(19) Octrooicentrum Nederland

(11) 2012187

(12) C OCTROOI

(21) Aanvraagnummer: **2012187**

(22) Aanvraag ingediend: **03.02.2014**

(51) Int.Cl.:
G01N 21/64 (2006.01) ***G02B 27/42*** (2006.01) ***G02B 27/00*** (2006.01) ***G02B 5/18*** (2006.01)

(43) Aanvraag gepubliceerd:
-

(47) Octrooi verleend:
06.08.2015

(45) Octrooischrift uitgegeven:
12.08.2015

(73) Octrooihouder(s):
Technische Universiteit Delft te Delft.
Stichting voor de Technische Wetenschappen te Utrecht.

(72) Uitvinder(s):
Sjoerd Stallinga te Delft.

(74) Gemachtigde:
ir. K.J. Metman te Amsterdam.

(54) **Diffractive optical element for localization microscopy.**

(57) A diffractive optical element for use in the emission light path of a fluorescence microscope is described wherein said fluorescent microscope is adapted to simultaneously measure the position of one or more emitters in a sample that is orthogonal to the plane of a substrate on which the sample is disposed and the wavelength of the light emitted by said one or more emitters on the basis of one or more diffraction orders formed by said diffractive optical element. The diffractive optical element comprises zones defined by a zone function in lateral coordinates in the plane of the diffractive optical element and is adapted for splitting the emission light into two or more diffraction orders.

Dit octrooi is verleend ongeacht het bijgevoegde resultaat van het onderzoek naar de stand van de techniek en schriftelijke opinie. Het octrooischrift komt overeen met de oorspronkelijk ingediende stukken.

NL C 2012187

NL20226-Vi/td

Diffractive optical element for localization microscopy

Field of the invention

The invention relates to a diffractive optical element for localization microscopy, and, in particular, though not exclusively, to methods for using a diffractive optical element for simultaneously measuring the axial position and emission colour of single emitters in localization microscopy, a localization microscopy system using such diffractive optical element and a computer program product for implementing such method.

Background of the invention

The invention relates to the field of microscopy, in particular to the field of super-resolution fluorescence microscopy. In ordinary (optical) microscopy the resolving power is limited to an amount on the order of the ratio between the wavelength of the light that is used in the image formation and the numerical aperture of the microscope. Different techniques for breaking this so-called diffraction limit ("super-resolution methods") have emerged over the last 10-15 years.

An important class of super-resolution microscopy techniques is the family of localization microscopy techniques, such as Photo-Activation Localization Microscopy (PALM) and STochastic Optical Reconstruction Microscopy (STORM). In these techniques the sample of interest is labelled with a set of fluorescent molecules which can be stochastically switched between a light emitting state ("on"-state) and a dark state ("off"-state) such that at any instant in time only a sparse subset of all fluorophores is in the on-state. This has the consequence that the individual fluorophores can be individually identified in the camera image.

The position of the individual emitters in the plane imaged onto the camera can then be determined with a precision, which is on the order of 10 nm, about 10-20 times smaller than the diffraction limit. Repeating this process, i.e. recording a movie of such sparse images and analyzing the images for the emitter locations, finally gives the positions of virtually all fluorophores and hence an image of the sample with details on the order of the localization precision rather than on the order of the diffraction limit.

In the field there is a need for measuring more parameters than just the position in the plane imaged onto the camera ("x and y-position"). For example, techniques that measure the position of the fluorophores in the direction orthogonal to the imaged plane ("axial position" or "z-position") can provide a 3D-representation of the sample with high resolution or distinguishing different types of fluorescent labels from each other may provide relevant information on the sample's spatial structure and (biological) function.

Ways for accomplishing axial position imaging and for distinguishing different types of fluorescent labels are known in the art. For example, the axial position may be determined using the astigmatism method wherein a cylinder lens in the imaging light path controllably introduces astigmatism for distorting the shape of the imaged spots into an elliptical shape. The ellipticity of the spot is a measure for the axial position. The defocus method makes use of the introduction of a relative defocus between the two duplicates wherein one of the images is focused slightly below the original focal plane and the other is focused slightly above the original focal plane. The ratio of the observed spot sizes of the two spots in the two sub-images originating from the same emitter is a measure for the axial position of the emitter. Finally, the double-helix point spread function method uses a phase plate in order to split the spot of an individual emitter into two adjacent sub-spots. The spots define a direction in the plane imaged onto the camera and the phase mask is designed such that the direction varies with the axial position of the emitter.

In order to distinguish different types of fluorescent labels a dichroic beamsplitter method may be used wherein light below a certain cut-off wavelength is directed in one direction and light above the cut-off wavelength in another direction. This method however can only be used in order to distinguish between up to three different colours.

Alternatively, diffraction grating may be used to distinguish between different colours. A diffraction grating is a phase plate with a height profile that depends on only one of the coordinates in the plane of the phase plate (e.g. the x-axis) and which furthermore is periodic (i.e. the height profile is repetitive). The period of repetition is called the pitch p of the grating. The diffraction grating splits the beam into discrete diffraction orders, labelled with an integer index m, such that each diffraction order has an angular deflection with respect to the incident beam with an angle α, where $\sin\alpha = m\lambda/p$. The angular separation depends on the wavelength and is therefore a measure for the emission colour.

The separate techniques mentioned above could be used in combination in order to measure axial position and emission colour. For example, a conventional diffraction grating (with large pitch/small angular deflection) in combination with a cylinder lens combines the above-mentioned astigmatic method with an emission colour measurement. However, in such situation two different optical components must be placed in/out of the light path, depending on which measurement is desired thereby increasing the overall complexity of the microscopy structure.

Another problem associated with the above-mentioned art relates to the fixed amount of astigmatism or relative defocus, which is added to the beam for the astigmatic or defocus method. The spot width and depth of focus both scale with the wavelength of the emission light, implying that the axial position detection has different characteristics (e.g. axial range) for different wavelengths. This will substantially increase the analysis of the image data when using emitters with many different emission wavelengths.

Hence, from the above it follows that there is a need in the art for an improved optical component, which allows the simultaneous measurement of the axial position and emission colour of single emitters in localization microscopy that does not exhibit the problems associated with prior art solutions.

Summary of the invention

As will be appreciated by one skilled in the art, aspects of the present invention may be embodied as a system, method or computer program product. Accordingly, aspects of the present invention may take the form of an entirely hardware embodiment, an entirely software embodiment (including firmware, resident software, micro-code, etc.) or an embodiment combining software and hardware aspects that may all generally be referred to herein as a "circuit," "module" or "system." Functions described in this disclosure may be implemented as an algorithm executed by a microprocessor of a computer. Furthermore, aspects of the present invention may take the form of a computer program product embodied in one or more computer readable medium(s) having computer readable program code embodied, e.g., stored, thereon.

Any combination of one or more computer readable medium(s) may be utilized. The computer readable medium may be a computer readable signal medium or a computer readable storage medium. A computer readable storage medium may be, for example, but not limited to, an electronic, magnetic, optical, electromagnetic, infrared, or semiconductor system, apparatus, or device, or any suitable combination of the foregoing. More specific examples (a non- exhaustive list) of the computer readable storage medium would include the following: an electrical connection having one or more wires, a portable computer diskette, a hard disk, a random access memory (RAM), a read-only memory (ROM), an erasable programmable read-only memory (EPROM or Flash memory), an optical fiber, a portable compact disc read-only memory (CD-ROM), an optical storage device, a magnetic storage device, or any suitable combination of the foregoing. In the context of this document, a computer readable storage medium may be any tangible medium that can contain, or store a program for use by or in connection with an instruction execution system, apparatus, or device.

A computer readable signal medium may include a propagated data signal with computer readable program code embodied therein, for example, in baseband or as part of a carrier wave. Such a propagated signal may take any of a variety of forms, including, but not limited to, electro-magnetic, optical, or any suitable combination thereof. A computer readable signal medium may be any computer readable medium that is not a computer readable storage medium and that can communicate, propagate, or transport a program for use by or in connection with an instruction execution system, apparatus, or device.

Program code embodied on a computer readable medium may be transmitted using any appropriate medium, including but not limited to wireless, wireline, optical fiber, cable, RF, etc., or any suitable combination of the foregoing. Computer program code for carrying out operations for aspects of the present invention may be written in any combination of one or more programming languages, including an object oriented programming language such as Java(TM), Smalltalk, C++ or the like and conventional procedural programming languages, such as the "C" programming language or similar programming languages. The program code may execute entirely on the user's computer, partly on the user's computer, as a stand-alone software package, partly on the user's computer and partly on a remote computer, or entirely on the remote computer or server. In the latter scenario, the remote computer may be connected to the user's computer through any type of network, including a local area network (LAN) or a wide area network (WAN), or the connection may be made to an external computer (for example, through the Internet using an Internet Service Provider).

Aspects of the present invention are described below with reference to flowchart illustrations and/or block diagrams of methods, apparatus (systems), and computer program products according to embodiments of the invention. It will be understood that each block of the flowchart illustrations and/or block diagrams, and combinations of blocks in the flowchart illustrations and/or block diagrams, can be implemented by computer program instructions. These computer program instructions may be provided to a processor, in particular a microprocessor or central processing unit (CPU), of a general purpose computer, special purpose computer, or other programmable data processing apparatus to produce a machine, such that the instructions, which execute via the processor of the computer, other programmable data processing apparatus, or other devices create means for implementing the functions/acts specified in the flowchart and/or block diagram block or blocks.

