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(54) Title: INTERFEROMETRIC SYSTEM WITH VARIABLE OPTICS FOR NON-COHERENT LIGHT SOURCE AND METHOD OF INTERFEROMETRIC SYSTEM ALIGNMENT

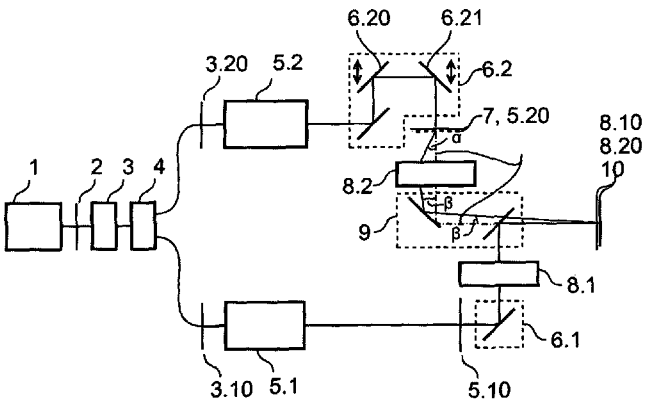

Fig.1

(57) Abstract: The invention relates to the interferometric system for creation of hologram with spatial carrier frequency comprising a source of radiation (1) with low coherence, behind which a field plane (2) optically conjugated with an output image plane (10) is arranged, a beam splitter (4) for splitting the beam of radiation into two separate branches of interferometer, an object branch with plurality of optical elements, a reference branch with plurality of optical elements, a diffraction grating (7), an extender (12), a transmission system of reflectors and a detector arranged in the output image plane (10), wherein the plurality of optical elements in the object branch includes a objective lens (5.12), wherein the objective lens used in the reference branch is not identical with the one used in the object branch, which means a significant financial saving.

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Interferometric system with variable optics for non-coherent source of radiation and method of interferometric system alignment

Field of the Invention

The present invention relates to interferometric system with variable optics for non-coherent radiation source for use in digital holographic microscopy to observe samples in reflected and transmitted radiation.

Background of the Invention

Present interferometric systems with separated object and reference branch have in common a beam splitter, which splits the beam of waves into two mutually coherent beams entering the object and reference branch. These present systems can be divided into three basic groups.

The first group comprises interferometric systems using mostly classic Mach-Zehnder or Michelson interferometers, where the axes of both branches are unified at the output of interferometer and the waves thus interfere at a zero angle. This solution allows usage of entirely non-coherent wave source as for example a classic light bulb, which has the advantage of removing the speckle pattern and allows in-depth discrimination of resulting image i.e. optical sections through sample. The drawback is that to obtain complete information about the object wave it is necessary to record at least 3 images with different phase shift, that has negative consequences. Firstly, the influence of flow in the environment and the influence of vibrations increase the noise in the resulting image and secondly, it is not possible to image fast processes. This solution is used by Krug&Lau, Horn and Mirau lens.

The second group consists of holographic systems. They use the same interfetometers as the previous group except that the axes of both branches are unified at the output of interferometer at sufficiently large non-zero angle so that the resulting interference structure has the spatial carrier frequency high enough to reconstruct the object wave from a single interferogram, thus a hologram, that is achieved by a simple tilt of the reflector or other optical element with similar function. Such interferometer is not achromatic and it is therefore not possible to use broadband waves, because the waves with various wavelengths enter the output plane at the same angle and the resulting interference structure has a different spatial frequency for each wavelength. The desired interference structure (fringes), when summed up, disappear from a large part of the field of view. An advantage of this solution is that the image may be completely reconstructed from a single record, a hologram. Another advantage is that the sampling frequency depends merely on the used detector, not on the arrangement of the holographic system. The solution is suitable for observation of dynamic processes. A drawback is then the necessity to use coherent or almost coherent waves, for example laser, so that the interference occurred in the whole field of view, that has the negative consequences such as presence of coherence noise and strictly limited possibility to observe sample immersed in a scattering environment. The field of view is 2x smaller than in the case of systems of the first group, which results from the holographic condition.

The third group consists of coherence-controlled achromatic holographic systems. This group eliminates the drawback described in the second group in that the waves with different wavelengths enter the output plane of interferometer at various angles so that the resulting interference structure would have the same and sufficiently high spatial frequency (fringe density) for each wavelength, so that it would be able to reconstruct the object wave in the whole field of view from a single interferometer, a hologram. This solution is achieved by a beam splitter represented herein by a diffraction grating. Diffraction order +1 enters the object branch and -1 enters the reference branch. Due to the angular dispersion of waves on diffraction grating, waves with different wavelengths exit at various angles and enter the condenser. Diffraction grating is imaged in each branch with respective imaging system into the output plane of interferometer, which provides the preservation of angular dispersion of waves in each of branches and gives precondition for formation of achromatic interference fringes. This solution comprises all advantages stated for the above-described first and second group and at the same time eliminates the mentioned drawbacks. However, one drawback is that the condenser and objective lens forming the imaging system in each branch have to be provided with two identical elements, i.e. for example in the arrangement of transmission microscope it is necessary to use four identical objective lenses for each magnification. A consequence is then the financial demand and limited space between lens and condenser for the lens with greater magnification. The size of field of view remains the same as in the second group. This solution is described in utility models CZ 8547 and CZ 19150 and is also known thanks to several publications which disclose the use of such interferometers in holographic microscopy in the reflection arrangement „Parallel mode confocal microscope" from 1999 or in transmitted light arrangement „Polychromatic coherent transfer function for a LCIM with achromatic fringes" from 2005.

Within the patent application CZ302491 a solution which partly eliminates the said drawbacks is described, namely interferometric system with spatial carrier frequency for imaging in broadband spectrum, which allows using classic condensers instead of illuminating objective lenses, because classic condensers do not require changing when replacing the observation lenses.

All the currently known solutions for achromatic holographic microscopes have in common one major drawback and that is necessity for the both branches of the holographic microscope to consist of identical optical elements arranged in the corresponding places in the optical beam path. Therefore the objective lens has to be changed if we want to change the magnification. This is necessary not only in the object branch, where the sample is observed, but in the reference branch as well. The consequences of the requirement to use identical objective lenses are high purchase costs.

Moreover, in the currently known systems it is necessary to put the reference sample having the same optical thickness as the object in the object branch into the reference branch. For each observed sample it is necessary to make a compensation reference sample.

The use of non-coherent light increases the microscope setting demands, because the less coherent light is used, the more difficult the setting of optical path length of both branches is due to the high sensitivity as well as the setting of the precise coincidence in lateral direction, which may not exceed the coherence length or respectively the coherence width within the given configuration of illumination.

Summary of the Invention

The invention relates to interferometric system for creation of hologram with spatial carrier frequency comprising a source of radiation with low coherence, behind which a field plane optically conjugated with the output image plane is arranged, a beam splitter for splitting the beam of radiation into two separated branches of interferometer, an object branch with plurality of optical elements, a reference branch with plurality of optical elements, a diffraction grating, an extender, a transmission system of reflectors and a detector arranged in the output image plane, wherein the system is configured in a way that the difference between the time of radiation propagation in the object branch and in the reference branch is shorter than the coherence time of the used radiation. This difference is measured from the field plane up to the output image plane, the magnification in the object and the reference branch from the field plane up to the output image plane is approximately the same and that the object output image created by the object branch in the output image plane and reference output image created by the reference branch in the output image plane substantially overlap, which provides the interference of radiation from both of the branches, wherein the plurality of optical elements in the object branch comprises the first objective lens, of which the optical properties include the nominal focal length and nominal numeric aperture characterized in that at least one of the said optical properties of any of the plurality of elements of reference branch differs from the said corresponding optical properties of the first objective lens.

In a preferred embodiment the plurality of optical elements of reference branch comprises the second objective lens, wherein at least one of the said optical properties of the second objective lens differs from the optical properties of the first objective lens.

In another preferred embodiment the plurality of optical elements of reference branch does not include the objective lens.

As it is apparent, in this arrangement of interferometric system according to the present invention there is no identical objective lens used in the object branch, which means a significant financial savings.

The mentioned drawbacks of known solutions are further eliminated by the method of interferometric system alignment characterised in that it comprises a step of placing the reticle pattern into the optical beam path, then in any order, for the each of the branches separately, a step of recording the image of reticle pattern created merely by the object branch and a step of recording an image of reticle pattern created merely by the reference branch, further a step of comparing the sizes of both of these images and subsequently a step of changing the magnification by the means of an element with variable focal length.

