Wet Mounts

A wet mount slide is the most common type of slide preparation for microscope work. Wet mount slides are used to view living organisms, as well as liquid substances of all kinds. They are also used for any sort of specimen that needs to be kept moist.

What is a wet mount?

In a wet mount, the specimen is suspended in a drop of liquid (usually water) located between slide and cover glass. The water refractive index of the water improves the image quality and also supports the specimen. In contrast to permanently mounted slides, wet mounts can not be stored over extended time periods, as the water evaporates. For this reason, a wet mount is sometimes also referred to as a "temporary mount" to contrast it from the "permanent mounts", which can be stored over longer times. The permanently mounted slides use a solidifying mounting medium, which holds the cover glass in place. The naming can be a bit problematic, because it is also possible to make wet mounts that can store over extended time periods. These are special cases, however.

Different types of wet mounts

Wet mounts can be made using several different kinds of liquids. Water, immersion oil and glycerin (glycerol) can be used, with water probably being the most commonly used. The source of the water is quite important, especially when observing living specimens. If you use water with a wrong osmotic potential (ie. too much or too little salt and mineral content), then there is the danger of damaging the specimen. A too high salt content can result in the specimen to lose too much water. Too low a salt content, and the specimen may swell and burst.

- **Using water from the natural habitat of the organism:** In the case of water organisms, such as algae or ciliates, the liquid water should come directly from the sample. In this case the organism is immersed in its own natural environment. The microscopist uses a dropper to place a drop of pond water directly on the microscope slide.
- **Using 0.9% salt water:** In some cases water from the natural habitat may not be available. This is the case when observing bacteria or molds grown on petri-dishes. Yoghurt bacteria, for example, need to be diluted a lot before being able to observe them, otherwise they are too dense to be observed as single cells. In this case it is necessary to mix some salt (NaCl) into some water to ensure an optimal osmotic potential. This "physiological saline", as it is called, can be made by dissolving 9 grams of table salt (NaCl) in 1 liter of water (or 0.9g Nacl in 100ml of water).
- **Using tap water:** If one wants to observe non-living specimens, such as dust samples, sand grains, or thin section cuts of plant material, then it is also possible to use regular tap water. These specimens are not osmotically sensitive. If the specimen is observed without water, in a dry condition, then the resolution and image quality may not be sufficiently high. I advise you to try out both to see the difference.

- **Using immersion oil:** Some wet mounts are not made with water, but by using immersion oil. Immersion oil is usually placed on top of the cover glass. In this case the specimen does not get into contact with the oil. It is also possible to submerge the specimen in the oil, however. Heat-fixed bacteria can be observed directly by placing a drop of immersion oil on the specimen, without cover glass. The oil-immersion objective is then rotated directly into the oil for observation. It goes without saying, that this procedure can only be used for specimens that do not contain water (and are, therefore, not living). It also only works for specimens that stick to the glass slide – there is no cover glas. If you need to observe these specimens with a lower magnification (ie. no immersion objective), then one needs to use a cover glass, of course. Other specimens, such as synthetic textile fibers, are hydrophobic in nature, and do not like to be mixed with water. They tend to float on top of the water drop and this can be cause for air bubbles. In this case I also recommend to use immersion oil and a cover glass to keep the sample flat.
- **Pure glycerin or glycerin-water mixtures:** Glycerin has a strong tendency to withdraw water from the sample. For this reason it also acts as a preservative. On the down side, the glycerin may therefore cause the specimen to shrink and deform. Especially algae and other water organisms are sensitive to dehydration. Other specimens, such as sectioned or microtomed plant material are not as sensitive. The reason why glycerin is used is because of its high refractive index. This may be necessary to see certain structures. If a lower refractive index is needed, then one should mix some water into the glycerin. It is possible to seal the glycerin mount by applying nail polish to the sides of the cover glass. This will hold the cover glass in place for longer time periods. This is then an example of a wet mount, which was made into a permanent mount.

