Development of a low-temperature immersion microscopy technique for ice research

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Abstract

Perennial ice can be studied for many purposes, including paleoclimate records or rheological properties. For most of those purposes, the ice microstructure must be studied, often through optical microscopy. The aim of this work is to assess the viability of immersion microscopy for the study of ice microstructures. It consists of using an oil between the objective lens and the specimen, to increase image resolution. Immersion microscopy is a technique well-developed for the investigation of diverse materials, but it has so far not been explored for ice research. Here we investigate the challenges and advantages of that technique. The main challenge is related to the selection of the immersion oil itself, which must satisfy a number of criteria, ranging from refractive index and viscosity to toxicity and reactivity. We identify pure silicone oil (dimethicone) as a simple and safe option for immersion microscopy of inner ice structures. Among its advantages, it provides higher resolution (compared to standard 'dry' microscopy) and it can be simultaneously used as a long-term coating to prevent undesired sublimation of the ice-sample surfaces. For the observation of surface structures, however, another type of oil with higher refractive index should be used.

Ice and its microstructure

Ice is formed of water molecules (H_2O) arranged in a crystalline lattice structure (Hobbs, 1974). Generally, in materials science the crystalline lattice is considered the most fundamental level of the microstructure. That is complemented by other three levels (Bunge and Schwarzer, 2001), listed here in descending size scale:

- First, the larger phase structure, which comprises all the various amorphous and crystalline components of the material. In the case of natural ice, the phase structure includes air bubbles, pores, clathrate hydrates, all kinds of impurities and inclusions and the ice matrix itself (Petrenko and Whitworth, 1999; Faria and others, 2018).
- Second, the grain structure, i.e. the crystalline domains, or crystallites, delimited by grain boundaries.
- Finally, there is the defects structure (also known as grain sub-structure), which includes all kinds of defects of the crystalline lattice (other than grain boundaries), e.g. dislocations, sub-grain boundaries, dislocation walls, slip bands, stacking faults, etc.

It must be understood that these microstructural levels are intertwined in space. For instance, the defects structure comprises sub-nanoscopic crystalline lattice imperfections (e.g. vacancies and interstitials) as well as grain boundaries on the scale of the grain structure. Likewise, the phase structure encompasses microinclusions of nanometric size as well as pores of many centimetres or even metres in length.

A key point about all those intertwined microstructural levels is that they evolve and interact with each other. This happens because the ice polycrystal is not perfect: it contains defects and interfaces that evolve and exchange energy among themselves, within the ice and with the environment, in a process called Structure–Form–Environment Interplay (SFEI; Faria and others, 2009; Faria and others, 2018). Examples of such interactions range from sublimation and sintering to recrystallization and recovery. As a result, the ice microstructure is constantly evolving at a pace that is strongly dependent on the temperature, applied stresses and other environmental conditions.

All those microstructural interactions and evolution imply that a sound microscopic study of polycrystalline ice requires a proper preparation and preservation of the ice samples, in order to minimize such changes and preserve the original ice microstructure. Common preservation measures include, among others, maintaining the temperature of the ice samples as cold as possible, producing ice sections with thicknesses smaller than the average grain size, sublimating the ice surfaces in a controlled way and preserving those surfaces with a film of oil or other medium that prevents further sublimation. Such preservation measures pose a challenge for ice microscopy, which have motivated the development of the methodology presented in the next sections.

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The objective of this work is to assess the viability of a microscopy method that has remained unexplored for the study of ice microstructures to date, called oil immersion microscopy. In the following sections, we will introduce the foundations of the technique and develop a special methodology for its application to ice microscopy. Further, we will assess the pros and cons of that new method, determining in which situations it may be advantageous over traditional (dry) microscopy.

Immersion microscopy

Optical microscopy has long established itself as the primary technique to study the microstructure of ice. Its robustness (with the basic workings relying solely on optics and a few mechanical parts) and its low magnification, when compared to more sophisticated microscopy techniques, makes it ideal for use at the low temperatures needed to preserve the ice samples and the typically large grains of polycrystalline ice.

The most common objective lenses used in optical microscopy are 'dry' objective lenses, which work with air between the specimen and the lens. They offer the most trivial way of observing the bare ice surface. Nevertheless, ice samples are often covered with an oil film to protect their surfaces and prevent further sublimation. For example, in the Microstructure Mapping technique (Kipfstuhl and others, 2006), the first (lower) surface of the sample is covered with a thin film of silicon oil and sealed on a glass plate. The second (upper) surface is treated in the same way but always after the first mapping has been done, to avoid the optical distortion caused by the strong refraction of an oil–air interface.