These computer program instructions may also be stored in a computer readable medium that can direct a computer, other programmable data processing apparatus, or other devices to function in a particular manner, such that the instructions stored in the computer readable medium produce an article of manufacture including instructions which implement the function/act specified in the flowchart and/or block diagram block or blocks.

The computer program instructions may also be loaded onto a computer, other programmable data processing apparatus, or other devices to cause a series of operational steps to be performed on the computer, other programmable apparatus or other devices to produce a computer implemented process such that the instructions which execute on the computer or other programmable apparatus provide processes for implementing the functions/acts specified in the flowchart and/or block diagram block or blocks.

The flowchart and block diagrams in the figures illustrate the architecture, functionality, and operation of possible implementations of systems, methods and computer program products according to various embodiments of the present invention. In this regard, each block in the flowchart or block diagrams may represent a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the blocks may occur out of the order noted in the figures. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustrations, and combinations of blocks in the block diagrams and/or flowchart illustrations, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.

It is an objective of the invention to reduce or eliminate at least one of the drawbacks known in the prior art. In particular, it is an objective of the invention is to provide means and a method for measuring the axial position and emission colour of single emitters in localization microscopy simultaneously in a simplified way.

In an aspect, the invention may relate to a diffractive optical element for use in the emission light path of a fluorescence microscope, wherein said fluorescent microscope is adapted to simultaneously measure the position of one or more emitters in a sample that is orthogonal to the plane of a substrate on which the sample is disposed and the wavelength of the light emitted by said one or more emitters. Said position and wavelength being determined on the basis of one or more diffraction orders, in particular, the shape and/or position of said one or more diffraction orders, formed by said diffractive optical element, wherein said diffractive optical element comprises zones defined by a zone function in the lateral coordinates in the plane of the diffractive optical element, wherein said diffractive element is adapted for splitting the emission light into two or more diffraction orders, such that the phase added to the emission light depends on the difference between the ratio of the zone function in the lateral coordinates and the wavelength of said emitted light and the largest integer smaller than said ratio; and, wherein said zone function is defined as a sum of Zernike polynomials in the lateral coordinates such that at least one Zernike polynomial of first order is non-zero and that at least one Zernike polynomial of second order is non-zero.

The diffraction element is a single optical component that can be placed in the optical path of a localization microscope that allows simultaneously measuring the axial position and emission colour of single emitters in localization microscopy. The diffraction element substantially extends the functionality of the localization microscopy in a simple way, i.e. without increasing the structural complexity of the microscope setup and its operation.

The diffraction elements according to the invention are configured to add a variable amount of astigmatism or defocus to the light beam that is proportional to wavelength.

The use of one or more non-zero first order (Zernike) polynomials in the zone function of the diffraction element may result in one or more laterally displaced diffraction orders, thereby generating a set of non-zero diffraction orders (sub-spots) with a lateral separation in proportion to the wavelength.

Further, the use of one or more non-zero second order (Zernike) polynomials in the zone function of the diffraction element may generate one or more non-zero diffraction orders (sub-spots) that are distorted by either defocus and/or astigmatism in proportion to the integer label m of the order, and in proportion to the wavelength. This proportionality allows determination of axial position of individual emitters thereby using the astigmatic or bifocal axial position measurement method.

In an embodiment, a second order Zernike polynomial may have a circular form x^2+y^2 for generating one or more diffraction orders with defocus.

In an embodiment, a second order Zernike polynomial may have a hyperbolic form x^2-y^2 for generating one or more diffraction orders with horizontal/vertical astigmatism (focal lines at 0 deg and 90 deg with the x-axis)

In an embodiment, a second order Zernike polynomial may have a hyperbolic form xy for generating diffraction orders with diagonal astigmatism (focal lines at +45 deg and - 45 deg with the x-axis).

In case of astigmatism, in an embodiment, horizontal/vertical astigmatism (i.e. hyperbolic second order

Zernike polynomial) may be used in combination with a lateral separation of orders in one of the diagonal directions (i.e. a first order Zernike polynomial). In this way the area occupied by the set of sub-spots is as small as possible.

In an embodiment, when using the diffraction element according to the invention, in a (fluorescence) localization microscope, two or more diffraction orders (sub-spots) may be generated at the camera whereby each diffraction order (sub-spot) may be deformed by an amount of defocus and/or by an amount of astigmatism that is proportional to the order m (m = ...,-2,-1,0,1,2,...).

In an embodiment, in case of defocus, the two or more sub-spots corresponding to the two or more diffraction orders have a relative defocus wherein the distance between the two sub-spots may provide a measure for the emission wavelength of one or more emitters, and the ratio of the size of the sub-spots may provide a measure for the axial position of the one or more emitter.

In an embodiment, in case of astigmatism, at least one of the sub-spots corresponding to at least one of the diffraction orders may be deformed by astigmatism (i.e. all but the 0th diffraction order).

In an embodiment, the axial position may be determined on the basis of the ellipticity of the spot shape of the one or more sub-spots with on-zero astigmatism. The ellipticity of a spot shape may be easily determined by processing the image of a spot shape using conventional image processing techniques.

In an embodiment, at least part of the functional dependence of the phase profile on the difference between the ratio of the zone function and the wavelength and the largest integer smaller than this ratio may have a sawtooth shape. The use of such functional dependence may result in a blazed type of diffractive optical element, which generates the +1st order and, optionally, the 0th order. The generation of the optional 0th order depends on the height of the saw tooth profile..

In an embodiment, at least part of the functional dependence of the phase profile on the difference between the ratio of the zone function and the wavelength and the largest integer smaller than this ratio may have a binary shape. A diffraction element with such functional dependence generates at least the -1st and +1st order and, optionally, the 0th order. The generation of the optional 0^{th} order depends on the phase difference between the two phase levels,

In an embodiment, at least part of the functional dependence of the phase profile on the difference between the ratio of the zone function and the wavelength and the largest integer smaller than this ratio may have a sinusoidal shape, which only generates the -1st and +1st orders and, optionally, the 0th order. The generation of the optional 0^{th} order depends on the amplitude of the sinusoidal phase profile

In an embodiment, at least part of the functional dependence of the phase profile on the difference between the ratio of the zone function and the wavelength and the largest integer smaller than this ratio may have any other shape, constructed as a sum of a predetermined number of Zernike polynomials in the lateral coordinates such that at least one Zernike polynomial of first order is non-zero and that at least one Zernike polynomial of second order is non-zero. The Zernike polynomials may be selected on the basis of the desired distribution of light over the different diffraction orders.

The diffractive optical element according to the invention may be realized by (conventional) semiconducting manufacturer techniques. In an embodiment, the required height profile of the diffractive optical element may be etched or embossed into or onto a transparent medium such as a glass, quartz or a plastic compound. In another embodiment, the required height profile of the diffractive optical element may be etched or embossed into or onto a reflective medium such as a metal or a substrate with a multilayer coating for reflection. In yet another embodiment, the diffractive optical element may be realized using an electronically addressable phase modulation device ("Spatial Light Modulator", SLM), such as a deformable mirror or a liquid crystal device.

In an embodiment the two or more sub-spots are generated simultaneously by the diffractive optical element. In another embodiment different sub-sets of the two or more sub-spots may be generated sequentially by modifying the phase modulation pattern of the electronically addressable phase modulation device. In yet another embodiment the phase modulation pattern of the electronically addressable phase modulation device alternates between blazed patterns that generate a positive first (1^{st}) order or a negative first (-1^{st}) order by reversing the signature of the sawtooth profile.

In an aspect, the invention may relate to a diffractive element as described above in a microscopy system, preferably a localization microscopy system.

In an aspect, the invention may relate to a microscopy system using a diffractive optical element as described above.

In another aspect, the invention may relate to an image-processing module for use with a diffractive optical element as described above, wherein said diffraction element may generate two or more diffraction orders (sub-spots) at a camera of said fluorescent microscope whereby each diffraction order (sub-spot) may be deformed by an amount of defocus and/or by an amount of astigmatism that is proportional to the order m ($m = \ldots,-2,-1,0,1,2,\ldots$), wherein said image-processing module may be configured for: receiving one or more images of one or more diffraction orders originating from said diffractive optical element; and,

in case of defocus, determining the distance between two or more sub-spots corresponding to two or more diffraction orders that have a relative defocus, wherein the distance between the two sub-spots provides a measure for the emission wavelength of at least one emitter; and, determining the ratio of the size of the sub-spots wherein said ratio provides a measure for the axial position of said at least one emitter; or, in case of astigmatism, determining the distance between two or more sub-spots corresponding to two or more diffraction orders that have no-zero astigmatism, wherein the distance between the two sub-spots provides a measure for the emission wavelength of at least one emitter; and, determining the ellipticity of the spot shape of at least one sub-spot with no-zero astigmatism, wherein said ratio provides a measure for the axial position of said at least one emitter.

In yet another aspect, the invention may relate to a method for simultaneously measuring the position of one or more emitters in a sample that is orthogonal to the plane of a substrate on which the sample is disposed and the wavelength of the light emitted by said one or more emitters on the basis of one or more diffraction orders formed by an diffractive optical element as described above.

In an embodiment, said method may comprise: measuring the deformation of one or more diffraction orders (sub-spots) caused by an amount of defocus and/or by an amount of astigmatism for determining the axial position of at least one emitter; and, measuring the distance between two or more sub-spots corresponding to two or more of said deformed diffraction orders for determining the emission wavelength of said at least one emitter.

