

Description

Title of Invention: FOCUSING DEVICE, FOCUSING METHOD, FOCUSING PROGRAM AND MICROSCOPE

Technical Field

[0001] The present invention relates to a focusing device, a focusing method, a focusing program and a microscope, and is suitable when observing a tissue section, for example.

Background Art

[0002] In a pathological diagnosis, a tissue section is fixed to a glass slide and a preparation is fabricated through a staining process and a sealing process. In general, when a storage term of the preparation becomes long, visibility of the preparation with the microscope deteriorates due to deterioration and color failure of a biological sample. Although there is a case in which a microscopic examination is performed to the preparation in facilities other than the facilities such as a hospital at which this is fabricated, the preparation is delivered by post in general and it takes a certain time.

[0003] In view of such circumstances, a device of saving the biological sample as image data is proposed (for example, refer to Patent Literature 1). In this device, focusing technology to focus on the biological sample based on contrast of the imaged image is adopted.

Citation List

Patent Literature

[0004] PTL 1: Japanese Patent Application Laid-Open No. 2009-175334

Summary of Invention

Technical Problem

[0005] The biological sample has a thickness. Therefore, in the above focusing technique, a process to shift a focal point at predetermined intervals in a depth direction of the biological sample, thereby searching an optimal focal position based on the contrast of the imaged image at the focal points is necessary.

[0006] For example, when the thickness of the biological sample on the preparation is 10 [mm] and a depth of field of an optical lens to condense light at the biological sample is 1 [mm], a process to search the optimal focal position from approximately 50 imaged images is necessary.

[0007] This takes considerable time until focusing, and efficiency of obtaining the biological sample as the image data significantly lowers.

[0008] In light of the foregoing, it is desirable to propose the focusing device, the focusing

method, the focusing program and the microscope capable of improving the efficiency of obtaining a subject image.

Solution to Problem

- [0009] In order to solve such an issue, according to the present invention, there is provided a focusing device, including an imaging device provided with a predetermined image forming surface predetermined separate from a main image forming surface of an object lens on which phase contrast images of an image formed on the main image forming surface are formed as an imaging surface, obtaining means for obtaining a distance between one image and the other image of the phase contrast images in a pixel unit from the imaging device, and determining means for determining a position on which a focus should be adjusted and the number of positions based on the distance of each pixel.
- [0010] Further, according to the present invention, there is provided a focusing method, including the steps of: obtaining a distance between one image and the other image of phase contrast images from an imaging device provided with a predetermined image forming surface predetermined separate from a main image forming surface of an object lens on which the phase contrast images of an image formed on the main image forming surface are formed as an imaging surface in a pixel unit; and determining a position on which a focus should be adjusted and the number of positions based on the distance of each pixel obtained in the obtaining step.
- [0011] Further, according to the present invention, there is provided a focusing program for causing a computer to execute obtaining a distance between one image and the other image of phase contrast images from an imaging device provided with a predetermined image forming surface predetermined separate from a main image forming surface of an object lens on which the phase contrast images of an image formed on the main image forming surface are formed as an imaging surface in a pixel unit, and determining a position on which a focus should be adjusted and the number of positions based on the obtained distance of each pixel.
- [0012] Further, according to the present invention, there is provided a microscope including a half mirror to divide light entering from an object lens into transmitted light and reflected light, a first imaging device for imaging a subject image formed on the object lens and projected on one of a transmission side and a reflection side of the half mirror, a field lens for relaying the subject image projected on the other of the transmission side and the reflection side of the half mirror backward, openings provided behind the field lens in a unit of pair and having sizes with which depth of field is larger than depth of field of the object lens, separator lenses provided behind the openings, respectively, for forming phase contrast images on a predetermined image forming

surface to which the field lens relays, a second imaging device provided with the pre-determined image forming surface as an imaging surface, and determining means for obtaining a distance between one image and the other image of the phase contrast images from the second imaging device in a unit of pixel and determining a position on which a focus should be adjusted and the number of positions based on the distance of each pixel.