In a preferred embodiment is included the step of positioning the reticle pattern into the optical beam path, then in any order, for each of the branches separately, the step of recording the image of reticle pattern created only by the object branch and step of recording the image of reticle pattern created only by the reference branch, further a step of determining the shift of these images with respect to each other and subsequently the step of reducing the mutual shift of image created by the object branch and image created by the reference branch in the image output plane by means of a deflector.

Included is preferably the step of measuring the *A* quantity in the first field of view section and the second field of view section, wherein the first field of view section is placed nearer to the intersection of the optical axis with the output image plane than the second filed of view section, subsequently the step of comparing these quantities *A* and then a step of changing the magnification using the element with variable focal length.

Quantity *A* is represented by the mean value of holographic signal, by the ratio of the value of the holographic signal to a holographic noise value, or by the ratio of the value of spectrum computed from a small area around the carrier frequency to the spectrum noise value.

Another preferred embodiment includes a step of measuring the quantity *A* value in the first position of deflector, a step of shifting the deflector, a step of measuring the quantity *A* value in the second position of deflector and a step of reducing the mutual shift of image created by the object branch and the image created by the reference branch in the output image plane by the means of deflector.

Another preferred embodiment includes a step of measuring the quantity *A* value when setting the first length of the extender, further a step of changing the optical path length of the extender, further a step of measuring the quantity *A* value when setting the second optical length of the extender and further a step of comparing these *A* values and subsequently reducing the difference between the time of radiation propagation in the object and the reference branch from the field plane up to the output image plane by the means of extender.

In a preferred embodiment, the above stated steps are performed repeatedly.

Further benefits and advantages of the present invention will become apparent after a careful reading of the examples of the invention embodiments with corresponding references to the respective accompanying drawings.

Description of the Drawings

Fig. 1 is a schematic illustration of a preferred embodiment of interferometric system

Fig. 2 is the first example of embodiment of object input imaging system

Fig. 3 is the second example of embodiment of object input imaging system

Fig. 4 is a schematic illustration of the second example of interferometric systemFig. 5 is a schematic illustration of the third example of interferometric system

Fig. 6 is a schematic illustration of the fourth example of interferometric system

Fig. 7 is a schematic illustration of the fifth example of interferometric system

Fig. 8 is a schematic illustration of the sixth example of interferometric system

Fig. 9 illustrates a method of holographic signal reconstruction

Fig. 10 illustrates a method of microscope alignment with setting the magnification of reticle pattern image

Fig. 11 illustrates a method of microscope alignment with setting the shift of reticle pattern image

Fig. 12 shows examples of images created during the deflector movement

Fig. 13 shows examples of images created during the change of optical path length of the extender

Exemplary Embodiments of the Invention

An example of preferred embodiment of interferometric system with variable optics for non-coherent source of radiation 1 is schematically illustrated in the fig. 1. It is an interferometric system for creation of hologram with spatial carrier frequency via low coherence illumination allowing confocal imaging by planar spatially non-coherent radiation source 1 in real time. This interferometric system consists of planar, temporally and spatially non-coherent radiation source 1, which can be for example white light source, behind which an optical system is usually arranged, referred to as a collector 3 and a beam splitter 4, designed as a divider cube. Optical splitter in optical system of the beam splitter 4 divides the incident illumination into the object branch and the reference branch. The object branch is the one comprising a sample, thus the observed object 5.14.

Object and reference branch comprise a set of optical elements, including for example reflector or lens as well as more complex optical elements, such as objective lens, tube lens, element with variable focal length, deflector, output imaging system, transmission system of reflectors, elements with fixed or adjustable optical path length or output system of reflectors.

In the example of fig. 1, a field plane 2 optically conjugated with the output image plane 10 and imaged into the image planes 3.10 and 3.20 of object and reference branch by the collector 3 and the splitter 4, is arranged between the source of radiation 1 and the collector 3. These image planes 3.10 and 3.20 may be imaged before or behind the input imaging systems 5.1 and 5.2, eventually also inside the input imaging systems 5.1 and 5.2. The object input imaging system 5.1 is arranged in the object branch. In the given example of embodiment a reference input imaging system 5.2 is arranged in the reference branch. The field plane 2 refers to a plane, in which an aperture is arranged, or in case of absence of field iris diaphgram it refers to any plane located between the source of radiation 1 and the splitter 4, which is optically conjugated with the output image plane 10.

Fig. 2 shows an example of embodiment of the object input imaging system 5.1 comprising the first condenser 5.11 and the first objective lens 5.12, which may be preferably provided with a tube lens, wherein the object plane of the microscope is arranged between the first condenser 5.11 and the first objective lens 5.12. The observed object 5.14 is inserted in this plane. It is therefore an arrangement, where the resulting image is obtained from radiation passing through the observed object. By the objective lens we mean the first imaging element arranged behind the observed object 5.14, which creates its reflection either in finite or infinite distance behind this imaging element, or a component intended for this purpose. In some examples of embodiments it is possible to use the objective lens also in the reference branch, where it does not serve to image the observed object 5.14.

In another embodiment shown in the fig. 3 is the system configured to use the radiation reflected from the observed object. In this arrangement the beam of radiation on semi-transparent reflector is at first directed through the first objective lens 5.12 onto the observed object 5.14, at the level of which the object plane 5.13 of the microscope is located. The reflected radiation then passes back through the first objective lens 5.12 and through the semi-transparent reflector, behind which it is further directed towards the output image plane 10.

The reference input imaging system 5.2 comprising the second condenser 5.21 and the second objective lens 5.22 is designed in similar way as it is described in the previous two examples illustrated in fig. 2 and fig. 3, except that the observed object 5.14 is not placed there. It is possible to place a reference sample into the reference plane corresponding to the object plane of the microscope 5.13 instead.

The first objective lens 5.12 of the object input imaging system 5.1 and the second objective lens 5.22 of the reference input image plane 5.2 differ from each other with at least one of the parameters comprising the exact focal length and exact numeric aperture. Alternatively, as it is described in other examples of embodiments, neither the reference input imaging system 5.2 nor the second objective lens 5.22 have to be included in the system.

Further in the example of the fig. 1 the input imaging systems 5.1 and 5.2 create secondary image planes 5.10 and 5.20 of image planes 3.10 and 3.20, wherein the secondary image planes 5.10 and 5.20 may be created anywhere behind the input imaging systems 5.1 and 5.2.

Interferometric system in this embodiment further comprises object output imaging system 8.1 in the output part of the object branch and reference output imaging system 8.2 in the output part of the reference branch.

The object secondary image plane 5.10 of the object input imaging system 5.1 and the output image plane 10 of interferometer as well as the reference secondary image plane 5.20 of the reference input imaging system 5.2 and the output image plane 10 of interferometer are optically combined by the output imaging systems 8.1 and 8.2 and thus create the output images 8.10 and 8.20 of the secondary image planes 5.10 and 5.20 in the output image plane 10 of interferometer.

The object transmission system 6.1 of reflectors and the reference transmission system 6.2 of reflectors are here realized by systems of reflectors and are arranged between the object input imaging system 5.1 and the object output imaging system 8.1 or between the reference input imaging system 5.2 and the reference output imaging system 8.2 respectively, wherein it is possible to position at least one of the elements of object transmission system 6.1 of reflectors between the elements of object input imaging system 5.1 or object output imaging system 8.1 as well as it is possible to place at least one of the elements of the reference transmission system 6.2 of reflectors between the elements of the reference input imaging system 5.2 or the reference output imaging system 8.2. The transmission systems 6.1, 6.2 of reflectors may comprise the extender 12, which is realized for example by a system of two reflectors 6.20 and 6.21. It applies for the extender 12 that the beam of radiation entering the reference transmission system 6.2 of reflectors at first exits in the direction parallel to the direction of the entering beam of radiation. Another extender 12 might be for example a so called optical wedge arranged behind the input imaging system 5.2 or 5.1 respectively.

Reference output imaging system 8.2 is in this embodiment a coherent optical unit, behind which the output system 9 of reflectors is arranged, as it is apparent from the fig. 1.

In the output image plane 10 of interferometer is then arranged a detector. It is usually designed as a CCD camera chip.

The reference branch in this embodiment comprises a diffraction grating 7 near the reference secondary image plane 5.20 of the reference input imaging system 5.2. In this embodiment a transmission diffraction grating 7 is used.

