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(54) **3-DIMENSIONAL CONFOCAL MICROSCOPY APPARATUS AND FOCAL PLANE SCANNING AND ABERRATION CORRECTION UNIT**

DREIDIMENSIONAL-KONFOKALES MIKROSKOP UND VORRICHTUNG ZUR VERSCHIEBUNG DER FOKUSEBENE UND ZUR KORREKTUR VON ABERRATIONEN

APPAREIL DE MICROSCOPIE CONFOCAL TRIDIMENSIONNEL ET UNITÉ DE DÉPLACEMENT DU PLAN FOCAL D'OBSERVATION ET DE CORRECTION DES ABERRATIONS

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## Description

### Technical Field

**[0001]** The present invention relates to a 3-dimensional confocal microscopy apparatus and a focal plane scanning and aberration correction unit. The present invention more particularly relates to a 3-dimensional confocal imaging apparatus which is manufactured by combining a so-called confocal microscope and an optical tweezers technique and can cope with problems which newly arise as the result of such combination, and a focal plane scanning and aberration correction unit which constitutes an essential part of the novel constitution of the apparatus.

### Background Art

**[0002]** In the field of biology or the like, conventionally, as described in the following patent literature 1, for example, there has been used a technique referred to as optical tweezers where a transparent micro bead is put into a liquid, the micro bead is captured at a focal point of a focused infrared laser beam, and DNA, a biological molecule, a cell or the like is manipulated in the liquid via the micro bead. Further, the following patent literature 2 discloses a technique where a resin-made micro structural body having a total length of 10 $\mu$ m prepared by a two-photon absorption stereolithography is used as an object, and a cell is manipulated or a force is measured by driving the object using optical tweezers in a liquid.

**[0003]** In such a technique which uses optical tweezers, an object (specimen) is extremely minute and hence, it is necessary to manipulate the object in a liquid while observing the object using a microscope. In such a case, the object which is to be manipulated using the optical tweezers can be observed only 2-dimensionally using a usual microscope and hence, there arises a drawback that it is difficult to grasp the vertical relationship between the object and a focal point of an optical trap so that an accurate manipulation cannot be performed.

**[0004]** On the other hand, as a microscope which enables the 3-dimensional observation of an object to be observed, conventionally, a confocal microscope has been used. In the confocal microscope, an excitation laser beam is focused and irradiated to a specimen (an object to be observed to which fluorescent staining is applied) through an objective lens, and a fluorescent light emitted from the specimen is made to pass through a pin hole arranged at a conjugated position with the objective lens and is imaged on a detector. Light emitted from regions other than a focal point of the objective lens is eliminated by the pin hole and hence, only light in a focal region can be detected. Then, by 2-dimensionally scanning a focal point of the excitation laser beam on the specimen using a pair of galvano mirrors and an acousto-optic modulator and hence, a thin cross-sectional image of the specimen in the vicinity of an area at a specified depth can be obtained. Further, by scanning the objective

lens or a specimen stage in the direction of an optical axis, cross-sectional images at different depths can be obtained. Accordingly, a 3-dimensional image of the specimen can be formed.

**[0005]** Further, with respect to the above-mentioned confocal microscope, as disclosed in the following patent literatures 3, 4, for example, there has been known a method where, to accelerate 2-dimensional scanning on a specimen, an excitation laser beam is split into a plurality of laser beams using a nipkow disc or a microlens array, and a plurality of regions on the specimen are simultaneously scanned. To achieve the substantially same purpose, as disclosed in the following patent literature 5, for example, there may be a case where a line-scanning-type scanning system where an excitation laser beam is scanned by focusing the laser beam in a line shape is used.

### **[0006]**

20 (PTL 1) JP-A-H8-234110  
 (PTL 2) JP-A-2009-294008  
 (PTL 3) JP-A-2009-210889  
 (PTL 4) JP-A-2010-160371  
 (PTL 5) JP-A-2010-164635

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**[0007]** Further prior art is EP1855141 A1 which discloses a laser scanning confocal microscope wherein the the tweezer light is coupled by the mirror 4 into the excitation/ observation light pass at the stage before the objective lens. A focus position of the excitation/observation light is controlled by movement of the objective lens. A focus position of even two tweezer lights is controlled in addition by units positioned outside the excitation/observation light path.

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**[0008]** Still further prior art DE102004034992 discloses a laser scanning confocal microscope comprising a pair of lenses for focal plane displacement is arranged between the objective lens and the fluorescent light imaging camera, the pair of lenses for focal plane displacement being constituted of an intermediate imaging lens which forms an intermediate image of the fluorescent confocal microscopy image of the specimen and an intermediate objective lens arranged such that a focal point of the intermediate objective lens overlaps with a focal point of the intermediate imaging lens on the same optical axis, and at least one lens out of these lenses being movable in an optical axis direction by a lens drive means.

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### Disclosure of the Invention

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**[0009]** As set forth above, by constituting the 3-dimensional confocal microscopy apparatus manufactured by combining a confocal microscope with an optical tweezers technique, a specimen can be observed 3-dimensionally using an optical tweezers technique. Accordingly, it is expected that the vertical relationship between the specimen and a focal point of an optical trap can be favorably grasped so that an accurate optical tweezers

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manipulation can be performed. However, an attempt to perform the 3-dimensional observation of the optically trapped specimen by combining the optical tweezers technique and the confocal microscope gives rise to a new drawback. This drawback is explained in conjunction with Fig. 1.

**[0010]** Fig. 1 shows an essential part of a 3-dimensional confocal microscopy apparatus manufactured by combining a confocal microscope with an optical tweezers technique in a simplified manner. In the drawing arranged on a left side of Fig. 1, a confocal imaging laser 1 passes through an objective lens 2, and forms a focal point on a focal plane 3. On the other hand, an optical tweezers laser 4 passes through the same objective lens 2 via a dichroic mirror 5, and captures a specimen 6 on the focal plane 3.

**[0011]** To perform the 3-dimensional observation of the specimen 6 in such a state, it is necessary to vertically scan the objective lens 2 in the confocal microscope. For example, when the objective lens 2 is moved to a lower position 7 as shown in the drawing arranged on a right side of Fig. 1, a focal plane 8 at a lower level can be observed. However, in such an operation, a focal point of the optical tweezers laser incident coaxially is also moved downward in an interlocking manner and hence, the specimen which is manipulated by the optical tweezers is also moved to a lower position 9 following the lowering of the focal point of the optical tweezers laser. That is, the conventional simple combination of the optical tweezers technique and the confocal microscope has a drawback that the 3-dimensional observation of the specimen without affecting the manipulation of the specimen using the optical tweezers cannot be realized.

**[0012]** Accordingly, it is an object of the present invention to overcome the above-mentioned drawback. To be more specific, it is an object of the present invention to provide a 3-dimensional confocal microscopy apparatus manufactured by combining a confocal microscope with an optical tweezers technique, wherein a 3-dimensional image of a specimen can be acquired without moving an objective lens and a focal point of an optical tweezers laser (specimen stage), in other words, without affecting an optical trap during the manipulation of the specimen using optical tweezers. It is another object of the present invention to ensure the accuracy of a 3-dimensional image of the specimen acquired in this manner.

(First invention)

**[0013]** The first invention is directed to a 3-dimensional confocal microscopy apparatus including: a light source for a first laser for confocal imaging excitation; a 2-dimensional scanning optical system for scanning the first laser on a specimen; an objective lens for focusing the first laser supplied from the light source on a fluorescent specimen which is an object to be observed; and a fluorescent light imaging camera for detecting a fluorescent confocal microscopy image based on a fluorescent light emitted

from the fluorescent specimen, wherein a pair of lenses for focal plane displacement is arranged between the objective lens and the fluorescent light imaging camera, the pair of lenses for focal plane displacement being constituted of an intermediate imaging lens which forms an intermediate image of the fluorescent confocal microscopy image of the specimen and an intermediate objective lens arranged such that a focal point of the intermediate objective lens overlaps with a focal point of the intermediate imaging lens on the same optical axis, and at least one lens out of these lenses being movable in an optical axis direction by a lens drive means, the 3-dimensional confocal microscopy apparatus further includes a computer having an arithmetic operation means for acquiring a 3-dimensional structural image of the specimen by correcting aberration of the fluorescent confocal microscopy image caused by the movement of the lens of the pair of lenses for focal plane displacement in real time using the fluorescent light imaging camera and aberration correction data which is acquired in advance by performing provisional measurement of a sample specimen, and a means which displays a corrected image obtained by the arithmetic operation means, and a beam splitter or a dichroic mirror is provided between the pair of lenses for focal plane displacement and the objective lens, and a second laser supplied from a light source for a second laser for optical tweezers passes through the objective lens via the beam splitter or the dichroic mirror and is focused on the specimen.

**[0014]** According to the first invention, in the 3-dimensional confocal microscopy apparatus manufactured by combining a confocal microscope with an optical tweezers technique, the pair of lenses for focal plane displacement is arranged between the objective lens and the fluorescent light imaging camera. By moving one lens of the pair of lenses for focal plane displacement in the optical axis direction by the lens drive means, as described later in detail in conjunction with Fig. 3, the focal plane can be displaced to planes at different heights in the specimen without moving the objective lens of the confocal microscope. Accordingly, even when the focal plane of the confocal microscope is displaced to planes at different heights in the specimen, a focal point of the optical tweezers laser (second laser) which is focused by the objective lens is not changed. Accordingly, the drawback that the present invention has can be overcome.

