(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
02 January 2020 (02.01.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/006101 A1

(51) International Patent Classification:
G02B 15/22 (2006.01)

(21) International Application Number:
PCT/US2019/039285

(22) International Filing Date:
26 June 2019 (26.06.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/763,694 27 June 2018 (27.06.2018) US

(71) Applicant: **CARNEGIE MELLON UNIVERSITY** [US/US]; 5000 Forbes Avenue, Pittsburgh, Pennsylvania 15213 (US).

(72) Inventor: **LANNI, Frederick**; 5000 Forbes Avenue, Pittsburgh, Pennsylvania 15213 (US).

(74) Agent: **CARLETON, Dennis M.**; Kacvinsky Daisak Bluni PLLC, 50 Doaks Lane, Marblehead, Massachusetts 01945 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) Title: IMMERSION FRONT-END LENS SYSTEM

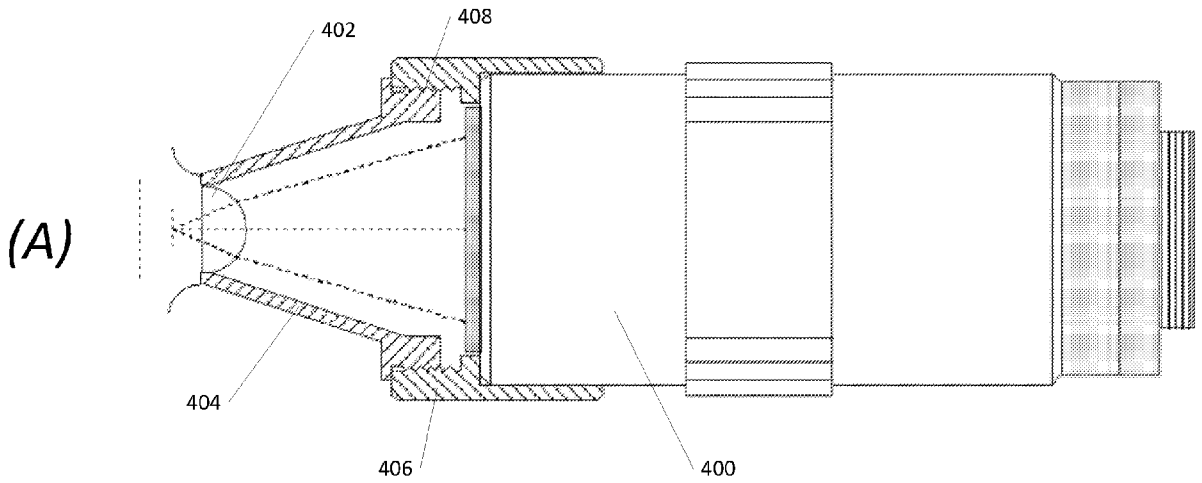


FIG. 4

(57) **Abstract:** An accessory optic is described which enables a non-immersed, long working distance microscope objective to be used as a higher-power immersion objective. This is particularly useful for biological imaging of specimens in an index-matching clarification medium. The accessory approach is the opposite of conventional unitized immersion objective design and presents several advantages over conventional design. The front lens element of the accessory may be selected to have precisely the refractive index of the specimen immersion medium and the accessory may be designed for use with immersion media that are incompatible with conventional immersion objectives. A dual accessory enables two such modified objectives to be optimized for light-sheet microscopy.

WO 2020/006101 A1

IMMERSION FRONT-END LENS SYSTEM

Related Applications

[0001] This application claims benefit of US Provisional Patent Application No. 62/763,694, filed June 27, 2018 entitled "Drop-In Immersion Front-End Lens System for Long Working-Distance Microscope", the contents of which are incorporated herein in their entirety.

Background of the Invention

[0002] Optical deep imaging in biological specimens (e.g. tissues, organs, embryos, biofilms, cell masses, artificial organs) is resurgent in a number of areas of biomedical science. In general, this practice requires the use of a microscope objective that is designed for immersion either in a culture medium (generally for living specimens) or in a clarifying medium (generally for preserved specimens).

[0003] For living specimens, water-immersion or silicone oil immersion objectives are optimal. For fixed (preserved) specimens, the highest optical transparency usually is obtained with specially formulated infiltration liquids. Dramatic advances, such as the Scale and CLARITY methods, have rendered centimeter-size objects such as whole embryos or the intact brain of a mouse sufficiently transparent for direct microscopic imaging without the need for cutting the tissue into sections. This places extraordinary demands on the immersion requirements and working distances of microscope objectives for deep imaging and has driven the design of special objectives with 3-8mm working distance corrected for the refractive index of ScaleA2 medium (n=1.387), CLARITY medium (n=1.453), or conventional oil immersion (n=1.518). These specialty objectives tend to be costly and most cannot be used with solvents or immersion media differing from the design formulations.

Summary of the Invention

[0004] Described herein is an accessory optic comprising a lens or lens assembly, for a low-power, non-immersed, long working distance microscope objective that transforms it into a higher-power immersion objective useful for deep imaging of specimens infiltrated with a clarifying medium or solvent. The accessory optic is easily attachable to the main

objective. The "add-on" design of the optic confers several advantages over traditional unitized immersion objectives. The accessory optic increases the resolution and magnification of the objective while retaining sufficient working distance for millimeter-depth imaging. Of equal importance, the accessory optic enables use of index-matching solvents that otherwise are incompatible with standard immersion objectives, including cases in which a cover glass cannot be used to separate the mounting medium of the specimen from the immersion medium of the lens.

[0005] The accessory optic may be embodied in the form of low-cost "kits". It is contemplated that two kits would be provided, but other types of kits may also be useful. The two kits include a Type I kit for deep imaging by conventional or confocal microscopy, and a Type II kit for deep imaging by 'light-sheet' microscopy, also known as selective-plane illumination microscopy (SPIM). The Type I kit comprises the accessory optic assembly with the front lens element selected to match, as closely as possible, the refractive index of the intended immersion medium. The Type I kits may comprise multiple accessory optics for use with different immersion media. The front optical element, which is a simple plano-convex lens, can be produced easily for this purpose from a range of optical glasses. The Type II kit comprises the accessory optics for a pair of objectives, but with a specially-configured pair of plano-convex lenses for optimal positioning of the two objectives for SPIM and optimal viewing of large specimens.

Brief Description of the Drawings

[0006] **FIG. 1** shows aplanatic refraction by a sphere.

[0007] **FIG. 2** shows the aplanatic condition implemented with immersion optics. The lens is shown as hemispheric, but may be any plano-convex lens, or generally, any singlet lens with a spherical outer surface.

[0008] **FIG. 3** is a schematic diagram showing placement of attachable accessory optic of the present invention with respect to a low-power, long working distance microscope.

[0009] **FIG. 4, View (A)** shows hardware components of the one embodiment of the accessory optic of the present invention having a singlet optic element. **View (B)** shows an embodiment having additional optical elements.

[0010] **FIG. 5** shows two alternate embodiments of the Type I kit. The accessory may be mounted on the objective, or separately on the microscope stand, or both.

[0011] **FIG. 6** shows a preferred embodiment of the accessory optic of the present invention having a magnetic mount to hold the accessory in place on a microscope objective.

[0012] **FIG. 7** shows a modified front-end lens pair in a symmetric 45° SPIM instrument with complete mechanical clearance for long-range horizontal specimen translation.

[0013] **FIG. 8** presents the schematic of lens configuration for symmetric light-sheet microscopy (selective-plane illumination microscopy, SPIM).

[0014] **FIG. 9, View (A)** shows a schematic view of Type II front-end lens pair, shown as PCX hyper-hemispheres. **View (B)** shows a schematic view of Type II front-end lens pair, shown as standard PCX hemispheres giving greater specimen clearance than in **View (A)**. **View (C)** shows a schematic view of Type II front-end lens pair, shown as conventional PCX lenses used with a right-angle prism.

[0015] **FIG. 10** shows two alternate embodiments of the type II kit.

Detailed Description of the Invention

[0016] Described herein is an accessory optic for a microscope objective and the use of the accessory optic in a microscope objective system for deep imaging of specimens immersed in refractive-index matching media. The use of the accessory optic with a low-power, non-immersed, long working distance microscope objective results in a moderate-power, moderate magnification, liquid-immersion objective system with sufficient working distance for use in deep imaging including selective plane illumination microscopy (SPIM).