Advantageous Effects of Invention

- [0013] The distance for each pixel between one image and the other image of the phase contrast images corresponds to information indicating irregularity of an imaging area (area appearing on the image forming surface of the object lens). Since the depths of field of the separator lenses are made larger than that of the object lens by the openings in the present invention, detailed irregularity of the subject may be recognized before focusing from the phase contrast images. Furthermore, the detailed irregularity of the subject may be recognized before focusing and by one imaging.
- [0014] Therefore, it is possible to appropriately determine the focusing position at which an entire subject appearing on the image forming surface of the object lens becomes sharp and the number thereof in consideration of the irregularity of the subject and the area according to the depth of field of the object lens in the present invention.
- [0015] As a result, the focal position may be determined at high speed and without loss according to the subject in the imaging area as compared to a method of imaging while shifting the focal point at predetermined intervals in the depth direction of the tissue section and searching the focal position based on the contrast of the imaged image. Therefore, the focusing device, the focusing method, the focusing program and the microscope capable of improving the efficiency of obtaining the subject image may be realized.

Brief Description of Drawings

- [0016] [fig.1] Fig. 1 is a schematic diagram illustrating a configuration of a microscope.
- [fig.2] Fig. 2 is a picture illustrating a subject image and phase contrast images of a tissue section.
- [fig.3] Fig. 3 is a schematic diagram illustrating irregularity of the tissue section.
- [fig.4] Fig. 4 is a schematic diagram illustrating disparity for each pixel between one image and the other image of the phase contrast images.
- [fig.5A] Fig. 5A is a schematic diagram used when illustrating determination of a focal position in a planar portion and in an inclined portion.
- [fig.5B] Fig. 5B is a schematic diagram used when illustrating determination of a focal position in a planar portion and in an inclined portion.
- [fig.6] Fig. 6 is a schematic diagram used when illustrating the determination of the

focal position according to a depth of field of an object lens.

[fig.7]Fig. 7 is a flow chart illustrating a focusing position determination procedure.

[fig.8]Fig. 8 is a schematic diagram used when illustrating an example of change of the depth of field.

[fig.9]Fig. 9 is a schematic diagram used when illustrating an example of dividing an imaging area in another embodiment.

Description of Embodiments

[0017] An embodiment for carrying out the invention is hereinafter described. Meanwhile, it will be described in the following order.

<1. Embodiment>

(1-1. Configuration of microscope)

(1-2. Configuration of focusing device)

(1-3. Focusing position determination procedure)

(1-4. Effect and the like)

<2. Another embodiment>

[0018] <1. Embodiment>

(1-1. Configuration of biological sample image obtaining device)

Fig. 1 illustrates a microscope 1 according to one embodiment of the present invention. The microscope 1 has a plate-shaped stage 11 on which a preparation PRT is arranged.

[0019] The preparation PRT is obtained by fixing a section of tissue such as connective tissue such as blood, epithelial tissue or both of them on a glass slide SG using a predetermined fixing method and sealing the same, and the tissue section is stained where appropriate. Staining includes not only the staining referred to as popular staining as represented by hematoxylin-eosin (HE) staining, Giemsa staining, Papanicolaou staining and the like, but also the staining referred to as special staining such as fluorescence in-situ hybridization (FISH) and immunoenzymatic technique.

[0020] A stage drive mechanism 12 is provided in the stage 11. The stage drive mechanism 12 is configured to drive the stage 11 in a direction parallel to a stage surface (X-Y axis direction) and in a direction orthogonal thereto (Z-axis direction). In general, an arresting unit (not illustrated) to arrest the preparation PRT at a fixed position is provided on the stage surface on a side on which the preparation PRT is arranged (hereinafter, also referred to as a preparation arranging surface).

[0021] A light source 13 is arranged on a side opposite to the preparation arranging surface of the stage 11. The light source 13 is configured to be able to apply light to illuminate the tissue section to which the popular staining is applied (hereinafter, also referred to as bright field illumination light) and light to illuminate the tissue section to which the

special staining is applied (hereinafter, also referred to as dark field illumination light) by switching them. However, it is also possible that only one of the bright field illumination light and the dark field illumination light may be applied. A condenser lens 14 of which light axis coincides with a normal to a standard position on the preparation arranging surface is arranged between the light source 13 and the stage 11.