The field plane 2 is imaged by the optics of the reference as well as the object branch into the output image plane 10, as it is apparent from the previous description, wherein the magnification between the field plane 2 and the output image plane 10 is identical for both branches in the perfectly aligned system. Therefore in practice the interferometric system has to be configured to reach at least approximately identical magnification, what can be defined as a difference of these magnifications, which further allows at least minimum interference of illumination. From the above-stated arises a requirement for the magnification of the output imaging systems 8.1 and 8.2 to compensate for the difference of the magnification of input imaging systems 5.1 and 5.2 as well as eventually, in other embodiments, also for the absence of reference input imaging system 5.2.

The duration of radiation propagation between the field plane 2 and the output image plane 10 does not differ in individual branches for more than the coherence length of used radiation, measured from the field plane 2 up to the output image plane 10. The difference of lengths of optical paths of individual branches is adjusted by the extender.

The extender 12 may be a sliding system of reflectors, using for example extension reflectors 6.20 and 6.21 illustrated in the fig. 1 or it may be an object with variable optical path length made of material having a different refractive index than the surrounding environment (glass, immersion liquid, etc.) or a combination of any of the mentioned options. An example of the extender 12 is illustrated in the fig. 1. Some extenders may be optionally arranged in the system or may be included in the imaging systems 5.1 and 5.2, transmission systems 6.1 and 6.2 of reflectors or output imaging systems 8.1 and 8.2.

Interferometric system may be configured so as the object input image (8.10) created by the object branch in the output image plane (10) and the reference output image (8.20) created by the reference branch in the output image plane (10) substantially overlap. It means that the overlap or mutual shift of the images still allows at least minimal interference of radiation. The setting is done by a deflector, which shifts one of the images. A deflector may be a separate element, as for example a deflection reflector, any part of any imaging system or any imaging system as a whole, for example the reference input imaging system 5.2.

The reference transmission system 6.2 of reflectors may be realized in many ways. Important thing is that it is configured so as the beam of radiation passing in the direction of the axis of the reference branch and diffracted by the diffraction grating 7 at an angle α enters the output imaging system 8.2 at the same angle α with respect to the optical axis of this output imaging system 8.2 and exists the output imaging system 8.2 with respect to its optical axis at an angle β and subsequently enters the output image plane 10 of interferometer with respect to its normal at the same angle β. Between the angles α and β it applies that $sin\beta = sin(\alpha)/m$, where m is the magnification of the output imaging system 8.2. Axis 11 of the zero order of the diffraction grating is identical with the axis of the object optical branch.

The object transmission system 6.1 of reflectors, which may be realized in various ways as well, has to be configured so as the beam of radiation passing the axis of object input imaging system 5.1 is directed through the object transmission system 6.1 of reflectors and through the output imaging system 8.1 to the output image plane 10 of interferometer in the direction of its normal.

The second exemplary embodiment of the interferometric system according to the present invention illustrated in the fig. 4 is an analogy of the above-described system illustrated in the fig.1 except that the output imaging systems 8.1 and 8.2 are divided into more optical units, between which the output system 9 of reflectors is arranged. The output imaging systems 8.1 and 8.2 may have a part of the system in common.

The third exemplary embodiment of interferometric system according to the present invention is illustrated in the fig. 5. It is an analogy of the above-described system illustrated in the fig. 1 except that the object output imaging system 8.1 is left out, however, the magnification between the field plane 2 and the output image plane 10 of interferometer stays the same for both branches and the field plane 2 is imaged in the object secondary image plane 5.1, thus in the output image plane 10 through systems 6.1 and 9 of reflectors.

Similarly as in the second example, between the parts of the reference output imaging system 8.2 an output system 9 of reflectors may be inserted in this example.

The fourth exemplary embodiment of interferometric system according to the present invention is illustrated in the fig. 6. It is an analogy of the above-described system illustrated in the fig. 1 except that the reference input imaging system 5.2 is left out, however, the magnification between the planes 2 and 10 remains the same for both branches.

Similarly as in the second example, between the parts of the output imaging systems 8.1 and 8.2 an output system 9 of reflectors may be inserted in this example.

The fifth exemplary embodiment of interferometric system according to the present invention is illustrated in the fig. 7. It is an analogy of the above-described system illustrated in the fig. 1 except that in both branches, in the reference as well as the object branch, a diffraction grating 7 is arranged. It is therefore an arrangement, where the first diffraction grating 7.1 is arranged near the object secondary image plane 5.10 of the object input imaging system 5.1 of the object branch and the second diffraction grating 7.2 is arranged near the reference secondary image plane 5.20 of the reference input imaging system 5.2 of the reference branch. The transmission systems 6.1 and 6.2 of reflectors may be realized in various ways.

It is important for the transmission systems 6.1 and 6.2 of reflectors to be adjusted so as the beam of radiation passing the axis of the reference branch and diffracted by the diffraction grating 7.1(7.2) at the angle α1(α2) enters the output imaging systems 8.1(8.2) at the same angle α1(α2) with respect to the optical axis of this output imaging system 8.1(8.2) and exits the output imaging system 8.1(8.2) with respect to its optical axis at the angle β1(β2) and then enters the output image plane 10 of interferometer with respect to its normal at the same angle β1(β2). Between the angles α1(α2) and β1(β2) it applies that $\sin\beta 1 = \sin(\alpha 1)/m1$ ($\sin\beta 2 = \sin(\alpha 2)/m2$), where *m1(m2)* is the magnification of the output imaging system 8.1(8.2).

It also applies here that the object secondary image plane 5.10 of the object input imaging system 5.1 of the object branch and the output image plane 10 of interferometer as well as the reference secondary image plane 5.20 of the reference input imaging system 5.2 of the reference branch and the output image plane 10 of interferometer are optically combined by the output imaging systems 8.1 and 8.2.

Similarly as in the second example, an output system 9 of reflectors may be inserted between the parts of imaging systems 8.1 and 8.2 in this example.

Similarly as in the fourth example, the reference input imaging system 5.2 may be left out in this example, while preserving the same magnification of the both branches of interferometer between the field plane 2 and the output image plane 10 of interferometer.

The sixth exemplary embodiment of interferometric system according to the present invention is illustrated in the fig. 8. It is an analogy of the above-described system illustrated in the fig. 1 except that the transmission diffraction grating 7 is replaced by a reflective diffraction grating. Reflective diffraction grating may be used in the all above described examples of embodiments.

By a combination of all mentioned embodiments many further arrangements suitable for various applications of interferometric systems with variable optics may be achieved.

In the all above described solutions, each arrangement is due to the low coherence of the used source very susceptible to precise alignment of the microscope, which lies in the setting of the identical optical path length of both branches, setting the mutual location of the optical beams of both branches in the output image plane 10 of interferometer (further described also as shifting the images of branches in lateral direction) and in the setting of identical magnification of images of the field plane 2 created by the branches of interferometer. An automatic procedure serves for this alignment, which lies in the searching for the maximum value of holographic signal using a controlled change of branch lengths using the extenders and controlled shifting of images of branches on each other in lateral direction by the means of deflectors, which may be a part of any of the imaging systems 5.1, 5.2, 8.1 and 8.2 and by the change of magnification of any of the imaging systems 5.1, 5.2, 8.1 a 8.2.