Advantages and disadvantage of a wet mount

Compared to permanently mounted slides, wet mounts do have certain advantages:

- **Quick preparation:** specimen fixation, dehydration and staining are not necessary (but possible, if required). For this reason, wet mounts are the first kind of mounts that students learn to make.
- **Few artifacts:** If there is no chemical and physical processing of the specimens before observation (no fixation), there are little artifacts and the specimens appear in their natural condition.
- **Living and moving:** It is possible to observe living and moving organisms. It is also possible to observe certain processes of life, such as feeding, cell division etc. (for water-based mounts)
- **Natural colors:** The colors are natural and not faded. The colors of permanently mounted specimens may fade over time.

Disadvantages of wet mounts include:

- **Movement:** The advantage of observing movement can also be a disadvantage. Due to the movement of the organisms it may be more difficult to take pictures or to make drawings. There is a solution to this problem: one can slow down ciliates and other

protozoa by adding a solution such as ProtoSlo, which increases the viscosity of the water.

- **Evaporation:** The heat of the lamp causes the water to evaporate more quickly. More water must be added under the cover glass from time to time.
- **Focus:** Some organisms may swim vertically in the water and therefore move in and out of focus. Here it is important not to use too much or too little water. Too little water may squeeze the specimen between cover glass and slide.
- **Storage:** Wet mounts can not be stored over a longer time.

Materials and Method

For making a wet mount you need these materials:

- **Microscope slides**
- **Cover glasses**
- **The specimen** to be observed: make sure that the specimen is sufficiently small and thin. Thick specimens must either be cut (microtomed) into sections, be squeezed or torn apart.
- **Water:** take care that the osmotic potential of the water is compatible with the specimen. For example, do not use fresh water with marine specimens, and vice versa. Use pond water (and not tap water) for observing pond organisms.
- **Droppers, pipette:** these are for transferring the water
- **Tweezers:** for handling the specimen, the cover glass and for adding water

If the specimen is already in water (algae, ciliates etc.) then you can proceed the following way:

- Place a small drop of sample fluid (containing the specimen) in the center of the microscope slide.
- Hold the cover glass on one side with the help of tweezers. Lower the cover glass onto the water drop at an angle.
- Then slowly lower the cover glass into the liquid. This will minimize disturbing air bubbles.
- Remove excess water with a paper towel. The cover glass should not float freely. The surface tension of the water should hold it in place. Alternatively you can add more water using a pipette or tweezers.

If the specimen is not in water:

- Place a small drop of water (without specimen) in the center of the microscope slide.
- Place the specimen into the water.
- Add some more water on top of the specimen and make sure that the specimen is completely submerged. Otherwise there is the possibility for air bubbles forming between cover glass and specimen. The remaining steps are the same as above.
- Hold the cover glass on one side with the help of tweezers. Lower the cover glass onto the water drop at an angle.

- Then slowly lower the cover glass into the liquid. This will minimize disturbing air bubbles.
- Remove excess water with filter paper or tissue paper. The cover glass should not float freely. The surface tension of the water should hold it in place. Alternatively you can add more water using a pipette or tweezers.
  If you are using a dry specimen (dust, insect parts, etc.), then place a small drop of tap water

How to prevent drying out

The heat of the microscope light will evaporate the water relatively quickly. There are several possibilities to counteract this:

- Keep adding more water from the side of the cover glass. Surface tension will pull the water in.
- Seal the sides of the cover glass with a thick layer of Vaseline (petroleum jelly). Press the cover glass against the slide so that the vaseline is able to seal off the water from the outside.
- Use nail polish to seal off the cover glass. This is used when making wet mounts with glycerin. Keep the glycerin drop very small. The nail polish will not stick to those parts of the cover glass and slide which came into contact with the glycerin.
- Use slides that have an indentation (concave) and are therefore able to hold more fluid. This only works for some samples because the liquid layer may be to thick. These slides are more expensive.
- Use two additional cover glasses to support a third cover glass left and right. These two cover glasses serve as a distance holder for the third cover glass. This way the third cover glass does not float freely on the liquid but is held in place by the two supporting glasses. More fluid can be stored in a stable manner.