- [0022] An object lens 15 of which light axis coincides with the normal to the standard position on the stage surface is arranged on the preparation arranging surface side of the stage 11. The object lens 15 is selected by a lens switch mechanism or manually from a plurality of object lenses with different magnification ratios. An imaging device 16 of which imaging surface is a main image forming surface of the object lens 15 is arranged behind the object lens 15.
- [0023] As control systems of the microscope 1, a stage drive controller 21, a lighting controller 22 and an imaging controller 23 are connected to the stage drive mechanism 12, the light source 13 and the imaging device 16, respectively, through data communication paths.
- [0024] The control system is a computer including a central processing unit (CPU), a read only memory (ROM), a random access memory (RAM) being a work memory of the CPU, an arithmetic circuit and the like.
- [0025] The stage drive controller 21 drive-controls the stage drive mechanism 12 to move (scan) the stage 11 in a stage surface direction (X-Y axis direction) such that the tissue section of the preparation PRT is allocated to a light condensing portion at which the light is condensed by the condenser lens 14.
- [0026] Further, the stage drive controller 21 drive-controls the stage drive mechanism 12 to move the stage 11 in the direction orthogonal to the stage surface (Z-axis direction (that is to say, a depth direction of the tissue section)) such that a part of the tissue section allocated to the light condensing portion is in focus of the object lens 15.
- [0027] The lighting controller 22 sets a parameter corresponding to a mode in which a bright field image should be obtained (hereinafter, also referred to as a bright field mode) or a mode in which a dark field image should be obtained (hereinafter, also referred to as a dark field mode) in the light source 13 and applies the illumination light from the light source 13. The parameter is, for example, strength of the illumination light, selection of a light source type and the like.
- [0028] Meanwhile, the illumination light in the bright field mode is visual light, in general. On the other hand, the illumination light in the dark field mode is the light including a wavelength to excite a fluorescent marker used in the special staining. Further, a background of the fluorescent marker is cut out in the dark field mode.
- [0029] When the illumination light is applied from the light source 13, the illumination light is condensed at the standard position on the preparation arranging surface of the stage

11 by the condenser lens 14. An image of the light condensing portion at which the light is condensed by the condenser lens 14 of the tissue section of the preparation PRT is formed in an enlarged manner on the image forming surface of the object lens 15, and the enlarged image is formed as a subject image on the imaging surface of the imaging device 16 by the object lens 15.

[0030] The imaging controller 23 sets the parameter corresponding to the bright field mode or the dark field mode in the imaging device 16 and obtains data of the subject image formed on the imaging surface of the imaging device 16. The parameter is, for example, start timing and end timing of an exposure and the like.

[0031] Further, there is a controller (hereinafter, also referred to as an integrated controller) 30 to control an entire microscope 1 as the control system of the microscope 1, which is connected to the stage drive controller 21, the lighting controller 22 and the imaging controller 23 through the data communication paths. The integrated controller 30 is the computer including the CPU, the ROM, the RAM, the arithmetic circuit, a storage medium and the like.

[0032] The integrated controller 30 waits a start instruction of the bright field mode or the dark field mode, and when receiving the start instruction, this provides an instruction that the control should be started in the mode corresponding to the starting instruction to the stage drive controller 21, the lighting controller 22 and the imaging controller 23.

[0033] Further, the integrated controller 30 stores the data of the enlarged image of the tissue section part obtained by the imaging device 16 in the storage medium each time the tissue section part of the preparation PRT is allocated in the stage drive controller 21.

[0034] Then, the integrated controller 30 waits a display instruction, and when receiving the display instruction, this reads the data corresponding to the enlarged image specified by the display instruction from the storage medium and provides the same to a source of the display instruction.

