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- (71) Applicant: MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.
[DE/DE]; Hofgartenstraße 8, 80539 München (DE).
- (72) Inventor; and
- (71) Applicant : DANIELS, Karen [US/US]; 267 Sweet Bay Pl., Carrboro, North Carolina 27510 (US).
- (72) Inventors: SCHROETER, Matthias; An der Bleichwiese 8, 37073 Goettingen (DE). HERMINGHAUS, Stephan; Brauweg 3, 37073 Göttingen (DE). LASKAR, Junaid; Am Schlehdorn 18, 37077 Göttingen (DE).
- (74) Agent: REHBERG HÜPPE + PARTNER PATENTANWÄLTE PARTG MBB; Robert-Gernhardt-Platz 1, 37073 Göttingen (DE).

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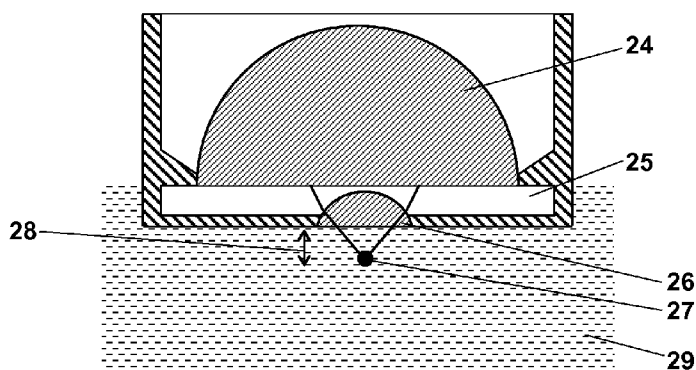


Fig. 5

(57) Abstract: A high refractive index immersion liquid (29) for immersing a front surface of an immersion objective lens (26) of a microscope comprises a solution of antimony tribromide (SbBr_3) dissolved in diiodomethane (CH_2I_2). The concentration of the antimony tribromide (SbBr_3) is not more than 50 % by weight of the solution.



HIGH REFRACTIVE INDEX IMMERSION LIQUID FOR SUPER-RESOLUTION 3D IMAGING USING SAPPHIRE-BASED ANAIL OPTICS

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a high refractive index immersion liquid for immersing a front
5 surface of an immersion objective lens of a microscope, and to a combination of such an
immersion liquid and an immersion objective lens. The present invention also relates to a kit for
preparing such an immersion liquid, and to a method of matching a high refractive index of such
an immersion liquid. Further, the present invention relates to a method of using such an
immersion liquid. Even further, the present invention relates to a microscope comprising an
10 immersion objective lens, particularly an immersion objective lens of both high numerical
aperture and long working distance. Finally, the present invention relates to an adaptive lens
comprising a cavity delimited by a curved deformable membrane in at least one direction and
filled with a high refractive index liquid.

PRIOR ART

15 **JP 2002-053839 A** discloses a high refractive index liquid for use in a microscope for enlarging
the NA (Numerical Aperture) of an object lens of the microscope. The high refractive index liquid
disclosed by JP 2002-053839 A is a liquid mixture of antimony tribromide and an organic
compound. The organic compound may be an alcohol, a glycol, an ether, an ester, a ketone, a
sulfurated hydrocarbon or nitrated hydrocarbon. In a first working example, the mixture consists
20 of 5 parts by weight of diethylene glycol and 334 part by weight of antimony tribromide. In a
second working example, the mixture consists of 5 part by weight of diethylene glycol and 50
parts by weight of antimony tribromide. The refractive indices of the two working examples
disclosed are reported as being 1.798 and 1.831. Diiodomethane is mentioned as a prior art

commercial high refractive index liquid here. In a comparative example presented, a high refractive index liquid is made of diiodomethane containing sulfur.

US 2008/0135808 A1 discloses a high refractive index immersion liquid for use in a fluorescence microscope. The immersion liquid comprises diiodomethane as its main ingredient, and a solid having a high refractive index such as sulfur. In fact, no other solid of high refractive index to be dissolved in the diiodomethane than sulfur is disclosed. A low auto-fluorescence of the high refractive index liquid is achieved in that its components are purified. This known high refractive index immersion liquid has a reported refractive index of 1.78. Due to the solid added, however, its transparency is low, and there is significant light scattering due to the fact that sulfur is not really dissolved in the diiodomethane but forms a colloidal dispersion. Further, even when sulfur of high purity is used, there still is significant auto-fluorescence.

EP 2 466 359 A1 discloses a high refractive immersion liquid for use in a microscope. This immersion liquid comprises an ionic liquid including a metal-halogeno complex anion containing bromine and antimony as a metal element, and a cation. The cation may be an imidazolium cation, a pyridinium cation, a pyrrolidinium cation or an ammonium cation. Particularly, the immersion liquid may be a 1:1 mixture of 1-butyl-3-methylimidazolium iodide and antimony tribromide. The refractive index of this particular immersion liquid is reported to be 1.80.

Robert Meyrowitz. A compilation and classification of immersion media of high index of refraction. *Am. Mineral.*, 40:398-409, 1955 reports various immersion media for use in identification of crystalline material. As a high index medium being a pure liquid he inter alia lists diiodomethane. Within 67 liquid solutions to be used as high index media he also discloses antimony tribromide dissolved in diiodomethane referring to prior publications in 1893 and 1933. The refractive index of antimony tribromide in diiodomethane reported here is 1.83. Using an immersion medium in the identification of a crystalline material does not involve the problems occurring in using a high refractive index immersion liquid in a microscope particularly in a super-resolution fluorescence microscope in which low auto-fluorescence and high transparency of an immersion liquid are highly relevant.

PROBLEM OF THE INVENTION

It is the problem of the present invention to provide a high refractive index immersion liquid, a combination of such an immersion liquid and an immersion objective lens, a kit for preparing such an immersion liquid, a method of matching a high refractive index of such an immersion
5 liquid to a high refractive index of a solid medium, a method of using such an immersion liquid, a microscope comprising an immersion objective lens, and an adaptive lens comprising a cavity delimited by curved deformable membrane in at least one direction and filled with such a high refractive index liquid, which allow for easily adjusting a high refractive index of the immersion
10 liquid or which make an advantageous use of an immersion liquid of an adjusted high refractive index, particularly in combination with an immersion objective lens of both high numerical aperture and long working distance.