[0017] The invention comprises at least two optics kits for microscopes. A Type I kit comprises an accessory immersion lens assembly which may be inserted between a non-immersed microscope objective and the specimen. A Type II kit comprises an optical setup for deep imaging by light sheet microscopy (also known as selective-plane illumination microscopy or SPIM) and incorporates a modification of the optics described in the Type I kit as the central optical component.

Type I Kit

[0018] The accessory device is referred to as an attachable immersion front end because it consists of a singlet lens, or a more complex lens assembly comprising a singlet lens and one or more secondary optics, that is inserted between a microscope objective and a specimen, and which is coupled to the specimen by an immersion medium. The

immersion medium may be the same medium in which the specimen is immersed or may be an iso-refractive immersion oil separated from the immersed specimen by a window or coverglass. In the ideal case, the refractive index of the immersion medium and the refractive index of the front lens element are equal. For practical reasons, the indices may be chosen to differ. In the simplest case, the accessory optic is a single plano-convex lens which may be a hemisphere or hyper-hemisphere.

[0019] This invention is related to the conventional design of high-power oil-immersion objectives in which a hyper-hemisphere and meniscus lens duplex constitute the built-in front end of the objective. In a conventional high-power oil-immersion objective, the front lens element is aplanatic. This is referred to as an Amici-type front end. Because of the unitized construction of conventional objectives, most only can be used with specific immersion media.

[0020] Additionally, in all conventional oil-immersion objectives the working distance generally is small, typically 60-500 μm , which limits their use in deep imaging. Recently, immersion objectives with moderate numerical aperture (NA) and 1-10 mm working distances have been introduced for large-scale (millimeter or centimeter) specimens rendered transparent with Scale, CLARITY, or other media. Some of these lenses are compatible with oil immersion but are generally not compatible with high-index organic liquids such as methyl salicylate.

[0021] The Amici-type front end of the present invention is a separate accessory optic that can be matched to any immersion medium by selection of the optical glass for manufacture. In practice, a standard glass is selected, and the refractive index of the immersion medium is adjusted slightly to match. When attachably connected to a long working-distance low-power objective, immersed working distances much greater than 1 mm can be attained. In its simplest form, the attachable front end consists of a centered plano-convex lens inserted between the specimen and the microscope objective with the curved surface of the lens facing the microscope objective and the flat surface of the lens coupled to the specimen by the immersion medium. Because the immersion medium and the lens have the same refractive index, the curved surface of the lens is the only additional refracting surface in the optical system. The plano-convex (PCX) lens may be a conventional thin lens, a thick lens, a hemisphere, or an Amici-type hyper-hemisphere. Without loss of generality, we refer to the drop-in lens as a hemisphere of radius R_S. Because of the index-matching fluid, the immersed surface of the lens may have arbitrary curvature. As such, the term "plano surface" as used herein should be interpreted as referring to a flat or

arbitrarily curved surface.

[0022] Aplanatic refraction occurs at a spherical glass surface. In this circumstance, a spherical wavefront passing through the surface is transformed by refraction into a precisely spherical wavefront with altered radius of curvature. In other words, the refraction introduces no spherical aberration, even for rays far outside the paraxial zone of the optical system. For a glass sphere of radius R_S and index of refraction n_g, aplanatic refraction occurs when a point source of spherical waves is positioned within the glass at a radius from the center equal to R_S/n_g. Rays exiting the opposite side of the sphere are refracted to form a spherical wave that is exactly centered on a back-projected collinear point outside the sphere at a radius equal to $n_g R_S$.

[0023] **FIG. 1** shows the aplanatic effect. For a glass sphere, the internal aplanatic surface is a centered spherical shell of radius R_S/n_g, and the external aplanatic surface is a centered spherical shell of radius $n_g R_S$. A point source located on the internal aplanatic surface is imaged as a virtual point source located at the collinear point on the external aplanatic surface. This occurs for all rays, independent of the ray angle θ^*, up to θ^*= 90 degrees.

[0024] In the example shown in **FIG.1**, a point source at O located at a distance $R_{int} = R_S/n_g$ from the center emits spherical waves. One ray is shown making an angle θ^* with the axis and making an angle θ_{max} after refraction at the spherical surface. The back-projection of the refracted ray intersects the axis at *VO*, the collinear point at a distance $R_{ext} = n_g R_S$ From the center. This is an exact result for any ray that exits the far side of the sphere. Therefore, *VO* is a perfect virtual object and the refracted wavefront exactly spherical and centered at this point.

[0025] Because $n_g > 1$ in all cases, $n_g R_s > R_S > R_S/n_g$. This applies equally to all plano-convex (PCX) lenses when immersed in an index-matching medium on the planar side. For a sphere, the point source would be located within the solid glass. However, it is common practice to truncate the sphere within the internal aplanatic radius to create a hyper-hemisphere, hemisphere, or other PCX lens so that the point source then may then be positioned on the inner aplanatic surface within the immersion medium. **FIG. 2** shows the aplanatic condition implemented with immersion optics, shown as a plano-convex hemisphere coupled to the specimen by a refractive index matching medium. This is referred to herein as the aplanatic condition.

[0026] In the attachable assembly optic of the present invention, the lens and specimen are positioned to satisfy two design conditions: (1) the focus condition of the unmodified microscope; and (2) the aplanatic condition of the inserted lens.

[0027] Condition (1) is met by positioning the PCX lens such that its external aplanatic surface is tangent to the in-focus specimen plane of the unmodified microscope. In this position, the distance between the apex of the convex lens and the in-focus specimen plane of the unmodified microscope is equal to $(n_g + 1)R_S$ where n_g is the refractive index of immersion medium and glass lens, and where R_S is the radius of curvature of the PCX lens surface.

[0028] Condition (2) is met by positioning the actual specimen in a plane tangent to the internal aplanatic surface of the PCX lens. This sets up a virtual object at the external aplanatic surface, and therefore at the in-focus specimen plane of the microscope. In this position, the distance between the apex of the convex lens and the specimen is equal to $(1 + {}^{1}/_{n_g})R_S$. If the PCX lens is a hemisphere, the immersed working distance is equal to the internal aplanatic radius, ${}^{R_S}/_{n_g}$.

[0029] For the purposes of this description W_0 is the working distance of the unmodified microscope objective (i.e., the distance between the objective and the in-focus object plane). Condition (1) is therefore equivalent to the relation:

$$W_0 = n_g R_S + R_S + D$$

where D is the separation distance between the apex of the hemisphere and the objective.

[0030] **FIG. 3** is a schematic diagram showing placement of attachable accessory optic of the present invention with respect to a low-power, long working distance microscope, enabling the instrument to be operated as a moderate-power microscope with sufficient immersion working distance for deep imaging and SPIM. This embodies the essential design conditions noted above.

[0031] **FIG. 4, View (A)** shows hardware components of one embodiment of the accessory optic of the present invention comprising a singlet accessory optic for use with a microscope objective (for example, a Mitutoyo 10X 0.28NA long working distance (LWD) objective). In this embodiment, the accessory optic comprises primary optic element 402, which may be a hemisphere, a hyper-hemisphere or other PCX lens, selected such that the point source (i.e., the specimen) may be positioned on the inner aplanatic surface of optic element 402, which would lie within the immersion medium. Primary optic element 402 is disposed in removable housing 404. Attachment member 406 may be attached to

microscope objective 400 such as to accept various housings 404 having various primary optic elements 402 with indices of refraction matching the index of refraction of the immersion medium. In alternate embodiments, (not shown) attachment member 406 may be attached to the microscope stand or other structures. Housing 404 may be connected to attachment member 406 via threaded arrangement or by any other known means of attachment, for example, via magnets mounted on attachment member 406 and housing 404. Nominally, the optic element 402 is fabricated from an optical glass selected to have a refractive index that matches the intended immersion medium. Attachment members 404 and 406 may also include precision alignment pins or trivets, adjustment screws, or other guides and fasteners not shown in the drawings.