**[0015]** That is, in the 3-dimensional confocal microscopy apparatus manufactured by combining the optical tweezers technique and the confocal microscope, a 3-dimensional image of a specimen can be acquired without moving the objective lens and a focal point of the optical tweezers laser (specimen stage) (in other words, without affecting an optical trap during a manipulation of the specimen using the optical tweezers).

**[0016]** Although explained in detail later in conjunction with Fig. 3, by moving one lens of the pair of lenses for focal plane displacement in the optical axis direction by

a lens drive means, a certain aberration is generated in a fluorescent confocal microscopy image in a microscope. However, such aberration is corrected in real time by an arithmetic operation means using the fluorescent confocal microscopy image and aberration correction data acquired in advance by performing the provisional measurement of a sample specimen and hence, an accurate 3-dimensional corrected image is displayed thus achieving the task of ensuring the accuracy of a 3-dimensional image of a specimen.

(Second invention)

**[0017]** The second invention is, in the 3-dimensional confocal microscopy apparatus according to the first invention, characterized in that the 2-dimensional scanning optical system includes a nipkow disc or a line scanning type scanning system.

**[0018]** According to the second invention, by using the nipkow disc or the line scanning type scanning system in 2-dimensional scanning of a laser beam on a specimen, a confocal image of a cross section of the specimen at an arbitrary depth can be acquired at a high speed. Accordingly, it is possible to acquire a cross-sectional image with no wobbling even in the case of a specimen during movement using optical tweezers or a specimen having a motion.

(Third invention)

**[0019]** The third invention is, in the 3-dimensional confocal microscopy apparatus according to the first invention or the second invention, characterized in that the lens drive means for the pair of lenses for focal plane displacement is one selected from a group consisting of a piezoelectric element, a magnetostrictive actuator and a voice coil.

**[0020]** According to the third invention, the lens drive means for the pair of lenses for focal plane displacement is a high-speed drive means such as a piezoelectric element, a magnetostrictive actuator or a voice coil and hence, a plurality of cross-sectional images at different depths in a specimen can be acquired at a high speed. Accordingly, in combination with a high-speed 2-dimensional scanning system of the second invention such as a nipkow disc or a line scanning-type scanning system, a 3-dimensional image of a specimen during the manipulation of the specimen using optical tweezers can be acquired at an extremely high speed.

(Fourth invention)

**[0021]** The fourth invention is, in the 3-dimensional confocal microscopy apparatus according to any one of the first invention to the third invention, characterized in that timing for driving the lens drive means for the pair of lenses for focal plane displacement and timing for acquiring an image by a fluorescent imaging camera are

adjusted so as to be synchronous with each other.

**[0022]** Further, as described in the fourth invention, by adjusting timing for driving the lens drive means for the pair of lenses for focal plane displacement and timing for acquiring an image by the fluorescent imaging camera so as to be synchronous with each other, timing at which the lens is stopped at a position where focusing is made at a specified depth of a specimen by driving a high speed scanning means and timing at which an image is imaged by a camera agree with each other and hence, a cross-sectional image of the specimen can be acquired without being affected by wobbling during high speed scanning.

(Fifth invention)

**[0023]** The fifth invention is, in the 3-dimensional confocal microscopy apparatus according to any one of the first invention to the fourth invention, characterized in that an arithmetic operation using an arithmetic operation means for acquiring a 3-dimensional structural image of a specimen is performed by a GPU (Graphics Processing Unit).

**[0024]** According to the fifth invention, a 3-dimensional image of the specimen can be formed at a high speed based on a plurality of cross-sectional images of the specimen at different depths by an image arithmetic operation using the GPU and is displayed. Accordingly, during the manipulation of optical tweezers performed by an observer, a viewer can perform the manipulation while observing a 3-dimensional image of a specimen at a point of time from an arbitrary viewpoint with arbitrary magnification.

(Sixth invention)

**[0025]** The sixth invention is, in the 3-dimensional confocal microscopy apparatus according to any one of the first invention to the fifth invention, characterized in that the 3-dimensional confocal microscopy apparatus further includes a beam splitter or a dichroic mirror which is arranged between the objective lens and the pair of lenses for focal plane displacement, and an imaging lens and a bright field imaging camera for imaging a 2-dimensional image of a specimen separated by the beam splitter or the dichroic mirror.

**[0026]** According to the sixth invention, a bright field image of a specimen which does not pass through the confocal optical system can be acquired by the bright field imaging camera different from the fluorescent light imaging camera. Accordingly, during the manipulation of the optical tweezers, a state of a non-luminescent portion of the specimen can be observed together with a 3-dimensional image of a fluorescent portion of the specimen.

(Seventh invention)

**[0027]** The seventh invention is directed to a focal

plane scanning and aberration correction unit to be incorporated into a 3-dimensional confocal microscopy apparatus comprises

a pair of lenses for focal plane displacement, the pair of lenses for focal plane displacement being constituted of an intermediate imaging lens which forms an intermediate image of a fluorescent confocal microscopy image of a specimen and an intermediate objective lens arranged such that a focal point of the intermediate objective lens overlaps with a focal point of the intermediate imaging lens on the same optical axis, and at least one lens out of these lenses being movable in an optical axis direction by a lens drive means, and

the focal plane scanning and aberration correction unit further comprises a computer having an arithmetic operation means for acquiring a 3-dimensional structural image of the specimen by correcting aberration of the fluorescent confocal microscopy image caused by the movement of the lens of the pair of lenses for focal plane displacement in real time using the fluorescent confocal microscopy image acquired by the fluorescent light imaging camera and aberration correction data which is acquired in advance by performing provisional measurement of a sample specimen, and a means which displays a corrected image obtained by the arithmetic operation means,

wherein the 3-dimensional confocal microscopy apparatus comprises a light source for a laser; an objective lens for focusing the laser supplied from the light source on a fluorescent specimen; and a fluorescent light imaging camera for detecting a fluorescent confocal microscopy image based on a fluorescent light emitted from the fluorescent specimen, and wherein

the focal plane scanning and aberration correction unit is to be arranged between the objective lens and the fluorescent light imaging camera.

**[0028]** According to the seventh invention, a novel focal plane scanning and aberration correction unit is provided. This unit constitutes a characterizing portion of the 3-dimensional confocal microscopy apparatus of the present invention. Further, for example, it may be also possible to provide a utilization method where the focal plane scanning and aberration unit is incorporated into an existing product of a general-type confocal microscope. Still further, it may be also possible to provide a utilization method where the focal plane scanning and aberration unit is incorporated into an existing product of a 3-dimensional confocal microscopy apparatus manufactured by combining a confocal microscope and an optical tweezers apparatus.

#### Brief Description of Drawings

##### **[0029]**

Fig. 1 shows a drawback which a 3-dimensional confocal microscopy apparatus manufactured by simply combining an optical tweezers technique and a con-

focal microscope has.

Fig. 2 shows an embodiment of the 3-dimensional confocal microscopy apparatus manufactured by combining the optical tweezers technique and the confocal microscope of the present invention.

Fig. 3 explains the manner of operation and advantageous effects of a pair of lenses for focal plane displacement and the principle of aberration correction of a fluorescent confocal microscopy image brought about by such manner of operation and advantageous effects.

Fig. 4 shows a flowchart of a 3-dimensional confocal microscopy and manipulation process according to the present invention.

Fig. 5 shows a confocal imaged image of a specimen (fluorescent columnar structural body) of this embodiment before aberration correction (drawing on an upper side) and after aberration correction (drawing on a lower side).

Fig. 6 shows a bright field imaged image of a resin-made minute structural body for cell manipulation having a total length of 10 $\mu$ m which is prepared by a two-photon absorption stereolithography.

Fig. 7 shows a mode where a columnar projection portion which is a minute structural body is captured by optical tweezers in a liquid and a movable arm is driven using the 3-dimensional confocal microscopy apparatus of the present invention.

Fig. 8 shows a mode where a cell is manipulated while the cell and an operation of a micro structural body are observed in real time 3-dimensionally using the 3-dimensional confocal microscopy apparatus according to the present invention.

#### 35 Reference Signs List

##### **[0030]**

- 1: confocal imaging laser
- 2: objective lens
- 3: focal plane
- 4: optical tweezers laser
- 5: dichroic mirror
- 6: specimen
- 7: lower position
- 8: focal plane
- 9: lower position
- 10: excitation laser light source
- 11: confocal unit
- 12: objective lens
- 13: specimen
- 14: intermediate imaging lens
- 15: intermediate objective lens
- 16: confocal imaging camera
- 17: piezoelectric actuator
- 18: infrared laser light source
- 19: optical tweezers driving optical system
- 20: dichroic mirror

- 21: half mirror
- 22: bright field imaging camera
- 23: image processing process
- 24: cross-sectional image acquisition process
- 25: optical tweezers control process
- 26: columnar cross section
- 27: side surface
- 28: columnar cross section
- 29: side surface
- 30: body
- 31: fixed arm
- 32: movable arm
- 33: columnar projection portion
- 34: cell
- 35: minute structural body
- 36: movable arm
- 37: columnar projection portion
- 38: ring structure
- 39: cell which is object to be manipulated

#### Description of Embodiments

**[0031]** Next, a mode for carrying out the present invention including the best mode for carrying out the present invention is explained.

#### [3-dimensional confocal microscopy apparatus]

**[0032]** A 3-dimensional confocal microscopy apparatus according to the present invention includes the following constitutions (1) to (4).