[0035] In this manner, the microscope 1 is configured to store the tissue section of the preparation PRT as an image of a microscopic examination state, thereby saving information regarding the tissue section for a long period without deterioration of a state of fixing, staining and the like as compared to a case in which the preparation PRT itself is saved.

[0036] (1-2. Configuration of focusing device)

In addition to such configuration, the microscope 1 is provided with a focusing device 40. The focusing device 40 includes a half mirror 41, a field lens 42, an aperture mask 43, separator lenses 44A and 44B, an imaging device 45 and an imaging condition determination processor 46 as illustrated in Fig. 1.

[0037] The half mirror 41 is arranged between the object lens 15 and the imaging device 16

such that a light axis thereof coincides with the normal to the standard position of the stage surface and divides light entering from the object lens 15 into transmitted light and reflected light.

- [0038] The field lens 42 relays the subject image of the object lens 15 projected on a reflection side, for example, of the half mirror 41 backward (predetermined image forming surface). Subject light reflected by the half mirror 41 is condensed by the field lens 42, so that brightness around the field is inhibited from lowering.
- [0039] The aperture mask 43 is arranged behind the field lens 42 and has a pair of openings 43A and 43B on a plane orthogonal to a light axis of the field lens 42 at positions symmetrical about the light axis. Sizes of the openings 43A and 43B are adjusted such that depth of fields of the separator lenses 44A and 44B are larger than the depth of field of the object lens 15.
- [0040] The aperture mask 43 divides a subject light beam entering from the field lens 42 by the openings 43A and 43B. The divided light beams intersect with each other on an image forming surface of the subject light beam to be the light beams of which positional relationship changes back and forth of the image forming surface.
- [0041] The separator lenses 44A and 44B are separately arranged behind the pair of openings 43A and 43B, respectively. The separator lenses 44A and 44B shift to form images of the divided light beams divided by the corresponding openings 43A and 43B and form a pair of subject images (hereinafter, also referred to as phase contrast images) on the predetermined image forming surface to which the field lens 42 relays.
- [0042] Meanwhile, when the separator lenses 44A and 44B cause vignetting of the field lens 42, a part of the divided light beams is lost. Therefore, the separator lenses 44A and 44B are arranged closer to a central side of the field lens 42 so as not to cause the vignetting.
- [0043] The imaging device 45 is not a line sensor but an area sensor of which imaging surface is the predetermined image forming surface of the subject image relayed by the field lens 42.
- [0044] Herein, Fig. 2 illustrates a picture of the bright field image imaged by the imaging device 16 arranged on the main image forming surface of the object lens 15 and the phase contrast images imaged by the imaging device 45 arranged on the predetermined image forming surface of the object lens 15. As is understood from Fig. 2, the bright field image formed on the imaging surface of the imaging device 16 (main image forming surface) is formed on the imaging surface of the imaging device 45 (predetermined image forming surface) as the phase contrast images by the separator lenses 44A and 44B.
- [0045] The imaging condition determination processor 46 is the computer including the ROM, the RAM, the arithmetic circuit and the like for the CPU in which the ROM

stores a program for determining an imaging condition (hereinafter, also referred to as an imaging condition determination program).

[0046] When an instruction that the imaging condition should be determined is provided by the integrated controller 30, the imaging condition determination processor 46 serves as a disparity obtaining unit 51 and a focusing position determining unit 52 as illustrated in Fig. 1, according to the imaging condition determination program.

[0047] The disparity obtaining unit 51 sequentially selects each pixel in one image, which should be a standard, of the phase contrast images (hereinafter, also referred to as a standard image) as a pixel, which is an object of interest (hereinafter, referred to as a pixel of interest).

[0048] Further, the disparity obtaining unit 51 detects a pixel corresponding to the pixel of interest (hereinafter, referred to as a relative pixel) from the other image, which should be referred to, of the phase contrast images (hereinafter, also referred to as a reference image) each time this selects the pixel of interest to obtain a distance between the pixel of interest and the relative pixel. The distance corresponds to the disparity.

