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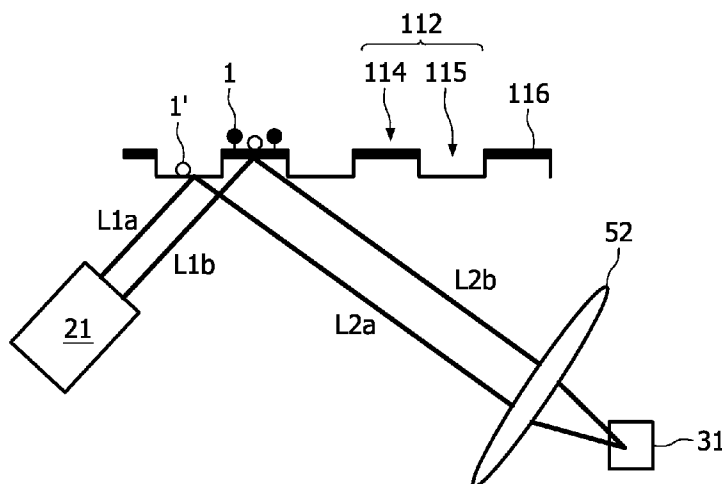


FIG. 3

(57) Abstract: The invention relates to a carrier (111) and a microelectronic sensor device for making optical examinations with the help of the reflection of an input light beam (L1). The contact surface (112) of the carrier (111) at which the reflection takes place comprises an optical phase structure, e.g. a pattern of pits (115) and lands (114), from which an input light beam (L1) is reflected as a plurality of output light beams (L2) with phase differences. In a preferred embodiment, the phase differences are adjusted such that the output light beams destructively interfere in zero-th order. For a clean contact surface, a minimal, ideally zero signal will therefore be measured in the output light beam. If target substances (1) are present at the contact surface (112), e.g. bound to binding sites (116) on the lands (114), this balance is disturbed by frustrated total internal reflection, and a corresponding non-zero signal can be detected.

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## A CARRIER FOR OPTICAL EXAMINATIONS WITH LIGHT REFLECTIONS

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The invention relates to a carrier for optical examinations, which comprises a contact surface where an incident light beam can be reflected. Moreover, it relates to a microelectronic sensor device comprising such a carrier.

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The US 2005/0048599 A1 discloses a method for the investigation of microorganisms that are tagged with particles such that a (e.g. magnetic) force can be exerted on them. In one embodiment of this method, a light beam is directed through a transparent material to a surface where it is totally internally reflected. Light of this beam that extends from the transparent material as an evanescent wave is scattered by microorganisms and/or other components at the surface and then detected by a photodetector or used to illuminate the microorganisms for visual observation. A problem of this and similar measurement devices is that the signal one is interested in is often very small in comparison to a large baseline signal, which makes accurate measurements difficult.

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Based on this situation it was an object of the present invention to provide means that allow for optical examinations at a contact surface and that preferably have a high accuracy and/or robustness.

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This object is achieved by a carrier according to claim 1, a microelectronic sensor device according to claim 2, and a use according to claim 15. Preferred embodiments are disclosed in the dependent claims.

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The carrier according to the present invention is intended for optical examinations, wherein the term "examinations" is to be understood in a broad sense, comprising any kind of manipulation and/or interaction of light with some entity to be

treated. The examinations may preferably comprise the qualitative or quantitative detection of target components comprising label particles, wherein the target components may for example be biological substances like biomolecules, complexes, cell fractions or cells.

5                   The carrier will usually be made from a transparent material, for example glass or poly-styrene, to allow the propagation of light of a given (visible) spectrum. It comprises a "contact surface" with an "investigation region" that has an optical phase structure by which different parts or sub-beams of an input light beam (or, equivalently, different input light beams) impinging on it (from within or outside the carrier) are  
10 reflected with a phase difference.

                  The term "contact surface" is chosen primarily as a unique reference to a particular part of the surface of the carrier, and though target components will in many applications actually contact and bind to said surface, this does not necessarily need to be the case. Moreover, the "investigation region" may be a sub-region of the contact  
15 surface or comprise the complete contact surface; it will typically have the shape of a substantially circular spot that is illuminated by the input light beam.

                  The optical phase structure may be realized in many different ways, some of which will be described in more detail with reference to preferred embodiments of the invention. In general, such a structure is defined by its effect on the reflection of  
20 impinging (preferably parallel) input light beams or different sub-beams of one broad input light beam, i.e. that there exist at least two (preferably adjacent) input light sub-beams with some finite, typically small diameter which enter in phase and which are reflected with a phase difference. Typically this phase difference is in the order of up to one wavelength of the incident light.

25                   The described carrier has the advantage that, when its contact surface is illuminated with an input light beam that covers the investigation region, at least one output light beam will be generated by reflection that comprises at least two sub-beams with a phase difference. This phase difference can be adjusted such that a desired interference occurs, particularly a destructive interference for a given angle. Thus the  
30 baseline signal that represents a nominal situation (e.g. with no target particles at the

contact surface) can be minimized or even made zero, allowing a sensitive detection of any deviations from this nominal situation.

The invention further relates to a microelectronic sensor device for optical examinations, comprising:

- 5 - A carrier of the kind described above, e.g. with an optical phase structure in the investigation region of a contact surface.
- A light source for emitting a light beam, called "input light beam" in the following, towards the investigation region of the carrier. The light source may for example be a laser or a light emitting diode (LED), optionally provided with some optics  
10 for shaping and directing the input light beam.

The microelectronic sensor device comprises as an essential component a carrier of the kind described above. Reference is therefore made to the description of this carrier for more information on the details and advantages of said microelectronic sensor device.