SOLUTION

The problem of the invention is solved by a high refractive index immersion liquid according to claim 1, by a combination of the immersion liquid and an immersion objective lens comprising
15 the features of claim 3, by a kit for preparing the immersion liquid comprising the features of claim 9, by a method of matching a high refractive index of the immersion liquid comprising the features of claim 11, by a method of using the immersion liquid comprising the features of claim 14, by a microscope comprising the features of claim 16, and by an adaptive lens comprising the features of claim 21. Preferred embodiments of the present invention are defined in
20 dependent claims 2, 4 to 8, 10, 12, 13, 15, 17 to 20, and 22.

DESCRIPTION OF THE INVENTION

A high refractive index immersion liquid for immersing a front surface of an immersion objective lens of a microscope comprises a solution of antimony tribromide (SbBr_3) dissolved in diiodomethane (CH_2I_2), wherein the concentration of the antimony tribromide is not more than
25 50 % by weight.

Surprisingly, adding this particular salt, antimony tribromide, to the well-known high refractive index immersion liquid, diiodomethane, further increases the refractive index of iodomethane up to 1.873 without significantly degrading the transparency of the immersion liquid or causing a

considerable Tyndall effect affecting the suitability of the high refractive index immersion liquid in high and super resolution microscopy. Auto-fluorescence is also no issue. This particularly applies, if both the antimony tribromide and the iodomethane are of high purity of at least 98 %, preferably of 99 % or better.

- 5 At 50 % by weight antimony tribromide in the immersion liquid of the present invention, a saturated solution of the antimony tribromide in the diiodomethane is reached, and first antimony tribromide crystals are formed at room temperature. Preferably, the antimony tribromide concentration in the high refractive index immersion liquid according to the present invention is below the saturation level of antimony tribromide in diiodomethane to avoid the
10 formation of any light scattering crystals in the immersion liquid.

With a concentration of the antimony tribromide of about 20 % by weight, the refractive index of the immersion liquid will be above 1.76. A relevant increase in refractive index is already achieved at lower concentrations of the antimony tribromide. Typically, the minimum concentration of the antimony tribromide will be 10 % by weight of the solution.

- 15 According to the present invention, in a combination of the immersion liquid according to the present invention and an immersion objective lens configured to be immersed into the immersion liquid, the immersion liquid matches the sapphire in refractive index.

- The immersion objective lens may particularly be made of sapphire, and it may be a truncated aplanatic immersion objective lens. More particularly, the immersion objective lens may be an
20 aNAIL. Such a combination according to the present invention may both have a high numerical aperture and a high working distance between a front surface of the immersion objective lens and an object or a sample of interest.

- Further, the combination according to the present invention may comprise a temperature control unit including a controlled temperature surface adjacent to a gap in front of the immersion
25 objective lens to be filled with the index matched immersion liquid.

A kit for preparing the immersion liquid according to the present invention includes an amount of antimony tribromide and an amount of diiodomethane. In the kit, the amount of the antimony tribromide by weight is not more than the amount of the diiodomethane. Typically, on the other

hand, the amount of the antimony tribromide is not less than a tenth of the amount of the diiodomethane. Thus, the kit allows for preparing the immersion liquid of the present invention at various particular refractive indices above 1.74 or above 1.76 and up to 1.873.

5 A method according to the present invention of matching a high refractive index of the immersion liquid according to the present invention to a high refractive index of a solid medium includes raising the concentration of the antimony tribromide for increasing the refractive index of the immersion liquid and lowering the concentration of the antimony tribromide for decreasing the refractive index of the immersion liquid. Further, the temperature of the immersion liquid may be raised for decreasing the refractive index of the immersion liquid, and lowered for
10 increasing the refractive index of the immersion liquid. Particularly, the concentration of the antimony tribromide may be used for a coarse adjustment of the refractive index of the immersion liquid, whereas the temperature may be used for fine-adjusting the refractive index.

The solid medium to which the high refractive index of the immersion liquid is matched according to the present invention may form a front surface of an immersion objective lens or
15 embed a sample of interest. The "solid medium forming the front surface of the immersion objective lens" does not refer to any coating of the immersion objective lens but to the material forming the immersion objective lens as such and determining the basic optical properties of the interface between the immersion objective lens and the immersion liquid.

The method according to the present invention of using the immersion liquid according to the
20 present invention in a microscope comprising an immersion objective lens comprises the steps of making the immersion objective lens of sapphire and filling a gap between a front surface of the immersion objective lens and a facing surface of a solid medium including a sample of interest with the immersion liquid matching the sapphire in refractive index. Alternatively, the gap filled with the immersion liquid of sapphire refractive index may be a gap between the
25 immersion objective lens and a facing surface of the sample of interest itself, or the sample of interest may even be floating in the immersion liquid covering the distance between the sample of interest and the immersion objective lens of sapphire. In all these cases, the present invention allows for making immersion objective lenses of sapphire, which are destined for use with immersion liquids of matching refractive index.

As the refractive index of the immersion liquid according to the present invention depends on the wavelength of the light more strongly than for solid transparent materials of high refractive index like, for example, sapphire, matching the refractive index of the immersion liquid according to the present invention always means matching the refractive index at one particular wavelength.

In the method of using according to the present invention, the immersion objective lens of the microscope may be made as a truncated aplanatic immersion objective lens in which the light passing the immersion objective lens is not to be refracted at the front surface but only at the back surface of the immersion objective lens. Such an aplanatic immersion objective lens requires the availability of an immersion liquid precisely matching its refractive index. The present invention provides such an immersion liquid and thus allows for designing a truncated aplanatic immersion objective lens of both a high numerical aperture (NA) and a long working distance (WD). For example, the NA may be at least 1.0 or and the WD may be at least 10 mm with a same immersion objective lens.

