PFS and Immersion Oils

A number of questions have been raised regarding which type of immersion oils to use with the PFS as well as why some oils seem to work better than others. While all Nikon oils will work with the PFS it is important to understand the reasons why you would recommend one type of oil versus another. In addition to using the correct oil you need to keep in mind that the sample itself can be a source of potential problems.

There are many different manufacturers of immersion oils with many choices of refractive index, dispersions, viscosities, drying properties, and temperature considerations. Fortunately if you use Nikon or Nikon recommended oils the potential for problems are minimal. Using oils from other manufacturers and simply making the selection based on the same "TYPE" designation can cause severe chromatic issues. See the following image provided by Alexey Khodjakov. Use only oils recommended by the microscope manufacturer or only after careful testing.



The two oils that Nikon provides are TYPE A and TYPE NF. The primary difference between these oils is the viscosity (thickness). In addition the NF oil is recommended for fluorescence applications because of its extremely low auto

fluorescence properties in applications where the excitation wavelengths are in the UV or near UV (340-400nm). Nikon NF oil has replaced Nikon DF oil. TYPE A immersion oil has a viscosity rating of 150cST (Centistokes) and TYPE B is 800cST. Nikon does not provide TYPE B immersion oil but the factory has tested Cargille 16484 and found it works well with Nikon objectives. I would still recommend caution when using this oil and it should be tested carefully for your application to insure no adverse optical affects. This oil has a viscosity of 1250cST.

The factory tested these oils with the PFS and found they all work well. The primary consideration for testing the performance of the immersion oils was to determine how well they maintain contact between the cover slip and the objective over extended periods of time at 37 degrees C.

At room temperature there should be very few issues because live cells are usually monitored for relatively short periods of time at this temperature. If you are doing multiple stage locations TYPE A oil has a tendency to separate from the cover slip if there are large differences in the X-Y locations. In this case higher viscosity oil might be preferable.

The factory tested TYPE A, NF, B and DF oils overnight at 37 degrees C with 50% humidity with the following results.

- 1. TYPE A separated from the coverslip because the viscosity decreased at the elevated temperature.
- 2. TYPE NF and DF had significant evaporation and the viscosity actually increased. These oils were found to work well at 37° C if the experiment is for an hour or so.
- 3. TYPE B was stable and experienced no noticeable changes.

There is a second consideration that must be addressed when working with live cells and that is the stability of the coverslip when using oil objectives. The potential for problems increases with the viscosity of the oil. Depending on the diameter of the front lens, the working distance of the objective, the viscosity of the oil and the type of specimen it is possible for the coverslip to "flex" or move as the PFS maintains focus. If you are using a slide with a coverslip that is simply held in place by the surface tension of a small drop of solution, the coverslip/water interface can easily move as the oil pressure changes when the PFS corrects for Z focus changes. If you notice the PFS focus light cycling very rapidly this may be the cause. Lower viscosity oils or sealing the edges of the coverslip can help alleviate this.

It is also very important to insure the slide, dish or chamber is held securely to the stage surface because the oil pressure could also cause movement with these types of specimens. The best solution for this is stage clips which are included with all Nikon stages. Tape will help but generally has very little holding power.