- 15 In a further development of the invention, the microelectronic sensor device further comprises a light detector for detecting a characteristic parameter of an output light beam that is generated by reflection of the input light beam at the contact surface, particularly in the investigation region of the carrier. Most preferably, said output light beam is generated by total internal reflection (TIR, including the case of  
20 frustrated total internal reflection, FTIR). To this end, the investigation region must comprise an interface between two media, e.g. glass and water, at which total internal reflection (TIR) can take place if the incident light beam hits the interface at an appropriate angle (larger than the associated critical angle of TIR). Such a setup may be used to examine small volumes of a sample at the TIR-interface which are reached by  
25 exponentially decaying evanescent waves of the totally internally reflected beam. Target components – e.g. atoms, ions, (bio-)molecules, cells, viruses, or fractions of cells or viruses, tissue extract, etc. – that are present in the investigation region can then scatter the light of the evanescent waves which will accordingly miss in the reflected light beam. In this scenario of a "frustrated total internal reflection", the output light beam of the  
30 sensor device will consist of the reflected light of the input light beam, wherein the small amount of light missing due to scattering of evanescent waves contains the desired

information about the target components in the investigation region. Thus the signal one is interested in (missing light) is very small in comparison to a large base signal, making accurate measurements difficult. The proposed application of an optical phase structure and the resulting interference in the output light beam helps in this situation to suppress  
5 the large base signal and to pronounce the small variations.

The aforementioned light detector is preferably arranged at a position where destructive interference occurs in the output light beam. As explained above, such a destructive interference can occur if a (parallel, coherent) input light beam impinges on the investigation region because the optical phase structure of this region induces phase  
10 differences in components (sub-beams) of the output light beam that later interfere.

In the following, further developments of the invention will be described that relate both to the carrier and the microelectronic sensor device of the kind described above.

In a first important further development, the optical phase structure on  
15 the contact surface of the carrier comprises a sequence of pits and lands. In this context, the terms "pits" and "lands" are used as it is for example known from the technology of compact discs (CDs) etc., i.e. for microscopic recesses (pits) and elevations (lands) with respect to the medium plane of a macroscopically flat surface. Ideally, the pits and lands extend in an area parallel to the medium plane of the contact surface, with approximately  
20 perpendicular steps/walls connecting neighboring pits and lands. When an input light beam with some finite width falls on such a pit-land structure, the reflected beam will consist of a plurality of sub-beams each of which is reflected by one single pit or land, wherein the sub-beams have phase differences due to the different optical lengths they had to travel to their reflection surface (pit or land).

25 Preferably, the optical path difference between neighboring pits and lands (with respect to a given angle of incidence) corresponds to approximately  $\lambda/2$ , i.e. the half of the wavelength  $\lambda$  of the light of the input light beam inside the medium (i.e.  $\lambda_0/2n$  with  $\lambda_0$  the wavelength in vacuum and  $n$  the refractive index of the medium; if no monochromatic input light beam is used, the wavelength  $\lambda$  refers to a characteristic  
30 wavelength of the beam, e.g. that of the peak or the center of gravity of the spectrum).

With such an optical path difference, destructive interference can occur at the zero-th order of the reflected output light beam, i.e. in the direction of "normal" reflection according to geometrical optics. The associated height (in z-direction) of the pits and/or the lands is chosen such that the desired (destructive) interference can take place in a satisfying manner while the carrier can still be produced with reasonable effort. This is typically the case if the height lies between about  $0.1 \cdot \lambda$  and about  $10 \cdot \lambda$ .

While a desired interference could already occur if there is only one pit and one land, it is preferred that the pits and lands are repeated for a plurality of times in one dimension or in two dimensions. Thus a comparatively broad input light beam is split during reflection in a plurality of output light sub-beams which have phase differences and can therefore mutually interfere.

In the aforementioned case, the repetition direction of the pits and lands may preferably be parallel or perpendicular to the plane of incidence of the input light beam. The corresponding interference patterns will then be oriented in a definite and advantageous way with respect to the input light beam.

The duty cycle of the lands preferably ranges between 10 % and 90 %, wherein said "duty cycle" is defined as the ratio of (a) the area of a land with respect to (b) the common area of said land and an associated (adjacent) pit. Typically, the description with a duty cycle is used if the whole pattern of pits and lands can be composed of identical, generic cells or tiles comprising one pit and one land each.

The duty cycle of the lands is preferably chosen such that a pit and an associated land reflect approximately the same amount of light (in the average or particularly with respect to an input light beam impinging under a given angle of incidence and with a given wavelength). This assures that the light reflected from said pits and lands can completely cancel by destructive interference if the phase difference is appropriate (i.e.  $\lambda/2$ ).

The width (grating land+pit period) determines the extent of the diffraction, i.e. the angles for the higher order beams with respect to the specularly reflected zero-th order beam. Typical values of the width are in the range of about one to several  $\mu\text{m}$ .

In many applications of the carrier or the microelectronic sensor device, target components in a sample shall selectively be detected. To this end the contact surface in the investigation region can be coated with binding sites for particular target substances. In a first particular embodiment of this approach, either the pits or the lands  
5 are coated with such binding sites for target substances. Binding will thus only occur at either the pits or the lands, thus creating an asymmetry in the amount of light reflected from these parts of the surface (such an asymmetry would usually not occur if both pits and lands would bind target substances in the same manner). The interference patterns that are observed in an output light beam after reflection from the pits and lands will  
10 therefore change, which can be detected and which provides desired information about the bound target substances (e.g. about their presence and/or amount).

