Immersion Oil and the Microscope FAQs

Aren't all immersion oils for microscope objectives basically the same?

No, there actually are many different types of oils with different refractive indices, viscosities, drying properties, dispersion numbers, acidity values, chemical stability and autofluorescence levels to name a few. In addition the properties change as the temperature varies and different optical techniques can require different types of oils.

What oil should I use?

In general, the best rule is to use the oil from the manufacturer of the objectives. The immersion oil specifications are selected to match the objective properties as determined by the optical designers. Each manufacturer uses different glass types and optical prescriptions in the design of their objectives, and as a result, their immersion oil specifications will vary slightly. Keep in mind that even small variations in refractive index and dispersion can have significant affect on image quality.

Can I use immersion oils from other manufacturers?

For many routine applications the answer is yes, but there are a number of factors to consider if you want to obtain the best performance from your microscope. These will be addressed separately in this document. For example, if your application is basic brightfield, most oils will work, but it is imperative that the oil have the correct refractive index as recommended by the microscope manufacturer. Normally this is 1.515 at 23°C. The best recommendation to avoid a problem is to take the time to compare images between the recommended oil of the microscope manufacturer and the new oil you are considering.

If you do decide to compare oils it is important to remember that you need to remove all traces of the first oil from the objective and slide before you test the second oil. You should not mix oils from different manufacturers as this will produce a poor quality image.

Can I standardize on one immersion oil for use with the various brands of microscope objectives?

You can use the same oil for different brands of microscopes, but as noted above, there will more than likely be some compromise in image quality. You need to ask yourself: "Can I detect the compromise?" "Does it matter for my application?" "What can be done in the selection process to minimize the potential negative effects?"

NIKON INSTRUMENTS INC.
Melville, NY USA
www.nikoninstruments.com nikoninstruments@nikon.net
1-800-52-NIKON

What are the most important factors to consider when selecting immersion oils?

Refractive Index: While all immersion oil properties are important, having the wrong refractive index oil will cause immediate loss of image sharpness due to spherical aberration. Immersion oils are typically specified to three decimal places at a specific temperature and wavelength. The typical immersion oil has a refractive index of 1.515 at 23°C when measured with green light at 546.1nm. Changes even in the third decimal place can affect image detail when working at the limit of resolution. Oil temperature can also impact image quality and the refractive index will change approximately 0.0004 for every 1°C. That means heat from the microscope lamp and room temperature can certainly have adverse affects on image quality. This also includes working with specimens that might require lower than normal temperatures.

- ✓ ***Refractive Index***
- ✓ ***Dispersion***
- ✓ ***Viscosity***
- ✓ ***Acid Value***

Dispersion: Ideally the immersion oil should match the refractive index of all wavelengths of the light spectrum to the same design specification of the optical glasses used in the objective. This property of the oil is referred to as the dispersion value. Dispersion is usually designated by the Abbe number (Abbe vD or Abbe ve). DIN/ISO standards specify that immersion oil have a (ve) value from 39 to 49 at 23°C. In simple terms, the dispersion value determines the amount of refraction of all the different wavelengths of light that enter the objective. If refraction across the light spectrum is not constant, it results in chromatic aberration. Most manufacturers consider the Abbe number proprietary, so it may not be readily available. You may need to experiment with a few different dispersion numbers to determine which works best for your application.

It is important to note that the dispersion properties for visible spectrum oils may not necessarily provide the best results for monochromatic applications, such as fluorescence, especially at the lower wavelengths near or below 400nm.

Viscosity: The decision on which viscosity oil to use is usually based on the preference of the user and has no real impact on image quality. In general, lower viscosity oils such as Type A, (150 cSt) will minimize air bubbles and work well with short working distance oil objectives, but the thinner oil tends to spread out quickly across the slide, requiring more oil. Higher viscosity oils like Type B, (1250 cSt) are more popular because they do not run and can be used with objectives that have longer working distances and when oiling condensers.

Acid Value: The acid value of immersion oil should be very low. Synthetic oils typically have low acidic values, but high acidity over time can affect the metal parts of the objective and more importantly, the objective lens seals. This is another reason to use the microscope manufacturer's oil as they test very carefully to insure that the oil will not harm the metal or seals on their objectives. As an additional precaution, regardless of the oil type, it is good practice to remove excess oil with clean lens paper from the objective between sessions.