Holographic signal may be derived from the light interference theory. Interference structure emerging in any point P (given by the vector $\boldsymbol{q_t}$ beginning in the intersection of the output image plane 10 with the axis 11 of the zero order of diffraction grating) of the output image plane 10 for exposure via one point of the field plane 2 and with frequency $\boldsymbol{\nu}$ may be described by a relation [1, 2]

$$\begin{aligned} i(q_t,\nu) &= |u_1(q_t,\nu) + u_2(q_t,\nu)exp(2\pi i f_o x)|^2 = \\ &= |u_1(q_t,\nu)|^2 + |u_2(q_t,\nu)exp(2\pi i f_o x)|^2 + u_1(q_t,\nu)u_2^*(q_t,\nu)exp(-2\pi i f_o x) + \\ &\quad + u_1^*(q_t,\nu)u_2(q_t,\nu)exp(2\pi i f_o x), \end{aligned} \quad (1)$$

where $u_1(q_t,\nu)$ is a complex amplitude of the first branch wave and $u_2(q_t,\nu)exp(2\pi i f_o x)$ is a complex amplitude of the second branch wave. Phasor $exp(2\pi i f_o x)$ indicates the phase shift resulting from the inclination of the interfering wave against the normal of the output image plane 10, wherein f_o is the carrier frequency of interferogram in the output image plane 10 and x is a coordinate in the output image plane 10. Assuming that the illumination in the field plane is completely spatially non-coherent, to express the interference structure formed by all radiation frequencies ν and all point of the field plane 2 it is possible to use Hopkins's formula [3]

$$I(q_t) = \iint_S \int_0^\infty |u_1(q_t,\nu)|^2 \, d\nu dS + \iint_S \int_0^\infty |u_2(q_t,\nu) exp(2\pi i f_o x)|^2 \, d\nu dS + \\ + exp(-2\pi i f_o x) \iint_S \int_0^\infty u_1(q_t,\nu) u_2^*(q_t,\nu) d\nu dS + \\ + exp(2\pi i f_o x) \iint_S \int_0^\infty u_1^*(q_t,\nu) u_2(q_t,\nu) d\nu dS. \quad (2)$$

The third term (or optionally also the fourth term, which is a complex conjugate of the third term) is in numerical image processing separated from the overall intensity by the Fourier methods using the carrier spatial frequency f_o, about which it is assumed that it depends neither on the position of the point of the field plane 2, nor on the radiation frequency. A term obtained in this manner is the searched holographic signal.

$$w(q_t) = \iint_S \int_0^\infty u_1(q_t,\nu) u_2^*(q_t,\nu) d\nu dS, \quad (3)$$

If both interferometer branches have identical optical path lengths, identical overall magnification while assuming that the interferometer is not aligned, ie. the beam axis of the first branch and the beam axis of the second branch do not intersect the output image plane 10 in the same point, the holographic signal according to [2] may be represented as

$$w(q_t; q_f) = \iint_S \int_0^\infty u_1(q_t - q_f, \nu) u_2^*(q_t,\nu) d\nu dS, \quad (4)$$

where q_f is the vector of mutual shift of both beams in the output image plane 10. The relation (4) describes the size of the holographic signal as well as the function of the mutual intensity assuming that the propagation time of beams in both branches is identical and reaches its maximum (including its module) if $q_f = 0$. Given that both shifted branches have the same overall magnification and do not have identical optical path lengths, for each radiation frequency a phase shift $\Phi(\nu)$ is formed and the holographic signal takes form of

$$w(q_t) = \iint_S \int_0^\infty u_1(q_t,\nu) u_2^*(q_t,\nu) exp[-i\Phi(\nu)] d\nu dS, \quad (5)$$

where $\Phi(\nu)$ is proportional to $(2\pi \Delta L \nu)/c$ due to the difference of optical path lengths ΔL and the speed of light in vacuum c. Module of the signal reaches its maximum, when optical path lengths of both branches are identical. In case the both branches of interferometer are shifted, have identical optical path lengths and different magnification, the holographic signal takes form of

$$w(q_t) = \iint_S \int_0^\infty u_1\left(\frac{m_1}{m_2} q_t, v\right) u_2^*(q_t, v)\, dv\, dS \quad (6)$$

where m_1 is the overall magnification of the first branch and m_2 is the overall magnification of the second branch. The relation (6) corresponds to the relation (4) for

$$\frac{m_1}{m_2} q_t = q_t - q_f. \quad (6.1)$$

Its values correspond to the function of the mutual coherence, however, in each field point for a different shift q_t, where $q_f = f(m_1, m_2, q_t)$. Assuming that in case of identical magnification the function of mutual coherence depends merely on the distance of respective points in the image plane, it is possible to measure the function by inducing different magnification using a single image with variable q_t. The module of the holographic signal then reaches its maximum, if $q_f(m_1, m_2, q_t) = 0$ [2].

Holographic signal is a suitable quantity for alignment of the microscope, because, as it has been demonstrated, it has a maximum module value for aligned microscope (identical optical path lengths of both branches, both branches have the same overall magnification and interfering waves are not mutually shifted in the output plane). If we consider the superposition of the described effects, the holographic signal may be generally defined as

$$w(q_t; q_f) = \iint_S \int_0^\infty u_1\left[\left(\frac{m_1}{m_2} q_t - q_f\right), v\right] u_2^*(q_t, v) \exp[-i\Phi(v)]\, dv\, dS \quad (7)$$

Reconstruction of the holographic signal is based on the Fourier filtering. This method is schematically illustrated in the fig. 9. Holographic record on the CCD is converted to 2-D by Fast Fourier Transform (FFT) into the spectrum of spatial frequencies. In this spectrum a window centred in the carrier frequency is made. The size of the window frame corresponds to the maximum frequency transferred by the lens. The beginning of coordinates in the 2-D frequency space is transferred to the centre of the window frame. Due to apodization this window is further multiplied by the weight function (e.g. Hanning function). Holographic signal is then obtained from the separated and multiplied spectrum of inverse FT, specifically using 2-D Inverse Fast Fourier Transform (IFFT). However, there are other suitable mathematical methods known to one skilled in the art. Thus the result is a digitized function w_D, which is a pretty good approximation of the real function of holographic signal w. The calculated function w_D will be called identically a holographic signal. The function w_D is a complex function, of which the absolute value is determined by the amplitude of holographic signal, while its phase corresponds to the phase difference caused by the difference of optical paths of the reference and object branch in the given point.

In each configuration of the microscope alignment it is possible to obtain the function w_D by the above described method and its mean value w_D will be referred to as A, ie.

$$A = \langle w_D \rangle = \sum_{i=0}^{N_x-1} \sum_{j=0}^{N_y-1} \frac{w_D(i,j)}{N_x \cdot N_y} \quad (8)$$

N_x and N_y is the size of the window in the x or y direction in pixels. We consider a microscope aligned when the value A reaches the maximum value. However, this invention is not limited to the criterion A in the form of mean value of holographic signal. Quantity A may be represented for example also by the mean value of holographic signal, mean value of the ratio of holographic signal to noise of holographic signal, value of the ratio of holographic signal to the noise of holographic signal, mean value of the spectrum in a small area around the carrier frequency, mean value of the ratio of the spectrum in a small are around the carrier frequency to the noise of spectrum values, ratio of the spectrum value in a small area around the carrier frequency or the spectrum value in a small area around the carrier frequency to the noise of spectrum values.

The method of automatic adjustment thus serves for alignment of the interferometer of a microscope and is divided into three steps. In the first step the alignment is carried out, during which a significant elimination of the influence of the magnification m_1/m_2 ratio, the size of the vector $\boldsymbol{q_f}$ and the phase shift $\phi(v)$ occurs, so as to provide at least minimal interference of radiation passing through the reference branch with the radiation in the object branch, thus a minimum value of the A referred to as A_{max} could be subtracted by the setting of optimal values m_1, m_2,$\boldsymbol{q_f}$,$\phi(v)$. In this step measuring the value of quantity A is used for all operations. The final step is a long-term maintenance of the obtained maximum average value of quantity A, for example for experiments with live preparations, when it is necessary to eliminate the influences of the external environment (e.g. temperature fluctuations).

The first step is a coarse alignment of microscope, therefore for the completeness of the description we base it on a state, when the microscope is completely misaligned. The reticle pattern 13, the example of which is shown in the fig. 10, is mechanically inserted into the field plane 2 or its surroundings, eventually to the optically combined planes, e.g. image planes 3.10 and 3.20 or their surroundings, and is projected into the output image plane 10 of interferometer, thus on the CCD camera chip by both branches of interferometer. The images of reticle pattern 13 are imaged with various magnifications and are laterally shifted in relation to each other in the output image plane 10. It will be apparent to one skilled in the art that the reticle pattern 13 may have substantially any form.

The object branch comprises an object shutter and the reference branch comprises a reference shutter, which serve to prevent the transmission of radiation through the given branch. After closing the reference shutter, the automatically sharpened image of the reticle pattern 13 created merely by the object branch is transferred and recorded. Subsequently the shutters are switched into the opposite position and automatically sharpened image of the reticle pattern 13 created merely by the reference branch is recorded. It is possible to use sharpening of any optical imaging system of the corresponding branch for the automatic sharpening and it is possible to use suitable software method for evaluation of the image sharpness (e.g. VAR – method of variance of distribution of gray values). Further, the size of both recorded images $d_{13.1}$ and $d_{13.2}$ of the reticle pattern 13 is compared. If it does not apply that $d = 0$ $(d = d_{13.1}-d_{13.2})$ the magnification (focal length) of at least one of the imaging systems 5.1, 5.2, 8.1, 8.2 is changed and this difference is balanced, as it is shown in the flow diagram in the fig. 10. The change of magnification is usually carried out by the zoom objective lens or other zoom element, which may be constructed as any of the imaging systems 5.1, 5.2, 8.1, 8.2 or may be a part of it, thus by the any element with variable focal length. This method provides the default setting of the magnification values m_1 a m_2 for the second step of setting.