In the method of using according to the present invention, a temperature of the immersion liquid in the gap may be controlled for fine-tuning the refractive index of the immersion liquid.

A microscope according to the present invention comprises a sapphire-based immersion objective lens configured to be immersed into an index matched immersion liquid. The immersion objective lens may be a truncated aplanatic immersion objective lens, particularly a so-called aNAIL (aplanatic Numerical Aperture Increasing Lens). According to the present invention, the truncated aplanatic immersion objective lens may be designed to both have a high numerical aperture (NA) and a long working distance (WD). For example, the NA may be about 1.17 and the WD may be about 12 mm with a same immersion objective lens. Further, the microscope may comprise a temperature control unit including a controlled temperature surface adjacent to a gap to be filled with the index matched immersion liquid. The controlled temperature surface may be a separate surface. It may also be the front surface of the immersion objective lens immersed into the index-matches immersion liquid.

An adaptive lens according to the present invention comprises a cavity delimited by a curved, deformable and transparent membrane in at least one direction of an optical axis and filled with a high refractive index liquid comprising a solution of antimony tribromide dissolved in

diiodomethane, wherein the concentration of the antimony tribromide is at least 10 % and not more than 50 % by weight of the liquid. Due to the high refractive index of the solution of antimony tribromide dissolved in diiodomethane, such an adaptive lens according to the present invention provides for a strong variation in optical properties with comparatively small variations in the curvature of the transparent membrane. As the refractive index of the solution increases with increasing concentration of the antimony tribromide, the concentration of the antimony tribromide may particularly be in a range from 40 to 50 % by weight, i.e. the concentration may be close to a saturation concentration of the antimony tribromide in diiodomethane.

The adaptive lens according to the present invention may comprise a temperature control unit including a controlled temperature surface adjacent to the cavity filled with the high refractive index liquid. The temperature control unit allows for setting a particular temperature of the liquid and thus a particular refractive index of the liquid.

The cavity of the adaptive lens according to the present invention may be delimited by transparent membranes in both directions of the optical axis. Alternatively, it may be delimited by the deformable and transparent membrane in one of these directions only, whereas it is delimited by a solid material in the other direction. This solid material may be sapphire-based, and the high refractive index liquid may be composed to match the sapphire in refractive index.

Advantageous developments of the invention result from the claims, the description and the drawings. The advantages of features and of combinations of a plurality of features mentioned at the beginning of the description only serve as examples and may be used alternatively or cumulatively without the necessity of embodiments according to the invention having to obtain these advantages. Without changing the scope of protection as defined by the enclosed claims, the following applies with respect to the disclosure of the original application and the patent: further features may be taken from the drawings, in particular from the illustrated designs and the dimensions of a plurality of components with respect to one another as well as from their relative arrangement and their operative connection. The combination of features of different embodiments of the invention or of features of different claims independent of the chosen references of the claims is also possible, and it is motivated herewith. This also relates to features which are illustrated in separate drawings, or which are mentioned when describing them. These features may also be combined with features of different claims. Furthermore, it is

possible that further embodiments of the invention do not have the features mentioned in the claims.

The number of the features mentioned in the claims and in the description is to be understood to cover this exact number and a greater number than the mentioned number without having to explicitly use the adverb "at least". For example, if a lens is mentioned, this is to be understood such that there is exactly one lens or there are two lenses or more lenses. Additional features may be added to these features, or these features may be the only features of the respective product.

The reference signs contained in the claims are not limiting the extent of the matter protected by the claims. Their sole function is to make the claims easier to understand.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following, the invention is further explained and described with respect to preferred exemplary embodiments illustrated in the drawings. Figs. 1 to 5 and the related description including the list of references has been submitted for publication in Optics Express: Junaid M. Laskar et al.: "High refractive index immersion liquid for super-resolution 3D imaging using sapphire-based aNAIL optics".

Fig. 1 is a schematic of a liquid refractometer setup, based on the design of Nemoto [12]. The cuvette cross section is 20 mm x 10 mm, with optical measurements performed across the width $d = 10$ mm.

Fig. 2 shows a measurement of laser beam displacement δ by knife-edge scanning. The inset shows the measured power versus the knife position. The main plot shows fits to two Gaussian beam profiles (i.e. derivatives of the inset) for the angles $\theta = 0^\circ$ and 20° . The difference between the two maxima is the displacement δ (20°).

Fig. 3 (a)-(b) shows refractive indices of both pure diiodomethane and with dissolved antimony tribromide. Each panel compares the results for a single wavelength, as a

function of concentration and temperature. The refractive index for ordinary rays through sapphire is shown by dotted line for comparison.

Fig. 4 shows the transmittance as a function of wavelength for a cuvette of width $d = 10$ mm.

5 **Fig. 5** is a schematic of the design for simultaneous increase of NA (numerical aperture) and WD (working distance), using sapphire-based aNAIL lens system immersed in the refractive index matching liquid (solution of SbBr_3 in CH_2I_2). The aNAIL and backing objective lens system depicted is designed to have $\text{NA} = 1.17$ and $\text{WD} = 12$ mm.

10 **Fig. 6** shows one embodiment of an adaptive lens according to the present invention; and

Fig. 7 shows another embodiment of an adaptive lens according to the present invention.

DESCRIPTION OF THE DRAWINGS

15 Sapphire-based aplanatic numerical aperture increasing lenses (aNAIL) [1, 2] provide a promising route to achieve super-resolution 3D imaging [3], but their development requires a suitable refractive index-matching liquid ($n \sim 1.77$). A typical aNAIL design would include a truncated aplanatic solid immersion objective lens of plano-convex shape, made of high refractive index solid material [4–6] such as sapphire [7, 8]. To date, the lack of a suitable
20 immersion liquid has limited the application of aNAIL to subsurface microscopy of objects or samples immersed inside a refractive index-matched solid medium, without the possibility of depth-scanning [1, 2, 6]. Therefore, a refractive index-matched immersion liquid will allow for simultaneously harnessing both the high spatial resolution and the depth scanning capability of sapphire-based aNAILS [3]. However, a persistent challenge in the search for high refractive
25 index immersion liquids is to find one with both low absorbance and low scattering. The ideal liquid would provide optical transparency across the full spectrum from ultraviolet to near-infrared, as well as tunability to provide precise index-matching [9].

