(31) Convention Application No. 2 655 041

(32) Filed 4 Dec. 1976 in

(33) Fed. Rep. of Germany (DE)

(44) Complete Specification published 21 May 1980

(51) INT. CL.3 G02B 21/02 7/00

(52) Index at acceptance G2J 8L3

(54) IMMERSION OBJECTIVE FOR A MICROSCOPE

(71) We, CARL-ZEISS-STIFTUNG, a Foundation established under the laws of Germany, 7920 Heidenheim (Brenz), Wuerttemberg, Germany, trading as CARL ZEISS, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to an immersion objective for microscope for use with a plurality of optically different immersion substances

An immersion objective is defined as a microscope objective which includes a front element adapted for immersion contact with a liquid substance through which a microscopic object is to be observed. By said immersion contact the liquid substance completely fills the space between the front lens of the objective, and, with covered objects, the cover glass, or, with uncovered objects, the object itself. Through the use of immersion substances, it is possible to compute microscope objectives having a high numerial aperture with values larger than 1 (one).

There are various immersion substances the best known of which are glycerine and 30 cedar oil. Each of these substances has a different index of refraction. With objectives of higher aperture, the image quality depends to a high degree on the optical medium between the object and the front element of 35 the objective. For this reason, immersion objectives are corrected for a specific immersion substance, i.e., a certain object type is provided in several examples each for use with a different immersion substance. When using such an objective, corrected for a certain immersion substance, e.g. oil (n = 1.52), with an immersion substance of lower index of refraction, e.g., water (n = 1.33), image errors, particularly spherical aberra-tion, result, which with medium-strength and high-strength objectives leads to an intolerable deterioration of image quality.

Even with dry objectives, i.e., microscope objectives which are used without immersion

substance, the image quality of higher-aperture objectives depends greatly on the thickness of the object cover glass used. The cover glass thickness is standardized at 0.17 mm and the objectives are computed and corrected precisely on the basis of this value. In order not to have to restrict the cover glass thickness of specimens with dry objectives of higher aperture to dimension deviations which are too small and cannot as a rule be maintained, there are known correction mounts which permit adaptation to a plurality of cover glasses, the thickness of which deviate from said standardized thickness by varying the distance of optical components within the objective.

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With such so-called correction mounts, the front lens and another lens group of the microscope objective ordinarily are made displaceable relative to each other in the direction of the optical axis. The shifting of these lens groups relative to each other affects the spherical correction of the objective in such a way as to permit correction of the spherical aberration, caused by the deviation of the thickness of the cover glass used from the standard thickness. However, there result other image errors, such as coma, color errors, astigmatism which have to be accepted.

Such correction mounts previously were used only for adjustment to the thickness of the cover glass used.

There is known in the art a microscope objective for vertical illumination where the image-forming system is surrounded by a ring-shaped condenser through which the light is directed to the specimen. In order to avoid disturbing reflexes, there is located in front of the front lens of the objective a plane-parallel conically restricted glass plate whose thickness is at least that of the front lens. This plate is brought in contact with the specimen and thus directs the illumination optical path to the specimen in such a way that only the spot to be observed is illuminated, without producing disturbing reflections impairing the image contrast. The front glass plate permits illumination

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and observation of objects covered by a thick liquid layer, in that the plate is immersed in the liquid and directs the illumination optical path to the object directly and without disturbing surface reflexes.

The object of the present invention is to provide an immersion objective for a microscope which can be used with a plurality of optically different immersion substances, thereby reducing the large number of different microscope objectives designed for a specific immersion substance, but similar in design, without restricting the application of

the immersion objectives.

The object of the present invention is achieved by means of an objective comprising a tubular housing and a plurality of lenses having a common optical axis and mounted in the tubular housing, said objective including at least one optical element which is selectively displaceable realtive to said lenses in the direction of said optical axis and over a predetermined axial range of adjustability, whereby depending upon the immersion substance used a selective correction may be made for image defects arising out of the use of a particular one of said substances.

The immersion substance which completely fills the space between object and front lens of the objective may be considered as a liquid plate. When increasing the index of refraction n of such a liquid plate with respect to a nominal value, there appears a spherical overcorrection of the objective; when decreasing the index of refraction with respect to said nominal value there appears a spherical undercorrection. When changing the thickness d of such a liquid plate, the spherical overcorrection in-

creases as the d-value increases, and decreases as the d-value decreases.

If, for example, an objective constructed according to the prior art and corrected for a certain immersion oil is to be used with an immersion substance of lower index of refraction (e.g., water) the thickness of the immersion space decreases by the ratio of the indici of refraction of said substances during the focusing action of the microscope equipped with said objective. The above considerations show that than spherical aberration results.