[0032] **FIG. 4, View (B)** shows an alternate embodiment wherein housing 404 houses both the primary optical element 402 and one or more secondary optical elements 410, for example, a meniscus lens or corrective lenses. **View (A)** of **FIG. 5** shows an alternate embodiment of the invention wherein the primary optical element 402 is disposed in a housing 502 separate from the microscope objective. The accessory may also comprise one or more secondary optical elements 410 housed in housing 404 which is attached to the microscope objective 400. Housing 404 may be attached directly to the microscope objective 400 or may be attached via an attachment member, similar to attachment member 406 shown in **FIG. 4, View (A)**. **View (B) of FIG. 5** shows an alternate embodiment wherein the microscope objective 400 is disposed off-axis with respect to the primary optical element 402. Additional lenses such as 410 may be mounted on objective 400 as shown in the configuration of **View (B)** of **FIG. 5**.

[0033] **FIG. 6** shows a preferred embodiment of the present invention *in situ* on a microscope objective. In this embodiment, attachment member 406 is attached to microscope objective 400 via a plurality of set screws 502 creating a frictional attachment between attached member 406 and microscope objective 400. Housing 404 slides on the end of microscope objective 400 and, in one embodiment, may be held in place via magnetic mounts 408 on attachment member 406, which mate with magnetic mounts on housing 404 (not shown). In other embodiments, magnetic mounts 408 may attract a steel ring configured as part of housing 404. Attachment member 406 and housing 404 may be composed of any suitable material, however, the portion of housing 404 which holds the primary optical element 402 should be composed of a material resistant to any adverse effects brought about by the intended immersion medium. Additionally, any cement or seals holding optical element 402 within housing 404 should also be resistant to any

adverse effects brought about by the immersion medium. The set screws 502 in this case, are composed of nylon to avoid damage to microscope objective 400 but may be composed of any suitable material.

[0034] **Effect of the attachable front-end accessory optic on aperture and magnification.** The resolution of a microscope increases with the numerical aperture (*NA*) of the objective. *NA* is defined as the refractive index of the immersion medium multiplied by the sine of the largest angle at which light rays may enter the objective, that is $NA = n_{imm} \sin \theta_{max}$. The maximum ray entry angle for the unmodified microscope is set by the numerical aperture of the non-immersed objective as:

$$NA = (1.000) \sin \theta_{max} = \sin \theta_{max}$$

[0035] When the hemisphere is positioned so that Conditions (1) and (2) hold, a ray can be traced exiting the hemisphere that is coincident with the marginal ray that enters the objective at the unaltered angle θ_{max}. The back-trace of this ray defines the maximum ray angle in the immersed specimen, and therefore the *NA* of the modified system. Referring to **FIG. 1,** if θ^* is defined as the ray angle in the immersed object space corresponding to this marginal ray, then:

$$\frac{\sin \theta^*}{R_S} = \frac{\sin \theta''}{\frac{R_S}{n_g}}$$

$$\text{therefore:} \quad \sin \theta^* = n_g \sin \theta''$$

$$n_g \sin \theta'' = (1.000) \sin \theta'$$

$$\text{therefore:} \quad \sin \theta^* = \sin \theta' \qquad \text{so: } \theta^* = \theta'$$

$$\frac{\sin \theta_{max}}{R_S} = \frac{\sin \theta'}{n_g R_S}$$

$$n_g \sin \theta_{max} = \sin \theta'$$

$$\text{therefore:} \quad n_g \sin \theta_{max} = \sin \theta^* \qquad \text{so: } \theta_{max} = \theta''$$

$$NA^* = n_g \sin\theta^* = n_g(n_g \sin\theta_{max}) = n_g^2 NA$$

[0036] Therefore, the immersed object and hemisphere, when used in the aplanatic condition, increases the numerical aperture by the square of the immersion index. This result is independent of R_S.

[0037] In the spherical system, the point object at R_{int} and virtual object at R_{ext} are always collinear with the center of the hemisphere. Therefore, a paraxial offset of the object from the microscope axis by a distance h_o at R_{int} produces a proportional offset, h_{vo}, of the virtual object at R_{ext}:

$$\frac{h_o}{R_{int}} = \frac{h_{vo}}{R_{ext}}$$

$$M^* = \left(\frac{h_{vo}}{h_o}\right) M_o = \left(\frac{R_{ext}}{R_{int}}\right) M_o = \frac{M_o n_g R_S}{\frac{R_S}{n_g}} = n_g^2 M_o$$

[0038] This shows that the immersed hemisphere also increases the magnification by the square of the immersion index.

[0039] Because both NA^* and M^* vary with immersion index squared, the system pupil radius is unaffected:

$$a_p^* = f_2 \frac{NA^*}{M^*} = f_2 \frac{{n_g}^2 NA}{{n_g}^2 M_0} = f_2 \frac{NA}{M_0} = a_p$$

[0040] In epi-fluorescence microscopy, increasing the magnification of the objective with a constant back pupil boosts the illumination irradiance by $\left(\frac{M^*}{M_0}\right)^2$ because the same total input power is concentrated into a more de-magnified field of view. Increasing the NA boosts the solid angle over which emission enters the objective by $\left(\frac{NA^*}{NA}\right)^2$. In the image, the total detected fluorescence from an object therefore increases by the product of these factors:

$$\frac{F^*}{F} = \left(\frac{M^*}{M_0}\right)^2 \left(\frac{NA^*}{NA}\right)^2 = \left(n_g{}^2\right)^2 \left(n_g{}^2\right)^2 = n_g{}^8$$

[0041] If, for example, the hemisphere is made from BK-7 glass, having an n_g = 1.52:

$$\frac{F^*}{F} = (1.52)^8 = 28.5$$

[0042] The theory derived here shows that NA^*, M^* and $\frac{F^*}{F}$ do not depend on the radius of the hemisphere, therefore R_s is nominally a free design parameter within limits.

[0043] Incorporation of a meniscus lens following the front element in a traditional duplex aplanatic arrangement does not change the immersed working distance, but further increases the system NA, as shown in **View (B)** of **FIG. 4**. The design parameters of the duplex (spacing, thickness, two radii, refractive index and dispersion) allow for some compensation of aberration. The disadvantage of the duplex is reduced clearance at the front end of the microscope objective.

[0044] For the duplex front end, shown in **View (B)** of **FIG. 4**, the aplanatic condition increases the achievable numerical aperture:

$$NA^* = n_1{}^2 n_2 NA$$

where n_1 is the index of the front element, and n_2 is the index of the meniscus. Nominally, both indices will be 1.52, therefore, in principle, $NA^* \sim (1.52)^3 NA = (3.51)NA$.

Type II Kit

[0045] The attachable accessory optic described as the Type I Kit is also useful in the imaging method known as light-sheet microscopy or selective-plane illumination microscopy (SPIM). Here, the acronym SPIM is used to represent the imaging method. In particular, the accessory optic enables implementation of symmetric SPIM optics utilizing a pair of modified objectives for immersion in high-index solvents that are incompatible with commercially available objectives with unitized construction. Furthermore, the achievable immersed working distances allow for complete mechanical clearance of planar specimens translated through the optical-sectioning plane of the SPIM instrument. This is

shown in **FIGS. 7, 8, Views (A-C)** of **FIG. 9** and **View (B)** of **FIG. 10** in a symmetric 45° SPIM setup.

[0046] Mechanical clearance is a major design problem in SPIM instruments, particularly symmetric setups for the high-resolution method known as "diSPIM" (dual-view SPIM). The essential design condition for the symmetric accessory kit is that the centers of the front-end hemispheres are separated by $\sqrt{2}$ times the inner aplanatic radius, so that the lenses cannot be complete hemispheres, but must be cut and abutted.

[0047] **FIG. 7** shows an attachable dual-lens front end in a symmetric 45° SPIM instrument with complete mechanical clearance for long-range horizontal specimen translation. In practice, the refractive index of the immersion medium is the same as or matches closely with the refractive index of the hemispheric lenses and the specimen. As such, rays are not deviated at the lens-medium boundary. The front-end optics are shown as a specially-configured pair of plano-convex lenses which sets up the correct intersection of the optic axes of the two LWD objectives and provides clearance for the immersed specimen. This is the basic optic configuration for a Type II kit.