A promising candidate solvent is the organic liquid diiodomethane (CH_2I_2), which is one of the liquids with highest known refractive index values ($n = 1.74$). While other high refractive index liquids exist (phenyldi-iodoarsine ($\text{C}_6\text{H}_5\text{AsI}_2$) with $n = 1.85$ and selenium monobromide (Se_2Br_2) with $n = 2.1$ [10]), diiodomethane has the key advantage of being commercially available. In addition, diiodomethane is an excellent solvent, and many liquid formulations using salts dissolved in diiodomethane are reported to increase the refractive index [10] and are even available commercially (Cargille labs, Series M, $n = 1.8$). However, the strong light scattering and high absorbance of these formulations render them insufficiently transparent for high-resolution optics applications. A lack of knowledge of a salt formulation to increase the refractive index while maintaining optical transparency has caused diiodomethane to remain under-utilized as a preferred immersion solvent liquid, despite its inertness with many minerals (including sapphire) [10].

The refractive index of an optical medium is typically proportional to its mass density, as described by the Lorentz-Lorentz equation [11]; this suggests that salts containing heavy elements would be promising candidates. In addition, a large electronegativity difference between the salt cation and anion typically predicts improved solubility. Guided by these principles, we screened four different salts – antimony tribromide (SbBr_3) [10], antimony trichloride (SbCl_3), barium chloride (BaCl_2), and bismuth trichloride (BiCl_3) – as potential solutes in CH_2I_2 . Of this list, BaCl_2 and BiCl_3 were found to have poor solubility due to small cation-anion electronegativity differences. Despite the excellent solubility of SbCl_3 , the refractive index increased only by 0.02, due to the low atomic weight of chlorine. Therefore, only SbBr_3 was found to be a suitable candidate: for concentrations between 20 wt% and saturation in liquid CH_2I_2 , it achieves a refractive index as high as $n \sim 1.873$. In addition, this solution shows excellent optical transparency and low scattering in the wavelength ranges $\lambda < 350$ nm and 450 nm $< \lambda < 1060$ nm. Below, we present measurements quantifying the concentration, temperature, and wavelength dependence of the index of refraction, transmittance, and scattering of these liquid solutions.

We prepare liquid solutions of different concentrations (wt%) from a powder sample of SbBr_3 (99% pure, Alfa Aesar) dissolved in the liquid CH_2I_2 (99% pure, Sigma-Aldrich). To obtain a solution of given wt%, we mix the two components in the desired weight ratio, first on an electrical shaker for six hours at $T = 22^\circ \text{C}$ and then in a centrifuge for 3 hrs at 800 rpm at $T = 15^\circ \text{C}$. We then separate the upper (supernatant) liquid solution from the precipitate (assumed

to contain chemical impurities) collected at the bottom of the centrifuge tube. For a 50 wt% concentration at room temperature ($T = 22^\circ \text{C}$), crystals are also formed that remain in equilibrium with the supernatant (saturated) solution. Note that the chemical handling of both components requires significant care. Antimony tribromide is harmful if swallowed (H302) or inhaled (H332), and is hygroscopic (absorbs water). Diiodomethane is harmful if swallowed (H302), causes skin irritation (H315), and serious eye damage (H318), and also may cause respiratory irritation (H335).

We perform index of refraction measurements using a liquid refractometer based on the design of Nemoto [12], as shown schematically in in **Fig. 1**. The apparatus determines the displacement δ of a laser beam, due to passing through a liquid-filled cuvette rotated by an angle θ with respect to the beam. The center of the laser beam is determined by scanning a knife edge across its profile. As shown in the inset of **Fig. 2**, the light intensity profile $P(x)$ is well-described by an error function, as expected for a single-mode laser. We identify the center of the beam as the location x at which $P(x)$ rises fastest, as obtained by numerical differentiation. Sample Gaussian beam profiles dP/dx are shown in Fig. 2, characterized by the beam width w at which the power falls by a factor of $1/e^2$ [13, 14]. Throughout the measurements, we control the temperature of the liquid to $\pm 0.05^\circ \text{C}$ by encapsulating the quartz cuvette (Hellma Analytics) inside a rectangular copper block containing channels connected to a refrigerated water bath circulator (Neslab RTE 111). The copper block is mounted on a rotation stage with a vernier scale which provides an angular reproducibility of ± 5 arcmin.

Following Nemoto [12], the refractive index n of the liquid can be calculated from the following Equation 1

$$n = n_0 \sin \theta \sqrt{1 + \left[\frac{\cos \theta}{\sin \theta - \Delta/d} \right]^2}$$

where n_0 is the refractive index of air (the empty cuvette), d is the width of the cuvette, and $\Delta \equiv \delta - \delta_0$ is the relative displacement of the Gaussian peak for the liquid-filled cuvette relative to the empty cuvette.

To obtain n , we repeat the same measurement at 7 different incident angles $\theta = (\pm 10^\circ, \pm 20^\circ, \pm 30^\circ, +40^\circ)$, corresponding to both clockwise and counter-clockwise rotation of the cuvette with

respect to the incident light. The average of these 7 measurements provides the value of n for each combination of wavelength, temperature, and SbBr_3 concentration. We repeat this process for 3 wavelengths of light ($\lambda = 473, 532, \text{ and } 633 \text{ nm}$), 5 temperatures from 15°C to 40°C , and 3 concentrations (20 wt%, 33.5 wt%, and saturation concentration).

5 Equation 1 already reduces systematic errors due to geometric imperfections of the cuvette by measuring all values of δ against the empty cuvette. In addition, we have analyzed the systematic errors in our setup and determined that the precision of the rotation stage and the imperfect parallelism of the cuvette sidewalls are the largest sources of error. These combined effects result in a refractive index measurement error of ± 0.003 . In practice, we find that we are
10 able to measure the refractive index of water and ethanol to within ± 0.001 of values reported in the literature [15–17].