[0049] Meanwhile, as a method of detecting a corresponding point, a method of searching a block having a pixel value most similar to that of a block of interest around a point of interest, for example, from the other image of the phase contrast images, which should be referred to, by a normalized correlating method and setting the center of the block the corresponding point is applied.

[0050] In this manner, the disparity obtaining unit 51 is configured to obtain the distance (disparity) between the standard image and the reference image in a pixel unit.

[0051] Relationship is such that the smaller the distance (disparity) is, the further forward a focal point of the object lens 15 is located, and on the other hand, the larger the distance (disparity) is, the further backward the focal point is located. Therefore, the distance between each pixel of the standard image and the relative pixel of the reference image corresponds to information indicating irregularity of an imaging area (area appearing on the image forming surface of the object lens 15) AR of the tissue section of the preparation PRT, as illustrated in Fig. 3.

[0052] Herein, relationship between the position of each pixel of the standard image of the phase contrast images of the tissue section illustrated in Fig. 2 and the distance between the pixel and the relative pixel of the reference image is illustrated in Fig. 4 as a graph. A light portion of the graph in Fig. 4 represents a front side and a dark portion represents a back side. From Fig. 4, it is understood that the irregularity of the tissue section part projected on the image forming surface of the object lens 15 is reflected and an end of a portion of the tissue section part is turned up.

[0053] A focusing position determining unit 52 determines a position on which the object lens 15 should be focused (hereinafter, also referred to as a focusing position) based on

the position of each pixel of the standard image and the distance between the pixel and the relative pixel of the reference image.

- [0054] Specifically, a center of gravity of the largest area out of areas in which the pixels with difference (gap) in the distance between the same and the relative pixel smaller than a threshold are adjacent to each other is set as the focusing position. That is to say, a portion with high density and small irregularity in the imaging area (area appearing on the image forming surface of the object lens 15) becomes the focusing position.
- [0055] The focal position is provided to the stage controller 21 through the integrated controller 30, a moving amount of the focal point of the object lens 15 relative to the focal position (hereinafter, referred to as a defocus amount) is calculated by the stage controller 21, and the stage 11 is moved.
- [0056] As described above, the depths of field of the separator lenses 44A and 44B are adjusted to be larger than that of the object lens 15. Therefore, the irregularity of the tissue section image formed on the imaging surface of the imaging device 45 (predetermined image forming surface) for determining the imaging condition is represented in more detail than that of the tissue section image formed on the imaging surface of the imaging device 16 (main image forming surface) for imaging.
- [0057] Therefore, the focusing position determining unit 52 may determine a position at which an entire subject image (tissue section image) appearing on the image forming surface of the object lens 15 becomes sharp as the focusing position in more detail as compared to a case in which the image of the imaging device 16 is used, by using the image of the imaging device 45 (phase contrast images).
- [0058] However, there is a case in which the tissue section is sealed between the glass slide and the cover glass in a twisted state, as illustrated in Fig. 5, for example. In this case, when the imaging area (area AR appearing on the image forming surface of the object lens 15) is a relatively flat portion (Fig. 5A), the entire subject image of the imaging area (partial image of the tissue section appearing on the image forming surface of the object lens 15) becomes sharp at the focusing position determined by the focusing position determining unit 52.
- [0059] However, when the imaging area is an inclined portion (Fig. 5B), a width in a depth direction of the subject image in the imaging area (hereinafter, also referred to as an imaging depth) is larger than the depth of field of the object lens 15. Therefore, even when the focal point of the object lens 15 is moved to the focusing position determined by the focusing position determining unit 52, a part of the subject image in the imaging area is lost or defocused.
- [0060] Therefore, the focusing position determining unit 52 is configured to determine a plurality of focal positions for the imaging area, as illustrated in Fig. 6, when the difference between the maximum value and minimum value in the distance between

each pixel of the standard image and the relative pixel of the reference image is larger than the threshold set for the difference.