In the aforementioned embodiment, the areas (lands or pits) that are not coated with binding sites may optionally comprise a "compensation coating" for adapting their reflectivity to that of the coated areas. This measure takes into account that the  
15 reflectivity of a surface region is changed when it is coated with some material, for example with the binding sites. A prevailing balance between the reflections from the pits and lands will therefore usually be disturbed if either the pits or the lands alone are coated. To avoid this, the balance of the reflectivities is re-established with the help of the compensation coating. (It should be noted that the adaptation with a compensation  
20 coating refers to the nominal situation with empty binding sites and that a disturbance of the balance of reflectivities due to the binding of target substances is desired.)

In another embodiment of the invention, the pits are at least partially filled with a spacer material having a refractive index smaller than that of the carrier material, preferably a refractive index close to that of the sample liquid. Thus the conditions for  
25 total internal reflection at the bottom pit interface can be kept. Practical examples of suitable spacer materials comprise sol-gel materials which, after hardening, result in a silica-comprising matrix with a refractive index close to 1.4.

Filling the pits with a spacer material will therefore not affect the reflection of an input light beam at the (bottom) face of the pit. The spacer material  
30 preferably fills the pits completely, yielding a smooth, planar contact surface at the height of the lands.

In the aforementioned case, the whole investigation region is preferably coated with binding sites. Such a uniform surface-wide coating with binding sites can considerably easier be produced (e.g. by ink-jet printing) than a patterned coating with binding sites covering for example only lands and sparing pits. Due to the spacer material  
5 in the pits, the binding sites above pits will not affect the reflection of an input light beam by the pits. Thus the binding of target substances to said binding sites will have no effect either, in contrary to the binding of target substances above the lands.

The invention further relates to the use of the carrier and/or the microelectronic device described above for molecular diagnostics, biological sample  
10 analysis, or chemical sample analysis, food analysis, and/or forensic analysis. Molecular diagnostics may for example be accomplished with the help of magnetic beads or fluorescent particles that are directly or indirectly attached to target molecules.

These and other aspects of the invention will be apparent from and elucidated with reference to the embodiment(s) described hereinafter. These  
15 embodiments will be described by way of example with the help of the accompanying drawings in which:

Figure 1 schematically shows a microelectronic sensor device with a  
20 carrier according to the present invention;

Figure 2 illustrates the principle of destructive interference due to an optical phase structure;

Figure 3 shows an optical phase structure realized by pits and lands;

Figure 4 shows two consecutive steps of the production of a pit-land  
25 structure with binding sites in the pits;

Figure 5 shows an alternative pit-land structure with a spacer material in the pits;

Figure 6 illustrates a two-dimensional pit-land pattern;

Figure 7 illustrates a one-dimensional pit-land pattern;

30 Figure 8 illustrates the orientation of the one-dimensional pit-land pattern perpendicularly to an input light beam;

Figure 9 illustrates the orientation of the one-dimensional pit-land pattern parallel to an input light beam.

5 Like reference numbers or numbers differing by integer multiples of 100 refer in the Figures to identical or similar components.

Though the present invention will in the following be described with respect to a particular setup (using magnetic particles and frustrated total internal reflection as measurement principle), it is not limited to such an approach and can  
10 favorably be used in many different applications and setups.

Figure 1 shows a general setup with a microelectronic sensor device according to the present invention. A central component of this microelectronic sensor device is the carrier 11 that may for example be made from glass or transparent plastic like poly-styrene. The carrier 11 is located next to a sample chamber 2 in which a sample  
15 fluid with target components to be detected (e.g. drugs, antibodies, DNA, etc.) can be provided. The sample further comprises magnetic particles, for example superparamagnetic beads, wherein these particles are usually bound as labels to the aforementioned target components. For simplicity only the combination of target  
20 components and magnetic particles is shown in the Figure and will be called "target particle" 1 in the following. It should be noted that instead of magnetic particles other label particles, for example electrically charged or fluorescent particles, could be used as well.

The interface between the carrier 11 and the sample chamber 2 is formed by a surface called "contact surface" 12. This contact surface 12 is coated with capture  
25 elements, e.g. antibodies, which can specifically bind the target particles.

The sensor device comprises a magnetic field generator 41, for example an electromagnet with a coil and a core, for controllably generating a magnetic field at the contact surface 12 and in the adjacent space of the sample chamber 2. With the help of this magnetic field, the target particles 1 can be manipulated, i.e. be magnetized and  
30 particularly be moved (if magnetic fields with gradients are used). Thus it is for example possible to attract target particles 1 to the contact surface 12 in order to accelerate their

binding to said surface, or to wash unbound target particles away from the contact surface before a measurement.

The sensor device further comprises a light source 21 that generates an input light beam L1 which is transmitted into the carrier 11 through an "entrance window" 14. As light source 21, a laser or an LED, particularly a commercial DVD ( $\lambda = 658 \text{ nm}$ ) laser-diode can be used. A collimator lens may be used to make the input light beam L1 parallel, and a pinhole of e.g. 0.5 mm may be used to reduce the beam diameter. The input light beam L1 arrives at the contact surface 12 at an angle larger than the critical angle  $\theta_c$  of total internal reflection (TIR) and is therefore totally internally reflected in an "output light beam" L2. The output light beam L2 leaves the carrier 11 through another surface ("exit window" 15) and is detected by a light detector 31. The light detector 31 determines the amount of light of the output light beam L2 (e.g. expressed by the light intensity of this light beam in the whole spectrum or a certain part of the spectrum). The measured sensor signals S are evaluated and optionally monitored over an observation period by an evaluation and recording module (not shown) that is coupled to the detector 31.