[0048] **FIG. 8** shows a schematic representation of a lens configuration for symmetric light-sheet microscopy (selective-plane illumination microscopy, SPIM). In this configuration, the inner aplanatic surfaces of both front-end lenses (shown in **FIG. 8** as dashed circles) intersect the optic axis of each objective at a common point in the specimen. In the diagram, OA1 and OA2 are the optic axes of the two microscope objectives, intersecting at the vertex V. C1 and C2 mark the hemispheric centers of the front-end lenses. The triangle C1-V-C2 is a right isosceles triangle in which the leg length is equal to the inner aplanatic radius R_S/n_g, and the hypotenuse length is greater by $\sqrt{2}$. Therefore, the center-to-center distance of the two hemispheres is (1.4142) R_S/n_g. Also in this configuration, the inner aplanatic surface of each lens is tangent to the optic axis of the other lens at the point of intersection. At this point the surface tangents of the two aplanatic spheres are intersecting normal planes.

[0049] In **FIG. 7**, the front-end lenses are shown as hemispheres, but there is leeway in which accessible *NA* is traded off for working distance. This is shown in **Views (A-C) of FIG. 9** as paired hyper-hemispheres, as shown in **View (A)** for greatest *NA* and smaller clearance, versus paired hemispheres, as shown in **View (B),** with greater clearance but reduced maximal *NA*. **View (C)** of **FIG. 9** shows how attachable front-end optics may be

implemented in a symmetric SPIM system using only standard optical components. In the example, two PCX lenses are immersed to a right-angle prism which is immersed to the specimen. In **View (C)**, the refractive index of the prism is selected to match the index of the intended clarification medium for the specimen. Immersion oil or immersion medium (not shown) is also used between the PCX lenses and the prism.

[0050] In practice, the front-end lenses may be disposed in a housing , as shown in **Views (A-B)** of **FIG. 10** that is kept stationary such as to keep the lenses aligned with the microscope objectives. The specimen may then be moved with respect to the lenses and microscopes to allow deep imaging by light sheet microscopy. **View (A)** of **FIG. 10** shows an alternate embodiment for conventional or confocal microscopy, not for SPIM, wherein a single plano-convex lens is used as the primary optical element, while V**iew (B)** of **FIG. 10** shows a second alternate embodiment optimized for SPIM wherein dual, truncated plano-convex lenses are used as the primary optical element, as described above.

I Claim:

1. An accessory optic for attachment to a microscope objective or microscope stand comprising:

an attachment member for attachment to the microscope objective or microscope stand;

a lens housing attached to or integral with the attachment member; and

a plano-convex lens mounted in the housing.

2. The accessory optic of claim 1 wherein the plano-convex lens is a hemispheric or hyper-hemispheric lens.

3. The accessory optic of claim 2 wherein the accessory optic is positioned on the microscope objective such that an external aplanatic surface of the plano-convex lens coincides with a focus plane of the microscope objective.

4. The accessory optic of claim 3 wherein an intended immersed specimen is placed at an inner aplanatic surface of the plano-convex lens.

5. The accessory optic of claim 4 wherein accessory optic is positioned on the microscope objective such that the axis of the microscope objective passes through a center of curvature of the plano-convex lens and is perpendicular with a plano surface of the plano-convex lens.

6. The accessory optic of claim 4 wherein the accessory optic is positioned on the microscope objective such that the axis of the microscope objective passes through a center of curvature of the plano-convex lens and is not perpendicular with a plano surface of the plano-convex lens.

7. The accessory optic of claim 4 wherein the accessory optic increases the numerical aperture of the microscope objective.

8. The accessory optic of claim 1 further comprising one or more secondary lenses positioned between the microscope objective and the plano-convex lens.

9. The accessory optic of claim 3 wherein the microscope objective is a non-immersion objective and further wherein the accessory optic converts the microscope objective to an immersion objective.

10. The accessory optic of claim 5 wherein the plano-convex lens is selected such that the refractive index of the plano-convex lens approximates the refractive index of an immersion medium in which the accessory optic is intended to be immersed.

11. The accessory optic of claim 1 wherein the lens housing attaches to the attachment member via magnetic coupling.

12. A kit comprising:

an attachment member for attachment to a microscope objective or microscope stand;

a plurality of lens housings capable of being removably attached to the attachment member, each lens housing having a plano-convex lens mounted therein;

wherein each of the plano-convex lenses has a different refractive index.

13. An accessory optic set for attachment to two microscope objectives arranged in a symmetric 45° configuration comprising:

a lens housing configured to be placed between the two microscope objectives and a specimen; and

an optic element mounted in the housing comprising dual plano-convex lenses arranged such that the centers of the plano-convex lenses are separated by $\sqrt{2}$ times the radius of an inner aplanatic surface of the lenses;

wherein the two microscope objectives are arranged such as to have orthogonal co-planar axes configured to intersect at a point where the inner aplanatic surfaces of the dual plano-convex lenses intersect.

14. The accessory optic of claim 13 wherein the dual plano-convex lenses are truncated hemispheric or hyper-hemispheric lenses.

15. The accessory optic of claim 13 wherein the dual plano-convex lenses comprise two plano-convex lenses coupled to adjacent sides of a right-angle prism.

16. The accessory optic of claim 15 wherein the dual plano-convex lenses are coupled to adjacent sides of the right-angle prism via an immersion medium.

17. An accessory optic comprising:

a lens housing configured to be placed between a microscope objective and a specimen; and

an optic element mounted in the housing comprising a plano-convex lens.

18. The accessory optic of claim 17 wherein the plano-convex lens comprises a hemispheric or hyper-hemispheric lens.

19. The accessory optic of claim 17 further comprising:

an attachment member for mounting on the microscope objective or a microscope stand; and

one or more secondary lenses mounted in the attachment member.

20. The accessory optic of claim 17:

wherein the optic element comprises dual hemispheric or hyper-hemispheric lenses arranged such that the centers of the lenses are separated by $\sqrt{2}$ times the radius of an inner aplanatic surface of the lenses; and

wherein two microscope objectives are arranged such as to have orthogonal co-planar axes configured to intersect at a point where the inner aplanatic surfaces of the dual plano-convex lenses intersect.