In an embodiment, in case of defocus, said axial position may be determined on the basis of the ratio of the size of the sub-spots.

In an embodiment, in case of astigmatism, said axial position may be determined on the basis of the ellipticity of the spot shape of at least one sub-spot with no-zero astigmatism.

The invention may further relate to a computer program product, comprising software code portions configured for, when run in the memory of a computer, executing the method steps as described above.

The invention will be further illustrated with reference to the attached drawings, which schematically will show embodiments according to the invention. It will be understood that the invention is not in any way restricted to these specific embodiments.

Brief description of the drawings

Fig. 1 depicts a localization microscopy system using an diffractive optical element according to an embodiment of the invention.

Fig. 2 depicts a high-level schematic of a process for forming an image of the sample using localization microscopy.

Fig. 3 depicts a diffractive element according to an embodiment of the invention.

Fig. 4A and **4B** depict a zone function for a diffractive element and the associated diffraction orders according to an embodiment of the invention.

Fig. 5A and **5B** depict a zone function for a diffractive element and the associated diffraction orders according to another embodiment of the invention.

Fig. 6 is a block diagram illustrating an exemplary data processing system for use in systems and methods as described within this application.

Detailed description

Fig. 1 depicts a microscopy system using an diffractive optical element according to an embodiment of the invention. In particular, **Fig. 1** depicts a schematic of a system **100** that may be used for super-resolution microscopy and in particular for localization microscopy. The microscopy system may relate to any type of localization microscopy system including the Photo-Activation Localization Microscopy (PALM) system and STochastic Optical Reconstruction Microscopy (STORM) system.

The system may comprise a microscope objective **106** comprising an optical lens system over a substrate holder (not shown) comprising a substrate **102.** A sample **104** positioned on the substrate may comprise a biological or molecular structure that is selectively labeled with one or more emitters **103**, e.g. florescent molecules, which can be stochastically switched between a light emitting and dark state such that at any instant in time a sparse subset of all emitters is in the on state. An emitter may be positioned in the sample at a location (x,y,z), wherein x,y are the coordinates in the plane of the substrate and the axial coordinate z is the coordinate normal to the plane of the substrate.

The optical lens system of the microscope object may be configured to focus light from one or more light sources **108,110** onto the sample and to image light originating from an emitter onto a camera **118.** The first light source may use a switching light source for exposing the sample with light while the second light source may be used to expose the sample with (laser) light for exciting the emitters. In another embodiment, one switching light source may be used that both exposes the sample and excites the emitters. Semi-transparent mirrors may be used for reflecting light from a first light source to the microscope objective and transmitting light originating from an emitter to the diffraction element.

In case localization of emitters in the sample is required, the emitter light originating from the emitters is imaged by the optical system of the microscope objective and one or more further optical elements (not shown) onto the image plane of a digital camera **118.** In that case, the diffractive optical element is not in the path of the light. An image-processing module **126** on a computer **120** that is connected to the camera may instruct the camera to take multiple images (a movie) of the randomly emitting emitters. Thereafter, the image-processing module may process the images in order to render an image of all emitters, wherein individual emitters are imaged with a resolution down to 10 nm.

Fig. 2 depicts a high-level schematic of a process for forming an image of the sample using localization microscopy. In this process (that may be executed by the image-processing module connected to the camera of the microscope), a sequence of images **202** of sparse subsets of emitting emitters is taken (step **202**). Because only a small subset of the whole set of emitters is emitting, the individual emitting emitters are visible at each image. These images are subsequently processed by the image-processing module (step **204**), wherein for each image the individual locations $\mathbf{206_{1-4}}$ of emitting emitters are localized. Repeating this process for a large number of images may render precise localization of all emitters and hence an image with details of the order of the localization precision rather than the diffraction limit. As the emitters are selectively attached to an underlying structure (e.g. a molecule, DNA, etc.), the shape of the underlying labelled structure may become visible as can be seen in the STORM picture of **Fig. 2.**

If the axial position and colour of the individual emitters need to be obtained, a special diffractive optical element **166** may be placed before the camera so that the light originating from the emitters is diffracted by the optical diffractive element in one or more diffraction orders $\mathbf{122}_{1,2}$ and imaged in the image plane of the camera. An example of an image **124** of a (non-diffracted) zero diffraction order and two first order diffraction orders associated with light of an emitter is depicted in **Fig. 1.**

As will be described hereunder in more detail, the diffractive optical element **116** is configured to generate one or more diffraction orders wherein the shape and, in some embodiment, the position of at least part of said one or more diffraction orders are directly dependent on the axial position of the emitter and the wavelength of the light that the emitter is emitting. By analysing the shape (and position) of the imaged diffraction orders, the axial position and color of a single emitter can be simultaneously determined. To that end, the image-processing module may be configured to process images of the diffraction orders in order to determine the shape and position thereof, and link the shape and location information to a particular axial position and wavelength. By determining the axial position and wavelength of all or at least a substantial part of all emitters, a color 3D image may be constructed.

As will be described hereunder in more detail, in certain embodiments, the diffractive element may be implemented as an electronically addressable phase modulation device ("Spatial Light Modulator", SLM), such as a deformable mirror or a liquid crystal device. In that case, the image-processing module may be configured to control the electronically addressable phase modulation device for generating one or more phase modulation patterns comprising one or more zone functions as described below. To that end, the image-processing module may be connected via an interface **128** to the electronically addressable phase modulation device.

Fig. 3 depicts a diffractive element according to an embodiment of the invention. In particular, **Fig. 3(A)** and **3(B)** depict a cross-section and a top-view respectively of a diffractive element for measuring axial position and the wavelength of a light emitting emitter to an embodiment of the invention. As will be shown hereunder in more detail the diffraction element in **Fig. 3** is configured for measuring the axial position and wavelength according to the astigmatic method with sub-spots separated in the diagonal direction.

As shown in **Fig. 3(A)** the diffractive element may comprise a transparent substrate **302**. The diffractive optical element according to the invention may be described on the basis of a curved (non-linear) zone function W(x,y) and a profile function h(t), wherein x,y are Cartesian coordinates in the plane of the element and t is the in-zone coordinate. The zone function of the optical element is shown in **Fig. 3(B)** and coincides with the top-view of the diffractive element. In **Fig. 3(B)** one zone may be defined by a first region **312_1** (of a first height indicated in black) and an associated second region **312_2** (of a second height indicated in white). These regions correspond to regions **304_1** and **304_2** in **Fig. 3A**.

A point (x,y) in the plane of the element falls in zone j if:

$$j \leq \frac{W(x,y)}{\lambda_0} \leq j+1$$

wherein λ_0 represents the nominal wavelength of the incident light. Within each zone the in-zone coordinate t may vary according to:

$$t=\frac{W(x,y)}{\lambda_0}-floor\left(\frac{W(x,y)}{\lambda_0}\right)$$

where floor(x) is the largest integer equal or smaller than x. The phase that the optical element adds to light with wavelength λ may be expressed as:

$$\Phi(x,y)=\frac{2\pi h(t)}{\lambda}=\frac{2\pi}{\lambda}h\left(\frac{W(x,y)}{\lambda_0}-floor\left(\frac{W(x,y)}{\lambda_0}\right)\right)$$

The transmission function of the element follows with some mathematical manipulations as:

$$T(x,y)=\exp\left(i\Phi\left(x,y\right)\right)=\sum_{m=-\infty}^{\infty}A_m\exp\left(\frac{2\pi mW\left(x,y\right)}{\lambda_0}\right)$$

wherein:

$$A_m=\int_0^1 dt\exp\left(\frac{2\pi ih\left(t\right)}{\lambda}-2\pi imt\right)$$

Every diffraction order m therefore has an amplitude A_m and a phase $2\pi mW(x,y)/\lambda_0$. The diffraction efficiency of order m is $|A_m|^2$. Here, the diffraction efficiencies depend on the actual wavelength λ, and the added phase profile only on the nominal wavelength λ_0. Different profile functions may be used to form the height profile of the zones. For example, in an embodiment the profile function may be defined as a blaze:

$$h\left(t\right)=h_{\max}t,\quad 0<t\leq 1$$

In another embodiment, the profile function may be defined as a binary function:

$$h\left(t\right)=\begin{cases}0, & 0<t\leq t_0\\ h_{\max}, & t_0<t\leq 1\end{cases}$$

wherein t_0 is defined as the so-called duty cycle. In yet another embodiment, the profile function may be defined as a sinusoidal function:

$$h(t)=h_{\max}\cos(2\pi t),\quad 0<t\leq 1$$

or any other shape.

The selection of the zone function determines the behaviour of the diffraction orders. For example, the zone function:

$$W(x,y)=\frac{\lambda_0 x}{p}$$

gives rise to straight zones of width p oriented along the y-axis, and provides a description of a conventional diffraction grating with pitch p. In the prior art the angular separation of two sub-spots of a diffraction order is used as a measure for the emission colour.

In an embodiment, a zone function may be defined by the curved zone function as defined by:

$$W(x,y)=\frac{\lambda_0 x}{p}+b\left(x^2+y^2\right)$$

This curved zone function providing zones that have a circular shape and give rise to diffraction orders with defocus.

In another embodiment, a zone function may be defined by the curved zone function:

$$W(x,y)=\frac{\lambda_0 x}{p}+2bxy$$

This curved zone function may give rise to zones of hyperbolic shape and results in diffraction orders with diagonal astigmatism (focal lines at +45 deg and - 45 deg with the x-axis).