Fortunately, for those cases where the sample has already been covered with oil, there is another type of objective lens that demands a liquid environment (usually some type of transparent oil) between the specimen and the lens. When a high viscosity oil is dropped on the surface of the sample and the tip of the objective is dipped into the oil drop, a meniscus is formed (Fig. 1). The surface tension will maintain the meniscus stable and the air layer will have been replaced by a medium with higher refractive index. This technique is called immersion microscopy. It was first developed by Giovanni Amici (1786–1863) and remarkably improved by Ernest Abbe (1840–1905; Ockenga, 2015).

A conventional optical microscope can be used for immersion microscopy, as long as a special immersion objective lens is employed. The objective lenses are one of the most important elements of an optical microscope. They are to a great extent responsible for the magnification and resolution of the images, greatly conditioning the results obtained with the microscope. Even if they are intricate systems formed by a set of high-quality lenses, objectives can generally be described by a reduced set of parameters. The two most important parameters are the numerical aperture and

the magnification. Numerical aperture (NA) describes the ability of the lens to gather light. It is defined as (Abbe, 1881):

$$NA = n \cdot \sin(\theta) \tag{1}$$

where n is the refractive index of the intermediate medium and θ is half of the angle of the vertex of the cone. The numerical aperture is closely related to the resolution of an objective lens, which is the distance between two adjacent objects needed to distinguish them as two independent identities. Thus, the higher the resolution, the more detail will be visible in the images. The limit of resolution can be calculated with the following formula (Abbe, 1873):

$$R = \frac{\lambda}{2\,NA} \tag{2}$$

where λ is the wavelength of the light used.

The resolution has acquired quite an importance in the era of digital images, as high-resolution digital zoom can be used to compensate optical magnification because, as it can be seen from (2), the resolution has no dependence on magnification.

Application to ice

The main objective of this work is to determine the advantages and usefulness of the immersion microscopy technique for the study of ice microstructures at low temperatures in relation to traditional dry microscopy. Two factors affecting microscopy will be tested in this work: the illumination and whether the target of study is at the surface or inside the ice sample. To this aim, we compare here the following objective lenses:

HC Pl FLUOTAR 63×/1.3 OIL (Leica[TM])

HC PL FLUOTAR L 50×/0.55 (Leica[TM])

HC PL FLUOTAR L 100×/0.75 (Leica[TM])

Immersion oil selection

The biggest challenge related to the immersion microscopy technique might be related to the selection of a suitable immersion oil and the determination of its physical properties at low temperatures. For instance, the standard microscopy oil provided for our objective, identified as Leica[TM] Type N Immersion Liquid, unmixes and crystallizes at temperatures below 18 °C. Likewise, most oils used for immersion microscopy solidify or crystallize at low temperatures.

On the other hand, a thin layer of silicone oil (also known as dimethicone, dimethyl silicone or polydimethylsiloxane) is often
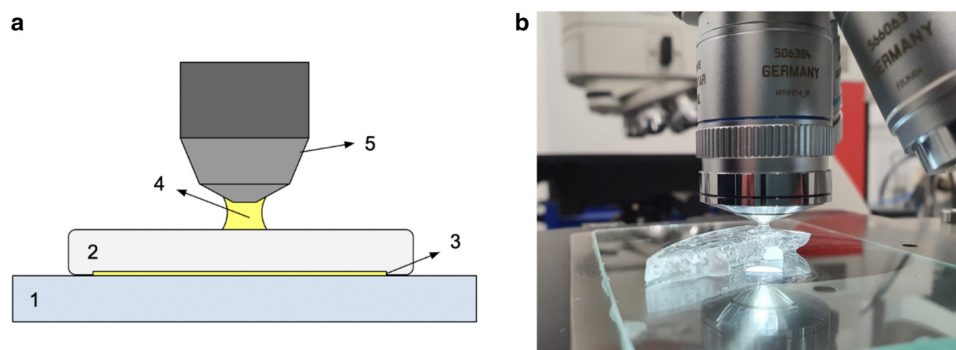

Figure 1. Illustration (a) and image (b) of an ice sample at the microscope and the formation of the meniscus (1 – sample holder, 2 – ice section (sample), 3 and 4 – oil, 5 – objective).

used to preserve ice samples and to prevent further sublimation, as already mentioned (Kipfstuhl and others, 2006). Its molecular formula is $CH_3[Si(CH_3)_2O]_iSi(CH_3)_3$, where the index i defines the length of the polymer molecule, which in turn determines the viscosity of the oil. The bigger the i, the longer the molecule and the higher the viscosity. When commercialized, these oils are categorized by their kinematic viscosity at 'standard' temperature (generally set between 20 and 25 °C), which is usually measured in centistokes (cSt = $mm^2 s^{-1}$).