#### **[0033]**

- (1) A 3-dimensional confocal microscopy apparatus including: a light source for a first laser for confocal imaging excitation; a 2-dimensional scanning optical system for scanning the first laser on a specimen; an objective lens for focusing the first laser supplied from the light source on a fluorescent specimen which is an object to be observed; and a fluorescent light imaging camera for detecting a fluorescent confocal microscopy image based on a fluorescent light emitted from the fluorescent specimen, wherein
- (2) a pair of lenses for focal plane displacement is arranged between the objective lens and the fluorescent light imaging camera, the pair of lenses for focal plane displacement being constituted of an intermediate imaging lens which forms an intermediate image of the fluorescent confocal microscopy image of the specimen and an intermediate objective lens arranged such that a focal point of the intermediate objective lens overlaps with a focal point of the intermediate imaging lens on the same optical axis, and at least one lens out of these lenses being movable in an optical axis direction by a lens drive means,
- (3) the 3-dimensional confocal microscopy apparatus further includes a computer having an arithmetic operation means for acquiring a 3-dimensional struc-

tural image of the specimen by correcting aberration of the fluorescent confocal microscopy image caused by the movement of the lens of the pair of lenses for focal plane displacement in real time using the fluorescent confocal microscopy image acquired by the fluorescent light imaging camera and aberration correction data which is acquired in advance by performing provisional measurement of a sample specimen, and a means which displays a corrected image obtained by the arithmetic operation means, and

(4) a beam splitter or a dichroic mirror is provided between the pair of lenses for focal plane displacement and the objective lens, and a second laser supplied from a light source for a second laser for optical tweezers passes through the objective lens via the beam splitter or the dichroic mirror and is focused on the specimen.

**[0034]** In the three-dimensional confocal microscopy apparatus having the above-mentioned constitution, the objective lens, the intermediate imaging lens and the intermediate objective lens which constitute a pair of lenses for focal plane displacement and, further, other various lenses described later in examples may be constituted from a single lens body, and may be constituted of a plurality of lens bodies which are arranged parallel to each other on the same optical axis and are accommodated in a cylindrical housing.

[First constitutional part of 3-dimensional confocal microscopy apparatus]

**[0035]** In the above-mentioned constitution (1), "2-dimensional scanning optical system" is an optical system for acquiring a thin cross-sectional image of a specimen in the vicinity of a specific depth by 2-dimensionally scanning a focal point of excitation laser beams on the specimen, and known various two-dimensional scanning optical system can be used. However, particularly, a nipkow disc or a line-scanning-type scanning system can be preferably used.

**[0036]** The nipkow disc is, as is well known, a rotary disc on which a large number of pin holes are arranged in a vortex shape, for example, and can acquire a confocal image of a specimen with high-speed scanning performance by multi-beam scanning. However, the use of only such a rotary disc has drawbacks such as an insufficient optical amount or a so-called poor S/N ratio and hence, there has been also known a nipkow disc of an improved type where a micro lens array disc having a large number of micro lenses at positions corresponding to pin holes formed in a nipkow disc is formed into the integral structure with the nipkow disc, and both discs are synchronously rotated. Further, a dichroic mirror may be incorporated between the above-mentioned nipkow disc and micro lens array disc. The "nipkow disc" according to this invention includes all of discs of the above-

mentioned types.

**[0037]** The line-scanning-type scanning system is disclosed in patent literature 5 or the like and is known. The line-scanning-type scanning system is a scanning optical system where an excitation laser beam is scanned in a focused manner in a line shape and, for example, an illumination light focused in a line shape is irradiated to a specimen, the illumination light in a line shape is scanned in the predetermined direction on the specimen, and light generated in an illuminated region on the specimen is detected by an optical detector thus acquiring 2-dimensional image data of the specimen.

**[0038]** The objective lens which focuses the first laser on the specimen which is an object to be observed is a fixed lens. However, a lens which is configured to be movable along an optical axis can achieve such an object when the lens is used without moving the lens at the time of using the lens and hence, such a lens can be also used as the objective lens of the present invention in this context.

[Second constitutional part of 3-dimensional confocal microscopy apparatus]

**[0039]** In the above-mentioned constitution (2), an intermediate imaging lens and an intermediate objective lens which constitute the pairs of lenses for focal plane displacement are arranged such that a focal point of the intermediate imaging lens and a focal point of the intermediate objective lens overlap with each other on the same optical axis, and at least one lens is movable in the optical axis direction by the lens drive means. That is, either one of lenses is fixed and the other lens is made movable or both lenses are made movable. However, when both lenses are made movable, an accurate control of the drive means becomes difficult. Accordingly, it is preferable that either one of lenses is fixed and the other lens is made movable. It is more preferable that the fixed intermediate imaging lens is arranged on an objective lens sides, and the movable intermediate objective lens is arranged on a fluorescent imaging camera side.

**[0040]** Although a kind of the drive means which moves the lens is not limited, it is preferable to adopt a means capable of moving a lens at a high speed under an arcuate control. As such a drive means, for example, any one of a piezoelectric element, a magnetostrictive actuator and a voice coil which are well known is named.

**[0041]** Due to the reason set forth with respect to the fourth invention in the above-mentioned column "advantageous effects of the invention", it is preferable that timing of driving the lens drive means in the pair of lenses for focal plane displacement and timing of acquiring an image by the fluorescent imaging camera are adjusted so as to be synchronous with each other.

[Third constitutional part of 3-dimensional confocal microscopy apparatus]

**[0042]** The meaning of "arithmetic operation for acquiring a 3-dimensional structural image of the specimen" in the above-mentioned constitution (3) is explained in conjunction with Fig. 3 by simplifying the basic constitution of the 3-dimensional confocal microscopy apparatus. In Fig. 3, an uppermost lens expressed as "Objective Lens 3" is a fixed objective lens for focusing a laser for confocal imaging excitation on the specimen, and lenses expressed as "Objective Lens 2" and "Objective Lens 1" arranged below "Objective Lens 3" are respectively the fixed intermediate imaging lens and the movable intermediate objective lens.

**[0043]** When the intermediate objective lens is at the position shown in Fig. 3, focal points of both lenses overlap with each other on a focal plane expressed as "Image 2". In this case, a focal point of the objective lens (Objective Lens 3) agrees with a specimen stage expressed as "Sample". Accordingly, assuming that the specimen is a body having a checkerboard pattern in plane shown on a right side in Fig. 3, an imaged image having a usual size (Image 2) is transmitted to a fluorescent imaging part expressed as "Confocal Unit" on a lowermost side in Fig. 3.

**[0044]** On the other hand, when the intermediate objective lens is moved to the upper position expressed as "Image 1" and approaches the intermediate imaging lens, the focal points of both lenses overlap with each other on a focal plane expressed as "Image 1" and hence, the focal point of the objective lens is shifted to an upper position beyond the specimen stage. Accordingly, the magnification of the lens is enlarged so that an enlarged imaged image which is the body having a checkerboard pattern is transmitted to the fluorescent light imaging part. To the contrary, when the intermediate objective lens is moved to a lower position expressed as "Image 3" so that the intermediate objective lens is moved away from the intermediate imaging lens, the focal points of both lenses overlap with each other on a focal plane expressed as "Image 3" and hence, the focal point of the objective lens is shifted to a lower position which does not reach the specimen stage. Accordingly, a contracted imaged image of the body having a checkerboard pattern is transmitted to the fluorescent imaging part.

**[0045]** In this manner, when the intermediate objective lens is made to approach the intermediate imaging lens or to move away from the intermediate imaging lens to obtain a 3-dimensional structural image of the specimen, the deviation from designed focal lengths of both lenses is generated so that a fluorescent confocal microscopy image including aberration such as enlargement or contraction of the image is generated in the fluorescent imaging camera. Accordingly, it is necessary to acquire an accurate 3-dimensional structural image of the specimen by correcting such an aberration.

**[0046]** Accordingly, by performing the provisional

measurement of a sample specimen such as the above-mentioned body having a checkerboard pattern, for example, the relationship of the degree of aberration such as enlargement or contraction of a fluorescent confocal microscopy image corresponding to a moving direction and a moving distance of the intermediate objective lens is acquired by a computer in advance as aberration correction data, and the aberration of the fluorescent confocal microscopy image based on the displacement of a focal plane in the pair of lenses for focal plane displacement is calculated and is corrected in real time thus accurately acquiring a 3-dimensional structural image of the specimen.

**[0047]** The above-mentioned calculation for acquiring the 3-dimensional structural image of the specimen can be preferably performed using a known GPU. The GPU is an auxiliary arithmetic operation unit or a dedicated circuit which is dedicated to 3-dimensional image processing in the computer.

[Fourth constitutional part of 3-dimensional confocal microscopy apparatus]

**[0048]** The above-mentioned constitution (4) is provided for combining the optical tweezers technique with the confocal microscope. A second laser for optical tweezers is introduced onto an optical path between the pair of lenses for focal plane displacement and the objective lens in the confocal microscope via the beam splitter or the dichroic mirror and is focused on the specimen after passing through the objective lens.

**[0049]** The beam splitter is an optical part which reflects some of incident light and divides a light flux by allowing other portion of the incident light to pass therethrough. The optical part where the intensity of reflection light and the intensity of transmitting light are approximately 1:1 is referred to as a half mirror, and this half mirror is also included in the beam splitter. The dichroic mirror is an optical part which reflects light having a specified wave length and allows lights having other wave lengths to pass therethrough.

[Other constitutional part of 3-dimensional confocal microscopy apparatus]

**[0050]** The 3-dimensional confocal microscopy apparatus can further include, besides the above-mentioned respective constitutional parts, a beam splitter or a dichroic mirror which is arranged between the objective lens and the pair of lenses for focal plane displacement, and an imaging lens and a bright field imaging camera for imaging a 2-dimensional image of the specimen separated by the beam splitter or a dichroic mirror.