[0061] Specifically, the imaging area is divided into a plurality of layers (hereinafter, also referred to as imaging layers) falling within the depth of field of the object lens 15, and portions with high density and small irregularity in the image layers are determined to be the focusing positions. Meanwhile, a value corresponding to an opening amount (that is to say, the depth of field) set for aperture opening for the object lens 15 and the like is set as the threshold.

[0062] (1-3. Focusing position determination procedure)

Next, a focusing position determination procedure in the imaging condition determination processor 46 is described with reference to a flowchart illustrated in Fig. 7.

[0063] The imaging condition determination processor 46 starts the focusing position determination procedure when the instruction that the imaging condition should be determined is provided, proceeds to a first step SP1 to obtain the phase contrast images of the subject (tissue section) projected on the image forming surface of the object lens 15 from the imaging device 45, and proceeds to a second step. The imaging condition determination processor 46 obtains the distance (disparity) between each pixel of the standard image and the relative pixel of the reference image from the phase contrast images in the second step SP2 and proceeds to a third step SP3.

[0064] The imaging condition determination processor 46 obtains the difference between the maximum value and the minimum value in the distance between each pixel of the standard image and the relative pixel of the reference image in the third step SP3, and proceeds to a fourth step SP4 to compare the difference obtained in the third step SP3 with the threshold.

[0065] Herein, when the difference obtained in the third step SP3 is not smaller than the threshold, the imaging condition determination processor 46 judges that the imaging depth of the subject image in the imaging area is larger than the depth of field of the object lens 15 and proceeds to a fifth step SP5. The imaging condition determination processor 46 divides the imaging area into the layers (imaging layers) falling within the depth of field of the object lens 15 in the fifth step SP5 and proceeds to a sixth step SP6.

[0066] On the other hand, when the difference obtained in the third step SP3 is smaller than the threshold, the imaging condition determination processor 46 judges that the imaging depth of the subject image in the imaging area is smaller than the depth of field of the object lens 15 and proceeds to the sixth step SP6 without the fifth step SP5.

[0067] The imaging condition determination processor 46 determines the portion with the high density and the small irregularity as the focusing position in the imaging area or each of the imaging layers obtained by dividing the imaging area in the sixth step SP6

to finish the focusing position determination procedure.

[0068] In this manner, the imaging condition determination processor 46 is configured to determine one or a plurality of focusing positions for the imaging area.

[0069] (1-4. Effect and the like)

In the above configuration, the focusing device 40 forms the phase contrast images on the imaging surface of the imaging device 45 from the openings 43A and 43B having sizes with which the depths of field of the separate lenses 44A and 44B are larger than that of the object lens 15 by the separator lenses 44A and 44B provided behind the openings 43A and 43B, respectively (refer to Fig. 3).

[0070] Then, the focusing device 40 obtains the distance between the standard image, which is one of the phase contrast images, and the reference image, which is the other of them, in a pixel unit (refer to Fig. 4) and determines the position on which the focus should be adjusted and the number thereof based on the distance of each pixel (refer to Fig. 7).

[0071] As described above, the distance between each pixel of the standard image and the relative pixel of the reference image corresponds to the information indicating the irregularity of the imaging area (area appearing on the image forming surface of the object lens 15) AR (Fig. 3). Since the depths of field of the separator lenses 44A and 44B are set to be larger than that of the object lens 15 by the openings in the focusing device 40, the detailed irregularity of the subject may be recognized before focusing from the phase contrast images. Furthermore, the detailed irregularity of the subject may be recognized by one imaging.

[0072] Therefore, in the focusing device 40, the focusing position at which the entire subject appearing on the image forming surface the object lens 15 becomes sharp and the number thereof may be appropriately determined in consideration of the irregularity of the subject and the area corresponding to the depth of field of the object lens 15.

[0073] As a result, the focal position may be determined at high speed and without loss according to the subject in the imaging area as compared to a method of imaging while shifting the focal point at predetermined intervals in the depth direction of the tissue section and searching the focal position based on contrast of the imaged image.

[0074] Since a fluorescent period of the fluorescent marker is limited to a certain period in the dark field mode, the fact that the focal position may be determined at high speed and without loss according to the subject in the imaging area is especially useful in a viewpoint of improving efficiency of obtaining the tissue section image.

