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(54) **MICROSCOPE, ESPECIALLY LASER SCANNING MICROSCOPE**

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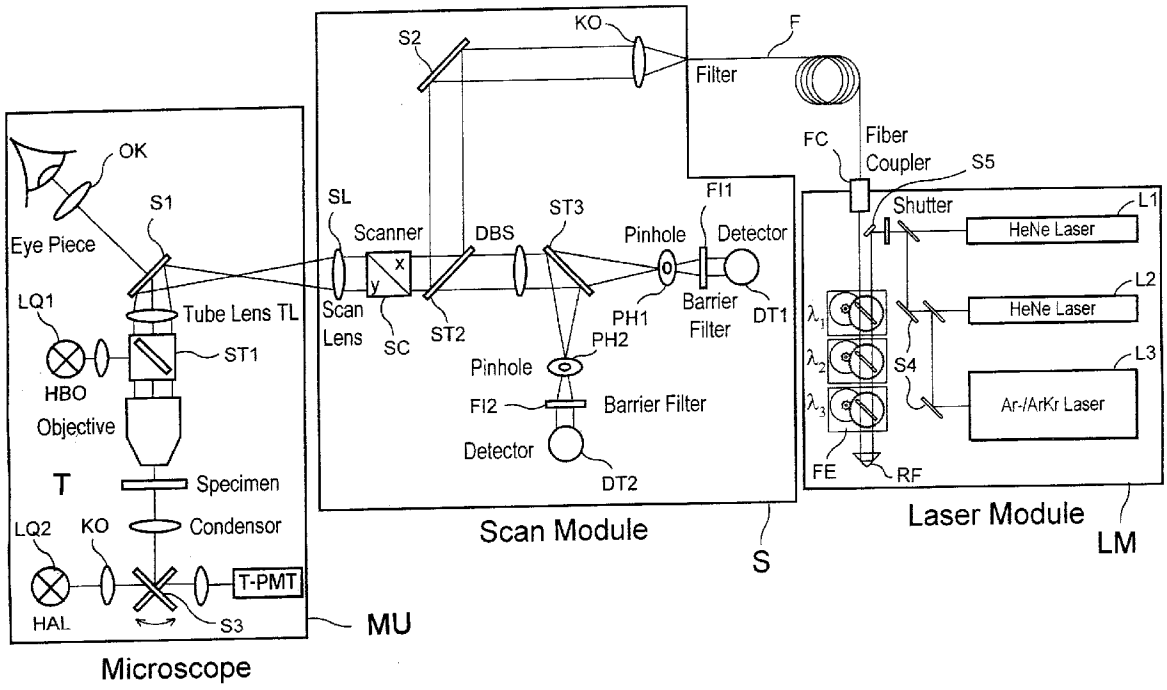
(57) **ABSTRACT**

A microscope, especially a laser scanning microscope, with illumination over one wavelength and/or a plurality of wavelengths, wherein a controlling of the intensity of at least one wavelength is carried out by at least one rotatable interference filter which is arranged in the illumination beam path, wherein the at least one wavelength is at least partially reflected out of the illumination beam path and a plurality of filters for different wavelengths can be arranged one behind the other in the illumination beam path.