We find that SbBr_3 dissolved in CH_2I_2 can meet and exceed the index of refraction of sapphire over a broad range of temperatures, visible wavelengths, and concentration greater than 20 wt%. **Fig. 3** presents the measured values of n as a function of wavelength (panels a-c),
15 temperature (plot abscissa), and concentration (line series). In each case, the refractive index can be increased by either changing the concentration (more dissolved salt corresponds to higher n) or the temperature (higher temperature decreases n). In applications, preparing a solution of known concentration is more convenient for coarse tuning, and temperature is more convenient for fine tuning in-situ. The largest value we measure is $n = 1.873$, for saturated
20 solutions at low temperature and short wavelength.

Optical transparency, which can be degraded by both light scattering and absorption, plays a crucial role in determining the utility of an immersion liquid. We characterize these properties of the $\text{SbBr}_3\text{-CH}_2\text{I}_2$ solutions, and compare with the two commonly-used commercial high refractive index immersion liquids (Cargille M Series with $n = 1.71 \pm 0.0005$ and $n = 1.80 \pm 0.0005$). **Fig. 4**
25 shows the transmittance spectra, measured from the near-ultraviolet to the near-infrared. The illumination source is an Ocean Optics tungsten-halogen light source (model HL-2000-FHSA-LL with output power 4.5 mW) and transmitted light is recorded on an Ocean Optics spectrometer (model HR2000+). For all five liquid solutions, there is a significant absorption band centered around 410 nm (blue). In addition, there are several coincident absorption bands located at $\lambda =$
30 725, 887, and 1037 nm, which possibly arise due to the common solvent CH_2I_2 used for all five liquids. Furthermore, we observe a Tyndall effect at all the three wavelengths, with stronger

scattering for the Cargille liquids than for our SbBr_3 solutions. This suggests that Mie scattering is present, caused by colloidal particles with a size on the same order as λ [18]. In order to quantify the amount of scattering, we measure the relative increase in beam width

$$\Delta w = \frac{w - w_0}{w_0}$$

- 5 , where w and w_0 are the beam-widths for the liquid and airfilled cuvettes, respectively. A large value of Δw signifies stronger scattering. For simplicity, all measurements are done at $T = 25^\circ\text{C}$ and for normal incidence ($\theta = 0^\circ$), using the knife-edge scanning method shown in Fig. 2. Results for the two wavelengths 473 and 633 nm are given in the following TABLE I.

TABLE I: Proportional increase (%) in the beam width, referenced against an empty cuvette

Liquid/liquid solution	Beam width increase(%)	
	$\lambda = 633 \text{ nm}$	$\lambda = 473 \text{ nm}$
Diiodomethane (CH_2I_2)	0	2.2
5 20 wt% SbBr_3 solution	0	9.5
33.5 wt% SbBr_3 solution	0	10.6
Cargille liquid ($n=1.71$)	1.2	16
Cargille liquid ($n=1.80$)	13.5	Opaque

At $\lambda = 633 \text{ nm}$, the beam width remains unaffected for the $\text{SbBr}_3\text{-CH}_2\text{I}_2$ liquid solutions and the Cargille liquid $n = 1.71$. Only the Cargille $n = 1.80$ liquid contains colloidal particles in the relevant size range. At $\lambda = 473 \text{ nm}$, the beam width also shows a concentration-dependent increase for the $\text{SbBr}_3\text{-CH}_2\text{I}_2$ liquid solutions. A likely source of particles in this diameter range is that hydrolysis with atmospheric humidity produces small antimony oxide crystals via the reaction $2\text{SbBr}_3 + 3\text{H}_2\text{O} \rightarrow \text{Sb}_2\text{O}_3 + 6\text{HBr}$ [19]. At the same wavelength, the Cargille liquid $n = 1.71$ shows an even stronger scattering, while the opacity of the $n = 1.8$ Cargille liquid does not even allow the measurement of the beam width.

Antimony tribromide (SbBr_3) dissolved in diiodomethane (CH_2I_2) is a strong candidate as an immersion liquid for sapphire-based aNAIL lenses. Together with a refractive index matched immersion liquid, these lenses allow for the simultaneous increase of both the numerical aperture (NA, the light-gathering power) and the working distance (WD) of an objective lens [2, 3]. This technique places the object or sample of interest at the aplanatic point of the spherical surface of the aNAIL in order to have an aberration-free focal spot. It can increase the NA of the backing objective lens by a factor of n_{aNAIL}^2 , up to the maximum achievable value n_{aNAIL} [1, 2].

Fig. 5 shows an aNAIL and a backing objective lens system designed for significantly increasing the NA (Numerical Aperture) of the backing objective lens from 0.619 to 1.17, while still maintaining a long working distance ($\text{WD} = 12 \text{ mm}$). The lens system depicted was designed using WinLens3D optical design software.

Because n is tunable via both concentration and temperature, the liquid formulation presented here could open new routes to creating adaptive lenses with tunable optical power [20]. In these applications, liquid lenses have the advantage of additionally allowing the shape of the lens to be tuned in order to adjust its focal length (and therefore the optical power), mimicking the mechanism of human eye. To date, the lack of an appropriate high- n liquid has limited the range of tunable optical power, as this depends on the difference of the refractive indices of the two immiscible liquids used to build the adaptive liquid lens [21–23].

Fig. 6 shows an embodiment of an adaptive liquid lens 1 according to the present invention. The adaptive lens 1 comprises a cavity 2 filled with the high refractive index liquid 3 according to the present invention and delimited by curved membranes 4 in both directions of an optical axis 5. The membranes 4 are both transparent and deformable. By pulling at the lens 1 in the direction of arrows 6, the radius of curvature of both the membranes and the liquid lens 1 will be decreased.