[0075] According to the above configuration, it becomes possible to determine the focusing position at high speed and without loss according to the subject in the imaging area, thereby realizing the focusing device 40 capable of improving the efficiency of obtaining the tissue section image.

[0076] <2. Another embodiment>

The tissue section is used as the biological sample in the above embodiment. However, the biological sample is not limited to this embodiment. A cell, a chromosome and the like may be used as the biological sample, for example.

[0077] The two separator lenses 44A and 44B are used in the above embodiment. However, the number of the separator lenses 44 is not limited to this embodiment. A plurality of separator lenses 44 may be used by making a pair of separator lenses 44A and 44B a unit (group). Meanwhile, in this case, it is necessary that the openings corresponding to the separator lenses 44 of each group are provided on the aperture mask 43.

[0078] The center of gravity of the largest area out of the areas in which the pixels with the difference (gap) in the distance from the relative pixel smaller than the threshold are adjacent to each other is determined as the position on which the focus should be adjusted in the above embodiment. However, the method of determining the position on which the focus should be adjusted is not limited to this embodiment.

[0079] For example, this may be the pixel of an average value of the distance between each pixel of the standard image and the relative pixel of the reference image. When there is a plurality of the pixels, they may be made the centers of gravity. In addition to this, a method of determining the position at which the entire subject image (tissue section image) appearing on the image forming surface of the object lens 15 becomes sharp may be applied.

[0080] In the above embodiment, the moving amount of the focal point (defocus amount) of the object lens 15 relative to the focal position is calculated by the stage controller 21 of the microscope 1. However, the focusing position determining unit 52 in the focusing device 40 may be used as a position to calculate the defocus amount in place of this embodiment.

[0081] Although the depth of field of the object lens 15 is fixed in the above embodiment, this may be variable. In the case of variable, the process in the above fifth step SP5 may be changed to another process.

[0082] Specifically, it is judged whether the difference between the maximum value and the minimum value in the distance (disparity) between each pixel of the standard image and the relative pixel of the reference image is smaller than a value corresponding to the maximum depth of field allowed for the object lens 15 but is not smaller than a value corresponding to the depth of field actually set for the object lens 15.

[0083] If a positive result is herein obtained, the imaging condition determination processor 46 enlarges the opening of the aperture (not illustrated) for the object lens 15 by a pre-determined amount to make the depth of field of the object lens 15 larger, as illustrated in Fig. 8. At that time, the imaging condition determination processor 46 sets exposure time for the imaging device 16 longer according to an enlarged amount and thereafter

proceeds to the sixth step SP6. On the other hand, if a negative result is obtained, the procedure proceeds to the sixth step SP6 without enlarging the aperture for the object lens 15.

[0084] Meanwhile, a mode in which when the difference between the maximum value and the minimum value in the distance (disparity) between each pixel of the standard image and the relative pixel of the reference image is not smaller than the value corresponding to the maximum depth of field allowed for the object lens 15, the process in the above fifth step SP5 is executed is possible.

[0085] In this manner, one or a plurality of focusing positions at which the entire subject image (partial image of the tissue section appearing on the image forming surface of the object lens 15) becomes sharp relative to the imaging area may be determined in further detail.

[0086] In the above embodiment, when the difference between the maximum value and the minimum value in the distance (disparity) between each pixel of the standard image and the relative pixel of the reference image is not smaller than the threshold, the imaging area is divided into the layers (imaging layers) falling within the depth of field of the object lens 15. However, a dividing mode is not limited to this embodiment.

[0087] For example, as illustrated in Fig. 9, the imaging area other than of the tissue section a portion broken in the depth direction may be divided into the imaging layers. In this manner, the process to image while focusing on the broken portion is eliminated, so that it becomes possible to further improving the efficiency of obtaining the subject image.

[0088] Although the phase contrast images are formed by the separator lenses 44A and 44B in the above embodiment, the forming method is not necessarily limited to this embodiment and another known one may be adopted.