Taking into account the above arguments, we conclude that our objective is to determine whether there is a dimethicone oil that, at the low temperatures of ice microscopy (between −10 and −30 °C), approaches the desired physical properties of the Leica[TM] Type N Immersion Oil at its ideal working temperature of 23 °C. Here we will focus on the following low-temperature properties of dimethicone, manufactured by Dow® under the name XIAMETER[TM] PMX-200 Silicone Fluid (DOW, 2020), to determine its suitability for low-temperature immersion microscopy: safety, chemical compatibility with ice surfaces and objective lenses, transparency (transmittance), refractive index and fluidity (viscosity).

The first properties can be easily assessed. Dimethicone is well-known to be non-toxic, odourless, inert, stable and hydrophobic. Owing to its non-toxicity, it is also used in surgical procedures (Yamada and others, 2019) and as a medium for live-cell imaging (Samuelsson, 2018). This represents a great advantage over other immersion liquids, which are often toxic. On the other hand, its inert and hydrophobic properties, combined with a very low surface tension (21 mN m^{-1}; DOW, 2020) make dimethicone particularly suitable for covering ice surfaces, since it can penetrate every microscopic groove or pit of the ice surface without leaving gaps or reacting with a potential quasi-liquid layer[1]. Finally, like all modern synthetic immersion oils, dimethicone does not affect the elements of immersion objective lenses and can remain in contact with the objective for long periods of time.

Suitability of optical properties

Being inert to the objective lenses is, however, not enough to qualify an oil as appropriate for microscopy: its optical properties must also be suitable. Fortunately, dimethicone is remarkably transparent and colourless. It appears crystal-clear with an ALPHA colour 5 (DOW, 2020), and its transmittance reaches 99% for layers of less than 1 mm thickness (Querry, 1987; Polyanskiy, 2022). Such properties are not expected to significantly vary with temperature (Wu and others, 2013).

Lastly, there remains to verify the most important optical property of the immersion oil, which is the refractive index. In our case, we had to determine if the refractive index of dimethicone at −22 °C has a similar value as that of Leica[TM] Type N oil at its ideal temperature of 23 °C (Table 1).

The problem that we faced in this research was the absence of data at low temperatures, as we only had the value of the refractive index at room temperature for the dimethicone (Table 1).

However, experience tells us that organic liquids in general possess refractive indices that usually are linearly dependent on temperature (Olson and Horne, 1973; Abbate and others, 1981; Faoro and others, 2018). Dimethicone is no exception to that rule, with a slope estimated as 3.1×10^{-4} units °C[-1] for a Midland Silicones[TM] MS-200 silicone oil of 350 cSt (Van Raalte, 1960). Based on that data, we estimate the value 1.4180 for the refraction index of dimethicone 350 at −22 °C (Table 1).

Table 1. Refractive indices of the materials studied at this paper at a number of temperatures of interest

Material	Reference temperature (°C)	Refractive index	Source
Leica[TM] Type N immersion oil	23	1.5180	Leica (2009)
Dimethicone 350 cSt	25	1.4034	DOW (2020)
Dimethicone 350 cSt	−22	1.4180	(own estimate)
Hexagonal ice Ih	−22.5	1.3113	Onaka and Kawamura (1983)

Even though the refractive index of dimethicone is lower than desired, it is close enough to the reference value of Leica[TM] Type N immersion oil (1.5180; Table 1) to allow a viability study of the method of immersion microscopy with ice.

Suitability of mechanical properties

We have already mentioned that its hydrophobic properties and low surface tension make dimethicone fit for covering ice surfaces, because it can penetrate every microscopic groove or pit on the surface. Nevertheless, this also means that, if the viscosity is too low, the silicone oil may eventually drain off the surface, leaving it unprotected. Likewise, during immersion microscopy, such a low-viscous oil may not stick well to the objective-surface gap, requiring frequent refilling. On the other hand, if the viscosity is too high, the oil will not spread well over the surface and the resulting protective layer may be too thick. The application of a high-viscous oil increases the risk of forming microbubbles, which may corrupt the protective layer and ruin the immersion microscopy images. Moreover, if the viscosity of the oil is too high, when the objective is moved, the friction between the oil and the ice could damage the sample. Therefore, if we need the oil for both applications, the immersion microscopy and protection of the sample surface, a well-balanced choice is needed. Even so, we have a comfortable margin of choice between the low- and high-viscosity limits. As it happens with the refractive index, we are interested in finding a silicone oil with a kinematic viscosity at $T = −22$ °C not too far apart from that of the Leica[TM] Type N oil at $T = 23$ °C, which is $\nu = 825$ cSt.