In the embodiment of the adaptive lens 1 according to **Fig. 7**, the cavity is delimited by a solid lens 7 made of sapphire in one direction of the optical axis 5 and by a membrane 4 in the other direction. The liquid 3 filled in the cavity 2 is refractive index matched to the material of the solid lens 7. By pulling at the membrane 4 in the direction of the arrows 6, the radius of curvature of the membrane 4 and thus one of the radii of curvature of the adaptive lens 1 will be altered. A temperature control unit may control the temperature of the solid lens 7 and thus of the liquid 3 contacting the solid lens 7 to ensure the refractive index match. Due to the chromatic properties of the liquid 3, a full refractive index match will only be achieved at a certain wavelength of the light passing through the adaptive lens 1 along the optical axis 5.

Finally, we close with the application that inspired our work on this immersion liquid formulation: the imaging of gems made from corundum minerals (ruby and sapphire), which have $n \sim 1.77$. High spatial resolution lenses such as those described above (aNAIL) will open new routes to studying the crystallization process during mineral formation [24] and quality in manufacturing industry (gemstone, watch etc.) [25]. Importantly, ruby also has a pressure-dependent fluorescence peak [26–28], which has recently been exploited as a tool for measuring the pressure field inside rubies themselves [29, 30].

We have demonstrated that antimony tribromide (SbBr_3) dissolved in diiodomethane (CH_2I_2), for concentrations between 20 wt% and saturation, provides a promising new high refractive index liquid formulation. At standard conditions for temperature and pressure (STP) and at wavelengths from near-ultraviolet to near-infrared, we observe that $n > 1.77$ (sapphire) is attainable. For optimized choice of parameters (low temperature, short wavelength, high concentration), we can achieve $n = 1.873$. In addition, this liquid is observed to have high transmission and low scattering for $\lambda < 350$ nm and $\lambda > 450$ nm. The tunability of this extremely high n liquid has several promising applications, particularly the development of sapphire based aNAIL optics for super-resolution 3D imaging, adaptive liquid lens, gemstone, watch industry etc.

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LIST OF REFERENCE NUMERALS

- 1 adaptive lens
- 2 cavity
- 3 liquid
- 4 membrane
- 5 optical axis
- 6 arrow
- 7 solid lens
- 11 mirror
- 12 incident angle
- 13 cuvette
- 14 power detector
- 15 knife edge (on x-translation stage)
- 16 Gaussian fit
- 17 CH_2I_2
- 18 20 wt% SbBr_3 in CH_2I_2
- 19 33.5 wt% SbBr_3 in CH_2I_2
- 20 saturated SbBr_3 in CH_2I_2
- 21 sapphire
- 22 Cargille $n=1.71$
- 23 Cargille $n=1.80$
- 24 backing objective lens
- 25 air medium
- 26 sapphire lens (aNAIL)
- 27 sample of interest at the aplanatic point
- 28 WD (working distance)
- 29 sapphire refractive index matching immersion liquid

CLAIMS

1. A high refractive index immersion liquid (29) for immersing a front surface of an immersion objective lens of a microscope, the immersion liquid (29) comprising a solution of antimony tribromide (SbBr_3) dissolved in an organic liquid, wherein the organic liquid is diiodomethane (CH_2I_2) and wherein the concentration of the antimony tribromide (SbBr_3) is not more than 50 % by weight of the solution.
2. The immersion liquid (29) of claim 1, wherein the concentration of the antimony tribromide (SbBr_3) is at least 10 % by weight of the solution.
3. A combination of the immersion liquid (29) of claim 1 or 2 and an immersion objective lens configured to be immersed into the immersion liquid, wherein the immersion liquid (29) matches the sapphire in refractive index.
4. The combination of claim 3, wherein the immersion objective lens is made of sapphire.
5. The combination of claim 3 or 4, wherein the immersion objective lens is a truncated aplanatic immersion objective lens.
6. The combination of claim 5, wherein the immersion objective lens is an aNAIL (26).
7. The combination of any of the claims 3 to 6, wherein the immersion objective lens has both a high numerical aperture (NA) of at least 1.0 and a long working distance (WD) of at least 10 mm.
8. The combination of any of the claims 3 to 7, and comprising a temperature control unit including a controlled temperature surface adjacent to a gap in front of the immersion objective lens to be filled with the index matched immersion liquid (29).
9. A kit for preparing the immersion liquid (29) of claim 1 or 2, the kit including an amount of antimony tribromide (SbBr_3) and an amount of an organic liquid, wherein the organic liquid is

diiodomethane (CH_2I_2) and wherein, by weight, the amount of the antimony tribromide (SbBr_3) is not more than the amount of the diiodomethane (CH_2I_2).

10. The kit of claim 9, wherein, by weight, the amount of the antimony tribromide (SbBr_3) is not less than a tenth of the amount of the diiodomethane (CH_2I_2).

11. A method of matching a high refractive index of the immersion liquid (29) of claim 1 or 2 to a high refractive index of a solid medium, wherein the concentration of the antimony tribromide (SbBr_3) is raised for increasing the refractive index of the immersion liquid (29) and lowered for decreasing the refractive index of the immersion liquid (29).

12. The method of matching of claim 11, wherein the temperature of the immersion liquid (29) is raised for decreasing the refractive index of the immersion liquid (29) and lowered for increasing the refractive index of the immersion liquid (29).

13. The method of matching of claim 11 or 12, wherein the solid medium is forming a front surface of an immersion objective lens or embedding a sample (26) of interest.

14. A method of using the immersion liquid of claim 1 or 2 in a microscope comprising an immersion objective lens, wherein the immersion objective lens is made of sapphire, and wherein a gap between a front surface of the immersion objective lens and a facing surface of a sample of interest or a solid medium including a sample of interest is filled with the immersion liquid (29) matching the sapphire in refractive index.

15. The method of using of claim 14, wherein a temperature of the immersion liquid (29) in the gap is controlled.

16. A microscope comprising an immersion objective lens, wherein the immersion objective lens is sapphire based and wherein the sapphire based immersion objective lens is configured to be immersed into an index matched immersion liquid (29).