It is proceeded similarly while securing the interference of radiation from the perspective of the size of the vector $\boldsymbol{q_f}$ being the vector of lateral shift of both beams in the plane of the output image plane 10 according to the relation (4). This is illustrated in the fig. 11. Again, images of the reticle pattern 13 are recorded separately by both branches and coordinates of the position of the object image 13.1 of the reticle pattern $[x_{13.1}\,;\,y_{13.1}]$ and the reference image 13.2 of the reticle pattern $[x_{13.2}\,;\,y_{13.2}]$ on the detector are determined. The positions of both images are subtracted from each other and multiplied by M constant, using which the mutual distance in pixels in the output image plane 10 of interferometer to the real distance $\Delta[\Delta_x\,;\,\Delta_y]$ is calculated, and about which the deflector moves. This will cause the shift of the images in the plane 10.

To provide the interference of radiation from the perspective of difference of optical paths of the interferometer branches, which is represented by the size of their mutual phase shift $\phi(\nu)$ according to relation (5), it is searched for the value *A*, which is higher than the determined minimum value A_{min}, by the means of change of the optical path of at least one of the extenders 12. The minimum value A_{min} may be easily determined by the manual alignment of the microscope as the value of the signal, when the interference structure becomes apparent on the detector. Optical path is changed continuously in the range of the motion of the extenders.

In the second step serving for fine adjustment it is necessary to find the maximum value of the quantity *A* for maximal achievable microscope alignment. It is carried out using three processes.

The first process serves to set the identical magnification of both branches of interferometer. If the magnifications are approximately the same, the interference of radiation at least around the intersection of the optical axis 11 with the output image plane 10 occurs after the previous step of setting, because as it follows from the relations (6) and (6.1), the holographic signal $w(\boldsymbol{q}_t)$ is not dependent on the magnification ratio $\frac{m_1}{m_2}$ for $\boldsymbol{q}_t$ equals zero, wherein $\boldsymbol{q}_t$ is thus a vector beginning in the intersection of the optical axis 11 with the output image plane 10. For interference to be homogenous in the field of view it is possible to compare the values of quantity *A* in the sections of the field of view in the intersection of the axis with the output image plane 10 and from the corner of the field of view while changing the magnification of at least one of the imaging systems 5.1, 5.2, 8.1, 8.2. It is easy to provide identical magnification in both of the branches of interferometer using this process.

The second process serves to reduce the mutual shift $\boldsymbol{q}_f$ of interfering waves, which is carried out by the means of shifting the deflector. In performing such 2D scan by the misalignment of the deflector around the optical axis and its repeating for various differences of the optical paths of the extender 12 it is possible to find the maximum of the quantity *A* of the scan always in the same position of the deflector regardless the difference of the optical paths. Examples of images resulting from such process are illustrated in the fig. 12 with the normalized record of the value of the quantity *A* for scans created by the misalignment of the deflectors with a 0,005 mm step. The difference of optical path of the branches of interferometer has been changed for a 0,005 mm step. The scan 6 comprises the maximum value of *A* in this measurement.

The maximum value *A* in the scan, corresponding to the $\boldsymbol{q}_f$, can be found using different approaches. Either by the creation of 2D scan and subsequently finding its maximum value *A*, or it is possible to use iterative methods suitable for finding the maximum value *A* in two-dimensional field (for example the gradient method).

The third process serves to eliminate the influence of different optical path lengths of the interferometer branches, thus the influence $\phi(\nu)$. The influence of size of the phasor $\phi(\nu)$ and thus of the difference of optical paths of both interferometer branches on the holographic signal $w(\boldsymbol{q}_t)$ according to the relation (5) may be fully compensated by the change of optical path length of at least one extender 12 by continuously subtracting the current value *A*, see the figure 13. The methods are illustrated on the 2D scans created by the deflecting system with a 0,001 mm step with various differences of the optical path lengths of the interferometer branches with a 0,001 mm step with identical magnifications of both interferometer branches. The scan 5 comprises the maximum value A_{max} – fully aligned state of the interferometer.

Three processes described in the second step are based on the fact that the method searches for sharp maximum of the quantity *A*. Therefore it can be performed gradually, combined and also applied in any order, to guarantee the finding of the maximum average value of the holographic signal *A*.

The third step includes a long-term maintenance of the aligned state of interferometer so as to maintain the maximum value A_{max}. The scanning is therefore performed in the determined time intervals with the determined step around the current position and the length of optical path of at least one extender 12 around its current optical path is changed by deflecting the deflector and the magnification of at least one of the imaging systems 5.1, 5.2, 8.1, 8.2 is changed around its current magnification.

This method of microscope alignment may be applied on many other known types of interferometric systems, for example all the types stated above in the background of the invention.

Although the invention has been described with respect to its preferred embodiment, many other adjustments and variants falling within the scope of the present invention are possible. Therefore it is assumed that the stated patent claims apply to these adjustments and variants falling within the actual scope of the present invention.

List of Reference Numbers

1	source of radiation
2	field plane
3	collector
3.10	object image plane
3.20	reference image plane
4	beam splitter
5.1	object input imaging system
5.2	reference input imaging system
5.11	first condenser
5.12	first objective lens
5.21	second condenser
5.22	second objective lens
5.13	object plane of the microscope
5.14	observed object
5.10	object secondary image plane
5.20	reference secondary image plane
6.1	object transmission system of reflectors
6.2	reference transmission system of reflectors
6.20	first extending reflector
6.21	second extending reflector
7	diffraction grating
8.1	object output image plane
8.2	reference output image plane
8.10	object output image
8.20	reference output image
9	output system of reflectors

10 output image plane

11 zero-order diffraction grating axis

12 extender

13 reticle pattern

13.1 object image of the reticle pattern

13.2 reference image of the reticle pattern

CLAIMS

1. The invention relates to interferometric system for creation of hologram with spatial carrier frequency comprising a source of radiation (1) with low coherence, behind which a field plane (2) optically conjugated with the output image plane (10) is arranged, a beam splitter (4) for splitting the beam of radiation into two separated branches of interferometer, an object branch with plurality of optical elements, a reference branch with plurality of optical elements, a diffraction grating (7), an extender (12), a transmission system of reflectors and a detector arranged in the output image plane (10), wherein the system is configured in a way that the difference between the time of radiation propagation in the object branch and in the reference branch is shorter than the coherence time of the used radiation, where this difference is measured from the field plane (2) up to the output image plane (10), the magnification in the object and reference branch from the field plane (2) up to the output image plane (10) is approximately the same and that the object output image (8.10) created by the object branch in the output image plane (10) and the reference output image (8.20) created by the reference branch in the output image plane (10) substantially overlap, thus providing the interference of radiation from both of the branches, wherein the plurality of optical elements in the object branch comprises the first objective lens (5.12), of which the optical properties include the nominal focal length and nominal numeric aperture characterized in that at least one of the said optical properties of any of the plurality of elements of reference branch differs from the said corresponding optical properties of the first objective lens (5.12).

2. Interferometric system according to claim 1 characterized in that the plurality of optical elements of the reference branch comprises the second objective lens (5.22), wherein at least one of the said optical properties of the second objective lens (5.22) differs from the said optical properties of the first objective lens (5.12).

3. Interferometric system according to claim 1, characterized in that the plurality of optical elements of the reference branch does not include the objective lens.

4. Interferometric system according to any of claims 1 to 3, characterized in that the object branch comprises the object input imaging system (5.1), which comprises the first condenser

(5.11), the observed object (5.14) in the object plane (5.13) of the microscope and the first objective lens (5.12) for creation of an image of the observed object (5.14) using the radiation passing through this observed object (5.14).

5. Interferometric system according to any of claims 1 to 3, characterized in that the object branch comprises the object input imaging system (5.1), which comprises the observed object (5.14) in the object plane (5.13) of the microscope and the first objective lens (5.12) for creation of the image of the observed object (5.14) using the radiation reflected from this observed object (5.14).

6. Interferometric system according to any of claims 1 to 5, characterized in that it comprises at least three of the plurality of imaging systems comprising an object input imaging system (5.1), a reference input imaging system (5.2), an object output imaging system (8.1) and a reference output imaging system (8.2).