In yet another embodiment, a zone function may be defined by the curved zone function:

$$W(x,y) = \frac{\lambda_0 x}{p} + b\left(x^2 - y^2\right)$$

This curved zone function may give rise to zones of hyperbolic shapes as well, but generates horizontal/vertical astigmatism (focal lines at 0 deg and 90 deg with the x-axis).

For these curved zone functions, the added phase profile only depends on the nominal wavelength λ_0. As a consequence, the angular deflection of the orders will scale with the actual wavelength λ. Also, the relative defocus of the orders or the distance between the astigmatic lines scales with the actual wavelength λ, which provides an accuracy of the axial position determination and a useful axial range that both scale with the actual wavelength λ.

In a further embodiment, the diffractive optical element may be configured to correct for the spherical aberration that arises from focusing above or below the nominal focal plane. This correction may be realized by adding suitable 4th order polynomials or by using the exact aberration function arising from such a misfocus.

For example, in an embodiment, the modification of the defocus zone function $\lambda_0 x/p$ to:

$$W(x,y) = \frac{\lambda_0 x}{p} + \Delta z\sqrt{n^2 - NA^2\frac{x^2+y^2}{R^2}}$$

allows for correction of spherical aberration of all orders. Here Δz is the relative defocus of the diffraction orders back-projected to object space, n is the refractive index in object space, NA is the microscope objective numerical aperture, and R is the radius of the beam incident on the diffractive optical element.

In general, in an embodiment, the optical diffraction element according to the invention may be characterized by a zone function in the beam cross-section area that may be described on the basis of so-called Zernike polynomials:

$$W(x,y)=\sum_{n}\sum_{m}C_{nm}Z_{nm}(x,y)$$

wherein the Zernike coefficients C_{nm} are given by:

$$C_{nm}=\frac{1}{\pi R^2(n+1)}\int_{x^2+y^2\leq R^2} dxdyW(x,y)Z_{nm}(x,y)$$

According to the invention at least one of the Zernike coefficients C_{11} (corresponding to the polynomial x) and C_{1-1} (corresponding to the polynomial y) is non-zero and at least one of the coefficients C_{20} (corresponding to the polynomial $2(x^2+y^2)-1$), C_{22} (corresponding to the polynomial x^2-y^2), and C_{2-2} (corresponding to the polynomial 2xy) is non-zero.

It is submitted that although the expressions above are described on the basis of Cartesian coordinates, other coordinate systems, e.g. polar coordinates, may be used to define the curved zone functions according to the invention.

The diffractive optical element as shown in Fig. 3(A) may be realized by (conventional) semiconducting manufacturer techniques. The required height profile of the diffractive optical element may be etched or embossed into or onto a transparent medium such as a glass, quartz or a plastic compound. Alternatively, the required height profile of the diffractive optical element may be etched or embossed into or onto a reflective medium such as a metal or a substrate with a multilayer coating for reflection.

The diffractive optical element may also be implemented as an electronically addressable phase modulation device ("Spatial Light Modulator", SLM), such as a deformable mirror or a liquid crystal device.

In an embodiment, the electronically addressable phase modulation device may be controlled by the image-processing module to generate a phase modulation pattern comprising zone functions as described above wherein the pattern may generate two or more sub-spots simultaneously onto the image plane of the camera.

In another embodiment, the electronically addressable phase modulation device may be controlled by the image-processing module to generate at least a first phase modulation pattern at a first point in time and a different second phase modulation pattern at a second point in time, wherein the first and second phase modulation pattern comprise one or more zone functions as described above. This way, the first and second phase modulation pattern are configured to generate different sub-sets of the two or more sub-spots onto the image plane of the camera. Hence, in this embodiment, the first and second phase modulation pattern may be generated sequentially by modifying the phase modulation pattern of the electronically addressable phase modulation device.

In yet another embodiment, the electronically addressable phase modulation device may be controlled by the image-processing module to generate a first blazed phase modulation pattern at a first point in time and second blazed phase modulation pattern at a second point in time wherein the first and second phase modulation pattern comprise one or more zone functions as described above. The first and second blazed phase modulation patterns may generate at least a positive first (1^{st}) order and at least a first negative first (-1^{st}) order. Switching between first and second blazed phase may be realized by reversing the signature of the sawtooth profile.

Typical values for the wavelength are in the visible range, typically between 400 nm and 750 nm. The required defocus/astigmatism values are in the range between 500 nm and 2000 nm in object space. This distance is the axial distance between focal points of different diffraction orders or focal lines of different diffraction orders. The wavelength of light and the refractive index of glass (around 1.5) imply typical step heights in the plane in the range of between 150 nm and 1500 nm. The zone width follows from the required angular separation which result in a distance between the sub-spots on the order of 2 to 10 times the spot width. This translates into about 4 to 20 zones that fit in the beam diameter at the diffractive optical element. Typically, the beam diameter is selected from a range between 5 and 20 mm, resulting in zone widths witin a range between 0.4 and 5 mm.

Fig. 4A and **4B** depict a zone function for a diffractive element and the associated diffraction orders according to an embodiment of the invention. The zone function is assumed to have a duty cycle of 50%, i.e. the white areas indicate a first height and the black area a second height. Adjacent pairs of white and black areas together constitute a single, curved zone of the diffractive optical element.

Fig. 4A depicts an example of a diffractive optical element according to the invention for measuring axial position according to the defocus method with sub-spots separated in the horizontal direction. The circle indicates the beam cross-section. **Fig. 4B** depicts the spot shapes of the -1st, 0th, and +1st diffraction orders **402,404,406** for different axial positions for the defocus case. As shown in this figure, in case of defocus, the two or more sub-spots corresponding to the two or more diffraction orders have a relative defocus wherein the distance between the two sub-spots may provide a measure for the emission wavelength of one or more emitters, and the ratio of the size of the sub-spots may provide a measure for the axial position of the one or more emitter. Hence, in this embodiment, the image-processing module may be configured to determine the two sub-spots and to measure the distance and the ratio of the two sub-spots.

Fig. 5A and **5B** depict a zone function for a diffractive element and the associated diffraction orders according to another embodiment of the invention.

Fig. 5A depicts a diffractive optical element according to the invention for measuring axial position according to the astigmatic method with sub-spots separated in the diagonal direction. The circle indicates the beam cross-section. **Fig. 5B** depicts typical spot shapes of the -1st, 0th, and +1st diffraction orders **502,504,506** for different axial positions for the astigmatic case. As shown in this figure, in case of astigmatism, at least one of the sub-spots corresponding to at least one of the diffraction orders may be deformed by astigmatism (i.e. all but the 0 th diffraction order), wherein the distance between the two sub-spot provides a measure for the emission wavelength of one or more emitters. The axial position may be determined on the basis of the ellipticity of the spot shape of the one or more sub-spots with on-zero astigmatism. The ellipticity of a spot shape may be easily determined by processing the image of a spot shape using conventional image processing techniques. Hence, in this embodiment, the image-processing module may be configured to determine the two sub-spots and to measure the distance and the ellipticiy of at least one of the two sub-spots.

Fig. 6 is a block diagram illustrating an exemplary data processing system that may be used in systems and methods as described with reference to **Fig. 1-5**. Data processing system **600** may include at least one processor **602** coupled to memory elements **604** through a system bus **606**. As such, the data processing system may store program code within memory elements **604**. Further, processor **602** may execute the program code accessed from memory elements **604**

via system bus **606.** In one aspect, data processing system may be implemented as a computer that is suitable for storing and/or executing program code. It should be appreciated, however, that data processing system **600** may be implemented in the form of any system including a processor and memory that is capable of performing the functions described within this specification.

Memory elements **604** may include one or more physical memory devices such as, for example, local memory **608** and one or more bulk storage devices **610.** Local memory may refer to random access memory or other non-persistent memory device(s) generally used during actual execution of the program code. A bulk storage device may be implemented as a hard drive or other persistent data storage device. The processing system **1100** may also include one or more cache memories (not shown) that provide temporary storage of at least some program code in order to reduce the number of times program code must be retrieved from bulk storage device **610** during execution.

Input/output (I/O) devices depicted as input device **612** and output device **614** optionally can be coupled to the data processing system. Examples of input device may include, but are not limited to, for example, a keyboard, a pointing device such as a mouse, or the like. Examples of output device may include, but are not limited to, for example, a monitor or display, speakers, or the like. Input device and/or output device may be coupled to data processing system either directly or through intervening I/O controllers. A network adapter **616** may also be coupled to data processing system to enable it to become coupled to other systems, computer systems, remote network devices, and/or remote storage devices through intervening private or public networks. The network adapter may comprise a data receiver for receiving data that is transmitted by said systems, devices and/or networks to said data and a data transmitter for transmitting data to said systems, devices and/or networks. Modems, cable modems, and Ethernet cards are examples of different types of network adapter that may be used with data processing system **1150.**

As pictured in **FIG. 6**, memory elements **604** may store an application **618.** It should be appreciated that data processing system **600** may further execute an operating system (not shown) that can facilitate execution of the application. Application, being implemented in the form of executable program code, can be executed by data processing system **600,** e.g., by processor **602.** Responsive to executing application, data processing system may be configured to perform one or more operations to be described herein in further detail.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" and/or "comprising," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

The corresponding structures, materials, acts, and equivalents of all means or step plus function elements in the claims below are intended to include any structure, material, or act for performing the function in combination with other claimed elements as specifically claimed. The description of the present invention has been presented for purposes of illustration and description, but is not intended to be exhaustive or limited to the invention in the form disclosed. Many modifications and variations will be apparent to those of ordinary skill in the art without departing from the scope and spirit of the invention. The embodiment was chosen and described in order to best explain the principles of the invention and the practical application, and to enable others of ordinary skill in the art to understand the invention for various embodiments with various modifications as are suited to the particular use contemplated.