A common practice in fluorescence microscopy is to refrigerate or freeze slides until they are viewed, and placing oil on the slides before they have had a chance to warm up and the condensation to evaporate can create severe spherical aberration and even cause some oils to become cloudy. If the slides are placed back in the freezer with oil on them for later viewing, the cold temperature could start precipitates to form, and if you add more oil at room temperature, different refractive index layers will form and result in a poor image.

One other very important factor that can complicate the issue is the specimen media and how far below the cover slip is the plane of focus. If you are working with fixed slides where the mounting media is the same RI as the oil and the actual specimen is close or touching the coverslip, the potential for spherical aberration is minimal. If your specimen is in liquid or you are focusing well below the coverslip, spherical aberration can be severe.

Correction collar objectives should be considered as a possible way to help compensate for the different penetration depths and varying refractive indices.

#MRD01991 CFI APO 100X Oil TIRF NA 1.49 WD

Keep in mind that when using higher viscosity oils on upright microscopes you may find that the slide will sometimes pull away from the stage when focusing due to the increased surface tension between the objective and slide. This can be a problem if you do not have a good slide holder or aren't using one.

On inverted microscopes the reverse is true. While the thicker oil will better maintain the gap between the objective and slide or chamber, focusing the objective upwards can push the specimen away from the stage surface making focusing more difficult, and cause significant problems with applications that use auto focus or TIRF systems. Simply clamping the specimen down to the stage solves this.

Will I damage my objective if I use another manufacturer's immersion oil?

If you are using modern microscopes and current immersion oils the answer is no (see the section above on Acid Value). But there is some basis for this if you have and older microscope and do not know the composition of the seals and cements used to mount the objective front lenses and how they will react with the chemical composition of today's synthetic oils.

When in doubt it is best to use the microscope manufacturer's oil or at least confirm with the manufacturer there are no potential problems with the seals or cements in older optics.

If temperature is so important, what can be done when working at physiological temperatures?

If you are doing live cell experiments that include microscope incubator boxes or stage top heaters that heat the specimen and objective to 37°C, you should consider an immersion oil that has the proper refractive index at this temperature to minimize spherical aberration. Cargille offers Type 37 oils for use in both the visible spectrum (Type 37 #16237) and lower fluorescence (Type 37DF #16239) wavelengths. These oils also have a higher viscosity which makes them ideal for use with inverted microscopes.

If you are viewing live cells for short periods and are not heating the objective and stage there probably will not be much benefit to using these special oils as the objective and stage act as heat sinks and the oil temperature will be closer to 25°C than 37°C. As always, you can compare the standard oils vs. Type 37 to see if there is any improvement in the image quality.

Can I mix oils?

The best rule is to NOT mix oils, especially if they are from different manufacturers. Do not mix standard oils and fluorescence oils, even from the same manufacturer. If you move slides from one microscope to another, make absolutely sure the oils are identical and from the same manufacturer. Cargille does state that you can mix their Type A and Type B oils to produce intermediate viscosities but consider this option only after careful testing.

What oils are recommended for fluorescence applications?

Most users think that as soon as you begin doing fluorescence you need special immersion oils. It is important to keep in mind that the majority of fluorescence is done in the visible spectrum. If the excitation wavelengths range from about 420nm to 800nm they are considered visible to near IR and standard Type A and Type B oils will usually work fine. An easy way to verify if the oil is contributing unwanted autofluorescence is to use a blank slide with the immersion oil above and below the cover slip to see if there is any background signal.

Different manufacturers use various designations for low or non-fluorescing immersion oils such as DF, HF, FF, LF, ELF and NF to name a few. It is important to check with the manufacturer for what wavelength range the oil is formulated. In some cases oils formulated for lower wavelength fluorescence applications can produce a weak green autofluorescence if used in the visible spectrum.

Nikon NF oil is recommended for near UV and UV fluorescence applications (below 420nm) as well as the visible spectrum with all Nikon oil objectives. **Nikon Type A** can be also be used for fluorescence applications from 420 – 800nm.

Cargille states that their HF oil has slightly more autofluorescence than their DF type oil, but the HF is halogen free.

A common misconception is to use FF oil for all microscope fluorescence applications. While this oil has virtually no autofluorescence it generally has a refractive index well below 1.515 which creates severe spherical aberration and produces poor images. It is excellent for photometric measurements in the UV, but not for imaging applications.