7. Interferometric system according to any of claims 1 to 6, characterized in that it comprises the object output imaging system (8.1) and the reference output imaging system (8.2), which have at least one common optical element.

8. Interferometric system according to any of claims 1 to 7, characterized in that the diffraction grating (7) is designed as a transmission diffraction grating (7).

9. Interferometric system according to any of claims 1 to 8, characterized in that it comprises an element with variable focal length.

10. Method of interferometric system alignment according to any of claims 1 to 9, characterized in that it comprises a step of placing a reticle pattern (13) into the optical axis of the beam, then in any order for each branch separately a step of recording the image of the reticle pattern (13) created by the object branch only and a step of recording the image of the reticle pattern (13) created by the reference branch only, further a step of comparing the sizes of these two images and then a step of changing the magnification using the element with variable focal length.

11. Method of interferometric system alignment according to any of claims 1 to 10, characterized in that it comprises a step of placing the reticle pattern (13) into the optical path of the beam, then in any order, for each of the branches separately, a step of recording

an image of the reticle pattern (13) created by the object branch only and step of recording an image of the reticle pattern (13) created by the reference branch only, further a step of shifting these images in relation to each other and then a step of reducing the mutual shift of the image created by the object branch and the image created by the reference branch in the output image plane (10) using a deflector.

12. Method of interferometric system alignment according to any of claims 1 to 11, characterized in that it comprises a step of measuring the value of quantity *A* in the first section of the field of view and the second section of the field of view, wherein the first field of view section is located closer to the intersection of the axis (11) and the output image plane (10) than the second field of view section, further a step of comparing the values of these quantities *A* and further a step of changing the magnification using the element with variable focal length, wherein the quantity *A* is represented by the mean value of holographic signal, ratio of the value of holographic signal to the noise of holographic signal, the value of the spectrum in a small area around the carrier frequency, or by the ratio of the value of the spectrum in small area around the carrier frequency to the noise of the spectrum values.

13. Method of interferometric system alignment according to any of claims 1 to 12, characterized in that it comprises a step of measuring the value of the quantity *A* in the first position of the deflector, subsequently a step of shifting the deflector, further a step of measuring the value of the quantity *A* in the second position of the deflector and then a step of comparing these quantities *A* and further a step of reducing the mutual shift of the image created by the object branch and the image created by the reference branch in the output image plane (10) using the deflector, wherein the quantity *A* is represented by the mean value of holographic signal, the value of ratio of the holographic signal to the noise of holographic signal, the value of spectrum in a small area around the carrier frequency or by the ratio of the value of spectrum in a small area around the carrier frequency to the noise of the spectrum values.

14. Method of interferometric system alignment according to any of claims 1 to 13, characterized in that it comprises a step of measuring the value of quantity *A* while setting the first length of extender (12), further a step of changing the path length of the extender (12) and a step of measuring the value of quantity *A* while setting the second optical path length of the extender (12) and subsequently a step of comparing these quantities *A* and

further by means of the extender (12) reducing the difference between the propagation time of radiation in the object and the reference branch from the field plane (2) up to the output image plane (10), wherein the quantity *A* is represented by a mean value of holographic signal, the ratio of the value of holographic signal to the noise of holographic signal, the value of spectrum in a small area around the carrier frequency, or by the ratio of the value of spectrum in a small area around the carrier frequency to the noise of the spectrum values.

15. Method of interferometric system alignment according to any of claims 10 to 14, characterized in that any of the steps described in claims 10 to 14 are performed repeatedly.

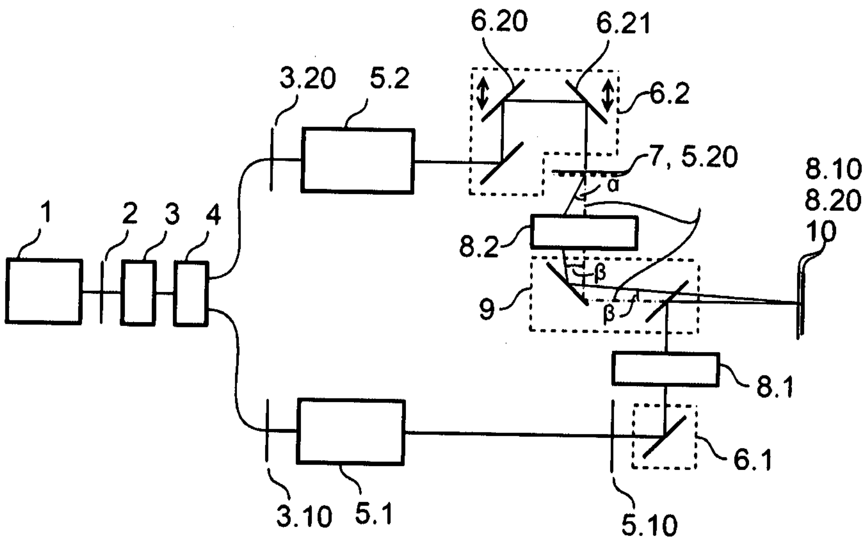


Fig.1

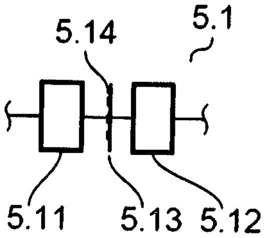


Fig. 2

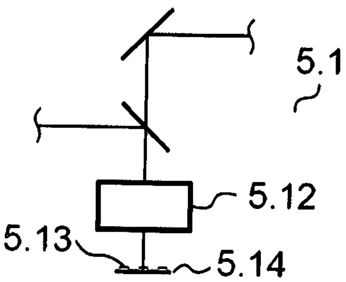


Fig. 3

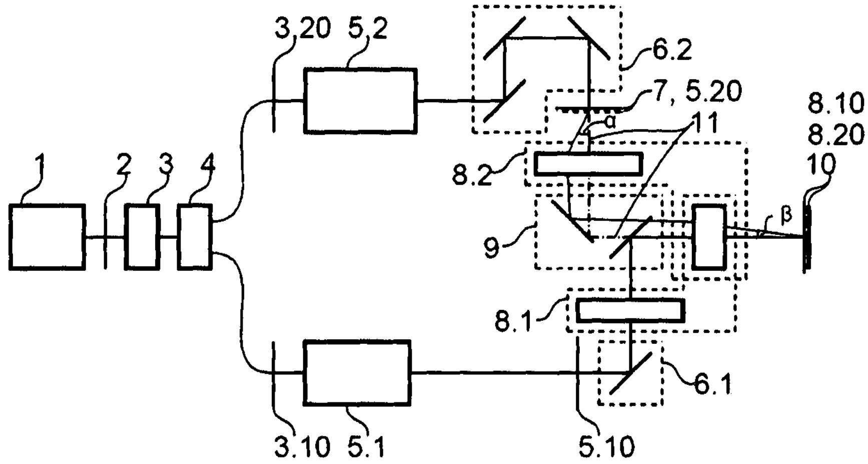
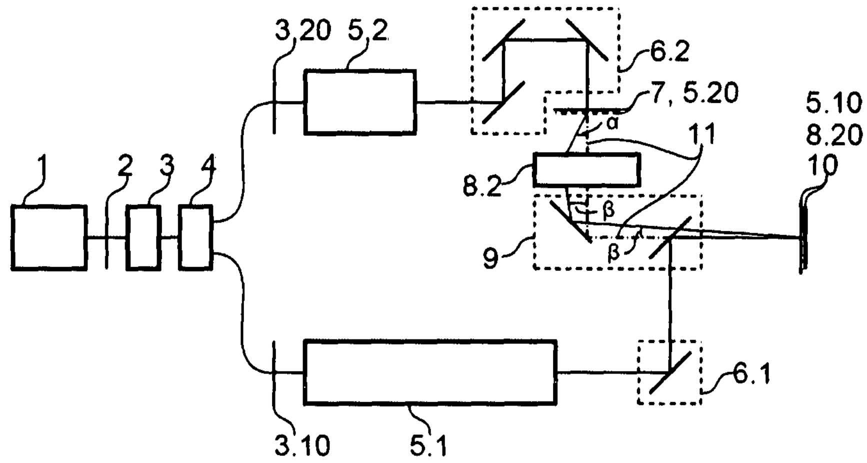