17. The microscope of claim 16, wherein the immersion objective lens is a truncated aplanatic immersion objective lens.

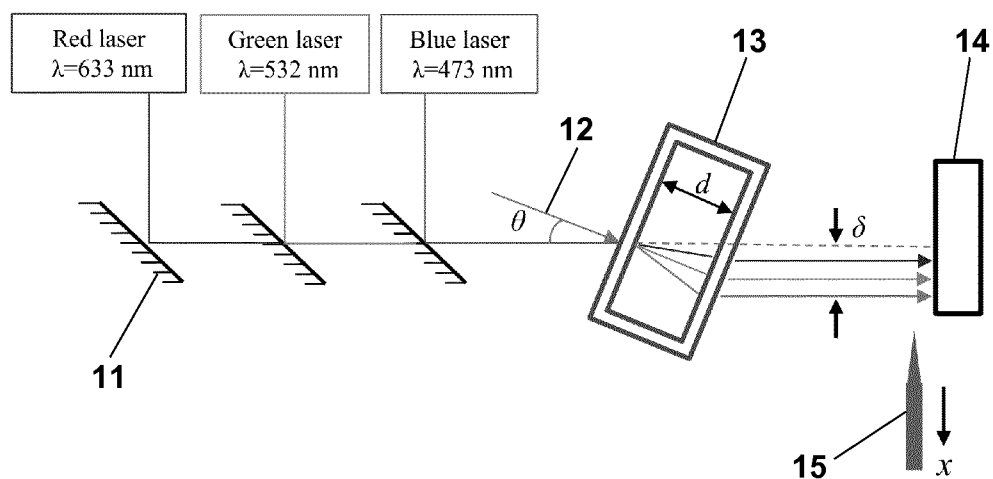
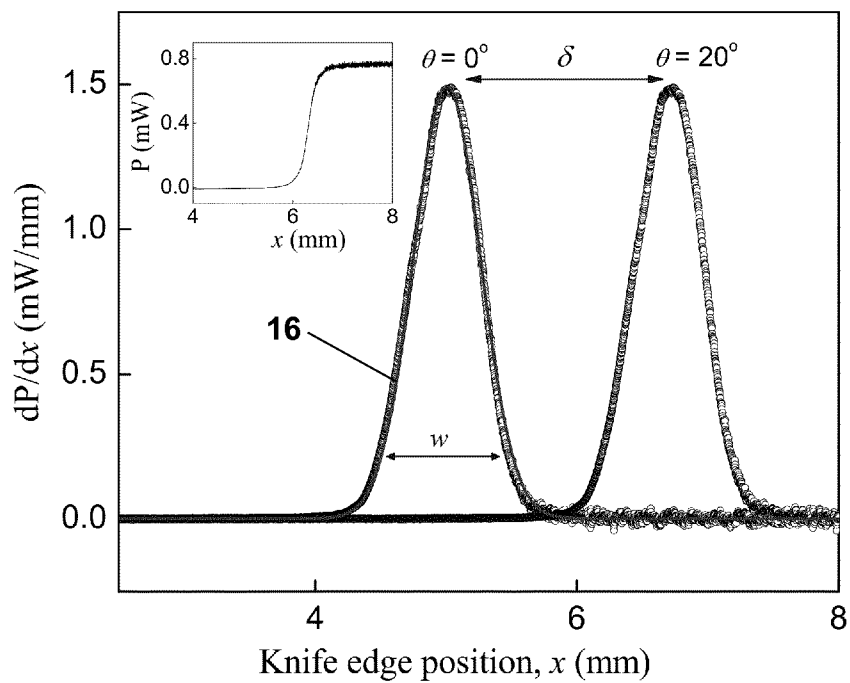
18. The microscope of claim 17, wherein the immersion objective lens is an aNAIL (26) .

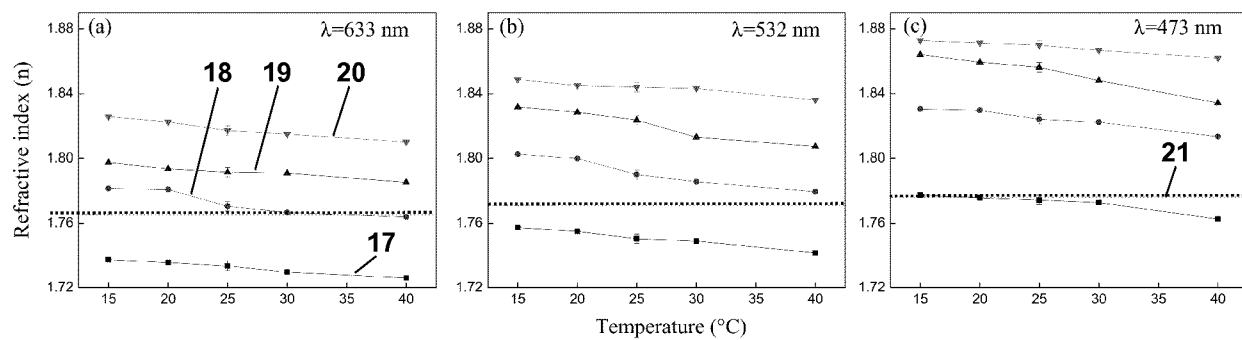
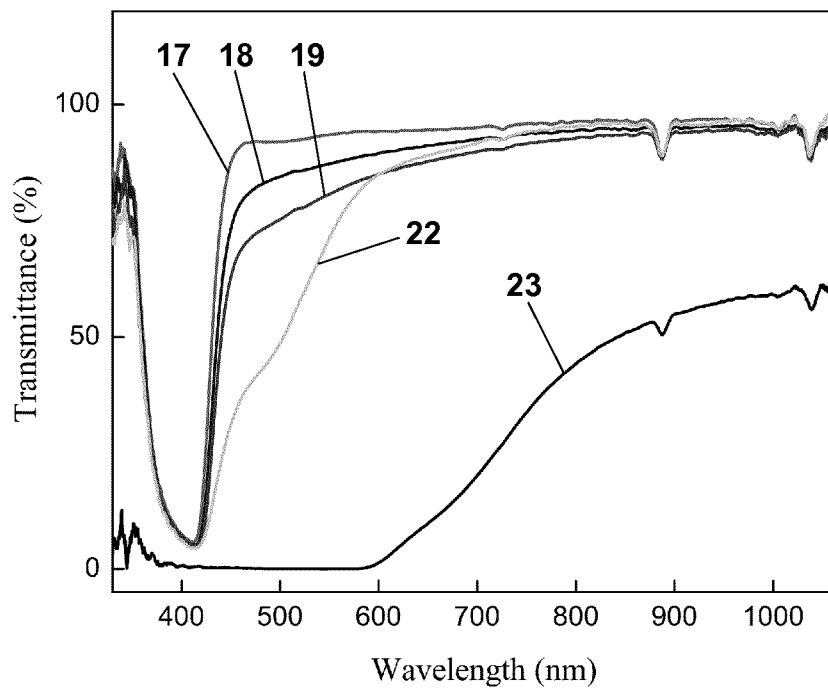
19. The microscope of any of the claims 16 to 18, wherein the immersion objective lens has both a high numerical aperture (NA) of at least 1.0 and a long working distance (WD) of at least 10 mm.