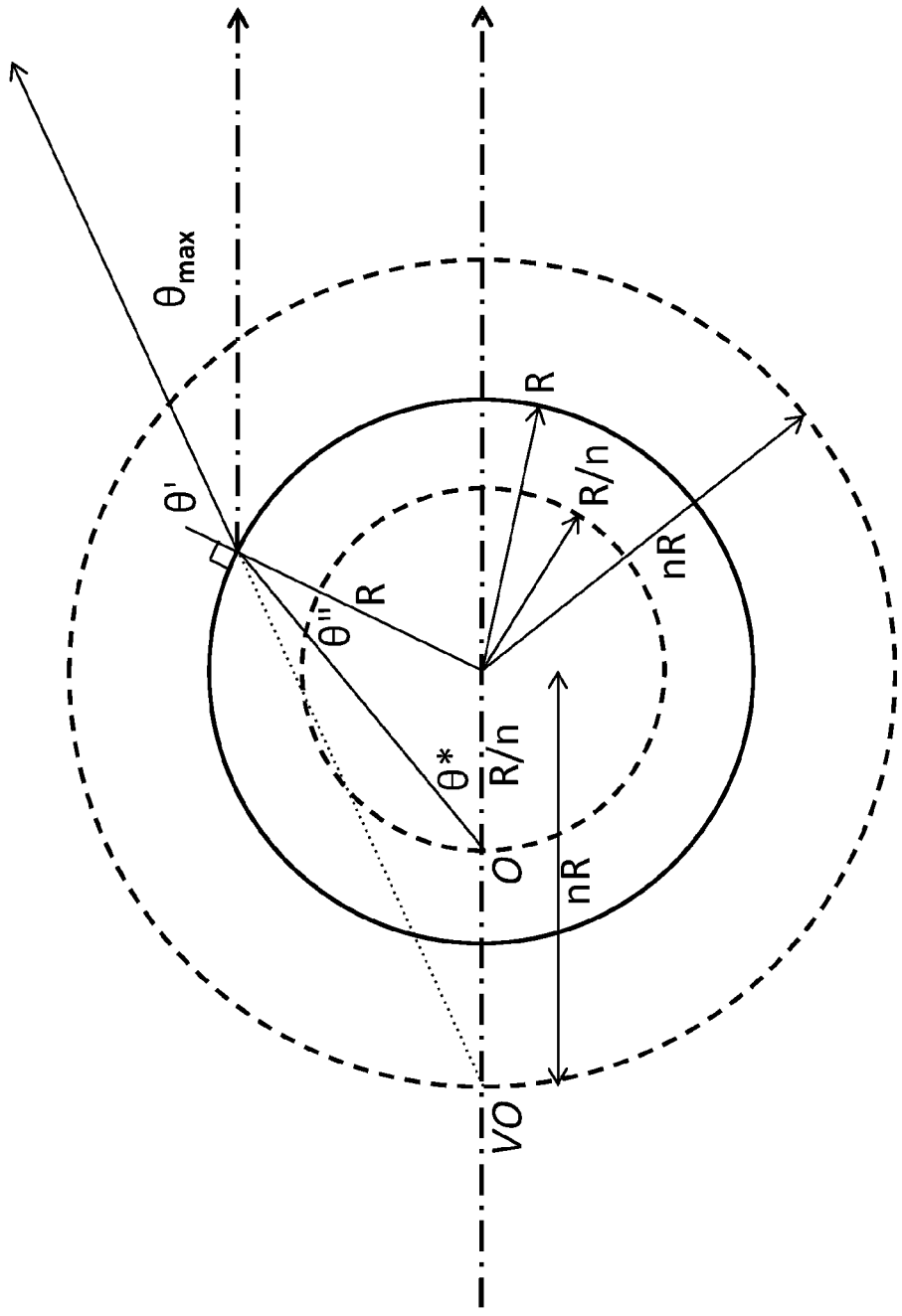


FIG. 1

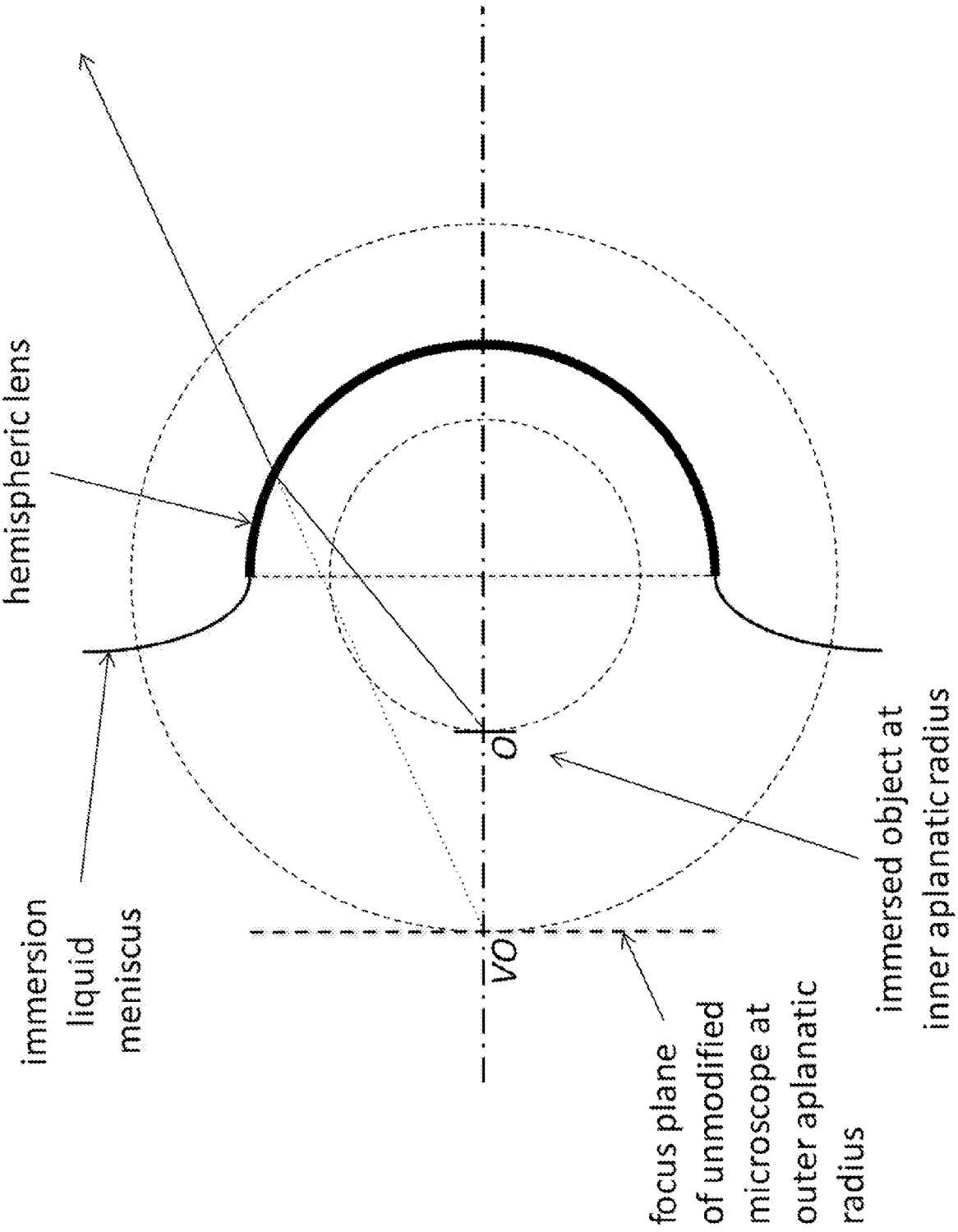

hemispheric lens
immersion liquid meniscus
O
VO
focus plane of unmodified microscope at outer aplanatic radius
immersed object at inner aplanatic radius