3.20
5.2
6.2
7, 5.20
α
11
8.10
8.20
10
1
2
3
4
8.2
9
β
8.1
6.1
3.10
5.1
5.10

Fig. 4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5.2
6.2
7, 5.20
α
11
5.10
8.20
10
1
2
3
4
8.2
β
9
β
6.1
3.10
5.1

Fig. 5

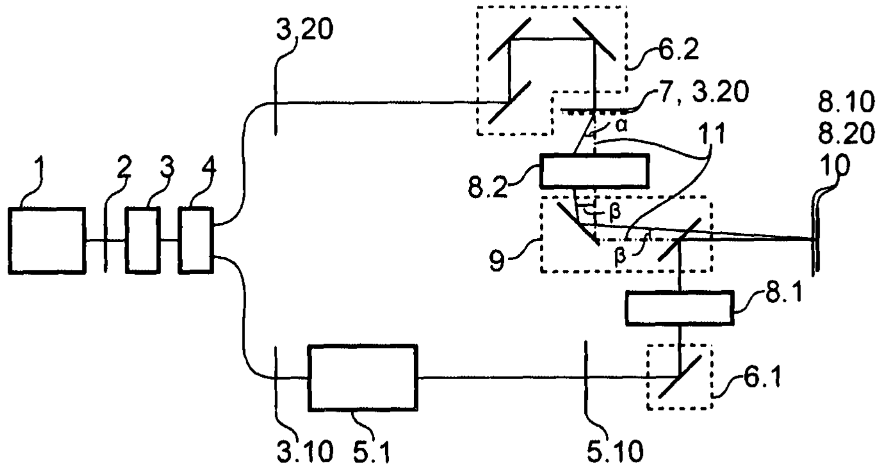
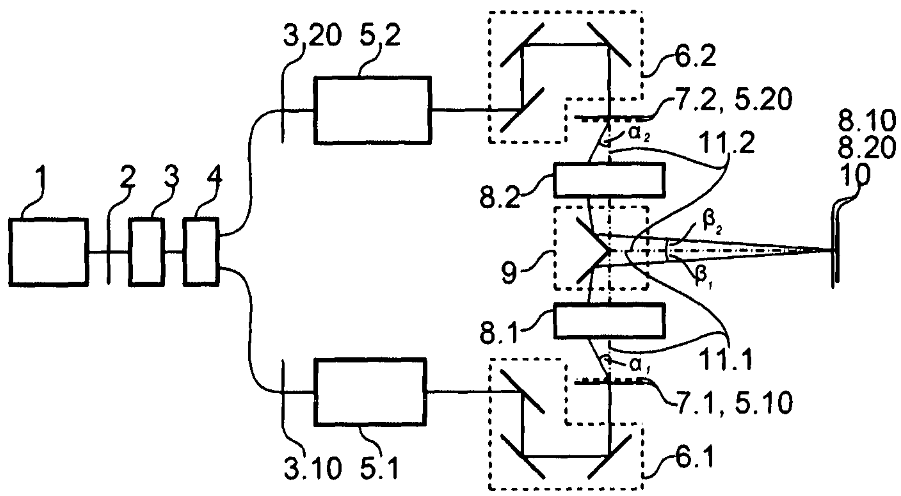

3.20
6.2
7, 3.20
α
11
8.10
8.20
10
1
2
3
4
8.2
β
9
β
8.1
6.1
3.10
5.1
5.10

Fig. 6


3.20
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6.2
7.2, 5.20
$α_2$
11.2
8.10
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10
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2
3
4
8.2
$β_2$
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11.1
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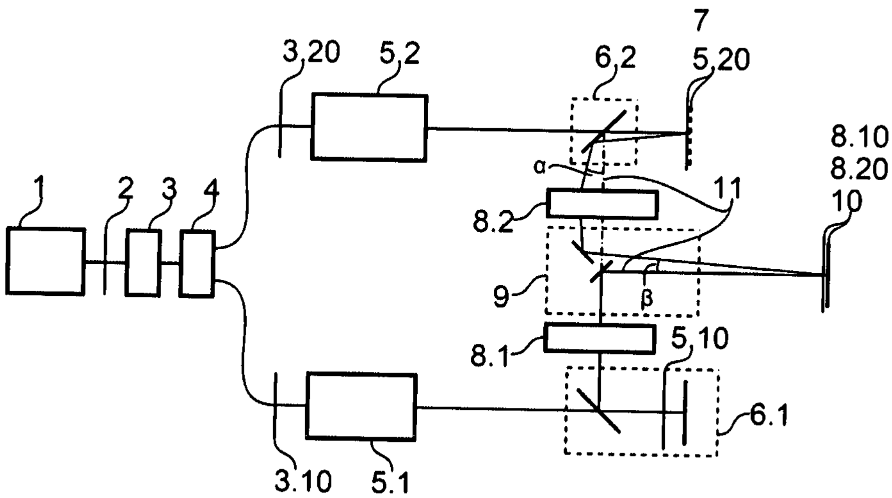
3.10
5.1
6.1

Fig. 7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β
8.1
5,10
6.1
3.10
5.1

Fig. 8

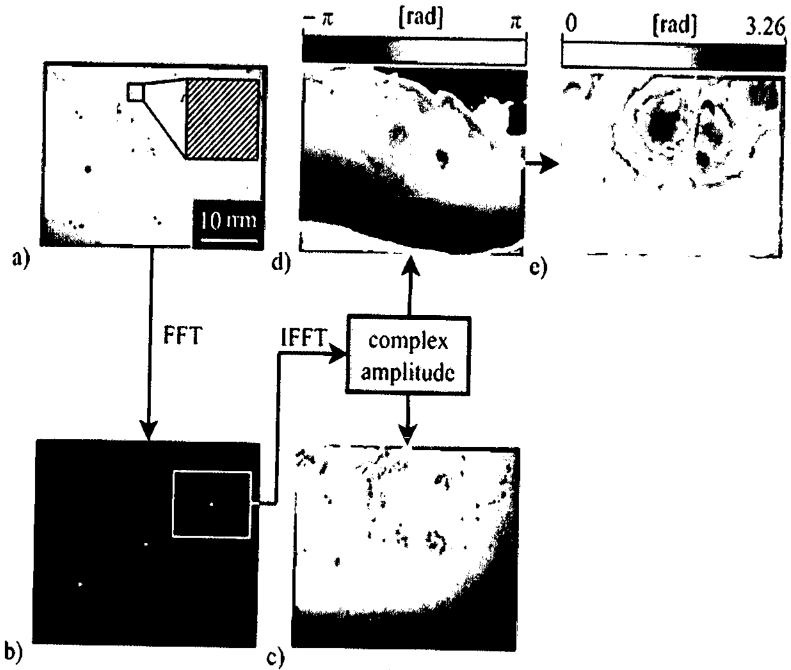
− π
[rad]
π
0
[rad]
3.26
10 nm
a)
d)
e)
FFT
IFFT
complex amplitude
b)
c)