It is possible to use the detector 31 also for the sampling of fluorescence light emitted by fluorescent particles 1 which were stimulated by the input light beam L1, wherein this fluorescence may for example spectrally be discriminated from reflected light L2. Though the following description concentrates on the measurement of reflected light, the principles discussed here can mutatis mutandis be applied to the detection of fluorescence, too.

The described microelectronic sensor device applies optical means for the detection of target particles 1. For eliminating or at least minimizing the influence of background (e.g. of the sample fluid, such as saliva, blood, etc.), the detection technique should be surface-specific. As indicated above, this is achieved by using the principle of frustrated total internal reflection (FTIR). This principle is based on the fact that an evanescent wave penetrates (exponentially dropping) into the sample 2 when the incident light beam L1 is totally internally reflected. If this evanescent wave then interacts with another medium like the bound target particles 1, part of the input light will be coupled into the sample fluid (this is called "frustrated total internal reflection"), and the reflected

intensity will be reduced (while the reflected intensity will be 100% for a clean interface and no interaction). Depending on the amount of disturbance, i.e. the amount of target particles on or very near (within about 200 nm) to the TIR surface (not in the rest of the sample chamber 2), the reflected intensity will drop accordingly. This intensity drop is a  
5 direct measure for the amount of bonded target particles 1, and therefore for the concentration of target particles in the sample. When the mentioned interaction distance of the evanescent wave of about 200 nm is compared with the typical dimensions of antibodies, target molecules and magnetic beads, it is clear that the influence of the background will be minimal. Larger wavelengths  $\lambda$  will increase the interaction distance,  
10 but the influence of the background liquid will still be very small. Another reason for the low background is that most biological materials have relatively low refractive indices near to the refractive index of water, i.e.  $n=1.3$ . The magnetic beads typically consist of a matrix material that has a significantly higher refractive index ( $n=1.6$ ) causing the outcoupling of the signal. Furthermore, the magnetic beads contain potentially light  
15 scattering magnetic or magnetizable grains.

The described procedure is independent of applied magnetic fields. This allows real-time optical monitoring of preparation, measurement and washing steps. The monitored signals can also be used to control the measurement or the individual process steps.

20 For the materials of a typical application, medium A of the carrier 11 can be glass and/or some transparent plastic with a typical refractive index of 1.52. Medium B in the sample chamber 2 will be water-based and have a refractive index close to 1.3. This corresponds to a critical angle  $\theta_c$  of  $60^\circ$ . An angle of incidence of  $70^\circ$  is therefore a practical choice to allow fluid media with a somewhat larger refractive index (assuming  
25  $n_A = 1.52$ ,  $n_B$  is allowed up to a maximum of 1.43). Higher values of  $n_B$  would require a larger  $n_A$  and/or larger angles of incidence.

Advantages of the described optical read-out combined with magnetic labels for actuation are the following:

- Cheap cartridge: The carrier 11 can consist of a relatively simple,  
30 injection-molded piece of polymer material.

- Large multiplexing possibilities for multi-analyte testing: The contact surface 12 in a disposable cartridge can be optically scanned over a large area. Alternatively, large-area imaging is possible allowing a large detection array. Such an array (located on an optical transparent surface) can be made by e.g. ink-jet printing of  
5 different binding molecules on the optical surface.  
The method also enables high-throughput testing in well-plates by using multiple beams and multiple detectors and multiple actuation magnets (either mechanically moved or electro-magnetically actuated).
  - Actuation and sensing are orthogonal: Magnetic actuation of the target  
10 particles (by large magnetic fields and magnetic field gradients) does not influence the sensing process. The optical method therefore allows a continuous monitoring of the signal during actuation. This provides a lot of insights into the assay process and it allows easy kinetic detection methods based on signal slopes.
  - The system is really surface sensitive due to the exponentially decreasing  
15 evanescent field.
  - Easy interface: No electric interconnect between cartridge and reader is necessary. An optical window is the only requirement to probe the cartridge. A contactless read-out can therefore be performed.
  - Low-noise read-out is possible.
- 20 A problem of the described microelectronic sensor device may arise from the fact that small signal changes from the target particles 1 have to be detected on a relatively large optical base line signal. Said optical base line signal originates from the large reflection from the contact surface 12. Due to this large optical base line signal, gain variations originating from temperature effects (drift) in the sensor, in the signal  
25 processing and optical light path will introduce large variations in the detected signal, which limits the achievable accuracy and detection limit of the biosensor. This is especially a problem during relatively long-time measurements for low target-concentrations. Moreover, demands on the dynamic range of the detection electronics are quite severe for high-sensitivity applications. Furthermore, spurious light sources  
30 from ambient and lighting may disturb the measurement. It is therefore desirable to achieve a low detection limit and a high accuracy without introducing unrealistic

demands on the optical light path and the signal processing electronics (stability, dynamic range).

To solve the aforementioned problems, it is proposed here to extinguish the reflected light in the nominal situation (when no target particles are bound to the contact surface 12) by applying an optical phase structure 51 to the carrier (e.g. a cartridge or a well plate). The implementation of this phase structure is such that when using a single parallel light beam, the specularly reflected light in the nominal situation is substantially suppressed by destructive interference at the sensitive area of the light detector 31. The presence of target particles 1 (e.g. magnetic labels) will change the balance in the phase structure, and thus give rise to an increased light intensity in the light detector 31, resulting in a non-zero signal. In this way the observed light amplitude for the reference measurement in the nominal situation will be very close to zero and the large optical baseline is compensated, leading to much reduced unwanted signal variations and thus much improved accuracy. Also, the required dynamic range of the detection electronics is significantly smaller.