20. The microscope of any of the claims 16 to 19, and comprising a temperature control unit including a controlled temperature surface adjacent to a gap in front of the immersion objective lens to be filled with the index matched immersion liquid (26).

21. An adaptive lens (1) comprising a cavity (2) delimited by a curved deformable transparent membrane (4) in at least one direction and filled with a high refractive index liquid (3), wherein the liquid (3) comprises a solution of antimony tribromide (SbBr_3) dissolved in diiodomethane (CH_2I_2) and wherein the concentration of the antimony tribromide (SbBr_3) is at least 10 % and not more than 50 % by weight of the solution.

22. The adaptive lens (1) of claim 21, and comprising a temperature control unit including a controlled temperature surface adjacent to the cavity (2) filled with the index matched immersion liquid (3).

**Fig. 1****Fig. 2**

**Fig. 3****Fig. 4**

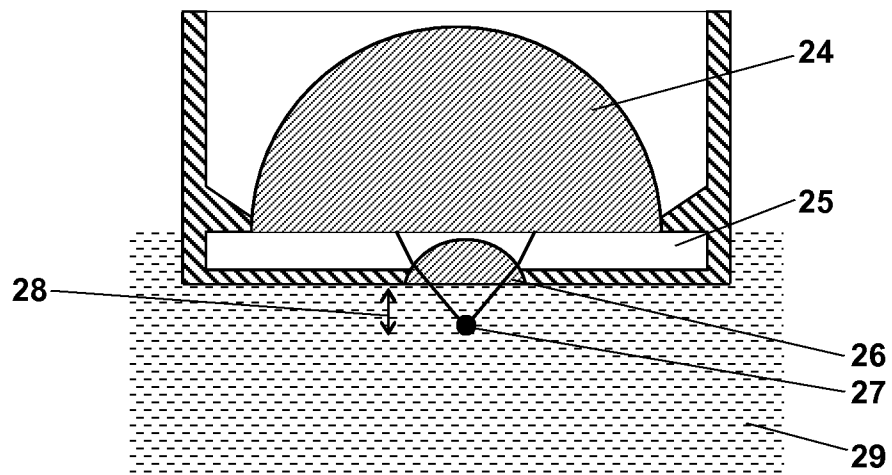


Fig. 5

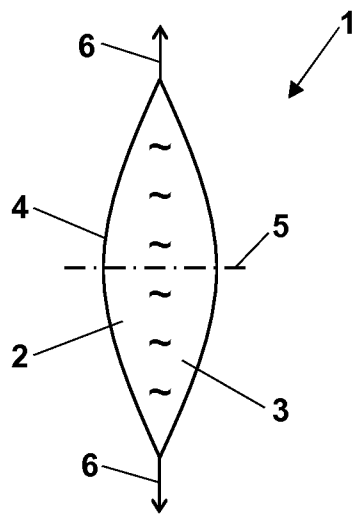


Fig. 6

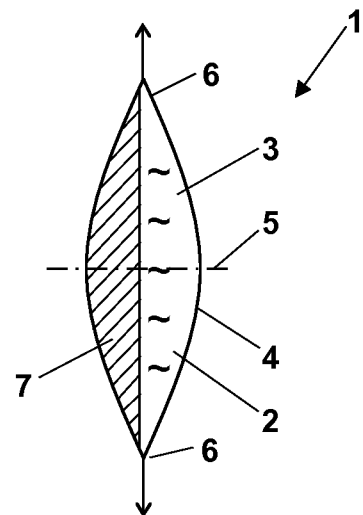


Fig. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/081209

A. CLASSIFICATION OF SUBJECT MATTER
INV. G02B21/33 G02B26/00
ADD.

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)
G02B

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROBERT MEYROWITZ: "A compilation and classification of immersion media of high index of refraction", AM. MINERAL., vol. 40, 1955, pages 398-409, XP055296755, cited in the application	1-13
Y	paragraphs [00II], [0J.1] -----	14,15
X	L.H. BORGSTRÖM: "BEITRAG ZUR ENTWICKLUNG DER IMMERSIONSMETHODE. II. VERWENDUNG VON ANTIMONBROMID. VERSUCHE MIT SELEN", COMM. GÉOL. FINLANDE BULL, vol. 101, 1933, pages 28-29, XP009191449, table 1 -----	1-13
Y		14,15
Y	US 2006/245087 A1 (TEARNEY GUILLERMO J [US] ET AL) 2 November 2006 (2006-11-02) claims 1, 16-18,75 ----- -/-	14,15



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

6 September 2016

Date of mailing of the international search report

22/11/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Rödig, Christoph

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/081209

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STEPHEN BRADLEY IPPOLITO, BB GOLDBERG; MS UNLU.: "High spatial resolution subsurface microscopy", APPL. PHYS. LETT., vol. 78, no. 26, 2001, pages 4071-4073, XP012028345, cited in the application figures 1, 3 -----	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2015/081209

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-15

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15

composition of of a high refractive index immersion liquid

2. claims: 16-20

microscope having a sapphire-based objective lens

3. claims: 21, 22

adaptive lens

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2015/081209

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006245087 A1	02-11-2006	US 2006245087 A1 WO 2004065994 A2	02-11-2006 05-08-2004