[Focal plane scanning and aberration correction unit]

**[0051]** The focal plane scanning and aberration correction unit according to the present invention is consti-

tuted of the above-mentioned second constitutional part and third constitutional part of the 3-dimensional confocal microscopy apparatus.

**[0052]** A representative usage of the focal plane scanning and aberration correction unit is a case where the focal plane scanning and aberration correction unit is incorporated into the 3-dimensional confocal microscopy apparatus manufactured by combining a confocal microscope with an optical tweezers technique. To be more specific, in the 3-dimensional confocal microscopy apparatus which includes the light source for a laser for confocal imaging excitation, the fixed objective lens which focuses the laser supplied from the light source on the fluorescent specimen, and the fluorescent imaging camera which detects a fluorescent confocal microscopy image based on fluorescent light emitted from the specimen, the focal plane scanning and aberration correction unit is arranged between the fixed objective lens and the fluorescent imaging camera.

Examples

**[0053]** Hereinafter, one example of the 3-dimensional confocal microscopy apparatus and the focal plane scanning and aberration correction unit according to the present invention is explained. The technical scope of the present invention is not limited by the following examples.

[Example 1: Constitution, manner of operation and advantageous effects of 3-dimensional confocal microscopy apparatus]

**[0054]** The constitution of the 3-dimensional confocal microscopy apparatus according to this example is shown in Fig. 2. In this 3-dimensional confocal microscopy apparatus, an excitation laser from an excitation laser light source 10 is divided and scanned through a confocal unit 11 of a nipkow disc type (CSU-X made by Yokogawa Electric Corporation), and is focused on a specimen 13 by an objective lens 12 (UPlanSApo100x made by Olympus).

**[0055]** The confocal unit 11 is of a type where a nipkow disc on a right side and a microlens array disc on a left side both shown in the cross-sectional view are formed into the integral structure, and both discs are synchronously rotated. A dichroic mirror is incorporated between the nipkow disc and the microlens array disc. Further, an imaging lens is provided below the dichroic mirror in the drawing.

**[0056]** A fluorescent light emitted from the specimen 13 by the excitation laser passes through a pair of lenses for focal plane displacement (UPlanSApo100x made by Olympus) which is arranged behind the objective lens 12 and is constituted of a fixed intermediate imaging lens 14 and a movable intermediate objective lens 15 which is arranged such that a focal point of the intermediate imaging lens 14 and a focal point of the intermediate ob-

jective lens 15 overlap with each other on the same optical axis, and is focused on a confocal imaging camera 16 (ImagEM: EMCCD camera made by Hamamatsu Photonics) which is a high sensitive fluorescent light imaging camera for detecting a fluorescent confocal microscopy image by an imaging lens of the confocal unit 11.

**[0057]** A piezoelectric actuator 17 (P-721 made by PI-Japan Corporation) is mounted on the intermediate objective lens 15. By driving the intermediate objective lens 15 in the optical axis direction with the actuation of the piezoelectric actuator 17, it is possible to displace an imaging focal plane in the vertical direction on the drawing without moving the objective lens 12 of the confocal microscope. The driving of the piezoelectric actuator 17 is performed at a high speed that approximately 30 slices of cross-sectional images can be acquired within 1 second at intervals of  $1\mu\text{m}$  of the specimen.

**[0058]** An infrared laser irradiated from an infrared laser light source 18 for optical tweezers passes through a pair of scanning mirrors of an optical tweezers driving optical system 19, and is focused on the specimen 13 by a dichroic mirror 20 arranged below the objective lens 12.

**[0059]** Further, a half mirror 21 is arranged below the objective lens 12 and, using this half mirror 21, a non-fluorescent image of the specimen 13 is focused on a bright field imaging camera 22 via the imaging lens thus enabling the observation of a bright field image.

**[0060]** Next, the flow of a process for processing data or the like in this embodiment is explained in conjunction with Fig. 4. The process is mainly constituted of three processes, that is, an image processing process 23, a cross-sectional image acquisition process 24, and an optical tweezers control process 25.

**[0061]** In the image processing process 23, firstly, an observation view point is instructed when an operator manipulates a mouse on a screen. A set of cross-sectional views of the specimen 13 which is sequentially transferred from the cross-sectional image acquisition process 24 and is updated is stored in a memory of a GPU (GTX295 made by NVIDIA) of a computer, pre-calibrated aberration correction calculation is applied to the respective cross-sectional images and, thereafter, a 3-dimensional image is formed from an observation view point instructed by volume rendering, and is displayed on a screen which constitutes a means for displaying a corrected image. A 3-dimensional image formed of  $512 \times 512 \times 512$  pixels can be displayed at a frame rate of 30 frames per second.

**[0062]** In the cross-sectional image acquisition process 24, firstly, the intermediate objective lens 15 is moved to a predetermined position by driving the piezoelectric actuator 17 and, thereafter, a confocal image of the focal plane is acquired by the confocal imaging camera 16, and is stored in the main memory. The confocal image is readily transferred to the set of cross-sectional images on the GPU memory in the image processing process 23. In this example, since the frame rate of the confocal imaging camera 16 is 30 frames per second, when the

specimen 13 is observed in cross sections at 10 layers, for example, a 3-dimensional image of the specimen 13 is, as a whole, updated 3 times for every second. Further, the confocal image in the main memory is recorded in a hard disc together with focal depth information and acquisition time information, and can be used for an analysis after an experiment is finished.

**[0063]** In the optical tweezers control process 25, firstly, a position of the optical tweezers is instructed by a manipulator using a 3-dimensional mouse on a screen, a scanning amount of the optical tweezers driving optical system 19 is calculated based on the instruction, and a focal point of the optical tweezers is moved.

**[0064]** Through the above-mentioned three processes, by freely manipulating an optical driving nano machine in a 3-dimensional space, positions and the deformation of the machine and an object to be manipulated can be 3-dimensionally observed in real time from an arbitrary direction.

[Example 2: Use of 3-dimensional confocal microscopy apparatus]

**[0065]** Fig. 5 shows a mode where a fluorescent columnar structural body having a diameter of  $3\mu\text{m}$  is subjected to the confocal observation using the 3-dimensional confocal microscopy apparatus of the example 1. When the above-mentioned aberration correction is not performed, the magnification of the lens is increased corresponding to the movement of a focal plane upward from a bottom surface of a specimen and hence, a columnar cross section 26 of the specimen becomes larger than an actual size so that a side surface 27 of the columnar structural body also appears in an inverse trapezoidal shape. However, when the real time correction is performed using the GPU, both a columnar cross section 28 and a side surface 29 of the columnar structural body can acquire a three dimensional image of an actual size.

**[0066]** Next, the driving of a minute structural body prepared by a two-photon absorption stereolithography and a manipulation of a cell are performed using the 3-dimensional confocal microscopy apparatus of the example 1. Fig. 6 is a 2-dimensional image of the minute structural body imaged by the bright field imaging camera 22. The structural body is constituted of a body 30, a fixed arm 31, a movable arm 32 which is operable to approach the fixed arm 31 or is movable away from the fixed arm 31, and a columnar projection portion 33 for capturing the movable arm 32 by optical tweezers. By capturing the body 30 and the columnar projection portion 33 by the optical tweezers and by driving the movable arm 32, a cell 34 can be manipulated. A mode where the movable arm 32 is driven by the optical tweezers is shown in sequence photographs shown in Fig. 7.

**[0067]** Further, Fig. 8 shows a 3-dimensional image where a minute structural body 35 and a columnar projection portion 37 having a diameter of  $1\mu\text{m}$  formed on a movable arm 36 are captured by optical tweezers, and

an attempt is made to grasp a cell 39 while driving the movable arm 36. The columnar projection portion 37 and the ring structure 38 of a mounting portion of the movable arm 36 are clearly observed so that it is safe to say that the resolution of sub micro order is obtained.

**[0068]** A specimen can be observed only in plane in the case of the conventional system and hence, it is difficult to grasp the vertical positional relationship between a cell and a minute structural body driven by optical tweezers so that the manipulation requires skills. By using the 3-dimensional confocal microscopy apparatus of the present invention, the 3-dimensional positional relationship between a minute structural body and a cell can be grasped at a glance so that the manipulability of the 3-dimensional confocal microscopy apparatus is greatly enhanced. In this manner, with the use of the 3-dimensional confocal microscopy apparatus of the present invention, the 3-dimensional confocal observation can be realized at an arbitrary view point simultaneously with the manipulation of an object to be observed such as a cell by driving a minute structural body using optical tweezers.

#### Industrial Applicability

**[0069]** According to the present invention, a 3-dimensional image of a specimen can be acquired during the manipulation of the specimen using optical tweezers without affecting an optical trap. Further, the accuracy of the 3-dimensional image of the specimen acquired in this manner can be also ensured.