If you feel your fluorescence application requires special oil, the best test is to compare different oils with your specimen. You can also check background signals (autofluorescence) by using a blank slide with immersion oil above and below the cover slip.

Do I need to use special oils with lasers?

If lasers are used for fluorescence applications such as bleaching, confocal, photoactivation and light scattering, the oils recommended for conventional fluorescence will generally work fine. Special consideration should be given to laser applications above 800nm as most oils have transmittance curves with peaks and valleys up to 1600nm and could cause performance issues at certain wavelengths. Poor transmission at certain wavelengths can cause the oil to heat up and change its refractive index at the point where the laser passes through the oil and cause beam distortion.

If the application is dependent on measuring light scatter you may want to consider oils formulated for low light scatter. Cargille offers several oils with relatively low scatter properties.

How does oil affect the Point Spread Function (PSF)?

PSF is a method used by many researchers to characterize the performance of the optical system, especially when doing high-end applications such as Z stacks and confocal. There are many papers available on the various factors that affect the PSF, but the wrong immersion oil can severely impact the PSF. So, special consideration should be given to all of the points mentioned in this document when selecting the immersion media.

Is there any immersion liquid recommended for use with water immersion objectives?

Most manufacturers offer water immersion objectives for doing live cell studies and a common problem with long term time lapse is the water evaporates before the experiment is complete. Cargille offers immersion liquids that can be substituted for water and has the same refractive index as water at both 23°C and 37°C depending on your application and time constraints.

How often should I remove the oil from my objectives?

Most modern oils are considered non-drying and can be left on the objectives when viewing multiple specimens with little or no problems, but there are some points to keep in mind. Dust and dirt in the air, and anything else that might be on the coverslip can accumulate in the oil and eventually degrade the image quality, especially in fluorescence. There is also the issue of acidity and the negative affects on seals and cements as noted earlier in this document.

This document is not meant to be a tutorial on cleaning objectives and each manufacturer has recommended procedures for cleaning their optics and should be consulted prior to cleaning any objectives.

With that in mind it is good practice to remove excess oil if you notice a loss in image sharpness or will not be using the oil objective for long periods of time. Be careful when using lens tissue to remove oil and do not put pressure on the objective while removing the oil because any grit in the oil could scratch the lens. It is better to use multiple sheets of tissue to gently remove the oil. ***Do not polish the objective lens with dry lens paper.*** Final cleaning should be done with a cleaning solution recommended by the microscope manufacturer and always in a sparing manner. Mounting media should immediately be removed before it can harden.

If you see deposits on the objective and are not comfortable with cleaning the objective you should contact a qualified service technician.

Does immersion oil have a storage shelf life and how should it be stored?

Immersions oils do have a shelf life and it is reduced after the container is opened. Depending on the medium type it can be as little as six months to as much as 10 years so check with the manufacturer where applicable.

When storing oils it is important that they be kept at or near room temperature. If not, they should be allowed to equilibrate to their rated temperature before use. Some oils will become cloudy or even form a precipitate if stored at too low a temperature (near or below 10^{o}C).

Immersion Oil Safety:

While most immersion oils used today are considered PCB-free, they do contain many different chemical compounds and some require more care in handling than others. It is good practice to obtain a copy of the Material Safety Data Sheet (MSDS) to make sure the oil complies with the safety standards in your facility.

General Comments:

As you can see from the above information selecting the correct immersion media can have a significant impact on the quality of your images. This document does not address the many optical issues that can affect the point spread function including specimen penetration depth and assumes the area of interest is directly below the coverslip.

There are many considerations for selecting the optimal immersion medium for your application and it is important to note that the best method is to carefully evaluate the different oils and compare the final images produced with your specimen and microscope. It can make the difference between a good image and a great image.

Immersion Oil Type A 50cc

Immersion Oil Type NF 50cc

Nikon Immersion Oils:

MXA20234	Immersion Oil Type A 50cc, PCB-Free, RI 1.515 at 23°C (Abbe Dispersion # 40.7)
MXA20235	Immersion Oil Type A 500cc, PCB-Free, RI 1.515 at 23°C
MXA22024	Immersion Oil Type NF 50cc, PCB-Free, RI 1.515 at 23°C (Abbe Dispersion # 42.5)