FIG. 2

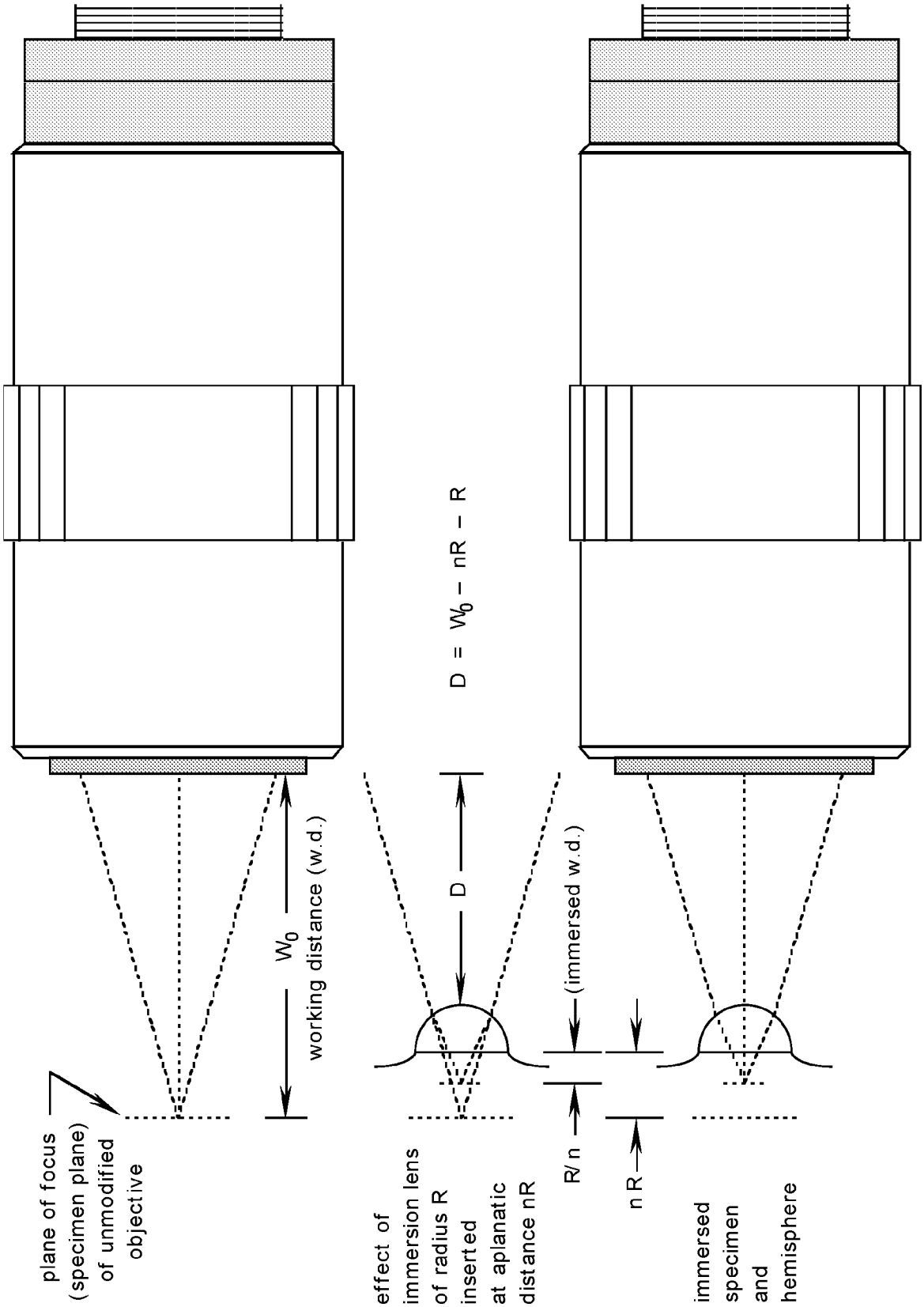
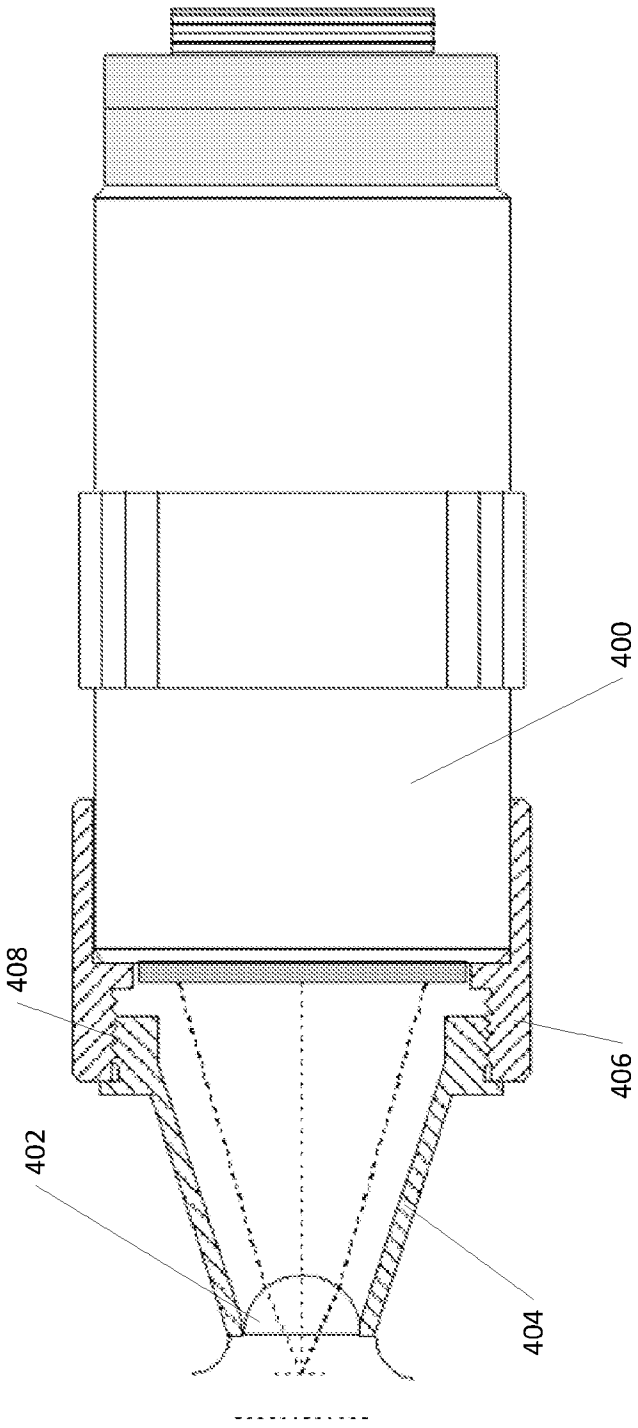
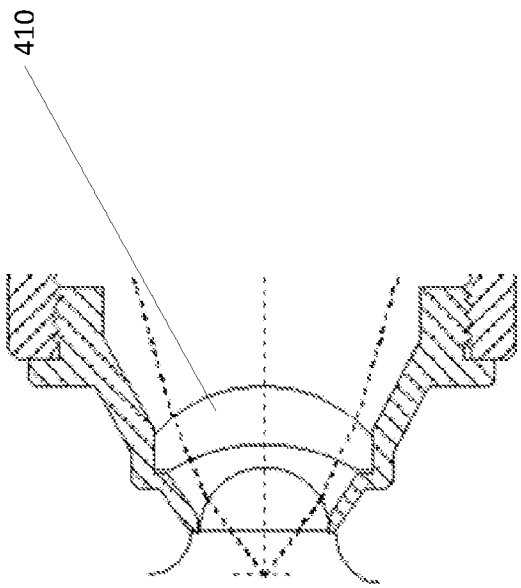

plane of focus
(specimen plane)
of unmodified
objective
W_0
working distance (w.d.)
D = W_0 – nR – R
D
(immersed w.d.)
effect of
immersion lens
of radius R
inserted
at aplanatic
distance nR
R/ n
n R
immersed
specimen
and
hemisphere

FIG. 3



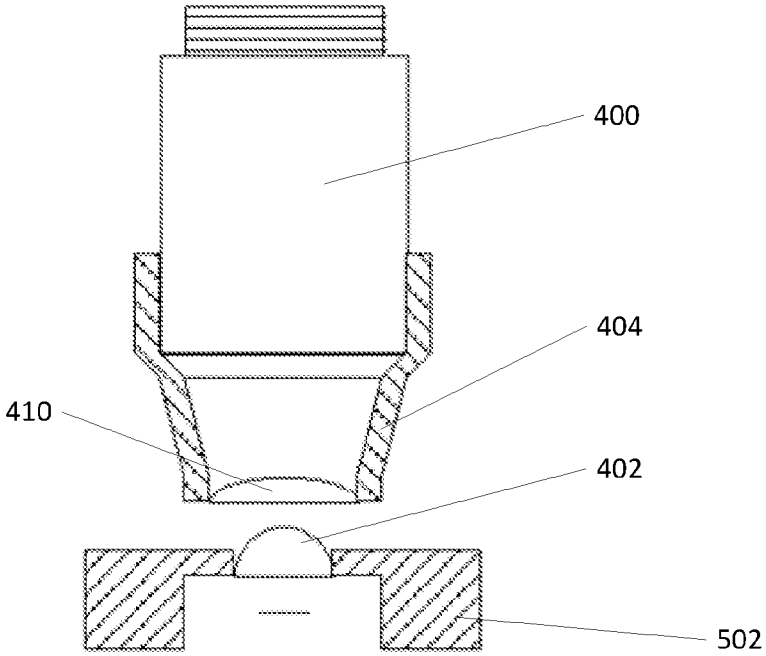
(A)



(B)

FIG. 4

(A)



(B)