#### Claims

1. A 3-dimensional confocal microscopy apparatus comprising: a light source for a first laser (1, 10) for confocal imaging excitation; a 2-dimensional scanning optical system for scanning the first laser on a specimen; an objective lens (2, 12) for focusing the first laser supplied from the light source on a fluorescent specimen which is an object to be observed; a fluorescent light imaging camera (16) for detecting a fluorescent confocal microscopy image based on a fluorescent light emitted from the fluorescent specimen; a pair of lenses (14, 15); lens drive means (17); a beam splitter or a dichroic mirror (20) and a light source (18) for a second laser; wherein the pair of lenses (14, 15) for focal plane displacement is arranged between the objective lens and the fluorescent light imaging camera, the pair of lenses for focal plane displacement being constituted of an intermediate imaging lens (14) which forms an intermediate image of the fluorescent confocal microscopy image of the specimen and an intermediate objective lens (15) arranged such that a focal point of the intermediate objective lens overlaps with a focal point of the intermediate imaging lens on the same

optical axis, and at least one lens out of these lenses being movable in an optical axis direction by the lens drive means (17),

the 3-dimensional confocal microscopy apparatus further includes a computer having an arithmetic operation means for acquiring a 3-dimensional structural image of the specimen by correcting aberration of the fluorescent confocal microscopy image caused by the movement of the lens of the pair of lenses for focal plane displacement in real time using the fluorescent confocal microscopy image acquired by the fluorescent light imaging camera and aberration correction data which is acquired in advance by performing provisional measurement of a sample specimen, and a means which displays a corrected image obtained by the arithmetic operation means, wherein

the beam splitter or a dichroic mirror (20) is provided between the pair of lenses for focal plane displacement and the objective lens, and the second laser supplied from the light source (18) for a second laser for optical tweezers passes through the objective lens via the beam splitter or the dichroic mirror and is focused on the specimen.

2. The 3-dimensional confocal microscopy apparatus according to claim 1, wherein the 2-dimensional scanning optical system includes a nipkow disc or a line scanning type scanning system.
3. The 3-dimensional confocal microscopy apparatus according to claim 1 or 2, wherein the lens drive means for the pair of lenses for focal plane displacement is one selected from a group consisting of a piezoelectric element, a magnetostrictive actuator and a voice coil.
4. The 3-dimensional confocal microscopy apparatus according to any one of claims 1 to 3, wherein timing for driving the lens drive means for the pair of lenses for focal plane displacement and timing for acquiring an image by a fluorescent imaging camera are adjusted so as to be synchronous with each other.
5. The 3-dimensional confocal microscopy apparatus according to any one of claims 1 to 4, wherein an arithmetic operation using an arithmetic operation means for acquiring a 3-dimensional structural image of a specimen is performed by a Graphics Processing Unit (GPU).
6. The 3-dimensional confocal microscopy apparatus according to any one of claims 1 to 5, wherein the 3-dimensional confocal microscopy apparatus further comprises a beam splitter or a dichroic mirror which is arranged between the objective lens and the pair of lenses for focal plane displacement, and an imaging lens and a bright field imaging camera

for imaging a 2-dimensional image of a specimen separated by the beam splitter or the dichroic mirror.

7. A focal plane scanning and aberration correction unit to be incorporated into a 3-dimensional confocal microscopy apparatus comprises lens drive means (17) and a pair of lenses (14, 15) for focal plane displacement, the pair of lenses for focal plane displacement being constituted of an intermediate imaging lens (14) which forms an intermediate image of a fluorescent confocal microscopy image of a specimen and an intermediate objective lens (15) arranged such that a focal point of the intermediate objective lens overlaps with a focal point of the intermediate imaging lens on the same optical axis, and at least one lens out of these lenses being movable in an optical axis direction by the lens drive means, and the focal plane scanning and aberration correction unit further comprises a computer having an arithmetic operation means for acquiring a 3-dimensional structural image of the specimen by correcting aberration of the fluorescent confocal microscopy image caused by the movement of the lens of the pair of lenses for focal plane displacement in real time using the fluorescent confocal microscopy image acquired by the fluorescent light imaging camera and aberration correction data which is acquired in advance by performing provisional measurement of a sample specimen, and a means which displays a corrected image obtained by the arithmetic operation means, wherein the 3-dimensional confocal microscopy apparatus comprises a light source for a laser (10); an objective lens (12) for focusing the laser supplied from the light source on a fluorescent specimen; and a fluorescent light imaging camera (16) for detecting a fluorescent confocal microscopy image based on a fluorescent light emitted from the fluorescent specimen, and wherein the focal plane scanning and aberration correction unit is to be arranged between the objective lens and the fluorescent light imaging camera.

#### Patentansprüche

1. Dreidimensionale, konfokale Mikroskopvorrichtung, aufweisend: eine Lichtquelle für einen ersten Laser für eine konfokale Bilderregung; ein zweidimensionales optisches Abtastsystem zum Abtasten eines ersten Musters durch den ersten Laser; eine Objektivlinse (2, 12) zum Fokussieren des ersten Lasers, der von der ersten Lichtquelle auf einem fluoreszierenden Muster angewendet wird, bei dem es sich um ein zu betrachtendes Objekt handelt; eine Fluoreszenzlicht-Bildkamera (16) zum Erfassen eines fluoreszenten, konfokalen Mikroskopbilds auf Basis

eines fluoreszierenden Lichts, das von dem fluoreszierenden Muster emittiert wird; ein Paar Linsen (14, 15); eine Linsenantriebseinrichtung (17); einen Strahlteiler oder einen dichromatischen Spiegel (20) und eine Lichtquelle (18) für einen zweiten Laser; wobei

das Paar Linsen (14, 15) für die Brennebenenjustage zwischen der Objektivlinse und der Fluoreszenzlicht-Bildkamera angeordnet ist, wobei das Paar Linsen für die Brennebenenjustage aus einer Zwischenbildlinse (14), die ein Zwischenbild des fluoreszenten, konfokalen Mikroskopbilds des Musters bildet, und einer Zwischenobjektivlinse (15), die so angeordnet ist, dass sich ein Fokuspunkt der Zwischenobjektivlinse mit einem Fokuspunkt der Zwischenbildlinse auf derselben optischen Achse überschneidet, und wobei zumindest eine dieser Linsen, die in einer Richtung der optischen Achse durch die Linsenantriebseinrichtung (17) bewegt werden kann, besteht,

wobei die dreidimensionale, konfokale Mikroskopvorrichtung ferner einen Rechner beinhaltet, der eine arithmetische Operationseinrichtung zum Erhalten eines dreidimensionalen Strukturbilds des Musters durch das Korrigieren von Bildfehlern des fluoreszenten, konfokalen Mikroskopbilds, die durch das Bewegen der Linse des Paares Linsen für die Brennebenenjustage entstehen, in Echtzeit unter Verwendung des fluoreszierenden, konfokalen Mikroskopbilds, das durch die Fluoreszenzlicht-Bildkamera erhalten wird, und von Bildfehler-Korrekturdaten, die im Voraus durch Durchführen einer vorläufigen Messung eines Beispielmusters erhalten werden, und eine Einrichtung, die ein korrigiertes Bild darstellt, das durch die arithmetische Operationseinrichtung erhalten wurde, aufweist, und wobei der Strahlteiler oder ein dichromatischer Spiegel (20) zwischen dem Paar Linsen zur Brennebenenjustage und der Objektivlinse angeordnet ist, und der zweite Laser, der von der Lichtquelle (18) für einen zweiten Laser für optische Pinzetten gespeist wird, über den Strahlteiler oder den dichromatischen Spiegel durch die Objektivlinse dringt und auf das Muster fokussiert wird.

2. Dreidimensionale, konfokale Mikroskopvorrichtung nach Anspruch 1, wobei das zweidimensionale optische Abtastsystem eine Nipkow-Scheibe oder ein Zeilenabtastsystem beinhaltet.

3. Dreidimensionale, konfokale Mikroskopvorrichtung nach Anspruch 1 oder 2, wobei die Linsenantriebseinrichtung für das Paar Linsen für die Brennebenenjustage eine ist, die aus einer Gruppe ausgewählt wird, die aus einem piezoelektrischen Element, einem magnetostriktiven Aktor und einer Schwingspule besteht.

4. Dreidimensionale, konfokale Mikroskopvorrichtung

nach einem der Ansprüche 1 bis 3, wobei der Zeitpunkt für das Antreiben der Linsenantriebseinrichtung für das Paar Linsen für die Brennebenenjustage und der Zeitpunkt für das Erhalten eines Bilds durch eine Fluoreszenz-Bildkamera so eingestellt werden, dass sie zueinander synchron sind.

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5. Dreidimensionale, konfokale Mikroskopvorrichtung nach einem der Ansprüche 1 bis 4, wobei eine arithmetische Operation unter Verwendung einer arithmetischen Operationseinrichtung zum Erhalten eines dreidimensionalen Strukturbilds eines Musters von einer Grafikverarbeitungseinheit (GPU) durchgeführt wird.

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6. Dreidimensionale, konfokale Mikroskopvorrichtung nach einem der Ansprüche 1 bis 5, wobei die dreidimensionale, konfokale Mikroskopvorrichtung ferner einen Strahlteiler oder einen dichromatischen Spiegel, der sich zwischen der Objektivlinse und dem Paar Linsen für die Brennebenenjustage befindet, und eine Bildlinse und eine Helffeld-Bildkamera zum Aufzeichnen eines zweidimensionalen Bilds eines Musters, das durch den Strahlteiler oder den dichromatischen Spiegel geteilt wird, aufweist.

