flows with flow 181 (for instance induced by a flow controller (not indicated). Analytes are indicated with reference 190. The micron scale birefringent entity 140 in the cavity 171 will in general also be arranged in a fluid, here indicated as second fluid 182. This second fluid 182 may be another or the same fluid as the analyte fluid 181. Preferably, the second fluid 182 is the same fluid as the analyte fluid 181 (but without analytes).
Figure 8a schematically depicts an embodiment of the feeding channel 170 comprising channel cavity 171, wherein the cavity 171 is arranged to host a plurality of micron scale birefringent entities 140 (here by way of example two). Hence, in such embodiment, the sensor system 100 employs a plurality of micron scale birefringent entities. For instance, in this way thermally induced false spikes may be filtered out. Preferably, the distance between adjacent micron scale birefringent entities 140, indicated with d, is preferably at least the diameter of the micron scale birefringent entities, preferably at least two times the diameter of the micron scale birefringent entities 140.

Figure 8b schematically depicts an embodiment of a sensor system arrangement 200, comprising a plurality of sensor systems 100, wherein the plurality of sensor systems 100 have a mutual analyte fluid feeding channel 170. However, each sensor system 100 comprises its “own” cavity 170, wherein each cavity 170 will in general hosts at least one micron scale birefringent entity 140. Such arrangement 200 may not only be used to sense analytes 190 but may also be used to track the analytes 190. The dashed line indicates the movement of the analyte 190.

Figure 9 schematically depicts non-limitingly some embodiments of the micron scale birefringent entity 140. Top left a cylinder is depicted, have an essentially constant diameter d1. In the top right, a truncated cone as micron scale birefringent entity 140 is depicted, having a smallest diameter d1 and a largest diameter d2. Bottom left, an elliptical cone is depicted, with a smallest diameter d1 at the top and bottom and a largest diameter d2 somewhere in between. The height of the micron scale birefringent entity 140 is indicated with reference h. Bottom right, a spherical micron scale birefringent entity is schematically depicted.

For the cylinder, both the ordinary and extraordinary axis are perpendicular to the cylinder axis. If one would take the cross-section of the cylinder (looking at it from above), the extraordinary and ordinary axis lie on that section, while the particle (micron scale birefringent entity) length axis is perpendicular to that plane.
Claims

1. A method for sensing with a sensor system an analyte in an analyte fluid, wherein the sensor system comprises (a) a micron scale birefringent entity, (b) a laser unit configured to generate polarized laser light, (c) optionally a polarization rotation device, wherein the laser unit and optional polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light, and (d) a detection unit, wherein the method comprises:
   a. feeding the analyte fluid along the micron scale birefringent entity while keeping with the laser light and the optional polarization rotation device the micron scale birefringent entity in an optical torque trap at a polarization rotation frequency;
   b. keeping the polarization rotation frequency at a sub-critical polarization rotation frequency, where the critical polarization rotation frequency is defined as the maximum frequency of the linear response regime between input polarization rotation frequency and micron scale birefringent entity rotation frequency; and
   c. measuring with the detection unit downstream of the micron scale birefringent entity the polarization of the laser light, and sensing a perturbation on the polarization of the laser light due to the analyte.

2. The method according to claim 1, wherein the analyte fluid is a liquid.

3. The method according to claim 1, wherein the analyte fluid is a gas.

4. The method according to any one of claims 1-3, wherein the laser unit is arranged to generate linearly polarized laser light.

5. The method according to any one of claims 1-3, wherein the laser unit is arranged to generate elliptically polarized laser light.

6. The method according to any one of the preceding claims, wherein the analyte is selected from the group consisting of a virus, a bacterium, and a cell.

7. The method according to any one of the preceding claims, wherein micron scale birefringent entity comprises quartz.

8. The method according to any one of the preceding claims, wherein micron scale birefringent entity comprises a cylindrically shaped quartz crystal.

9. The method according to any one of the preceding claims, wherein the laser unit comprises an IR laser.
10. The method according to any one of the preceding claims, wherein the polarization rotation device comprises a rotating wave plate device.

11. The method according to any one of the preceding claims, wherein the polarization rotation device comprises an electro-optical modulator in combination with a quarter-wave plate.

12. The method according to any one of the preceding claims, wherein laser unit and the optional polarization rotation device are configured to be able to vary the polarization rotation frequency at least in a range larger than 0 Hz and equal to or smaller than 500 Hz.

13. The method according to any one of the preceding claims, wherein the sub-critical polarization rotation frequency is selected from the range of 70-99%, especially 70-95 % of the critical polarization frequency.

14. The method according to any one of the preceding claims, wherein the micron scale birefringent entity is arranged in a second fluid.