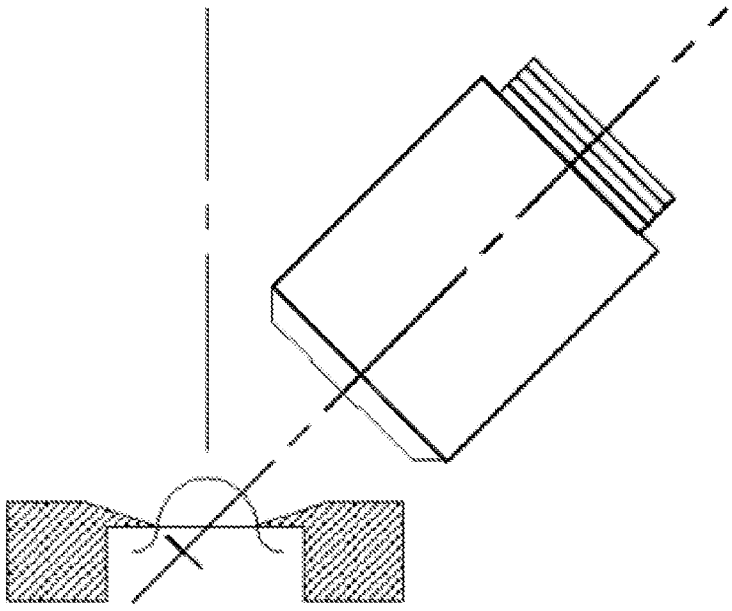


FIG. 5

FIG. 6

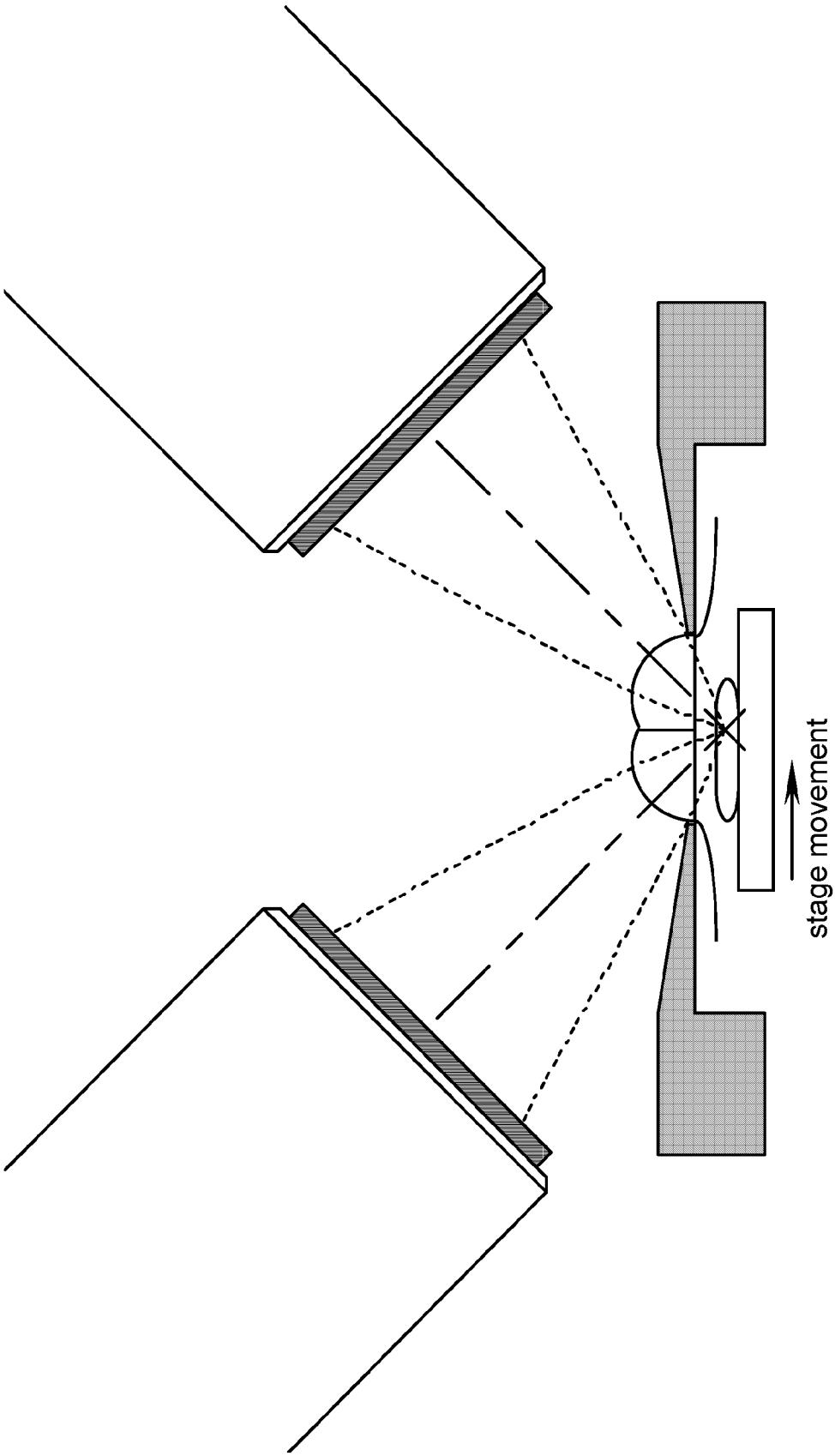


FIG. 7

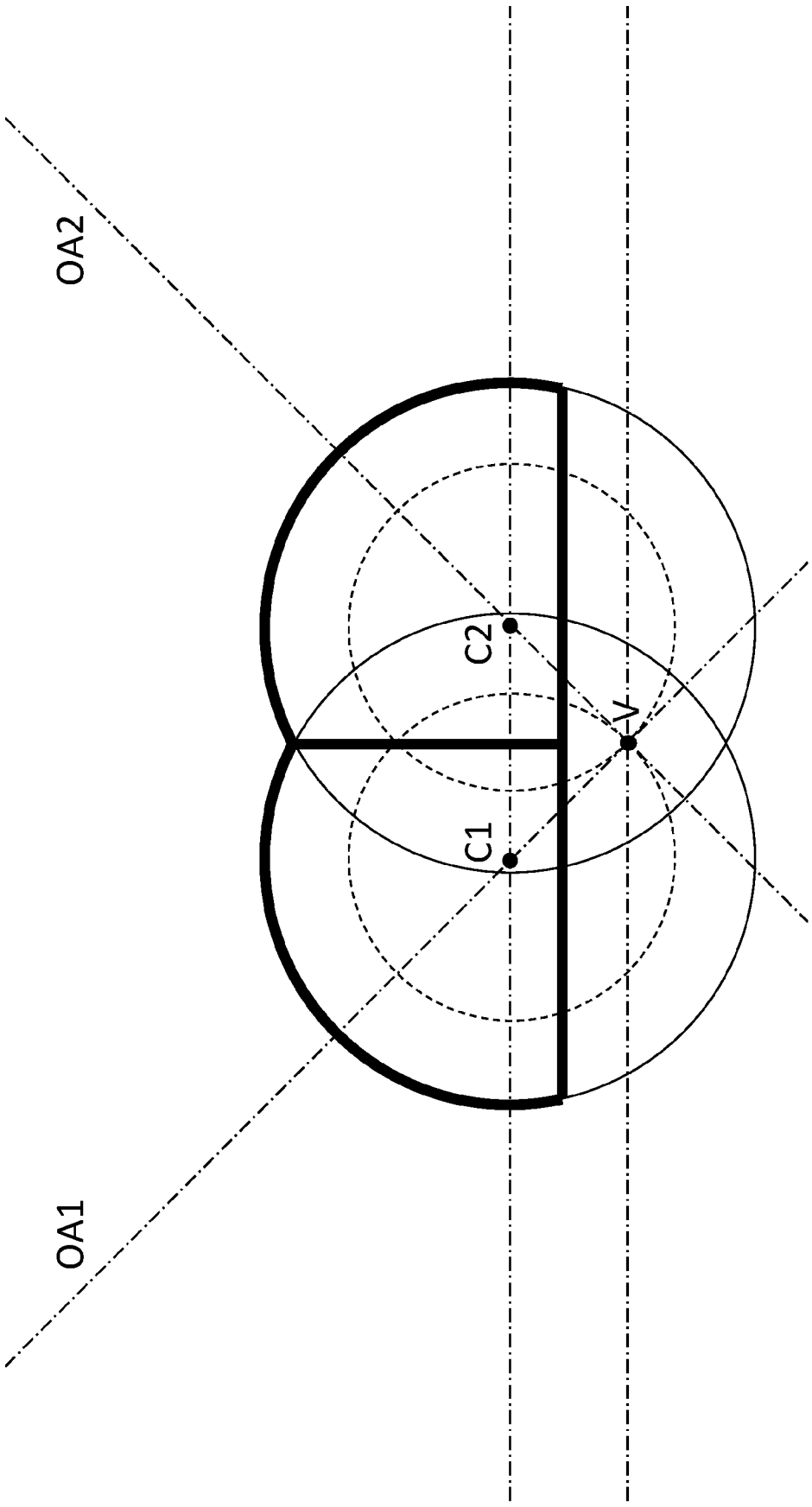
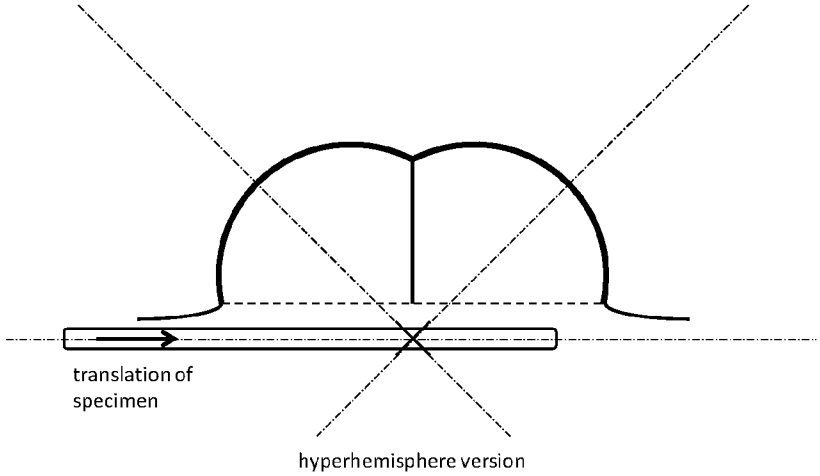
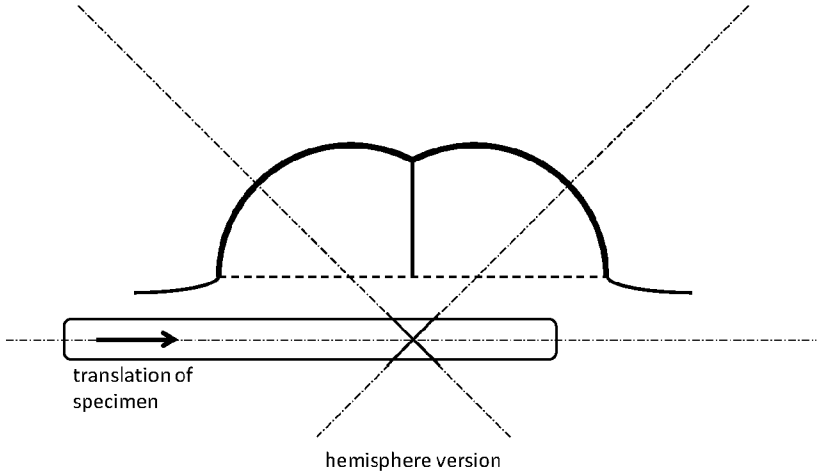