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7. Brennebenen-Abtast- und Bildfehler-Korrekturereinheit, die in eine dreidimensionale, konfokale Mikroskopvorrichtung integriert ist, und die eine Linsenantriebseinrichtung (17) und Folgendes aufweist:

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ein Paar Linsen (14, 15) für die Brennebenenjustage, wobei das Paar Linsen für die Brennebenenjustage aus einer Zwischenbildlinse (14), die ein Zwischenbild eines fluoreszenten, konfokalen Mikroskopbilds eines Musters bildet, und einer Zwischenobjektivlinse (15), die so angeordnet ist, dass sich ein Fokuspunkt der Zwischenobjektivlinse mit einem Fokuspunkt der Zwischenbildlinse auf derselben optischen Achse überschneidet, und wobei zumindest eine dieser Linsen in einer Richtung der optischen Achse durch die Linsenantriebseinrichtung bewegt werden kann, besteht, und  
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wobei die Brennebenen-Abtast- und Bildfehler-Korrekturereinheit ferner einen Rechner aufweist, der eine arithmetische Operationseinrichtung zum Erhalten eines dreidimensionalen Strukturbilds des Musters durch das Korrigieren von Bildfehlern des fluoreszenten, konfokalen Mikroskopbilds in Echtzeit unter Verwendung des fluoreszierenden, konfokalen Mikroskopbilds, das durch die Fluoreszenzlicht-Bildkamera erhalten wird, und von Bildfehler-Korrekturdaten, die im Voraus durch Durchführen einer vorläufigen Messung eines Beispielmusters erhalten werden,  
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und einer Einrichtung, die ein korrigiertes Bild

darstellt, das durch die arithmetische Operationseinrichtung erhalten wurde, aufweist, wobei die Bildfehler durch das Bewegen der Linse des Paares Linsen für die Brennebenenjustage entstehen,

wobei die dreidimensionale, konfokale Mikroskopvorrichtung eine Lichtquelle für einen Laser (10); eine Objektivlinse (12) zum Fokussieren des Lasers, der von der Lichtquelle auf ein fluoreszierendes Muster ausgesendet wird; und eine Fluoreszenzlicht-Bildkamera (16) zum Erfassen eines fluoreszierenden, konfokalen Mikroskopbilds auf Basis eines fluoreszierenden Licht, das von dem fluoreszierenden Muster emittiert wird, aufweist, und wobei die Brennebenen-Abtast- und Bildfehler-Korrekturereinheit zwischen der Objektivlinse und der Fluoreszenzlicht-Bildkamera angeordnet ist.

## Revendications

1. Appareil de microscopie confocale tridimensionnelle comprenant : une source de lumière pour un premier laser (1, 10) pour l'excitation d'imagerie confocale; un système optique de balayage bidimensionnel pour balayer le premier laser sur un spécimen ; une lentille d'objectif (2, 12) pour focaliser le premier laser fourni par la source de lumière sur un spécimen fluorescent qui est un objet devant être observé ; une caméra d'imagerie de fluorescence (16) pour détecter une image de microscopie confocale fluorescente sur la base d'une lumière fluorescente émise par le spécimen fluorescent ; une paire de lentilles (14, 15), des moyens d'entraînement de lentilles (17) ; un séparateur de faisceau ou un miroir dichroïque (20) et une source de lumière (18) pour un second laser ; dans lequel  
la paire de lentilles (14, 15) pour le déplacement de plan focal est agencée entre la lentille d'objectif et la caméra d'imagerie de fluorescence, la paire de lentilles pour le déplacement de plan focal étant constituée d'une lentille d'imagerie intermédiaire (14) qui forme une image intermédiaire de l'image de microscopie confocale fluorescente du spécimen et une lentille d'objectif intermédiaire (15) agencée de telle sorte qu'un point focal de la lentille d'objectif intermédiaire chevauche un point focal de la lentille d'imagerie intermédiaire sur le même axe optique, et au moins une lentille parmi ces lentilles étant mobile dans une direction d'axe optique grâce aux moyens d'entraînement de lentilles (17),  
l'appareil de microscopie confocale tridimensionnelle inclut en outre un ordinateur ayant des moyens d'opération arithmétique pour acquérir une image structurelle tridimensionnelle du spécimen en corrigeant l'aberration de l'image de microscopie confocale fluorescente causée par le mouvement de la

- lentille de la paire de lentilles pour le déplacement de plan focal en temps réel au moyen de l'image de microscopie confocale fluorescente acquise par la caméra d'imagerie de fluorescence et des données de correction d'aberration qui sont acquises à l'avance en effectuant une mesure provisoire d'un spécimen d'échantillon, et des moyens qui affichent une image corrigée par les moyens d'opération arithmétique, dans lequel le séparateur de faisceau ou un miroir dichroïque (20) est prévu entre la paire de lentilles pour le déplacement de plan focal et la lentille d'objectif, et le second laser fourni par la source de lumière (18) pour un second laser pour pinces optiques traverse la lentille d'objectif via le séparateur de faisceau ou le miroir dichroïque et est focalisé sur le spécimen.
2. Appareil de microscopie confocale tridimensionnelle selon la revendication 1, dans lequel le système optique de balayage bidimensionnel inclut une roue de Nipkow ou un système de balayage de type à balayage linéaire.
  3. Appareil de microscopie confocale tridimensionnelle selon la revendication 1 ou 2, dans lequel les moyens d'entraînement de lentilles pour la paire de lentilles pour le déplacement de plan focal sont ceux sélectionnés dans un groupe constitué d'un élément piézoélectrique, d'un actionneur magnétostrictif et d'une bobine acoustique.
  4. Appareil de microscopie confocale tridimensionnelle selon l'une quelconque des revendications 1 à 3, dans lequel le temps pour entraîner les moyens d'entraînement de lentilles pour la paire de lentilles pour le déplacement de plan focal et le temps d'acquisition d'une image par une caméra d'imagerie fluorescente sont ajustés de sorte à être synchrones l'un avec l'autre.
  5. Appareil de microscopie confocale tridimensionnelle selon l'une quelconque des revendications 1 à 4, dans lequel une opération arithmétique utilisant des moyens d'opération arithmétique pour acquérir une image structurelle tridimensionnelle d'un spécimen est effectuée par une unité de traitement graphique (Graphics Processing Unit-GPU).
  6. Appareil de microscopie confocale tridimensionnelle selon l'une quelconque des revendications 1 à 5, dans lequel l'appareil de microscopie confocale tridimensionnelle comprend en outre un séparateur de faisceau ou un miroir dichroïque qui est agencé entre la lentille d'objectif et la paire de lentilles pour le déplacement de plan focal, et une lentille d'imagerie et une caméra d'imagerie en champ clair pour imager une image bidimensionnelle d'un spécimen séparé par le séparateur de faisceau ou le miroir dichroïque.
  7. Unité de balayage de plan focal et de correction d'aberration devant être incorporée dans un appareil de microscopie confocale tridimensionnelle comprenant des moyens d'entraînement de lentilles (17) et une paire de lentilles (14, 15) pour le déplacement de plan focal, la paire de lentilles pour le déplacement de plan focal étant constituée d'une lentille d'imagerie intermédiaire (14) qui forme une image intermédiaire d'une image de microscopie confocale fluorescente d'un spécimen et d'une lentille d'objectif intermédiaire (15) agencée de telle sorte qu'un point focal de la lentille d'objectif intermédiaire chevauche un point focal de la lentille d'imagerie intermédiaire sur le même axe optique, et au moins une lentille parmi ces lentilles étant mobile dans une direction d'axe optique grâce aux moyens d'entraînement de lentilles, et l'unité de balayage de plan focal et de correction d'aberration comprend en outre un ordinateur ayant des moyens d'opération arithmétique pour acquérir une image structurelle tridimensionnelle du spécimen en corrigeant l'aberration de l'image de microscopie confocale fluorescente causée par le mouvement de la lentille de la paire de lentilles pour le déplacement de plan confocal en temps réel utilisant l'image de microscopie confocale fluorescente acquise par la caméra d'imagerie de lumière fluorescente et les données de correction d'aberration qui sont acquises à l'avance en exécutant une mesure provisoire d'un spécimen échantillon, et des moyens qui affichent une image corrigée obtenue par les moyens d'opération arithmétique, dans lequel l'appareil de microscopie confocale tridimensionnelle comprend une source de lumière pour un laser (10) ; une lentille d'objectif (12) pour focaliser le laser fourni par la source de lumière sur un spécimen fluorescent ; et une caméra d'imagerie de fluorescence (16) pour détecter une image de microscopie confocale fluorescente sur la base d'une lumière fluorescente émise par le spécimen fluorescent, et dans lequel l'unité de balayage de plan focal et de correction d'aberration doit être agencée entre la lentille d'objectif et la caméra d'imagerie de fluorescence.