As already coarsely (and not to scale) shown in Figure 1, the optical phase structure may be realized by a pit-land structure 51 of the contact surface 12, at least in the investigation region 13. The geometrical properties of this structure are chosen such that when illuminating it with a parallel input light beam L1, the specularly reflected light of the output light beam L2 cancels due to destructive interference on the sensitive area of the light detector 31. Typical pit-land periods (x-direction in Figure 1) are in the order of 1 to 5  $\mu\text{m}$ . Such a structure can consist of a 50% duty cycle phase grating with  $\lambda/2$  (or integer multiple) optical path difference between adjacent areas of the pits and lands, respectively (it should be noted that the term "optical path difference" already takes the effect of refractive index into account and is measured along the beam's propagation direction). This results in destructive interference of the zero-th order, i.e. the specularly reflected light, leading to a substantially zero intensity at the detector. The light diffracted into higher orders should be blocked from the detector.

In Figure 2, this operation principle is sketched (for clarity, relative scale is not correct). Upon reflection, the input light beam L1 sees a stepped phase profile 51, leading to a diffraction pattern with a suppressed zero-th order ( $n = 0$ ). An optional

lens 52 may be used to image the diffracted beam pattern onto e.g. a photo diode in the light detector 31. Unwanted higher orders are blocked from the detector 31 by a diaphragm 53.

Figure 3 schematically shows the main components of a microelectronic sensor device with an enlarged representation of the contact surface 112 with a sequence of pits 115 and lands 114 on a carrier 111. In this embodiment, the lands 114 carry a layer of binding sites 116 (e.g. anti-bodies) to which target particles 1 can bind (unbound target particles are denoted here with 1'). Due to the phase structure, the reflected light L2a, L2b from pit-land pairs in relative congruity is cancelled in the nominal situation in which no target particles are present at the contact surface 112. This makes this approach insensitive to tilt- and reflection- variations over the sensor surface as well as surface warpage. A compensation layer (not shown) may be applied to the pits 115 to compensate for the reflection difference due to the binding sites 116 on the lands 115. Alternatively, the duty cycle of the lands 115 may be adapted to a value different from 50 % (increasing the area of the lands 115 relative to that of the pits, i.e. a duty cycle > 50%, is usually necessary to compensate for a reduction of the reflectivity due to the binding sites 116).

When target particles 1 appear at the contact surface 112, the balance between the pit and land areas is changed which results in a net light intensity in the light detector 31. The net resulting signal is a direct measure for the target particle concentration on the contact surface.

In Figure 3, the binding site layer 116 is located on the lands 114, while the pits 115 do not bind target particles as no binding sites are applied there (or as they are blocked). Coating only the lands with target molecules can for example be achieved by application of a relatively stiff stamp to coat the top surface with binding sites, e.g. using micro contact printing (sufficient stiffness prevents bending of the stamp surface and hence contacting the pit areas).

It should be noted that the contributions of unbound sediment target particles 1' are suppressed in this approach as they mutually cancel for the pits and lands. Alternatively or in addition, sediment target particles 1' on both areas can be removed by a so-called washing step.

In an alternative approach, binding takes place in the pit area. Figure 4 illustrates in this respect a two-step process by which antibodies are applied in the pits 215 on a carrier 211. In step a) of this process, anti-bodies are applied over the whole contact surface 212 by e.g. ink-jet printing, yielding binding layers 216 and 217 on  
5 both lands 214 and pits 215, respectively.

In step b) the lands 214 are made "neutral" by cleaning (using e.g. mechanical or chemical means) or blocked, e.g. by applying a cover-layer via contact printing. Obviously extra compensation layers (not shown) may be applied for better balancing the reflections of the pit and land signals.

10 Unfortunately, the described approach is not compatible with inkjet printing of the binding sites (anti-bodies) on the contact surface, which is a preferred technique. Figure 5 therefore shows an alternative design of a carrier 311 with a contact surface 312. Here, the pits 315 of the grating structure are filled with a spacer material 318 having a refractive index different from that of the rest of the carrier 311  
15 (e.g. made of polystyrene). The spacer layer 318 can be applied via a separate deposition step using a mask. Binding sites are then applied uniformly over the whole contact surface. The spacer layer 318 in the pits 315 optically isolates the resulting binding layer 317 from the bottom areas of the pits and thus from the FTIR detection mechanism. In this way it is allowed to cover both the pits 315 and the lands 314 with  
20 capture probes and thus use an inkjet printing process; no surface patterning is needed. The part of the cover layer which is in contact with the sample fluid is preferably bio compatible.

In order to compensate in the nominal situation (no target particles bound) for a reflection difference between the pits 315 and the lands 314 caused by the  
25 spacer layer 318, the ratio between lands and pits can be adapted (resulting in a duty cycle of the lands different from 50%). Thus the zero-th order can be totally suppressed in the nominal situation, while the biomaterial can be applied without patterning, e.g. by inkjet printing.

In the area of the contact surface on the carrier, the pits and/or the lands  
30 may be organized in different ways. Figure 6 illustrates in this respect an organization in the investigation region 413 in two directions as a grid of square pits 415 (alternatively,

the pits could of course have other shapes, e.g. of circles, or the role of lands 414 and pits 415 could be exchanged). Figure 7 illustrates an organization in the investigation region 513 in one direction (e.g. as tracks of pits 514 and lands 515). Alternatively, other organizations could be realized, e.g. in concentric circles.

5                    In case of the organization in tracks shown in Figure 7, the orientation of the tracks in the investigation region 613 can be chosen to be perpendicular to the plane of incidence of the input light beam L1, as illustrated in Figure 8. This configuration leads to diffraction orders in the same plane as the incident and reflected light beams L1, L2, which may be beneficial for avoiding cross-talk to neighboring detection areas (i.e. when multiplexing is used).