[0089] It should be understood by those skilled in the art that various modifications, combinations, sub-combinations and alterations may occur depending on design requirements and other factors insofar as they are within the scope of the appended claims or the equivalents thereof.

Reference Signs List

- [0090] 1 Microscope
11 Stage
12 Stage drive mechanism
13 Light source
14 Condenser lens
15 Object lens
16, 45 Imaging device

21 Stage drive controller
22 Lighting controller
23 Imaging controller
30 Integrated controller
40 Focusing device
41 Half mirror
42 Field lens
43 Aperture mask
43A, 43B Opening
44A, 44B Separator lens
46 Imaging condition determination processor
51 Disparity obtaining unit
52 Imaging condition determining unit
PRT Preparation

Claims

- [Claim 1] A focusing device, comprising:
an imaging device provided with a predetermined image forming surface predetermined separate from a main image forming surface of an object lens on which phase contrast images of an image formed on the main image forming surface are formed as an imaging surface;
obtaining means for obtaining a distance between one image and the other image of the phase contrast images in a pixel unit from the imaging device; and
determining means for determining a position on which a focus should be adjusted and the number of positions based on the distance of each pixel.
- [Claim 2] The focusing device according to claim 1, wherein
when difference between a maximum value and a minimum value of the distance of the each pixel is not smaller than a value set based on a depth of field of the object lens, the determining means divides an imaging area into a plurality of layers falling within the depth of field of the object lens and determines a position on which the focus should be adjusted in each layer.
- [Claim 3] The focusing device according to claim 2, wherein
the determining means divides the imaging area other than a portion in which a subject is broken in a depth direction into a plurality of layers falling within the depth of field of the object lens.
- [Claim 4] The focusing device according to claim 1, wherein
the phase contrast images are phase contrast images of a biological sample part.
- [Claim 5] The focusing device according to claim 1, wherein
the determining means sets a center of gravity of a largest area out of areas in which pixels of which difference in distance is smaller than a threshold are adjacent to each other as the position on which the focus should be adjusted.
- [Claim 6] The focusing device according to claim 2, wherein
when the difference between the maximum value and the minimum value of the distance of the each pixel is not smaller than the value set based on the depth of field of the object lens, the determining means enlarges the depth of field of the object lens and sets exposure time for another imaging device provided with the main image forming surface

as an imaging surface according to an enlarged amount.

[Claim 7]

A focusing method, comprising the steps of:

obtaining a distance between one image and the other image of phase contrast images from an imaging device provided with a predetermined image forming surface predetermined separate from a main image forming surface of an object lens on which the phase contrast images of an image formed on the main image forming surface are formed as an imaging surface in a pixel unit; and

determining a position on which a focus should be adjusted and the number of positions based on the distance of each pixel obtained in the obtaining step.

[Claim 8]

A focusing program for causing a computer to execute:

obtaining a distance between one image and the other image of phase contrast images from an imaging device provided with a predetermined image forming surface predetermined separate from a main image forming surface of an object lens on which the phase contrast images of an image formed on the main image forming surface are formed as an imaging surface in a pixel unit; and

determining a position on which a focus should be adjusted and the number of positions based on the obtained distance of each pixel.

[Claim 9]

A microscope comprising:

a half mirror to divide light entering from an object lens into transmitted light and reflected light;

a first imaging device for imaging a subject image formed on the object lens and projected on one of a transmission side and a reflection side of the half mirror;

a field lens for relaying the subject image projected on the other of the transmission side and the reflection side of the half mirror backward;

openings provided behind the field lens in a unit of pair and having sizes with which depth of field is larger than depth of field of the object lens;

separator lenses provided behind the openings, respectively, for forming phase contrast images on a predetermined image forming surface to which the field lens relays;

a second imaging device provided with the predetermined image forming surface as an imaging surface; and

determining means for obtaining a distance between one image and the other image of the phase contrast images from the second imaging

device in a unit of pixel and determining a position on which a focus should be adjusted and the number of positions based on the distance of each pixel.