Fig. 1

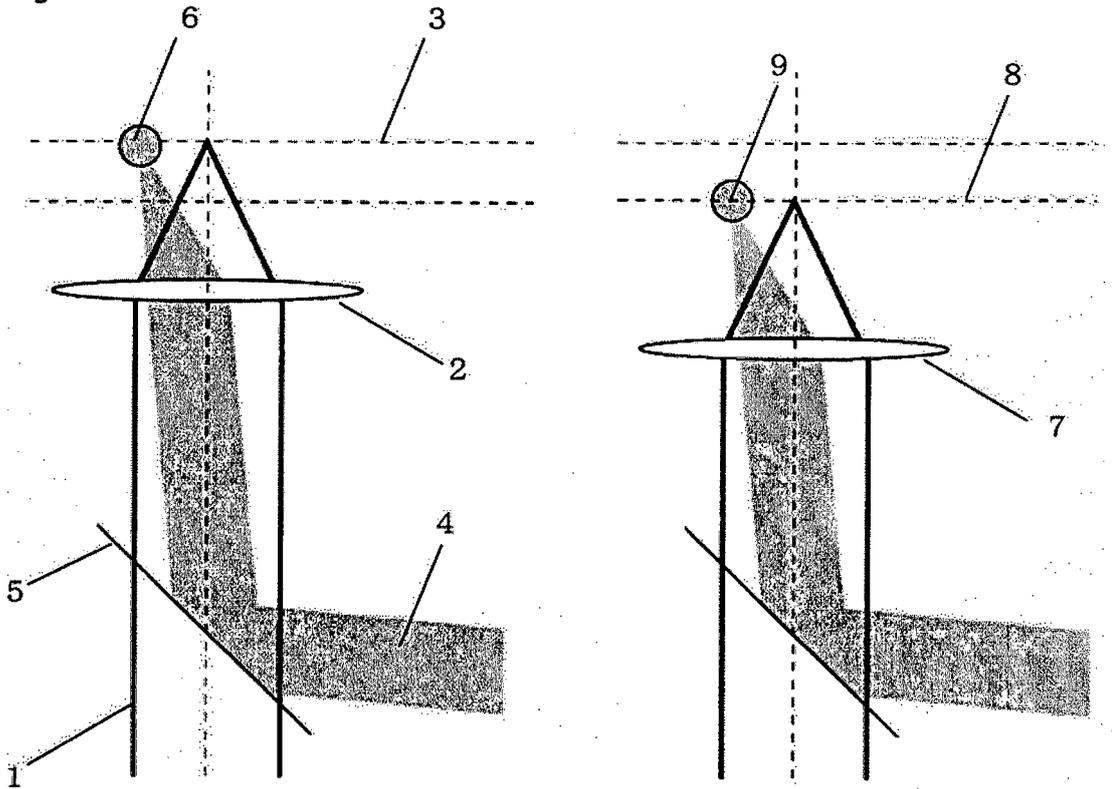


Fig. 2

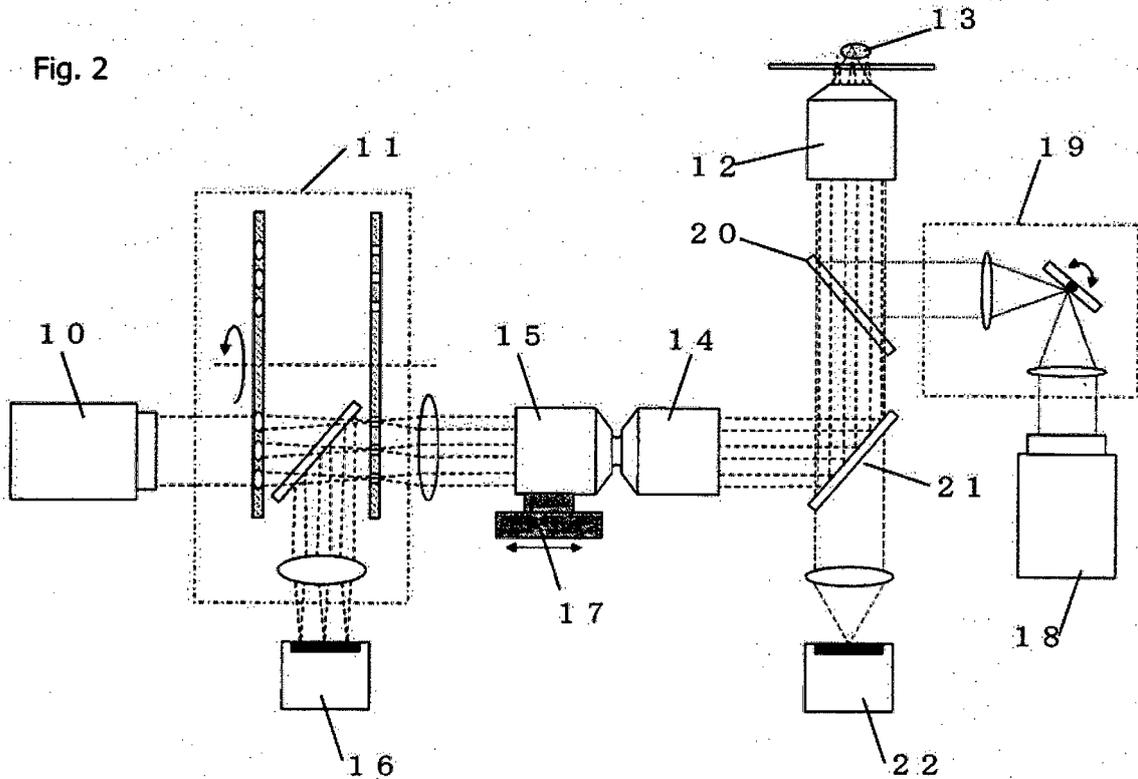


Fig. 3

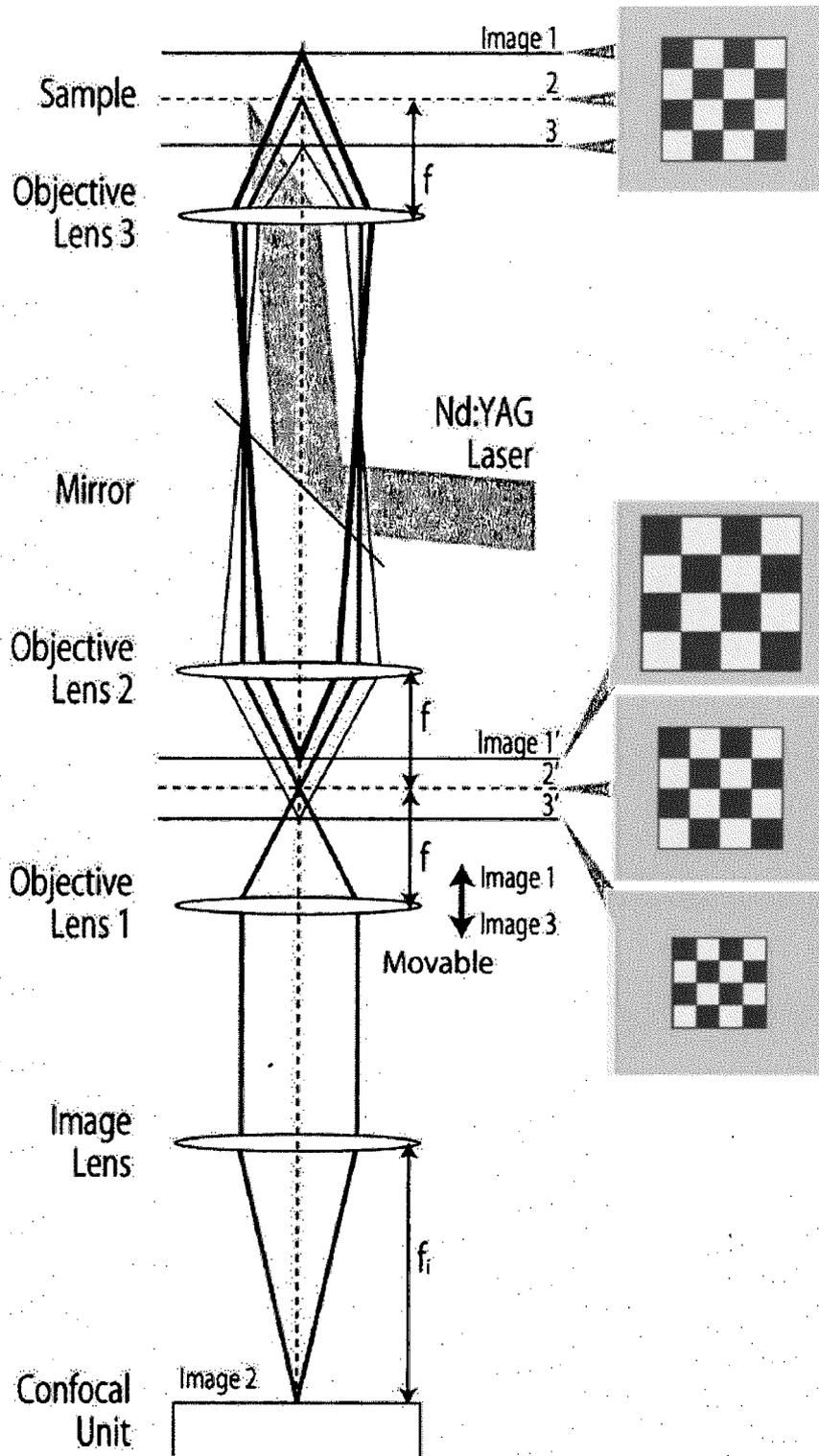


Fig. 4

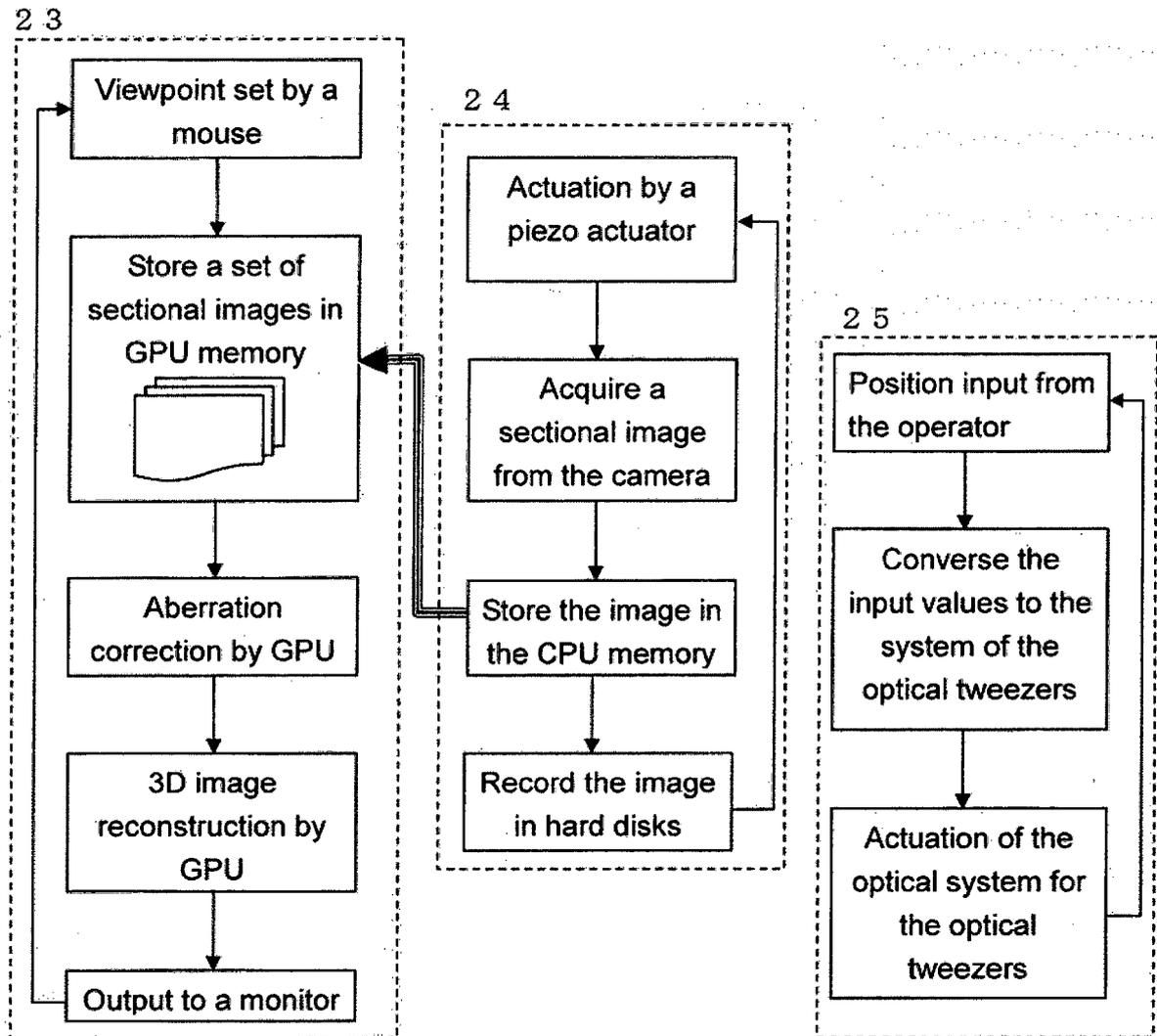


Fig. 5

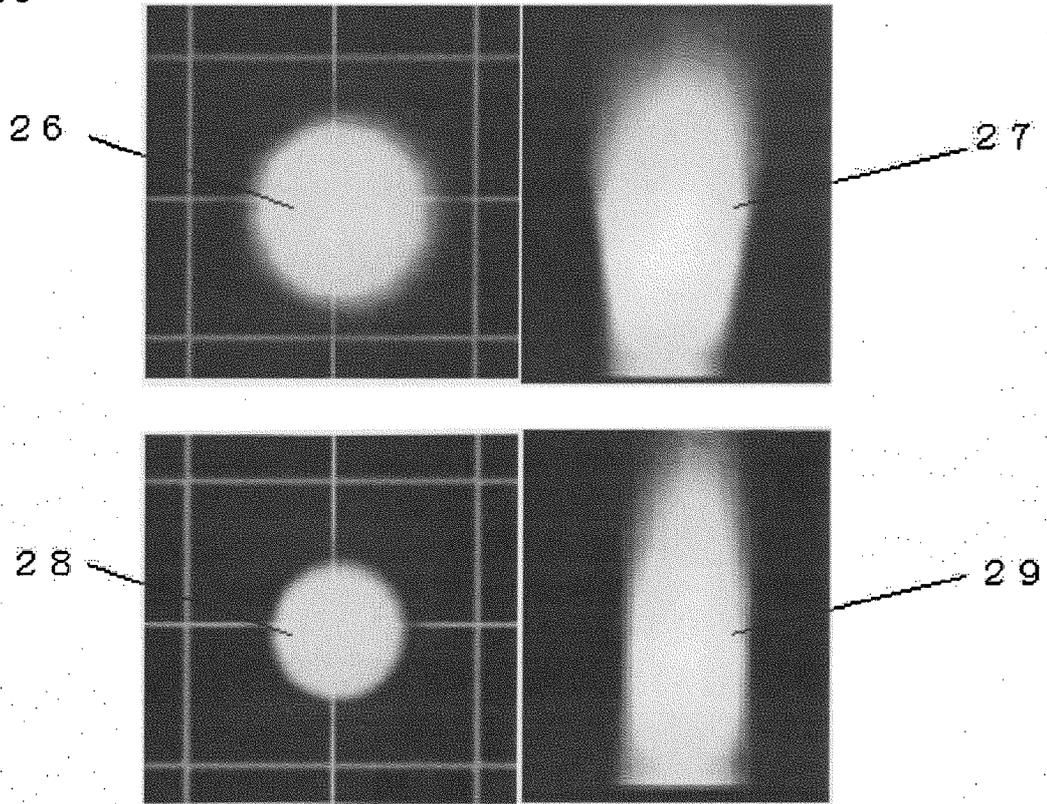