                    Tracks in the investigation region 713 that run parallel to the plane of incidence of the input light beam L1 are illustrated in Figure 9. They give rise to diffraction orders in the perpendicular direction. This has the advantage that diffracted orders are properly coupled out, avoiding possible problems with internal reflections.

15                    While the invention was described above with reference to particular embodiments, various modifications and extensions are possible, for example:

- The sensor can comprise any suitable sensor to detect the presence of magnetic particles on or near to a sensor surface, based on any property of the particles, e.g. it can detect via magnetic methods, optical methods (e.g. imaging, fluorescence, chemiluminescence, absorption, scattering, surface plasmon resonance, Raman, etc.),  
20                    sonic detection (e.g. surface acoustic wave, bulk acoustic wave, cantilever, quartz crystal etc), electrical detection (e.g. conduction, impedance, amperometric, redox cycling), etc.
- In addition to molecular assays, also larger moieties can be detected with  
25                    sensor devices according to the invention, e.g. cells, viruses, or fractions of cells or viruses, tissue extract, etc.
- The detection can occur with or without scanning of the sensor element with respect to the sensor surface.
- Measurement data can be derived as an end-point measurement, as well  
30                    as by recording signals kinetically or intermittently.

- The particles serving as labels can be detected directly by the sensing method. As well, the particles can be further processed prior to detection. An example of further processing is that materials are added or that the (bio)chemical or physical properties of the label are modified to facilitate detection.
- 5 - The device and method can be used with several biochemical assay types, e.g. binding/unbinding assay, sandwich assay, competition assay, displacement assay, enzymatic assay, etc. It is especially suitable for DNA detection because large scale multiplexing is easily possible and different oligos can be spotted via ink-jet printing on the optical substrate.
- 10 - The device and method are suited for sensor multiplexing (i.e. the parallel use of different sensors and sensor surfaces), label multiplexing (i.e. the parallel use of different types of labels) and chamber multiplexing (i.e. the parallel use of different reaction chambers).
- The device and method can be used as rapid, robust, and easy to use  
15 point-of-care biosensors for small sample volumes. The reaction chamber can be a disposable item to be used with a compact reader, containing the one or more field generating means and one or more detection means. Also, the device, methods and systems of the present invention can be used in automated high-throughput testing. In this case, the reaction chamber is e.g. a well-plate or cuvette, fitting into an automated  
20 instrument.

Finally it is pointed out that in the present application the term "comprising" does not exclude other elements or steps, that "a" or "an" does not exclude a plurality, and that a single processor or other unit may fulfill the functions of several means. The invention resides in each and every novel characteristic feature and each and  
25 every combination of characteristic features. Moreover, reference signs in the claims shall not be construed as limiting their scope.

## CLAIMS:

5

1. A carrier (11, 111, 211, 311) for optical examinations, comprising a contact surface (12, 112, 212, 312) with an investigation region (13, 113-713) that has an optical phase structure by which impinging input light beams (L1, L1a, L1b) are reflected with a phase difference.

10

2. A microelectronic sensor device for optical examinations, comprising  
a) a carrier (11, 111, 211, 311) having a contact surface (12, 112, 212, 312) with an investigation region (13, 113-713) that has an optical phase structure (51) by which impinging input light beams (L1, L1a, L1b) are reflected with a phase difference;

15

b) a light source (21) for emitting an input light beam (L1) towards the investigation region of the carrier.

3. The microelectronic sensor device according to claim 2, characterized in that it comprises a light detector (31) for detecting a  
20 characteristic parameter of an output light beam (L2) that is generated by reflection of the input light beam (L1) at the contact surface (12, 112, 212, 312), preferably by total internal reflection.

25

4. The microelectronic sensor device according to claim 3, characterized in that the light detector (31) is arranged at a position of destructive interference of components of the output light beam (L2) that have been reflected by the optical phase structure (51).

30

5. The carrier (11, 111, 211, 311) according to claim 1 or the microelectronic sensor device according to claim 2,

characterized in that the optical phase structure comprises a sequence of pits (115-715) and lands (114-714).

6. The carrier (11, 111, 211, 311) or the microelectronic sensor device  
5 according to claim 5,  
characterized in that the optical path difference between neighboring pits (115-715) and lands (114-714) corresponds to approximately  $\lambda/2$ , with  $\lambda$  being the wavelength of the light of the input light beam (L1).

10 7. The carrier (11, 111, 211, 311) or the microelectronic sensor device  
according to claim 5,  
characterized in that the pits (115-715) and lands (114-714) are repeated in one dimension or in two dimensions, and in that the repetition direction is parallel or perpendicular to the plane of incidence of the input light beam (L1).

15 8. The carrier (11, 111, 211, 311) or the microelectronic sensor device  
according to claim 5,  
characterized in that the duty cycle of the lands (114-714) ranges between 10% and 90%.

20 9. The carrier (11, 111, 211, 311) or the microelectronic sensor device  
according to claim 5,  
characterized in that the duty cycle of the lands (114-714) is such that pits (115-715) and lands (114-714) reflect approximately the same amount of light.

25 10. The carrier (11, 111, 211, 311) or the microelectronic sensor device  
according to claim 5,  
characterized in that the width of the pits (115-715) and/or of the lands (114-714) ranges between about 1  $\mu\text{m}$  and 10  $\mu\text{m}$ .

30

11. The carrier (111, 211) or the microelectronic sensor device according to claim 5,  
characterized in that either the pits (215) or the lands (114) are coated with binding sites (116, 217) for target substances (1).