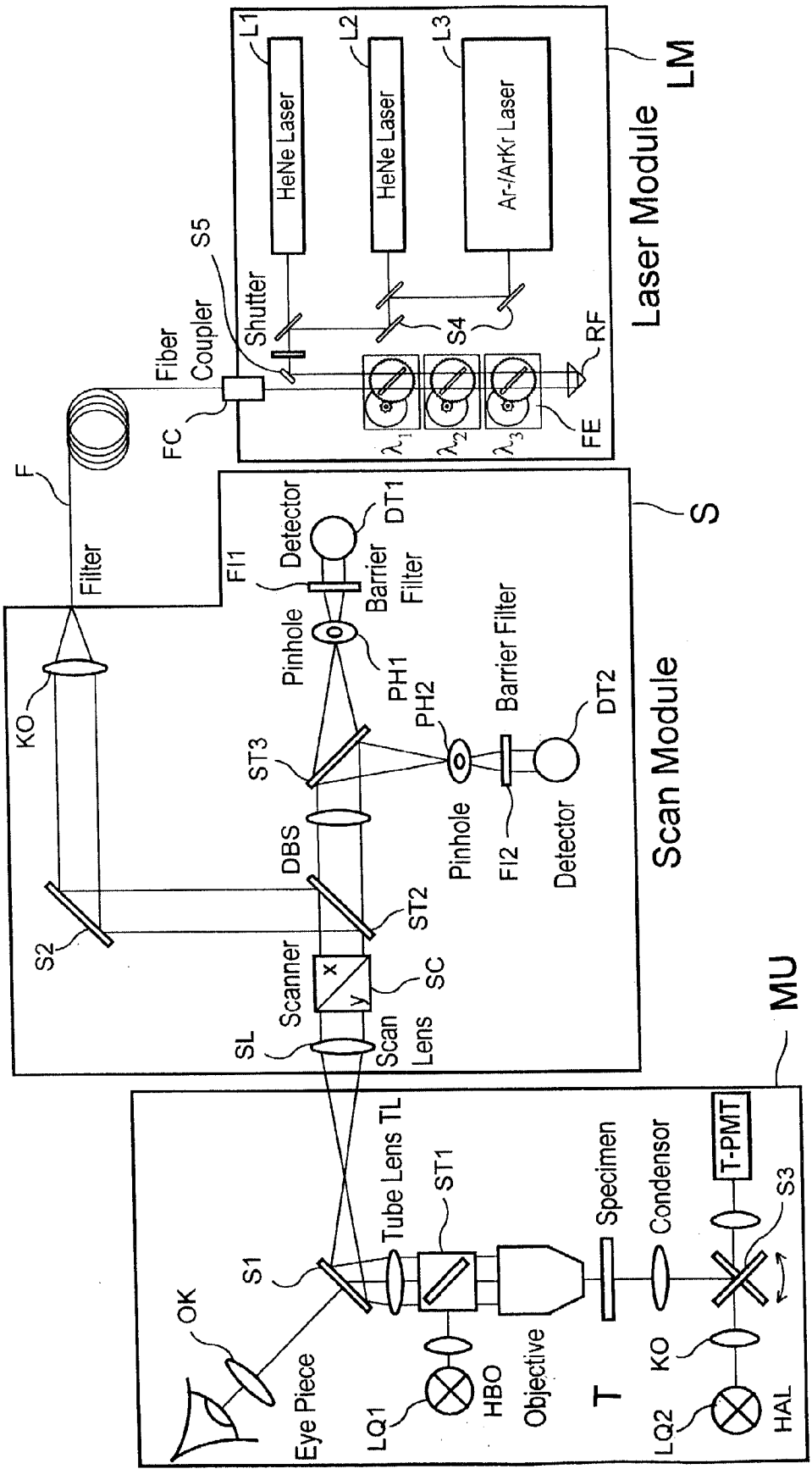

Laser Module
LM
L1
L2
L3
HeNe Laser
HeNe Laser
Ar-/ArKr Laser
S5
Shutter
Fiber
Coupler
FC
S4
RF
FE
F
Filter
S
Scan Module
Detector
DT1
Barrier
Filter
FI1
Pinhole
PH1
PH2
Barrier Filter
DT2
KO
ST3
DBS
Pinhole
FI2
Detector
S2
ST2
Scanner
SC
SL
Scan
Lens
MU
Microscope
Tube Lens TL
ST1
Specimen
Condensor
T-PMT
S3
S1
OK
Eye Piece
LQ1
HBO
Objective
T
KO
LQ2
HAL