According to the Technical Data Sheet of XIAMETER[TM] PMX-200 Silicone Fluid (DOW, 2020), the available dimethicone options that come closer to a kinematic viscosity of 825 cSt at $T = −22$ °C are those graded with 'standard' (room-temperature) viscosities of 200 and 350 cSt. The manufacturer's data indicate that both grades have a pouring point at −65 °C, meaning that they remain liquid within the temperature range of interest. The former should reach a viscosity of $\nu_{200}(T = −22$ °C$) \approx 600$ cSt, while the latter should be close to $\nu_{350}(T = −22$ °C$) \approx 1050$ cSt (see also Sutton, 2015). Thus, both grades are suitable, depending on the preferences of the user and the laboratory temperature: for temperatures down to −20 °C the silicone oil with grade 350 cSt may be more suitable, while for temperatures close to −30 °C and below it may be better to use the oil of grade 200 cSt.

It must be remarked, however, that such viscosity estimates are prone to variations, not only because of measurement uncertainties, but also because the polymeric molecules of a given oil are not all exactly of the same length. In practice, each oil consists of a distribution of molecular lengths. Certificates of analysis from the manufacturer estimate variations in the kinematic viscosity, mainly due to variations in the molecular length distribution, of the order of 10% (Schatz and Howden, 1995; Zilliac, 1996).

Therefore, in order to verify the estimated viscosity of dimethicone at low temperatures, we performed a very simple viscosity test with a sample of 350 cSt dimethicone. The test consisted in

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[1]Admittedly, there are a few rare cases of interaction with the ice surface, as reported by Kipfstuhl and others (2007); but such seldom reactions could not be reproduced and their causes remain unidentified.

emptying a syringe full of dimethicone at $-22\,^{\circ}\mathrm{C}$ under controlled time (Muniozguren-Arostegi, 2022). The result of this simple test showed the viscosity of 900 ± 200 cSt at $-22\,^{\circ}\mathrm{C}$, which is consistent with the values provided by the manufacturer.

Expected advantage and disadvantages of immersion microscopy on ice

Before describing the methodology applied for the low-temperature immersion microscopy of ice, some advantages and disadvantages of this technique can be anticipated:

- A higher resolution is expected with immersion microscopy, as the numerical aperture (NA) is directly related to the refractive index, and a high NA leads to high resolution. Nevertheless, the refractive index of dimethicone is lower than that of Leica$^{\mathrm{TM}}$ Type N Immersion Oil at $T = 23\,^{\circ}\mathrm{C}$, so the NA will not be as high as that specified by the objective.
- Due to a much smaller working distance for the oil objective ($WD_{\mathrm{oil}} = 0.16$ mm) than for the air objectives ($WD_{50\times} = 8$ mm and $WD_{100\times} = 4.7$ mm), it is not possible to observe the bottom surface of the ice sample with immersion microscopy using the current objective.
- The refractive index of hexagonal ice Ih at $T = -22.5\,^{\circ}\mathrm{C}$ is $n = 1.3113$ for light with a 546.1 nm wavelength (Table 1). This makes the refractive index of ice and dimethicone close to each other, so the refraction at their interface will be minimal. This leads to a lower background distortion when studying inner structures but, at the same time, makes it very difficult to observe the surface itself.
- Finally, stored ice samples are often protected by a silicon oil layer. If, in any case, those stored samples have to be reanalysed under the microscope, using the oil immersion microscopy with this existing oil seems quite a straightforward procedure.

Methodology

The aim of this work is to develop an immersion microscopy technique on ice, which implies, not only evaluating its viability and results, but also describing the procedure in detail, for reproducibility and future research.