5

12. The carrier (111, 211) or the microelectronic sensor device according to claim 11,  
characterized in that the areas that are not coated with binding sites comprise a compensation coating for adapting their reflectivity to that of the coated

10 areas.

13. The carrier (311) or the microelectronic sensor device according to claim 5,

15 characterized in that the pits (315) are at least partially filled with a spacer material (318) having a reflective index smaller than that of the carrier material.

14. The carrier (311) or the microelectronic sensor device according to claim 13,

20 characterized in that the whole investigation region (313) is coated with binding sites (316, 317).

15. Use of the carrier or the microelectronic sensor device according to any of the claims 1 to 15 for molecular diagnostics, biological sample analysis, or chemical sample analysis.

25

1/3

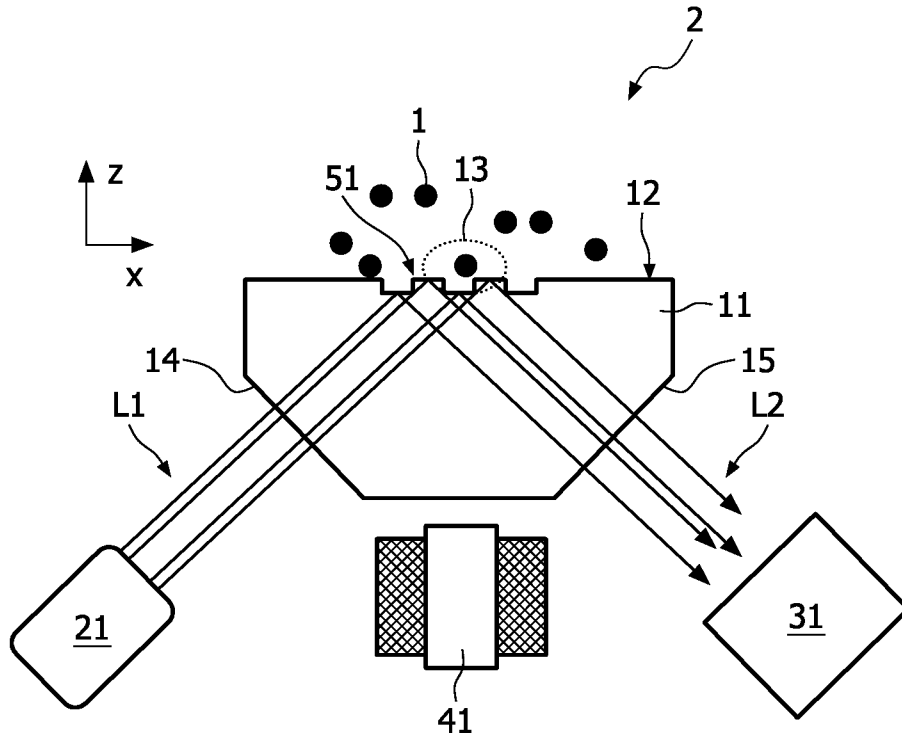


FIG. 1

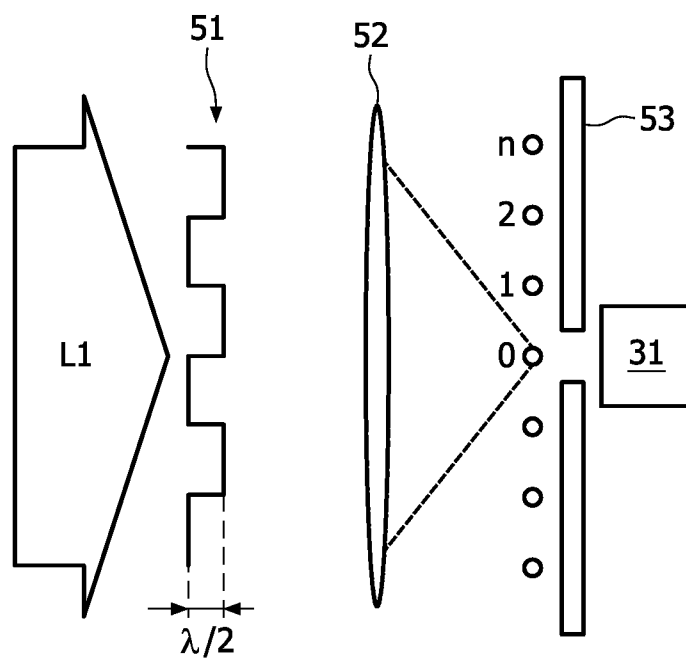


FIG. 2

2/3

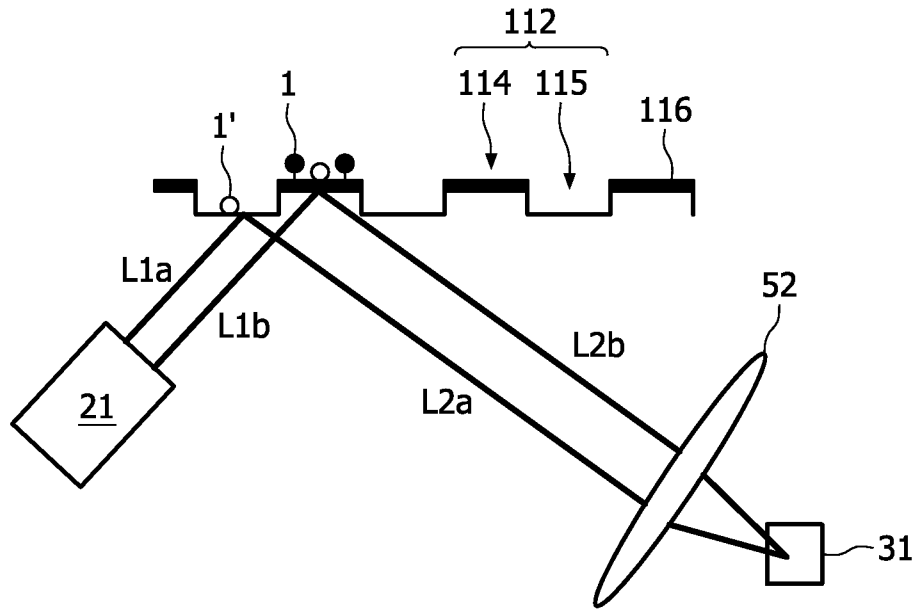


FIG. 3

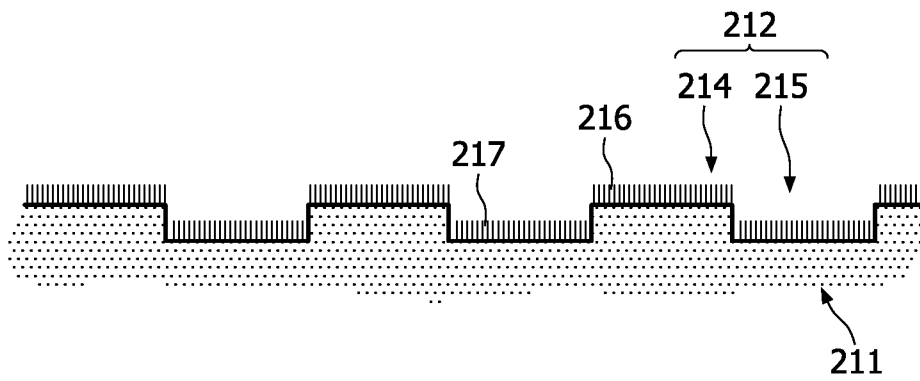


FIG. 4a

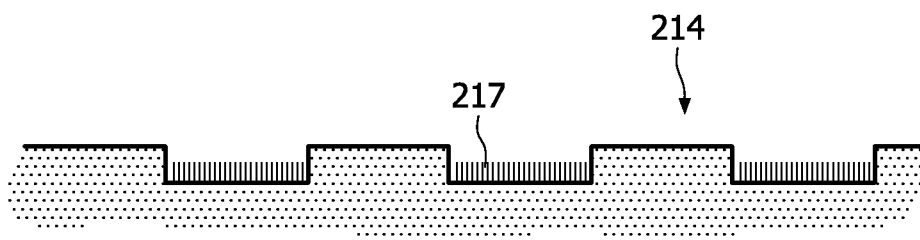


FIG. 4b

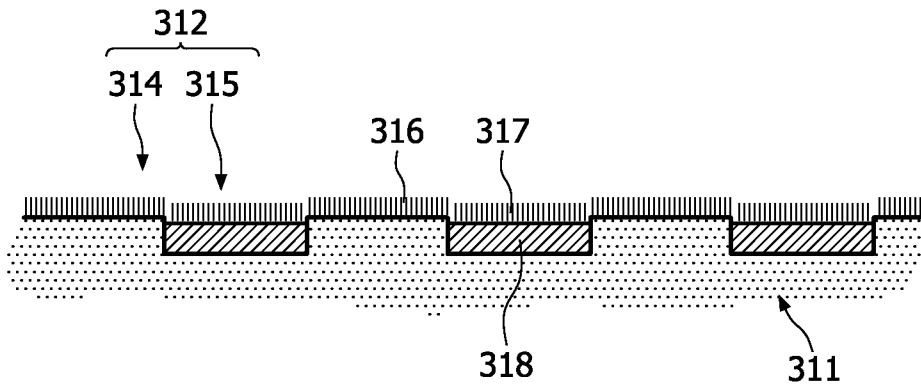


FIG. 5

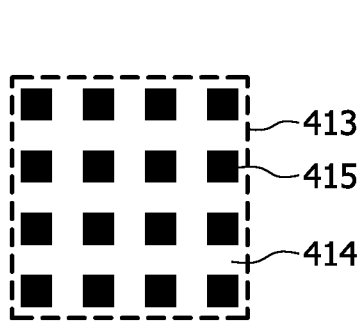


FIG. 6

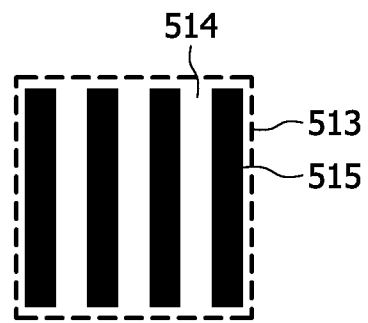


FIG. 7

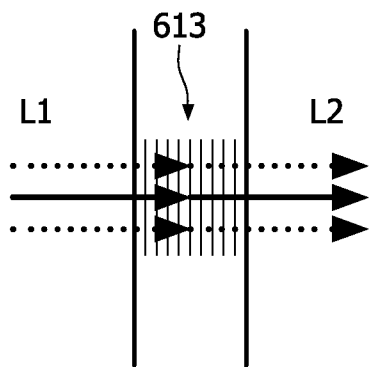


FIG. 8

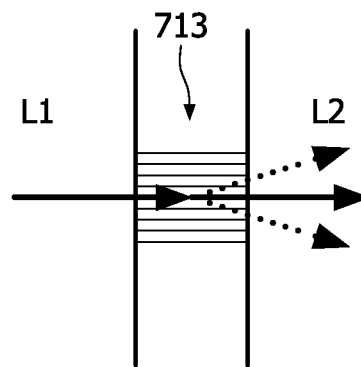


FIG. 9