FIG. 8

(A)


translation of
specimen
hyperhemisphere version

(B)


translation of
specimen
hemisphere version

(C)

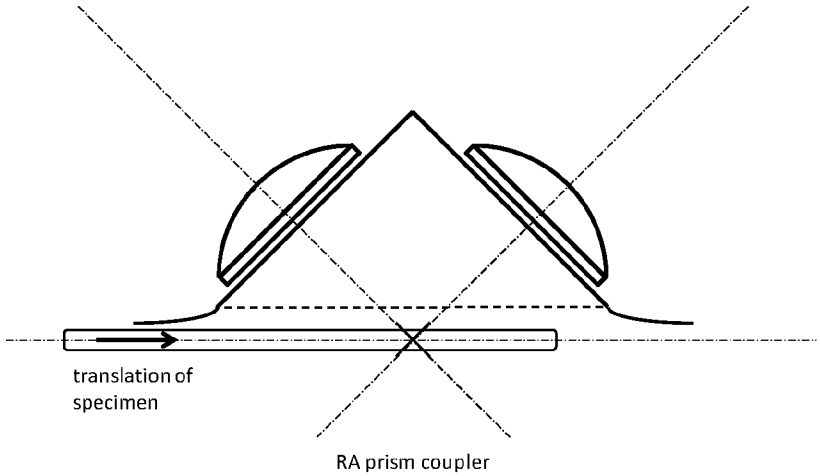
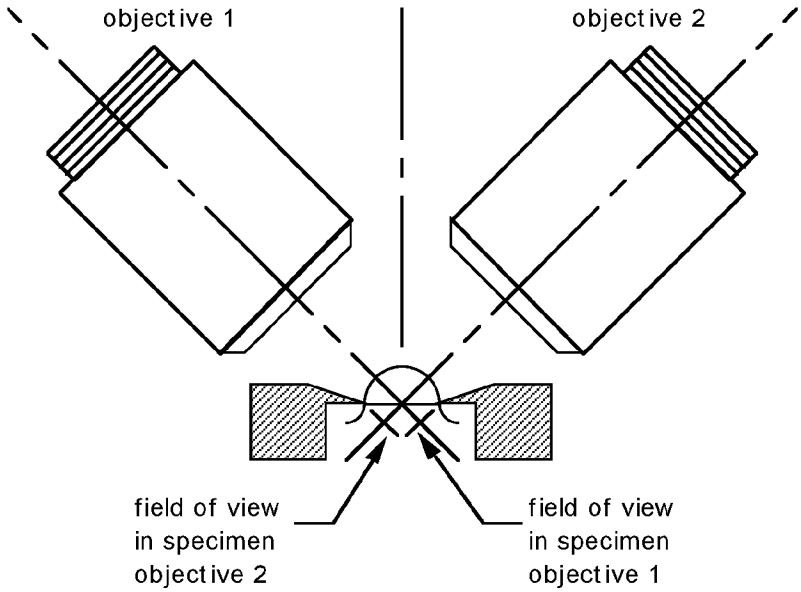

translation of
specimen
RA prism coupler

FIG. 9

(A)


objective 1
objective 2
field of view
in specimen
objective 2
field of view
in specimen
objective 1

(B)

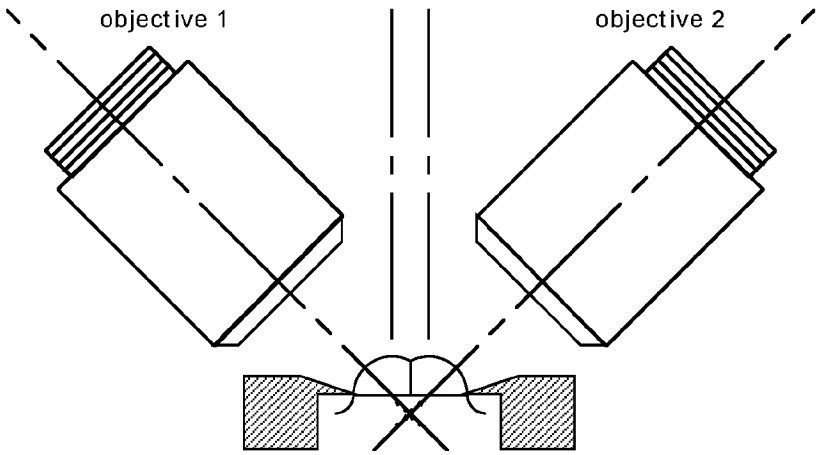

objective 1
objective 2

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/39285

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G02B 15/22 (2019.01)
CPC - G02B 15/22; G02B 13/0095; G02B 13/06; G02B 13/0055; G02B 13/02; G02B 27/0068; G02B 27/0075

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2005/0200947 A1 (Hirata et al.) 15 September 2005 (15.09.2005), entire document, para [0104], [0105], [0150], [0151]	1, 8, 12, 17, 19 --- 2, 11, 18 --- 3-7, 9, 10, 13-16, 20
Y --- A	US 2009/0002815 A1 (Hoult et al.) 01 January 2009 (01.01.2009), entire document, para [0004]	2, 18 --- 3-7, 9, 10
Y	US 2017/0168292 A1 (Carl Zeiss Meditec AG) 15 June 2017 (15.06.2017), entire document	11
A	US 2016/0187633 A1 (Applied Scientific Instrumentation Inc.) 30 June 2016 (30.06.2016), entire document	13-16
A	US 2017/0363864 A1 (MARGOLIS) 21 December 2017 (21.12.2017), entire document	1-20
A	US 6,215,586 B1 (Clark) 10 April 2001 (10.04.2001), entire document	1-20
A	US 2008/0279542 A1 (Frazier) 13 November 2008 (13.11.2008), entire document	1-20
A	US 2011/0115895 A1 (Huisken) 19 May 2011 (19.05.2011), entire document	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 September 2009	02 OCT 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774