Sample selection and cut. The first step is to select the piece of ice to be analysed. The ice sample studied here comes from one of the three ice cores from the Monte Perdido Glacier (42° 40' 50" N, 00° 02' 15" E; Central Pyrenees) extracted in autumn 2017 by the Pyrenean Institute of Ecology (Moreno and others, 2021). The selected sample is cut using a vertical electric band saw in order to get a final size that is smaller than the sample holder. In our case, the rectangular ice sample should not be larger than 4 cm wide and 6 cm long. In this particular case, the selected sample thickness was between 0.5 and 1 cm, which is large enough for comfortable handling, but smaller than the average grain size, which is coherent with the ice samples used for microstructural mapping (Kipfstuhl and others, 2006).

Preparation of the sample. The ice sample, already cut in a suitable size and shape, needs to be prepared for microscopy. This means that both surfaces of the ice section must be polished. That is achieved by smoothing the ice surface with a microtome, followed by sublimation in cold air for a period long enough to remove micro-scratches from the microtome blade. Additionally, sublimation also works as a thermal etching process that produces grooves where grain and subgrain boundaries meet the ice surface. In our case, a sublimation period of approximately 1 h at $-20\,^{\circ}\mathrm{C}$ was enough to produce smooth ice surfaces with clear (sub-)grain boundary grooves.

Selection of a point of interest using traditional objective lenses. If the ice sample is being analysed for the first time, inspection by traditional dry microscopy will precede the immersion microscopy. Traditional microscopy is simpler, faster and can be used to identify and locate structures that are worth later studying in detail through immersion microscopy.

Applying the oil and observation by an immersion objective lens. Once the zones and aims of study for the immersion microscopy have been fixed, we can go on using the oil. The nosepiece of the microscope has several 'dry' objectives alongside with the immersion one. For this part of the procedure, we are going to select the oil objective and set it out of focus, just above a point of interest, in order to let space between the sample and the optics. Using a pipette, we are going to put several drops of the oil in the area and submerge the objective into the oil (see a scheme of the set-up in Fig. 1). In case the sample has already had oil on its surface (e.g. because it has previously been analysed and preserved but we want to reanalyse it), we simply have to make sure that the surface has enough oil to form the meniscus necessary for the immersion microscopy.

Results

We have previously mentioned that in the era of digital images, the magnification of the optics is not determinant as it can be complemented with digital zoom. What is indeed most important is the resolution (that will limit the digital zoom) and the absolute size of the sample that appears in the photomicrograph. These characteristics remain constant for their corresponding objective lenses, and as the photos are rectangular, they will be expressed by two dimensions:

$$50\times \rightarrow (261.8 \pm 0.1)\,\mu\mathrm{m} \times (196.3 \pm 0.1)\,\mu\mathrm{m}$$
$$63\times \text{ OIL} \rightarrow (207.8 \pm 0.1)\,\mu\mathrm{m} \times (155.8 \pm 0.1)\,\mu\mathrm{m}$$
$$100\times \rightarrow (130.91 \pm 0.01)\,\mu\mathrm{m} \times (98.15 \pm 0.01)\,\mu\mathrm{m}$$

Surface structures

When we talk about surface structures, the most notable are grain and subgrain boundaries, followed by dislocation walls. As we have previously mentioned, these structures are present all over the ice, but are only observable on the sample surface in the form of surface grooves produced by sublimation during sample preparation.

The first comparison aims to determine which objective and type of illumination gives better results when observing the surface, the three candidates being: (i) transmitted (diascopic) light, (ii) incident (reflected/episcopic) light and (iii) the combination of both (Fig. 2).

It is evident that the combination of incident and transmitted light gives the best result in relation to illumination. If only transmitted light is used, the inner structures that are close to the surface lead to notable shadows, especially the bubble just above the triple junction. For a combined illumination, background bubble's shadow has been mitigated or even has disappeared for the 50× and 100× objectives.

When it comes to the objective lenses, the immersion objective leads to poorer results, because, as already mentioned, the similar refractive indices of ice and oil imply that the surface irregularities (e.g. grain-boundary grooves) will be fainter and the inner structures (e.g. bubbles) will be more noticeable.