CONCLUSIES

1. Optisch diffractie-element voor het gebruik in het pad van uitgezonden licht van een fluorescentiemicroscoop, waarbij de fluorescentiemicroscoop is aangepast voor het tegelijkertijd meten van de positie van een of meerdere emitters in een monster die orthogonaal is ten opzichte van het vlak van een substraat waar het sample op geplaatst is en het op basis van een of meerdere diffractie-ordes die gevormd worden door het optische diffractie-element meten van de golflengte van het licht dat wordt uitgezonden door de een of meerdere emitters,

waarbij het optisch diffractie-element een of meerdere zonefuncties omvat die gedefinieerd zijn door een of meerdere zonefuncties in laterale coördinaten in het vlak van het optisch diffractie-element;

waarbij het diffractie-element is aangepast voor het splitsen van het uitgezonden licht in een of meerdere diffractie-ordes zodanig dat de fase die aan het uitgezonden licht wordt toegevoegd afhangt van het verschil tussen de verhouding van de zonefunctie in de laterale coördinaten en de golflengte van het uitgezonden licht en de grootste integer die kleiner is dan de verhouding,

waarbij de een of meerdere zonefuncties gedefinieerd zijn als een som van Zernike polynomen in de laterale coördinaten zodanig dat ten minste een Zernike polynoom van de eerste orde niet gelijk aan nul is en tenminste een Zernike polynoom van de tweede orde niet gelijk aan nul is.

2. Optisch diffractie-element volgens conclusie 1

waarbij de fase een zaagtandvorm heeft voor het genereren van alleen een nulde diffractie-orde en een eerste diffractie-ordes; of, waarbij de fase een binaire vorm heeft voor het genereren van ten minste twee diffractie-ordes en, optioneel, een nulde diffractie-orde; of,

waarbij de fase een sinusvorm heeft voor het genereren van alleen twee eerste diffractie-ordes en, optioneel, een nulde diffractie-orde.

3. Optisch diffractie-element volgens conclusies 1 of 2 waarbij de Zernike polynomen gedefinieerd zijn door:

$$W(x,y)=\sum_n\sum_m C_{nm}Z_{nm}\left(x,y\right)$$

waarbij de Zernike coëfficiënten C_{nm} gegeven zijn door:

$$C_{nm}=\frac{1}{\pi R^2\left(n+1\right)}\int_{x^2+y^2\leq R^2} dxdyW(x,y)Z_{nm}\left(x,y\right)$$

en waarbij ten minste een van de eerste orde Zernike coëfficiënten C_{11} (corresponderend met de polynoom x) en C_{1-1} (corresponderend met polynoom y) niet gelijk aan nul is; en, ten minste een van de tweede orde Zernike coëfficiënten: C20 (corresponderend aan de polynoom $2(x^2+y^2)-1$), C_{22} (corresponderend aan de polynoom x^2-y^2 of C_{2-2} (corresponderend met de polynoom 2xy) niet gelijk aan nul is.

4. Optisch diffractie-element volgens een van de conclusies 1-3 waarbij een tweede orde Zernike polynoom een circulaire vorm x2+y2 heeft voor het genereren een of meerdere diffractie ordes met defocus.

5. Optisch diffractie-element volgens een van de conclusies 1-4 waarbij een tweede orde Zernike polynoom een

hyperbolische vorm x2-y2 heeft voor het genereren van een of meerdere diffractie ordes met horizontale/verticale astigmatisme (focuslijnen op 0 graden en 90 graden met de x-as).

6. Optisch diffractie-element volgens een van de conclusies 1-5 waarbij een tweede orde Zernike polynoom een elliptische vorm xy heeft voor het genereren van een of meerdere diffractie ordes met diagonaal astigmatisme (focuslijnen op +45 graden en -45 graden met de x-as).

7. Optisch diffractie-element volgens een van de conclusies 1-6 waarbij in het geval van defocus, de twee of meerdere sub-spots corresponderend met de twee of meerdere diffractie-ordes een relatieve defocus hebben, waarbij de afstand tussen de twee sub-spots een maat geven voor de uitgezonden golflengte van een of meerdere emitters en/of waarbij de verhouding van de grootte van de sub-spots een maat bepalen voor de axiale positie van de een of meerdere emitters.

8. Optisch diffractie-element volgens een van de conclusies 1-7 waarbij het element een transparant substraat omvat, bij voorkeur een glas, plastic of een kwarts substraat, waarin of waarop de zonefuncties gevormd worden; of, waarbij het element een reflecterend substraat omvat waarin en waarop de zonefuncties gevormd zijn.

9. Optisch diffractie-element volgens een van de conclusies 1-7 waarbij het element is geïmplementeerd als een elektronisch addreseerbare fasemodulatie-inrichting, bij voorkeur een spatiele lichtmodulator, een vervormbare spiegel of een vloeibare kristalleninrichting.

10. Optisch diffractie-element volgens conclusie 9 waarbij de elektronisch addresseerbare fasemodulatie-inrichting geconfigureerd kan worden voor het genereren van ten minste een eerste fasemodulatiepatroon op een eerste tijdstip voor het genereren van ten minste een eerste deel van de twee of meer diffractie-ordes en een tweede fasemodulatiepatroon op een tweede tijdstip voor het genereren van ten minste een tweede deel van de twee of meer diffractie-ordes.

11. Gebruik van een diffractie-element volgens een van de conclusies 1-10 in een microscoopsysteem, bij voorkeur een lokalisatie microscoopsysteem.

12. Een microscoopsysteem dat een optisch diffractie-element gebruik volgens een van de conclusies 1-10.

13. Beeldverwerkingsmodule voor gebruik met een optisch diffractie-element volgens een van de conclusies van 1-10, waarbij het diffractie-element twee of meerdere diffractie-ordes (sub-spots) genereert ter hoogte van de camera van de fluorescentiemicroscoop waarbij elke diffractie-orde (sub-spot) vervormt mag worden door een hoeveelheid defocus en/of door een hoeveelheid astigmatisme dat evenredig is met de orde m (m= …,-2,-1,0,1,2,…), waarbij de beeldverwerkingsmodule geconfigureerd is voor:

het ontvangen van een of meer beelden van een of meerdere diffractie-ordes die van het optisch diffractie-element vandaan komt; en,

in het geval van defocus, het bepalen van de afstand tussen twee of meerdere sub-spots die overeenkomen met twee of meerdere diffractie-ordes die een relatieve defocus hebben, waarbij de afstand tussen de twee sub-spots een maat geeft voor de uitgezonden golflengte van ten minste een emitter; en, het bepalen van de verhouding van de grootte van de sub-spots waarbij de verhouding een maat geeft voor de axiale positie van ten minste een transmitter; of,

in het geval van astigmatisme, het bepalen van de afstand tussen twee of meerdere sub-spots die overeenkomen met twee of meerdere diffractie-ordes die astigmatisme hebben, waarbij de afstand tussen de twee sub-spots een maat geeft voor de uitgezonden golflengte van ten minste een emitter; en, het bepalen van de ellipciteit van de spotvorm van ten minste een sub-spot met astigmatisme, waarbij de verhouding een maat geeft voor de axiale positie van de ten minste een emitter.

14. Beeldverwerkingsmodule volgens conclusie 13, waarbij het optisch diffractie-element een elektronisch addresseerbare fasemodelatie-inrichting is, waarbij de beeldverwerkingsmodule geconfigureerd is om de elektronisch addresseerbare fasemodelatie-inrichting te bestuderen om ten minste een eerste fase modulatie patroon op ten minste een eerste tijdstip te genereren voor het genereren van ten minste een eerste deel van twee of meerdere diffractie-ordes en een tweede fasemodulatie patroon op een tweede tijdstip voor het genereren van ten minste een tweede deel van de twee of meer diffractie-ordes.