15. The method according to claim 14, wherein the micron scale birefringent entity is arranged in the analyte fluid.

16. The method according to any one of the preceding claims, wherein the sensor system comprises an analyte fluid feeding channel, wherein the channel comprises a channel cavity in fluid connection with the fluid feeding channel, wherein the channel cavity is configured to host the micron scale birefringent entity and to allow free rotation of the micron scale birefringent entity, and wherein the fluid connection is configured to prevent migration of the micron scale birefringent entity from the channel cavity into the analyte fluid feeding channel.

17. The method according to any one of the preceding claims, wherein the method further involves a calibration procedure for determining the critical polarization rotation frequency of the micron scale birefringent entity.

18. The method according to any one of the preceding claims, wherein a plurality of sensor systems is applied, and wherein the method further comprises comparing the measurements of the sensor systems and filtering out noise spikes.

19. The method according to claim 18, wherein adjacent micron scale birefringent entities have a distance from each other of at least 1/2 the diameter of the micron scale birefringent entities, preferably a distance from each other of at least the diameter of micron scale birefringent entities.
20. A sensor system for sensing an analyte in an analyte fluid, wherein the sensor system comprises:
   a. an analyte fluid feeding channel, wherein the channel comprises a channel cavity in fluid connection with the fluid feeding channel, wherein the channel cavity hosts a micron scale birefringent entity and wherein the channel cavity is configured to allow free rotation of the micron scale birefringent entity in a fluid in the channel cavity;
   b. a laser unit configured to generate polarized laser light and optionally a polarization rotation device, wherein the laser unit and optional polarization rotation device are configured to rotate at a polarization rotation frequency the polarization of the laser light, and wherein the laser unit and the optional polarization rotation device are configured to keep with the laser light the micron scale birefringent entity in an optical torque trap at said polarization rotation frequency;
   c. a detection unit configured to measure the polarization of the laser light downstream of the micron scale birefringent entity and to sense a perturbation on the polarization of the laser light due to the analyte.

21. The sensor system according to claim 20, wherein the fluid connection is configured to prevent migration of the micron scale birefringent entity from the channel cavity into the analyte fluid feeding channel.

22. The sensor system according to any one of claims 20-21, wherein the channel cavity hosts a plurality of micron scale birefringent entities.

23. A sensor system arrangement, comprising a plurality of sensor systems according to any one of claims 20-22, and having a mutual analyte fluid feeding channel.

24. The sensor system arrangement according to claim 23, wherein the plurality of sensor systems comprise a mutual channel cavity hosting the plurality of micron scale birefringent entities.

25. The sensor system arrangement according to claim 23, wherein the mutual analyte fluid feeding channel comprises a plurality of channel cavities hosting the plurality of micron scale birefringent entities, respectively.
Fig 2

The diagram shows the relationship between $V(x)$ and $x$, with different regions defined by $\omega < \omega_c$, $\omega = \omega_c$, and $\omega > \omega_c$. The curves $E_1$ and $E_2$ represent different energy levels, and the circles indicate the motion of the system at various frequencies.
Fig 3a

Fig 3b

Fig 3c
Fig 4
Fig 9
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N21/21 G21K1/00 G01N15/14
ADD. G01N21/23 G01N21/03

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)
G01N 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, WPI Data, INSPEC, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>LA PORTA A ET AL: &quot;Optical torque wrench: angular trapping, rotation, and torque detection of quartz microparticles&quot;, PHYSICAL REVIEW LETTERS APS USA, vol. 92, no. 19, 14 May 2004 (2004-05-14), pages 190801-1-4, XP002610948, ISSN: 0031-9007 cited in the application figures 1a, 1e pages 190801-2, right-hand column, paragraph 2 pages 190801-3, left-hand column, paragraph 2 - right-hand column, paragraph 1; figures 3a-3c pages 190801-4, left-hand column, paragraph 2</td>
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Date of the actual completion of the international search: 9 August 2011
Date of mailing of the international search report: 19/08/2011

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<td>INMAN J; DEUFEL C; FORTH S; WANG M D: &quot;Viscous Drag Torque on a Rotating Nanofabricated Cylinder Near an Infinite Plane Boundary&quot;, BIOPHYSICAL JOURNAL, vol. 96, no. 3, 2 March 2009 (2009-03-02), page 293A, XP025919937, 1472-Pos, Board B316; page 293a, right-hand column, last paragraph</td>
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<tr>
<td>A</td>
<td>PARKIN SIMON ET AL: &quot;CHAPTER 19: Optical torque on microscopic objects&quot;, 1 January 2007 (2007-01-01), LASER MANIPULATION OF CELLS AND TISSUES (BOOK SERIES: METHODS IN CELL BIOLOGY), ELSEVIER/ACADEMIC PRESS, PAGE(S) 525 - 561, XP009138228, ISBN: 978-0-12-370648-5 page 548, paragraph 2; figures 18,19 page 554, paragraph 1; figure 25</td>
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