Inner structures

Inner structures can include a large variety of inclusions such as bubbles, microparticles, clathrate hydrates or other structures such as slip bands. In this subsection we study bubbles, as they are the most common and evident inner structures in our samples.
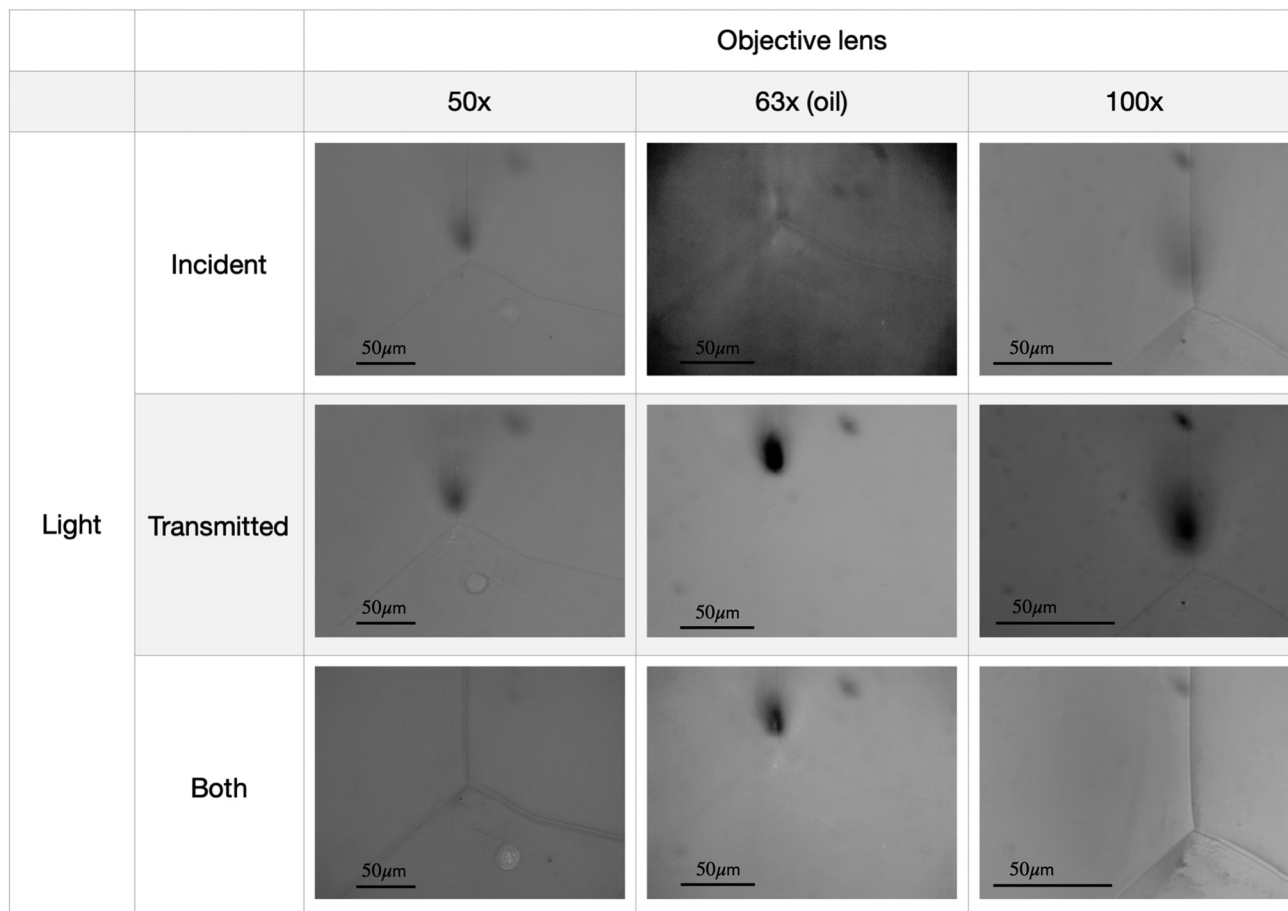
Figure 2. Comparison of the micrographs of three different objective lenses (standard 50× and 100× lenses without oil, and immersion 63× lens with oil) using three different illumination techniques for observing a triple junction (surface structure consisting of three grain-boundary grooves meeting at the centre of the image).

As we did for the surface structures, we are going to compare two different illumination techniques (incident and transmitted) with three different objectives (standard 50× and 100× lenses without oil, and immersion 63× lens with oil; Fig. 3).