15. Werkwijze voor het tegelijkertijd meten van de positie van een of meerdere emitters in een monster die orthogonaal staat op het vlak van het substraat waar het monster op geplaatst is en de golflengte van het licht dat uitgezonden wordt door de een of meerdere emitters op basis van de een of meerdere diffractie-ordes die gevormd worden

door een optisch diffractie-element volgens een van conclusies 1-10, de werkwijze omvattende:

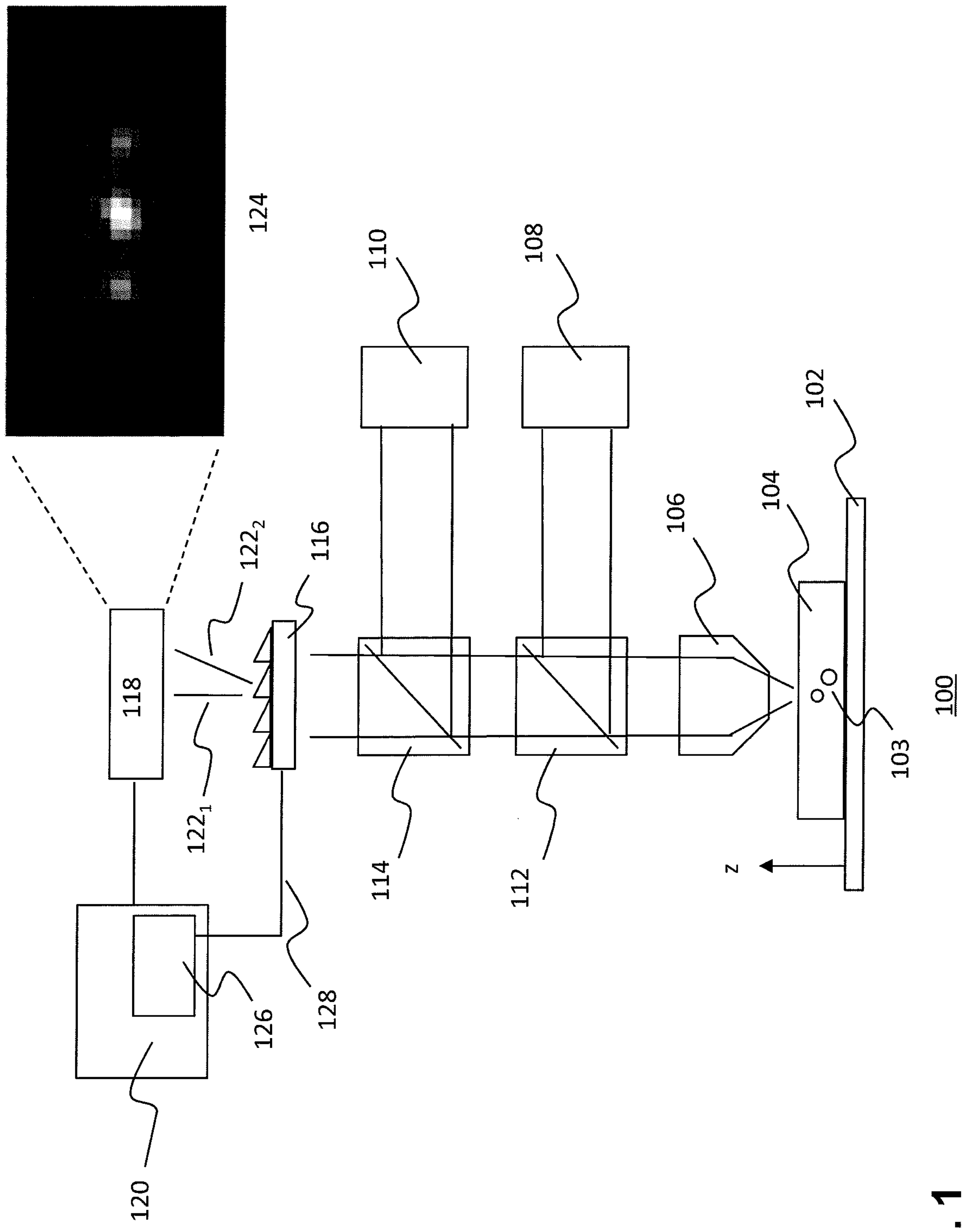
het meten van de deformatie van een of meerdere diffractie-ordes (sub-spots) veroorzaakt door een hoeveelheid defocus en/of door een hoeveelheid astigmatisme voor het bepalen van de axiale positie van ten minst een emitter; en,

het meten van de afstand tussen twee of meerdere sub-spots die overeenkomen met twee of meerdere vervormde diffractie-ordes voor het bepalen van de uitgezonden golflengte van de ten minste een emitter.

16. Werkwijze volgens conclusie 15 waarbij in het geval van defocus, de axiale positie wordt bepaald op basis van de verhouding van de grootte van de sub-spots

17. Werkwijze volgens conclusie 15 of 16 waarbij in het geval van astigmatisme, de axiale positie wordt bepaald op basis van de ellipticiteit van de spotvorm van ten minste een sub-spot met astigmatisme.

18. Computerprogrammaproduct omvattende delen van softwarecode die wanneer uitgevoerd in het geheugen van een computer, de werkwijzestappen volgens een van conclusies 15-17 uitvoeren.