FIG. 1

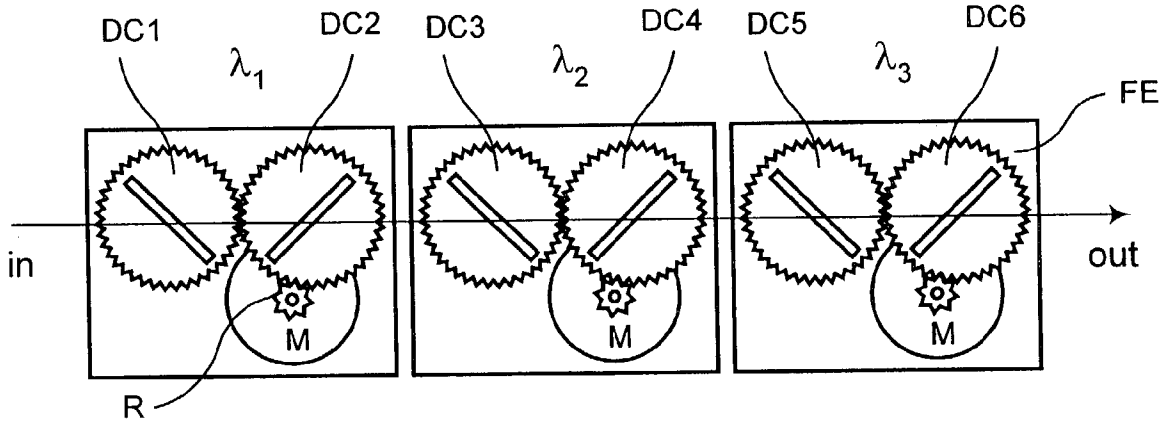

DC1
DC2
DC3
DC4
DC5
DC6
λ_1
λ_2
λ_3
FE
in
out
M
M
M
R

FIG. 2A

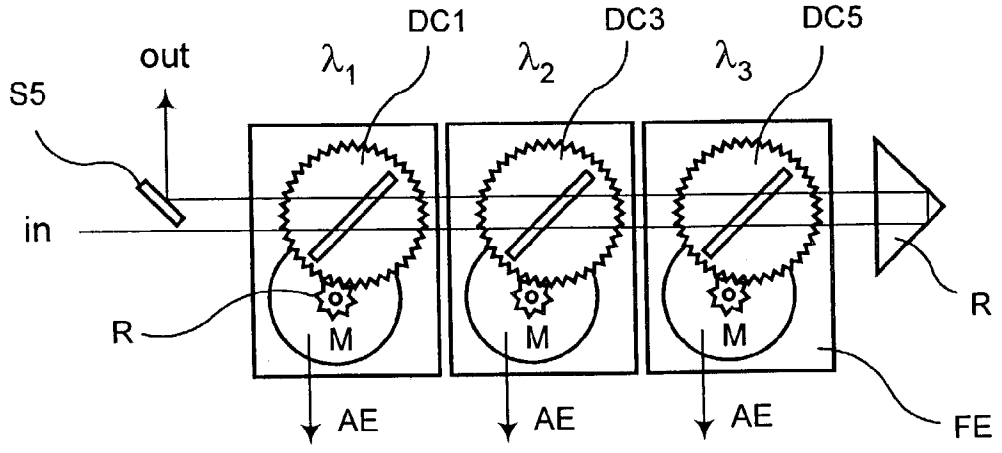

DC1
DC3
DC5
out
λ_1
λ_2
λ_3
S5
in
R
M
M
M
R
AE
AE
AE
FE

FIG. 2B

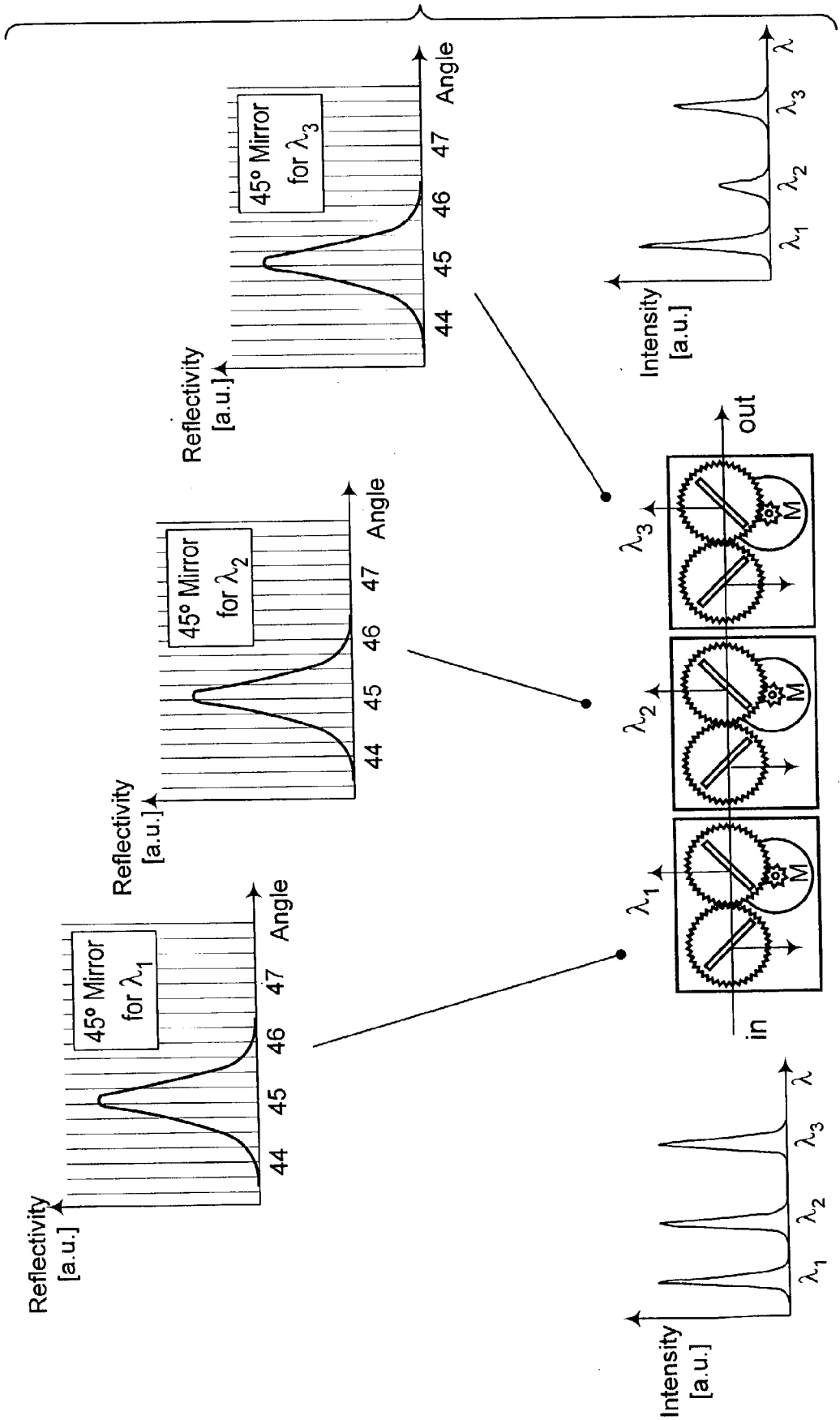

Reflectivity [a.u.]
45° Mirror for λ_1
44
45
46
47
Angle
Reflectivity [a.u.]
45° Mirror for λ_2
44
45
46
47
Angle
Reflectivity [a.u.]
45° Mirror for λ_3
44
45
46
47
Angle
in
out
M
λ_1
λ_2
λ_3
Intensity [a.u.]
λ_1
λ_2
λ_3
λ
Intensity [a.u.]
λ_1
λ_2
λ_3
λ

FIG. 3

MICROSCOPE, ESPECIALLY LASER SCANNING MICROSCOPE

BACKGROUND OF THE INVENTION cl a) FIELD OF THE INVENTION

[0001] The invention relates to laser scanning microscopes and, in particular, an improvement in such microscopes for controlling the intensity of one wavelength of illumination.

OBJECT AND SUMMARY OF THE INVENTION

[0002] The primary object of the invention is to provide an improvement in laser scanning microscopes where the intensity of one wavelength of illumination is controlled.

[0003] In accordance with the invention, a microscope, especially a laser scanning microscope, comprises means for providing illumination over at least one of a single wavelength and a plurality of wavelengths and means for controlling the intensity of at least one wavelength being carried out by at least one rotatable interference filter which is arranged in an illumination beam path. The at least one wavelength is at least partially reflected out of the illumination beam path.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] In the drawings:

[0005] **FIG. 1** illustrates a microscope unit and scan head of a laser scanning microscope;

[0006] **FIGS. 2***a* and **2***b* illustrate rotation of dichroics for influencing the wavelengths in accordance with the invention; and

[0007] **FIG. 3** illustrates in schematic and pictorial the effect of the invention on chosen wavelengths.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0008] A microscope unit M and a scan head S connected thereto are shown schematically in **FIG. 1**.

[0009] A light source LQ**1** with illumination optics illuminating the object on the microscope stage T via a beam splitter ST**1** in a conventional manner is provided in the microscope.

[0010] A swivelable mirror S**3** serves to switch to transmitted-light illumination by means of a light source LQ**2** via the condensor KO.

[0011] Observation through an eyepiece OK is carried out via a tube lens TL and a mirror S**1**. In addition, by means of this mirror or beam splitter S**1**, the scanning beam path is coupled in via the scanning lens SL and the scanner SC.

[0012] The light of a laser module LM is coupled in in the direction of the scanner SC via light conductor F, collimating optics KO, mirror S**2** and beam splitter ST**2**.