If we compare the pairs of micrographs, we can clearly see that the transmitted light leads to much better images for any of the three objective lenses, regardless of the type of objective. In the case of the 'dry' objectives with incident light, the bubble can
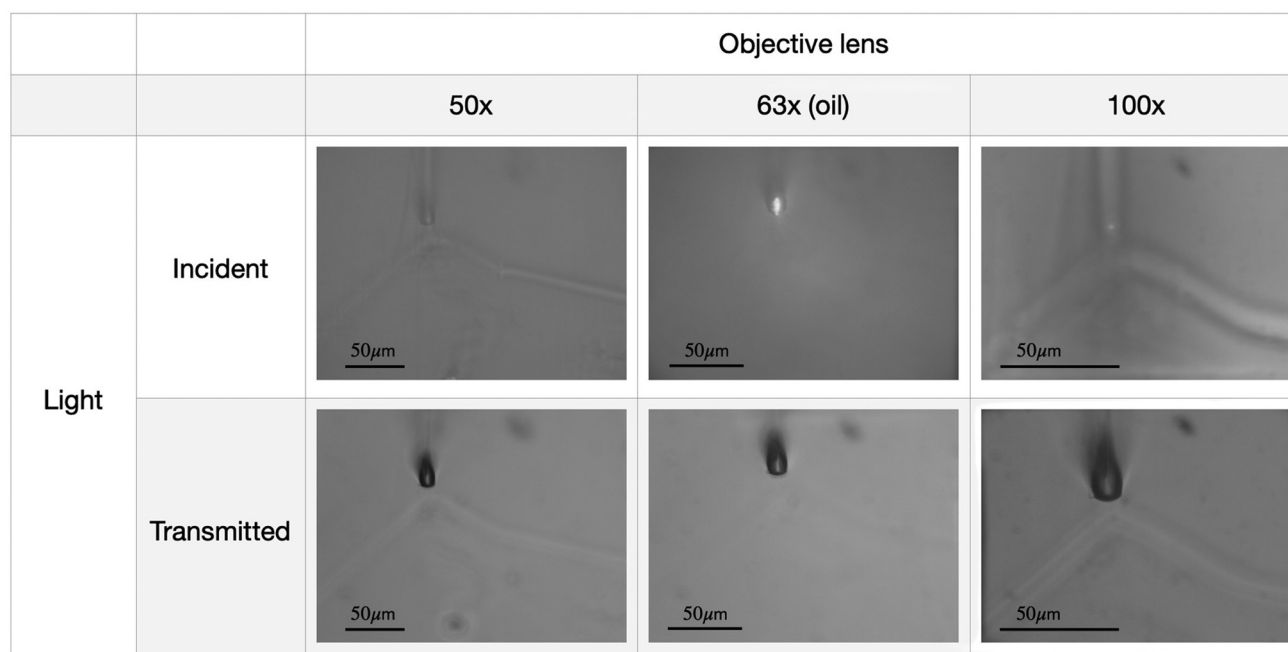

Figure 3. Comparison of the micrographs of three different objective lenses (standard 50× and 100× lenses without oil, and immersion 63× lens with oil) using two different illumination techniques for a bubble (inner structure).
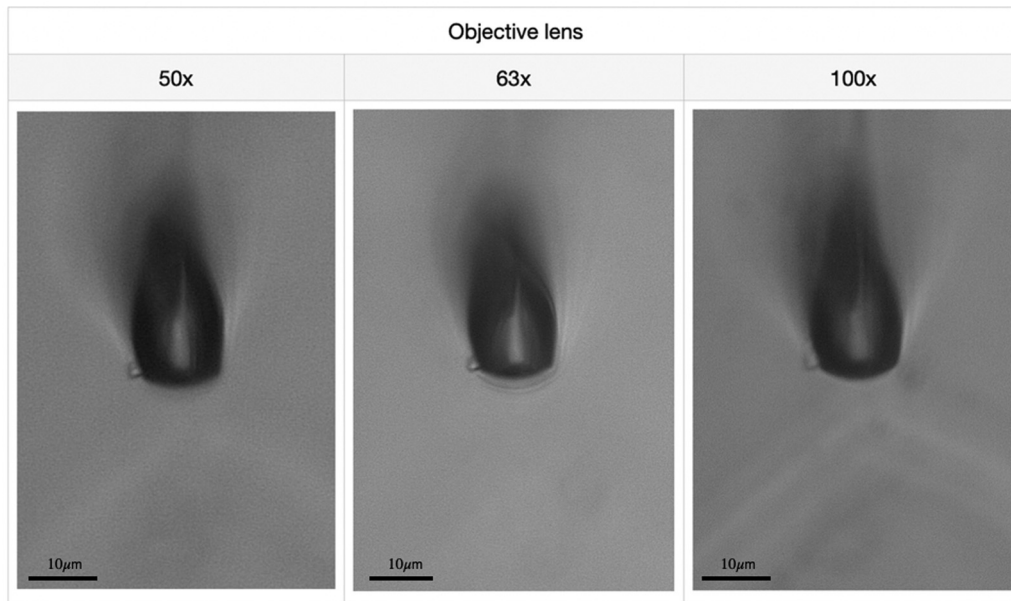
Figure 4. Bubble observed with three different objectives (standard 50× and 100× lenses without oil, and immersion 63× lens with oil), illuminated with transmitted light. The magnification of the optical devices has been compensated with digital magnification so that the bubble has a similar size for the three images. The dimensions of these photomicrographs are **(left):** (46 ± 4) μm × (69 ± 4) μm, **(centre):** (50 ± 3) μm × (75 ± 3) μm, and **(right):** (46.2 ± 0.2) μm × (69.2 ± 0.2) μm.