124
110
108
102
104
106
116
122_2
118
122_1
114
112
103
100
z
120
126
128

FIG. 1

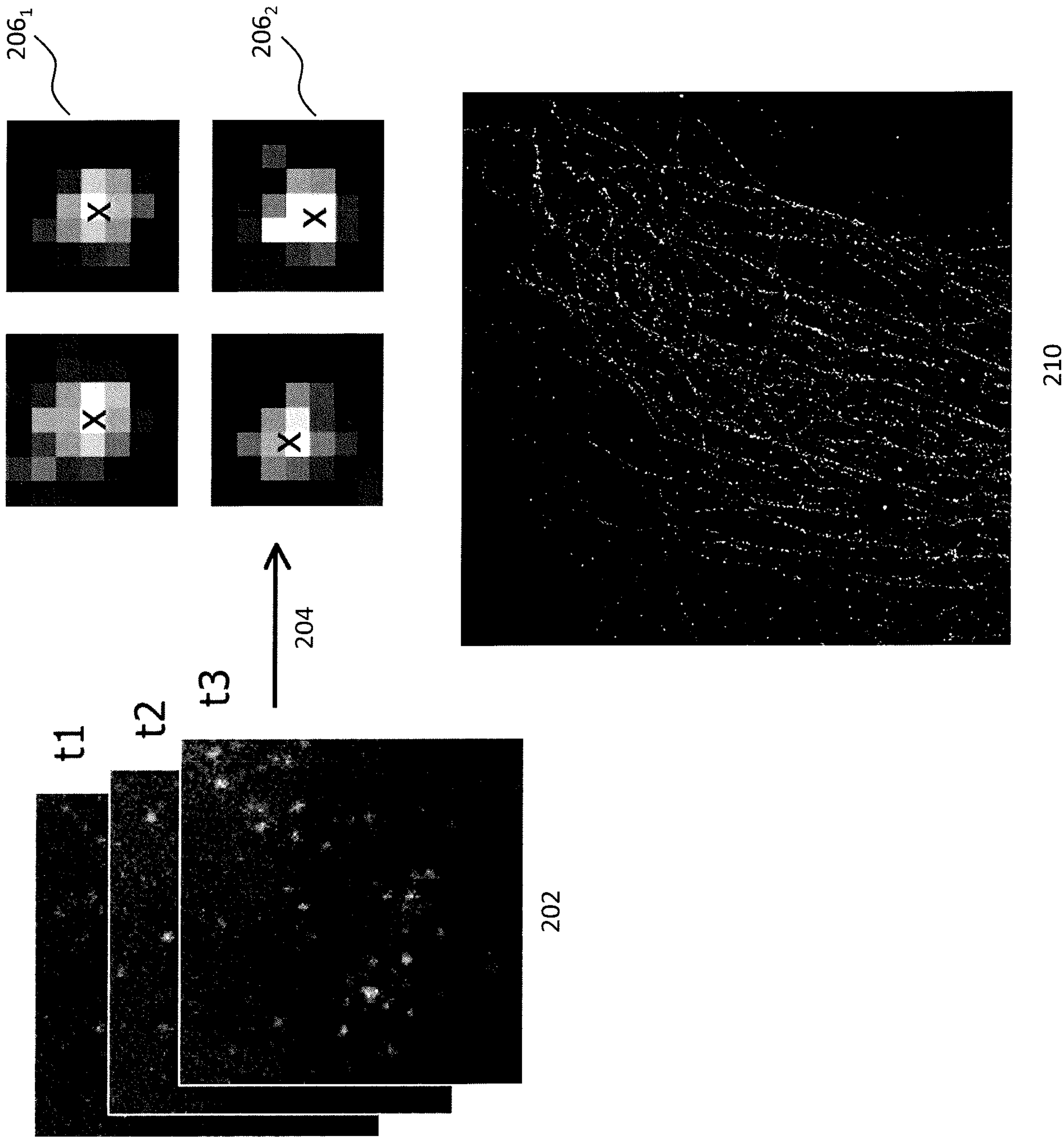


FIG. 2

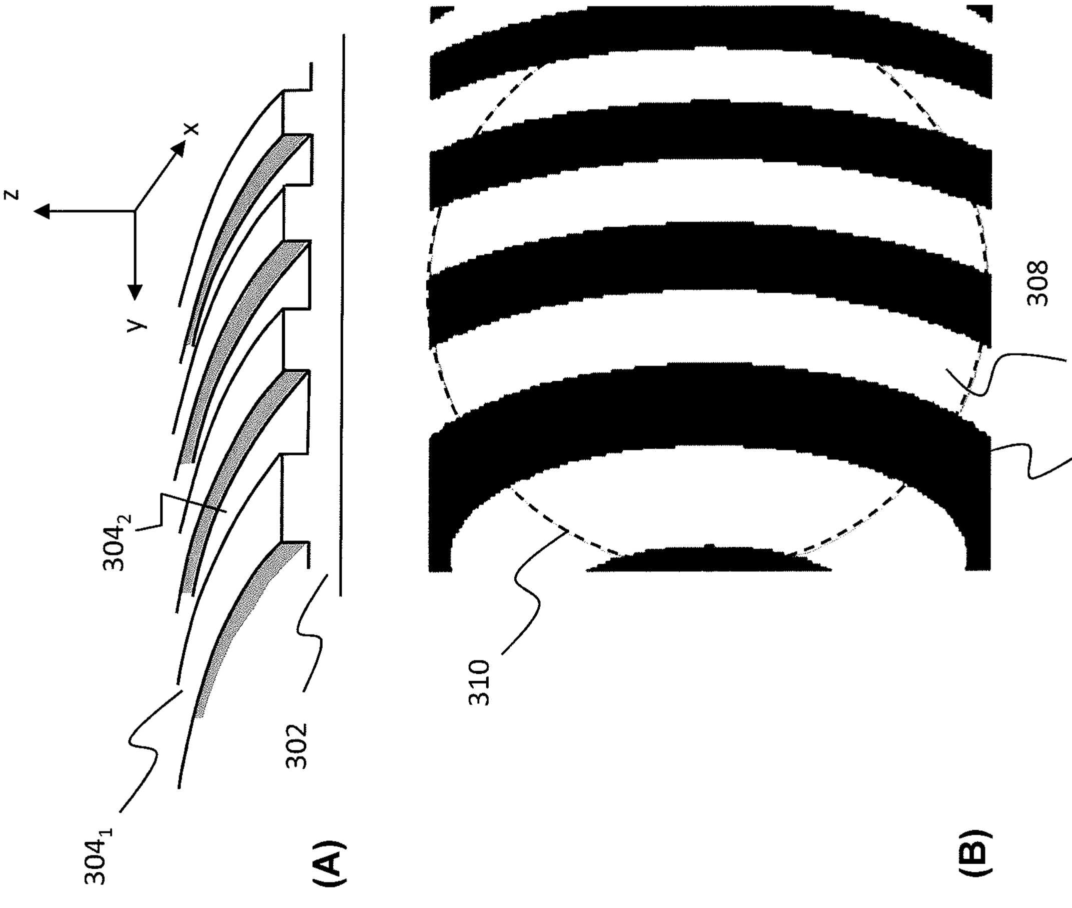


FIG. 3

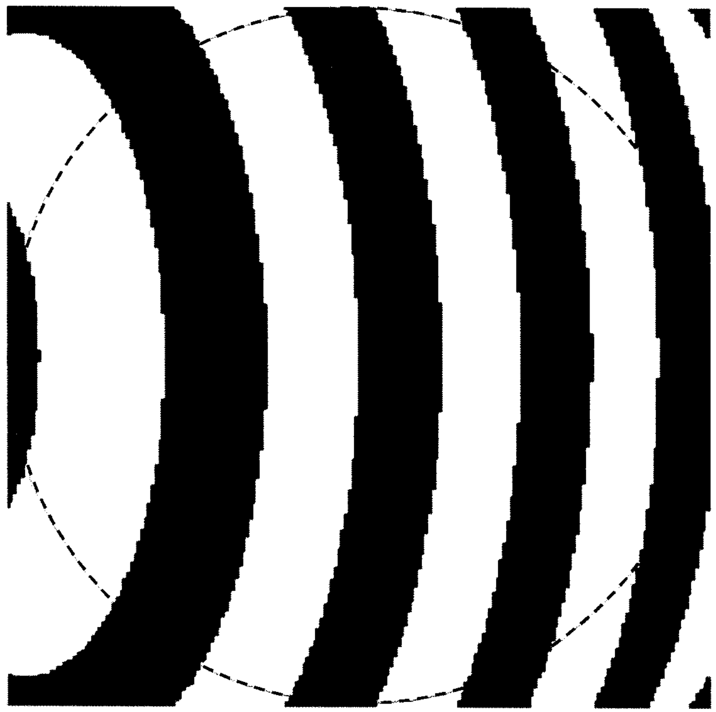


FIG. 4A

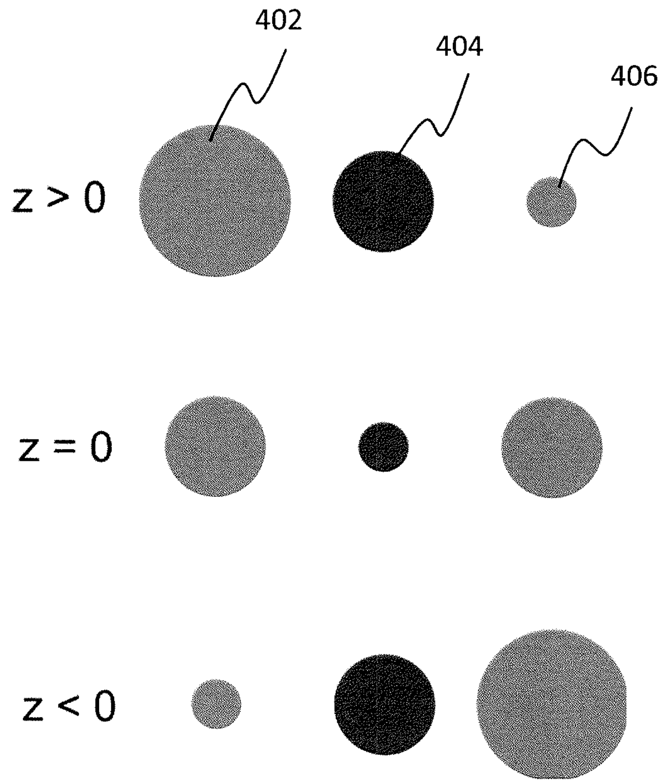


FIG. 4B

FIG. 5A

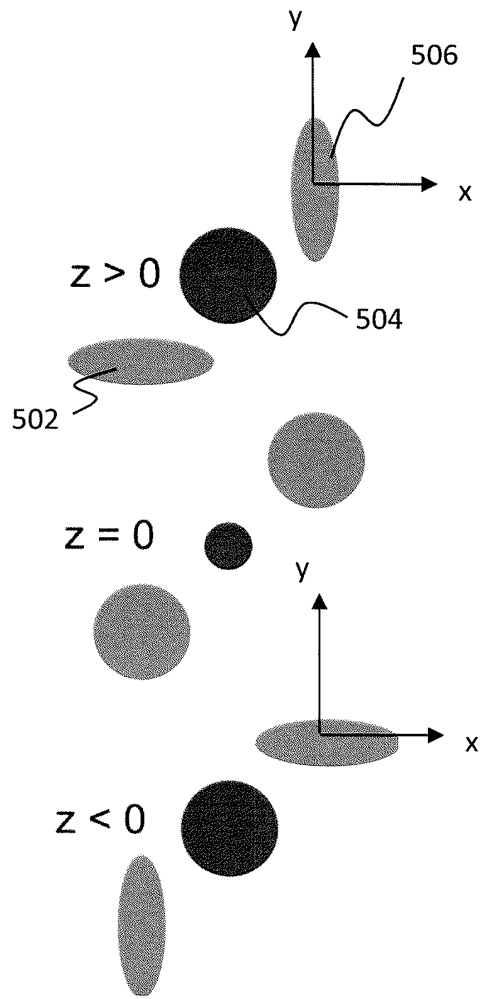


FIG. 5B

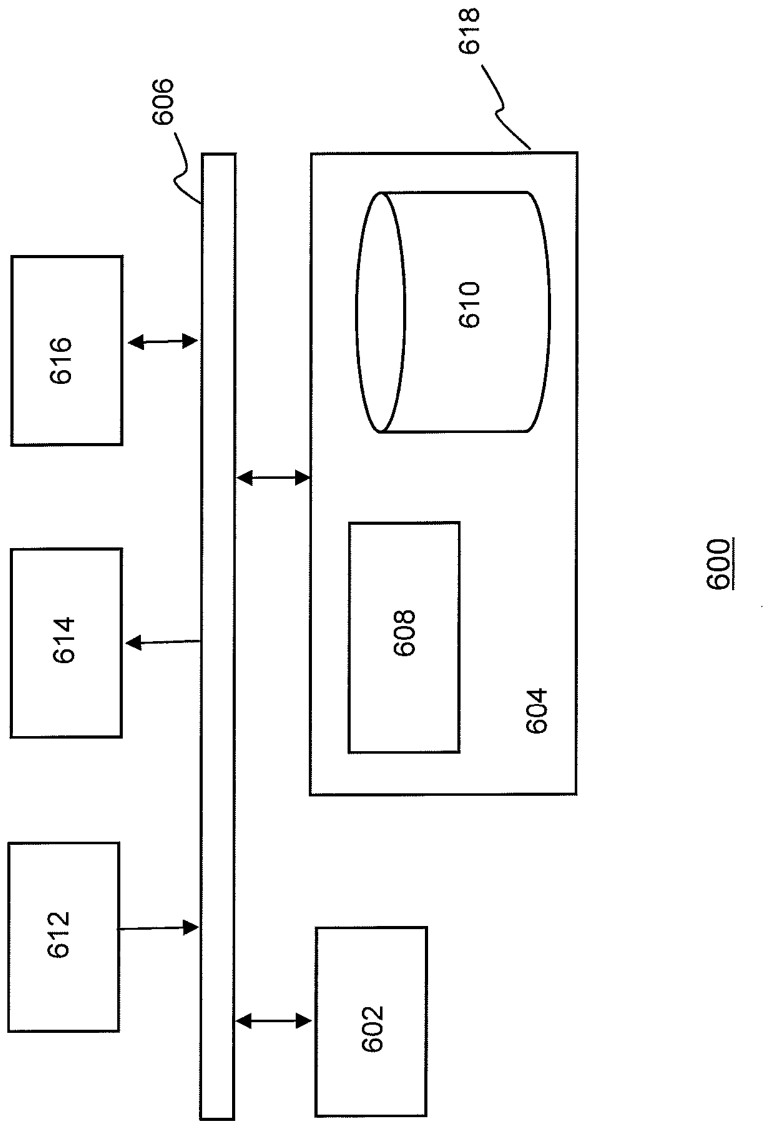


FIG. 6

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
	NL 20226-VI/mv
Nederlands aanvraag nr. **2012187**	Indieningsdatum **03-02-2014**
	Ingeroepen voorrangsdatum
Aanvrager (Naam) **Technische Universiteit Delft, et al**	
Datum van het verzoek voor een onderzoek van internationaal type **24-05-2014**	Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. **SN 62086**