It is an object of the present invention to provide a microscope objective which can be used with different immersion substances having different indices of refraction with-

out the resulting of image defects.

This object of the present invention is achieved in that the objective contains a lens group selectively displaceable in the direction of the optical axis of the objective with respect to the other lens groups of the objective, thereby influencing the spherical correction of the objective in such a way

that the image errors resulting when changing the immersion liquid are compensated.

The object of the present invention is achieved with particular advantage in that there is provided an objective comprising a tubular housing and a plurality of lens elements mounted in this housing and including a front lens, facing an object to be observed, all said lens elements having a common optical axis, a transparent plate carried by said housing and positioned in axially spaced relation to the front of said front lens, the distance between said plate and said front lens being selectable as one of a plurality of different distances depending upon the index of refraction of the immersion substance being used, whereby during normal focusing action of a microscope equipped with said objective, the axial space through the immersion substance to a microscopic object under examination is directly related to the refractive index of the immersion substance being used.

When changing the immersion substance between the microscope object and said front plate the spherical aberration resulting from the changed index of refraction n is compensated by the aberration caused by the changed thickness d of the immersion space. The image field correction of the microscopic objective itself is thereby not influenced and remains the same independent of the immersion substance used, since the objective is always used in the same

way.

Said transparent front plate is designed as a plane-parallel glass plate. Its thickness does not influence its effectiveness.

The new immersion objective is constructed in such a way that it includes externally accessible mechanical actuating means provided with a refractive-index scale showing markings for conventional immersion substances and serving for adjusting the distance between front lens and said 110 front plate. This actuating means preferably is an adjusting ring carried by the objective housing and mechanically rotatable about the optical axis of the objective. By rotating this ring the distance between front lens 115 and front plate is adjusted in accordance with the index of refraction n of the immersion substance used.

The invention will be explained in further detail below with reference to Figs. 1 to 3 120 of the accompanying drawings, in which:

Fig. 1 is a partial section through one embodiment of the new immersion objective;

Fig. 2 shows the space between the front lens of a new immersion objective and the 125 cover glass of an object in an enlarged section;

Fig. 3 shows the space illustrated in Fig. 2 when using an immersion substance of lower index of refraction.

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In Fig. 1, 1 denotes a microscope immersion objective comprising a front lens 2, two additional lens groups 3 and 4 and additional lens groups not shown. All lens groups are mounted in a tubular housing 5 which is movable upwards against the action of a spring (not shown) in the direction of the optical axis of the objective.

In axially spaced relation to the front
of front lens 2 of the objective, there is
arranged a plane-parallel glass plate 6 in
a mount 7. A mechanically rotatable adjusting ring is denoted by 8 and is provided with
an index mark 9. Adjustment ring 8 is connected with another ring 10 which has a
cutout 11 in the form of a curved slot. A
pin 12, rigidly connected to the cylinder
mount 5, slides on the curved slot in ring 10.

During rotation of adjusting ring 8, the cylinder mount 5 is moved in the direction of the optical axis via the curved slot 11 and the pin 12, in relation to the fixed mount 7 and the fixed ring 13. The ring 13 bears a refractive-index scale 14 showing marks for indici of refraction of various immersion substances.

During rotation of adjusting ring 8, the distance between the front lens 2 and the plate 6 is changed so that the index of refraction for the immersion substance selected on scale 14 lines up with index mark 9. Then the selected immersion medium is placed in the space between the front plate 6 and the object to be observed and focusing is accomplished by moving the objective with respect to the microscopic object.

The mode of operation of the new immersion objective is explained in detail by means of Figs. 2 and 3. In Fig. 2, 15 denotes the cover glass of a specimen to be examined. Between the front plate 6 of the objective 1 and the cover glass 15, there is an immersion substance 16 which in the example shown is oil with an index of refraction n = 1.52. By turning the adjusting ring 8 on tubular housing objective 1, the index mark 9 is lined up with mark 1.52 on the ring 13 which results in the distance 50 between the front lens 2 and the front plate 6 as shown in Fig. 2. During subsequent focusing on the specimen, a thickness d_1 of the immersion space results. As soon as this is achieved, a light beam 18 has the path 55 shown in Fig. 2.