barely be seen and the background distortion of the grain boundaries has a great impact, as one of the boundary grooves is just above the bubble. But for transmitted light, the bubble produces a much higher contrast for every objective. If we compare results of the three objective lenses obtained using transmitted illumination, the most notable difference between the results of traditional and immersion microscopy is how the background distortion originated by the grain boundaries almost disappears for the latter one.

In order to reach a conclusion about the resolution of the traditional and immersion microscopy, we need to study the images in detail. We have previously stated that the magnification of the optics is not that important, as in digital image acquisition it can be improved with digital zoom. So, as resolution is independent of magnification, we will compensate the optical magnification with digital zoom on the images taken with transmitted light so the bubble is the same size for the three cases. Also, aiming to make the resolution comparison easier, the images will be cropped, which means that the size of the ice shown has changed (Fig. 4).

Those images leave no doubt about the difference in resolution of the immersion and traditional microscopy. Indeed, on the image obtained with the oil objective all details are much sharper, which proves that we were able to maintain the theoretical advantages on the immersion microscopy for the case of investigating inner structures of the ice.

Conclusions

Seeing that, to our knowledge, the immersion microscopy technique had never been systematically explored to study ice, its viability for ice microstructural studies was the main interest of this work. It can clearly be appreciated at the results that immersion microscopy has certain advantages over standard 'dry' microscopy, especially for the observation of inner structures of ice.

A key question that arises from this study is if the use of oil is worth the complication. A general answer cannot be given, as it depends on the application. Evidently, when the ice sample is already covered with oil for protection, immersion microscopy is greatly simplified. Two main advantages offered by this technique are:

- The background distortion derived from surface structures is dramatically minimized when we are observing inner structures.
- The resolution of the images obtained by immersion objectives is notably higher than the resolution of those obtained by 'dry' air objectives.

In contrast, the observation of ice surface features by immersion microscopy was not satisfactory, as the ice surface features could be hardly identified. This can be attributed to the fact that the refractive index of the oil (1.4180) and ice (1.3113) are too similar. For surface studies, an oil with a much higher refractive index should be sought.

The oil viscosity was a particular concern of this study. The viscosity of the dimethicone 350 cSt at the cold temperatures of ice research is low enough for the oil to cover all the sample without producing an excessively thick layer, but high enough to immerse the objective while avoiding the oil draining off the sample surface. Additionally, the immersion objective is allowed to move while it is immersed. This is a key point of the development of the technique, because it might provide great advantage to observed ice samples that have already been stored with a protective oil coating to avoid over-sublimation. Bringing back those samples to the laboratory and observing the ice section covered with oil through 'dry' microscopy gives notably poor results. Therefore, immersion microscopy may offer advantages when it comes to re-observing stored samples.

The general conclusion about the immersion microscopy technique applied to ice studies is that it may offer certain advantages, but it does not replace standard dry microscopy. Instead, it has to be understood as a technique complementary to the standard microscopy. Among many useful applications of the immersion microscopy technique, we can mention the differentiation of slip bands and subgrain boundaries, as the technique drastically differentiates surface (subgrain boundary) and inner structures (slip bands). Also the investigation of microinclusions, clathrate hydrates, plate-like inclusions and other tiny microstructural elements inside the ice could benefit from this technique. It must be emphasized, however, that for such applications, an immersion objective with a longer working distance should be sought.

Owing to the desired stability of the oil meniscus (Fig. 1), the separation between the objective and the sample during immersion microscopy is critical, and consequently the immersion objective's working distance plays an even more important role than in dry microscopy. Immersion objectives with long working distances are capable of investigating inner structures deep into the ice, while objectives with short working distances are best suited for observing surface and near-surface structures. However, in the latter case, an oil (and its corresponding objective) with a much higher refractive index than that of ice is required to allow the good observation of ice surfaces.

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