Fig. 9

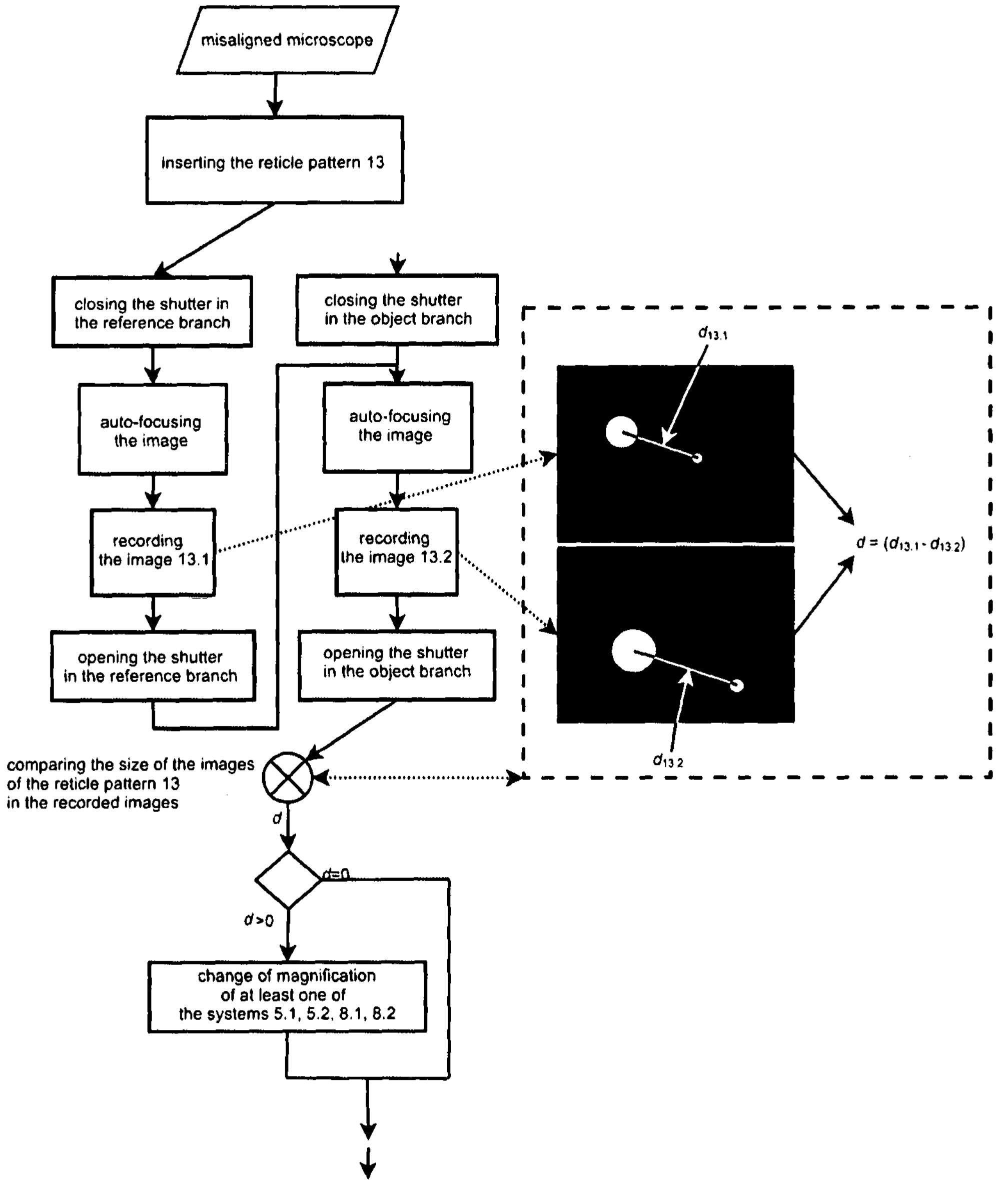
misaligned microscope
inserting the reticle pattern 13
closing the shutter in the reference branch
closing the shutter in the object branch
auto-focusing the image
auto-focusing the image
recording the image 13.1
recording the image 13.2
opening the shutter in the reference branch
opening the shutter in the object branch
d13.1
d13.2
d = (d13.1 - d13.2)
comparing the size of the images of the reticle pattern 13 in the recorded images
d
d=0
d>0
change of magnification of at least one of the systems 5.1, 5.2, 8.1, 8.2

Fig. 10

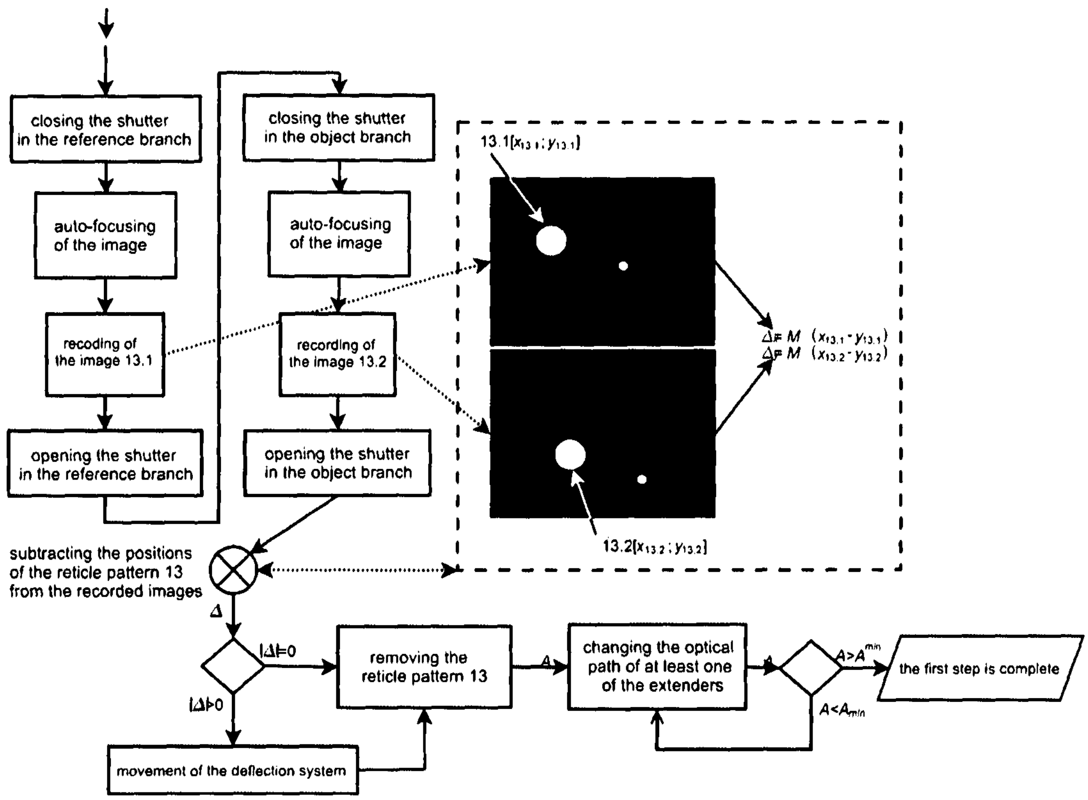
closing the shutter in the reference branch
auto-focusing of the image
recoding of the image 13.1
opening the shutter in the reference branch
closing the shutter in the object branch
auto-focusing of the image
recording of the image 13.2
opening the shutter in the object branch
13.1[x13.1; y13.1]
13.2[x13.2; y13.2]
Δ= M (x13.1 - y13.1)
Δ= M (x13.2 - y13.2)
subtracting the positions of the reticle pattern 13 from the recorded images
Δ
|Δ|=0
|Δ|≠0
removing the reticle pattern 13
A
changing the optical path of at least one of the extenders
A
A>A min
A<A min
the first step is complete
movement of the deflection system

Fig. 11

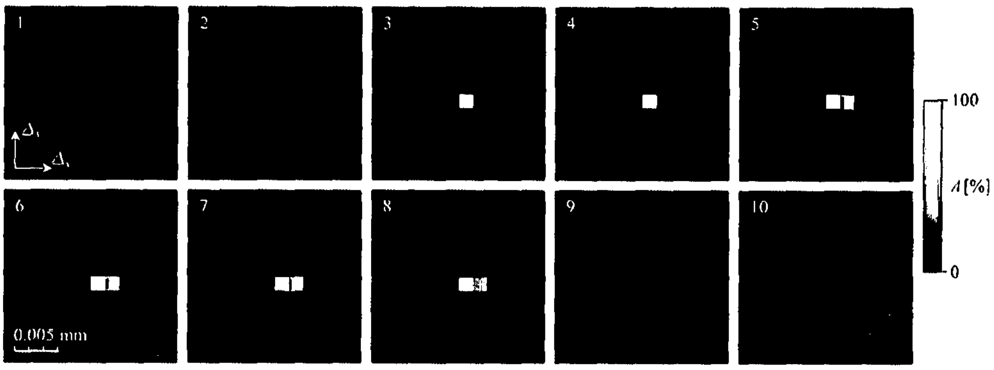

Fig. 12

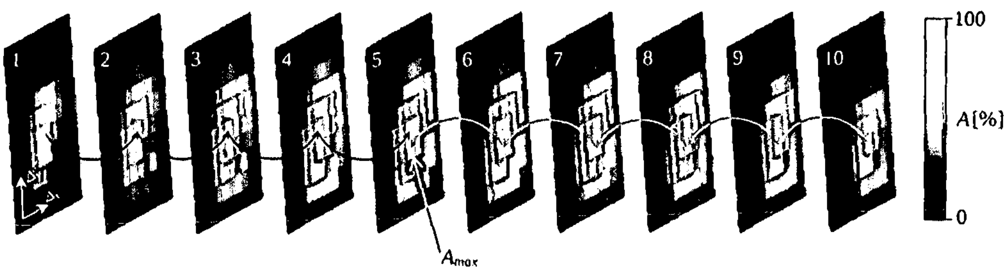

Fig. 13