I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)

Volgens de internationale classificatie (IPC)

G01N21/64 **G02B27/42** **G02B27/00**
G02B5/18

II. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimumdocumentatie	
Classificatiesysteem	Classificatiesymbolen
IPC	**G01N** **G02B**

Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

III.	☐	**GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES**	(opmerkingen op aanvullingsblad)
IV.	☐	**GEBREK AAN EENHEID VAN UITVINDING**	(opmerkingen op aanvullingsblad)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2012187

A. CLASSIFICATIE VAN HET ONDERWERP
INV. G01N21/64 G02B27/42 G02B27/00 G02B5/18
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte miminum documentatie (classificatie gevolgd door classificatiesymbolen)
G01N G02B

Onderzochte andere documentatie dan de mimimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)

EPO-Internal, WPI Data, INSPEC, COMPENDEX, BIOSIS

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	WO 2013/010859 A1 (IMAGINE OPTIC [FR]; LEVECQ XAVIER [FR]; ANDILLA JORDI [ES]) 24 januari 2013 (2013-01-24) * bladzijde 4, regel 12 - bladzijde 7, regel 27 * * bladzijde 10, regel 1 - bladzijde 20, regel 22; figuren 1A,1B,2C,3C,5A,5B * ----- -/--	1-18

[X] Verdere documenten worden vermeld in het vervolg van vak C.

[X] Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

° Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvrage vermeld

"E" eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid

23 september 2014

Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type

Naam en adres van de instantie
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

1

De bevoegde ambtenaar

Duijs, Eric

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2012187

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
A	ZHOU YAOPENG ET AL: "Adaptive optics two-photon scanning laser fluorescence microscopy", MEMS ADAPTIVE OPTICS V, SPIE, 1000 20TH ST. BELLINGHAM WA 98225-6705 USA, deel 7931, nr. 1, 10 februari 2011 (2011-02-10), bladzijden 1-8, XP060006656, DOI: 10.1117/12.875596 [gevonden op 2011-02-14] * samenvatting * * bladzijde 4, alinea 2 * -----	1-18
A	US 2013/034913 A1 (SEYFRIED VOLKER [DE] ET AL) 7 februari 2013 (2013-02-07) * alineas [0001], [0005], [0010], [0028] - [0030]; figuren 1,2 * -----	1

1

Formulier PCT/ISA/201 (vervolg tweede blad) (Januari 2004)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar de stand van de techniek

NL 2012187

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
WO 2013010859 A1	24-01-2013	EP 2742379 A1	18-06-2014
		FR 2978254 A1	25-01-2013
		WO 2013010859 A1	24-01-2013
US 2013034913 A1	07-02-2013	DE 102010016598 A1	27-10-2011
		EP 2561393 A1	27-02-2013
		JP 2013525770 A	20-06-2013
		US 2013034913 A1	07-02-2013
		WO 2011131401 A1	27-10-2011

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
SN62086	03.02.2014		NL2012187
International Patent Classification (IPC) INV. G01N21/64 G02B27/42 G02B27/00 G02B5/18			
Applicant Technische Universiteit Delft, et al			

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the application
- ☒ Box No. VIII Certain observations on the application

	Examiner
	Duijs, Eric

Application number

WRITTEN OPINION

NL2012187

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	1-18
	No:	Claims	
Inventive step	Yes:	Claims	
	No:	Claims	1-18
Industrial applicability	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION

Box No. VII Certain defects in the application

see separate sheet

Box No. VIII Certain observations on the application

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following document:

D1 WO 2013/010859 A1 (IMAGINE OPTIC [FR])

D2 ZHOU YAOPENG ET AL: "Adaptive optics two-photon scanning laser fluorescence microscopy", MEMS ADAPTIVE OPTICS V, SPIE, 1000 20TH ST. BELLINGHAM WA 98225-6705 USA, deel 7931, nr. 1, 10 februari 2011 (2011-02-10), bladzijden 1-8, XP060006656, DOI: 10.1117/12.875596 [gevonden op 2011-02-14]

2 **Lack of inventive step**

The present application does not meet the criteria of patentability, because the subject-matter of **claims 1-18** does not involve an inventive step:

2.1 **Independent claim 1**:

D1 is regarded as being the **prior art closest** to the subject-matter of **claim 1**, and discloses an

-- *Optisch diffractie-element* 200, 150 (FIGs. 1A, 1B; p. 10, l. 21-22; p. 11, l. 27-28, 31-32: "un miroir dédormable") *voor het gebruik in het pad van uitgezonden licht van een fluorescentiemicroscoop* 130 (p. 10, l. 21; p. 11, l. 12; p. 12, l. 5), *waarbij de fluorescentiemicroscoop* 130 *is aangepast voor het tegelijkertijd meten van de positie van een of meerdere emitters in een monster* 10 (p. 10, l. 2, 17-19) *die orthogonaal is ten opzichte van het vlak van een substraat* 131 (p. 11, l. 5) *waar het sample* 10 *op geplaatst is en het op basis van een of meerdere diffractie-ordes die gevormd worden door het optische diffractie-element* 200, 150 *meten van de golflengte van het licht* 2, 3 (p. 11, l. 19, 34) *dat wordt uitgezonden door de een of meerdere emitters,*

-- *waarbij het optisch diffractie-element* 200, 150 *een of meerdere zonefuncties omvat die gedefinieerd zijn door een of meerdere zonefuncties in laterale coördinaten in het vlak van het optisch diffractie-element* 200, 150 (p. 5, l. 24-31: "a déformation contrôlée est obtenue par une combinaison de polynômes de Zernike d'ordre azimutal pair"; p. 19, l. 3-5)*;*

-- *waarbij het diffractie-element* 200, 150 *is aangepast voor het splitsen van het uitgezonden licht* 2 (p. 11, l. 19) *in een of meerdere diffractie-ordes zodanig dat de fase die aan het uitgezonden licht* 2 *wordt toegevoegd afhangt van het verschil tussen de verhouding van de zonefunctie in de laterale coördinaten en de golflengte van het uitgezonden licht en de grootste integer die kleiner is dan de verhouding* (implicitly disclosed or at least obvious in view of FIGs. 5A, 5B; p. 16, l. 19 - p. 17, l. 25: "ourbes 54, 54 et 55 représentant ainsi les valeurs des différences entre les dimensions latérales en X et en Y (Wx - Wy) pour trois valeurs d'astigmatisme", "la relation bijective existant entre la différence Wx - Wy et la position axiale de la bille par rapport au plan focal objet"),

-- *waarbij de een of meerdere zonefuncties gedefinieerd zijn als een som van Zernike polynomen in de laterale coördinaten (p. 5, l. 24-31; p. 19, l. 3-5) zodanig dat en tenminste een Zernike polynoom van de tweede orde niet gelijk aan nul is* (p. 19, l. 5-6).

D1 generally states "une combinaison de polynômes de Zernike d'ordre azimutal pair" (p. 5, l. 24-25). Somme preferred Zernike polynomials are mentioned (p. 5, l. 26; p. 19, l. 5-12).

The subject-matter of **claim 1** therefore **differs** from this known "*Optisch diffractie-element*" in that "*ten minste een Zernike polynoom van de eerste orde niet gelijk aan nul is*" and is therefore new.

The **problem** to be solved by the present invention may therefore be regarded as to select suitable Zernike polynomials.

The **solution** proposed in **claim 1** of the present application cannot be considered as involving an inventive step for the following reasons:

D2 (p. 4, par. 2) points out the relevance of the first and second order Zernike polynomials. In view of **D1** (p. 5, l. 24-26; p. 19, l. 3-12) the skilled person would consider to use any combination of Zernike polynomials. He would find suitable polynomials via an obvious trial-and-error or iterative procedure (see also **D1**, p. 13, l. 9-13). He would arrive at the subject-mater of **claim 1** without involving an inventive step.

2.2 Dependent **claims 2-18** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of inventive step, see **D1** and in particular for:

-- **claim 9**: see **D1** (p. 11, l. 31-32; p. 19, l. 31 - p. 20, l. 3);

-- **claims 11, 12**: see **D1** (p. 7, l. 13-15).

Re Item VII

Certain defects in the application

3 The relevant background art disclosed in **D1 and D2** is not mentioned in the description, nor are these documents identified therein.

4 The features of the claims are not provided with **reference signs** placed in parentheses.

5 The reference signs not appearing in the description shall not appear in the drawings, and vice versa. This requirement is not met in view of the **reference sign 166** (p. 17, l. 5; not in FIG. 1). It appears that "166" should be replaced with "116".

Re Item VIII

Certain observations on the application

6 **Claim 1** defines "*het tegelijkertijd meten van de positie van een of meerdere emitters in een **monster** die orthogonaal is ten opzichte van het vlak van een substraat waar **het sample** op geplaatst is*" (emphasis added). It appears that the term "*sample*" should be replaced with the term "*monster*".

7 The vague and imprecise statements ("equivalents", "in combination with other claimed elements", "many modifications and variations", "spirit of the invention", "various modifications") in the description on p. 27, l. 25 - p. 28, l.

28 imply that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity when used to interpret them.