Fig. 6

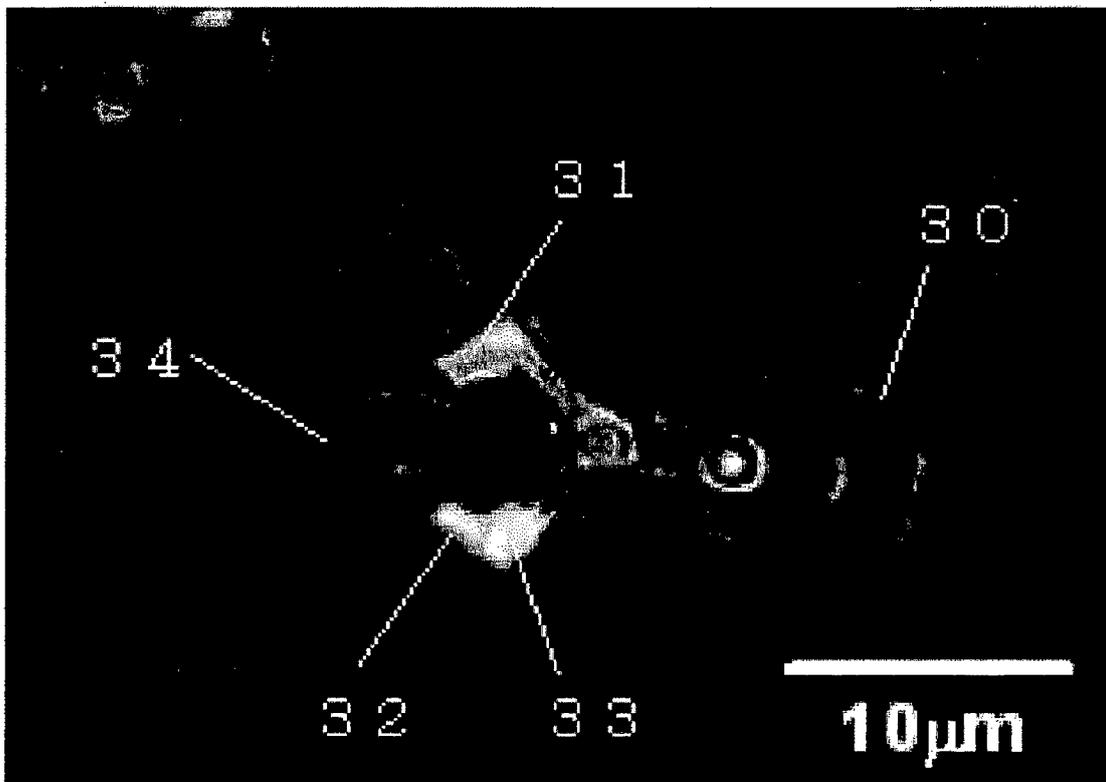
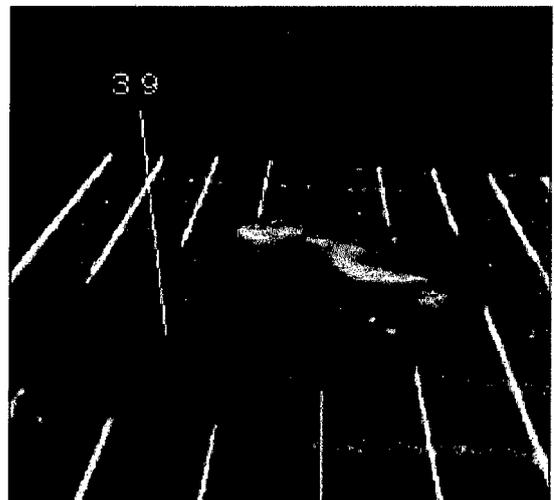
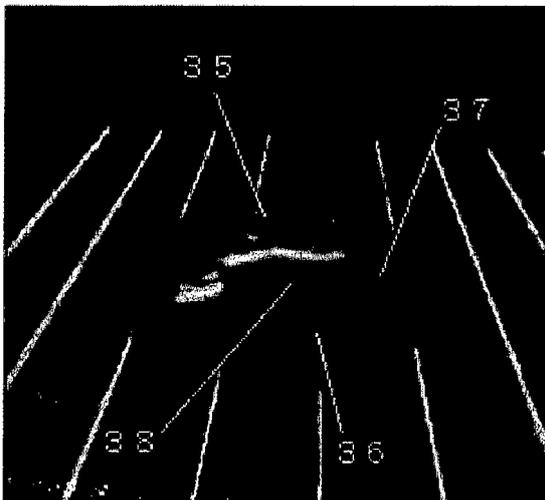


Fig. 7



Fig. 8



**REFERENCES CITED IN THE DESCRIPTION**

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