[0013] The light coming from the object travels through the scanner SC and dichroic beam splitter ST**2** in the direction of detection, represented herein by way of example by another beam splitter ST**3** for splitting into detection beam paths with pinholes PH**1**, **2**, filters Fl**1**, **2** and detectors DT**1**, **2**.

[0014] A plurality of lasers L**1**, L**2**, L**3** with different wavelengths are provided in the laser module; these lasers L**1**, L**2**, L**3** can also be multiline lasers. They are combined via mirrors and beam splitter S**4**, respectively, and coupled into a coupling-in unit FC in the light conductor F.

[0015] Before being coupled in, they pass a mirror S**5** and a filter unit FE, as is shown in **FIG. 2***b*, and are deflected, again via a filter unit FE, in the direction of the coupling-in unit by a reflector R.

[0016] Dichroics DC**1**, **2**, **3**, **4**, **5**, **6** which have a wavelength-dependent and angle-dependent reflectivity are arranged in the filter unit FE. This is shown by way of example in **FIG. 3** with reference to the angle-dependent reflectivity for the three wavelengths in which the mirror coating is optimized for 45 degrees, i.e., the greatest reflectivity for a determined wavelength occurs at 45 degrees. Therefore, the transmission is adjusted in a continuous manner for the respective wavelength by rotation. The rest of the wavelengths are not affected.

[0017] The optimization at 45 degrees is given by way of example; other angles could also be selected for the greatest reflectivity.

[0018] The light component that is reflected out is suppressed in a suitable manner, for example, by light traps. Since the rotation of the dichroics generates a beam offset, these dichroics are arranged in pairs for compensation.

[0019] In **FIGS. 2***a* and **3**, pairs of dichroics DC**1**, **2** for a wavelength λ1, DC**3**, **4** for a wavelength λ2, and DC**5**, **6** for a wavelength λ3 are arranged in a continuous beam path so as to be selectively reflecting and can accordingly influence these wavelengths and compensate the beam offset by means of the paired arrangement.

[0020] **FIG. 2***b* shows another arrangement as in **FIG. 1**, wherein the beam offset is compensated by passing twice through the dichroics DC**1**, DC**3**, DC**5** for the three wavelengths.

[0021] The driving means for the rotation of the dichroics are carried out in a manner familiar to the person skilled in the art, in this case, as is shown schematically in **FIGS. 2***a* and *b*, by toothed wheels to which the dichroics are fastened, wherein the toothed wheels of the pairs of dichroics in **FIG. 2***a* mesh with one another and accordingly ensure a coupled movement of the pairs of toothed wheels.

[0022] A pinion R which is driven by a motor M is provided for driving the toothed wheels. The driving of the motors can be carried out via a central control unit AE which, for example, controls a predetermined illumination and detection mode which includes the attenuation/adjustment of determined laser wavelengths.

[0023] In a multi-wavelength laser, or when a plurality of laser wavelengths are coupled into a microscope jointly, especially in a laser scanning microscope, one or more wavelengths can advantageously be adjusted, i.e., attenuated, continuously with respect to intensity. If the lasers should be exchangeable, a plurality of such interchangeable filter units with different wavelengths can also be provided.

[0024] The dichroics utilized herein are interference filters such as those supplied, for example, by Laseroptik GmbH, also in pairs for compensating beam offset. Further, dichro-

ics of the above-mentioned type can also be used advantageously for wavelength-dependent influencing of the detection beam path, for example, in **FIG. 1**, in a detection beam path following the beam splitter ST**2** for the suppression of especially unwanted wavelengths, for example, of the excitation wavelength in fluorescence detection.

[**0025**] While the foregoing description and drawings represent the preferred embodiments of the present invention, it will be obvious to those skilled in the art that various changes and modifications may be made therein without departing from the true spirit and scope of the present invention.

What is claimed is:

1. A microscope, especially a laser scanning microscope, comprising:

means for providing illumination over at least one of a single wavelength and a plurality of wavelengths; and

means for controlling the intensity of at least one wavelength being carried out by at least one rotatable interference filter which is arranged in an illumination beam path, said at least one wavelength being at least partially reflected out of the illumination beam path.

2. The microscope according to claim 1, wherein a plurality of filters for different wavelengths are arranged one behind the other in the illumination beam path.

3. The microscope according to claim 1, wherein pairs of filters with identical wavelength characteristics are provided for at least one wavelength for compensating the beam offset.

4. The microscope according to claim 1, wherein at least one filter is traversed twice in that a reflector returns the illumination light via the filter.

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