If, using the same objective 1, another immersion medium 17, for example water (n = 1.33) is to be used, the adjusting ring 8 is rotated till the index mark 9 is lined up with mark 1.33 on scale 14. The distance between the front lens 2 and the front plate 6 varies in the way shown in Fig. 3, i.e., it is reduced by the distance s. During the subsequent focusing, as shown by a comparison of Figs. 2 and 3, the specimen with cover

glass 15 is lifted slightly so that a thickness d_2 of the immersion space between front plate 6 and cover glass 15 results. Thickness d_2 is slightly larger than thickness d_1 of the immersion space in the example of Fig. 2. Thus the spherical aberration resulting from the reduced index of refraction n of immersion substance 17 is compensated by the one caused by changing from thickness d_1 to thickness d_2 of the immersion space.

In the case of optimum focusing, the light beam shown as an example in Figs. 2 and 3 has precisely the same path within the objective itself. This makes it evident that the image field correction of the microscope objective remains exactly the same when changing the immersion medium.

The new immersion objective also makes possible correction of the cover glass thickness. For this purpose, the adjusting ring 8 is slightly rotated until the necessary correction is effected. Now, when changing the immersion substance, the adjusting ring 8 is rotated starting from said correction posion.

With the new microscope objective it is also possible to correct for the cover glass thickness zero so that this objective can be used for examining specimens with or without cover glass. With the immersion, shown in Fig. 2, it makes virtually no difference whether a cover glass 15 is used, since both media 15 and oil 16 have nearly the same index of refraction. In case of Fig. 3 with the lower refractive medium 17, the thickness d_2 of the immersion space must be slightly increased when switching from examining a specimen with cover glass to examining a specimen without cover glass.

The objective shown in Figs. 1 to 3 may 105 be constructed with numerical apertures between 0.5 and 1. With objectives with a numerical aperture smaller than than 0.5, the error resulting from changing the immersion substance can be practically dis-110 regarded.

The new immersion objective has a very large correction range which includes all immersion media and reaches virtually from n=1.33 to an upper limit not further 115 defined.

Immersion objectives according to the present invention are preferably used in fluorescence microscopy. There it is important to use immersion substances with- 120 out any disturbing fluorescence of their own. When using the new objective, suitable liquids can be used as immersion substance regardless of their index of refraction. For example, it is possible to use liquid paraffin 125 as an immersion substance. Quite generally, the new immersion objectives make the user completely independent from the immersion substance, i.e., the range of application of an immersion objective is vastly increased 130

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over conventional objectives designed for a specific immersion substance, so that the number of different immersion objectives can be reduced.

WHAT WE CLAIM IS:-

1. An immersion objective for a microscope for use with a plurality of optically different immersion substances, said objective comprising a tubular housing and a plurality of lenses having a common optical axis and mounted in the tubular housing, said objective including at least one optical element which is selectively displaceable relative to said lenses in the direction of said optical axis and over a predetermined axial range of adjustability, whereby depending upon the immersion substance used a selective correction may be made for image defects arising out of the use of a particular one of said substance.

2. An immersion objective for a microscope for use with a plurality of optically different immersion substances, said objective comprising a tubular housing and a plurality of lens elements mounted in this housing and including a front lens facing an object to be observed, all said lens elements having a common optical axis, a transparent plate carried by said housing and positioned in axially spaced relation to the

front of said front lens, the distance between said plate and said front lens being selectable as one of a plurality of different distances depending upon the index of refraction of the immersion substance being used, whereby during normal focusing action of a microscope equipped with said objective under scope equipped with said objective, the axial space through the immersion substance to a microscopic object under examination is directly related to the refractive index of the immersion substance being used.

3. An immersion objective according to claim 2, in which said transparent plate is 45

of plane-parallel glass.

4. An immersion objective according to claim 2, including externally accessible mechanical actuating means for movably positioning said plate to select a given one of 50 different substances.

5. An immersion objective according to claim 4, wherein said mechanical means includes an externally exposed refractive-

index scale.

6. An immersion objective according to claim 4, in which said mechanical means is an adjusting ring carried by said housing and mechanically rotatable about the optical axis of the objective.

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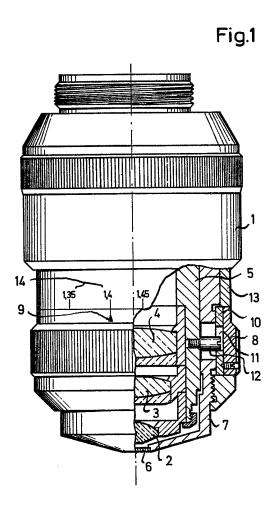
Printed for Her Majesty's Stationery Office by Burgess & Son (Abingdon), Ltd.—1980
Published at The Patent Office, 25 Southampton Buildings, London, WC2A 1AY
from which copies may be obtained.

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