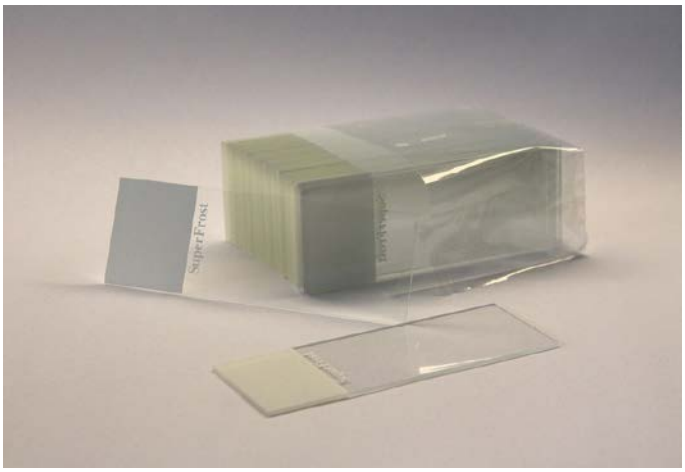
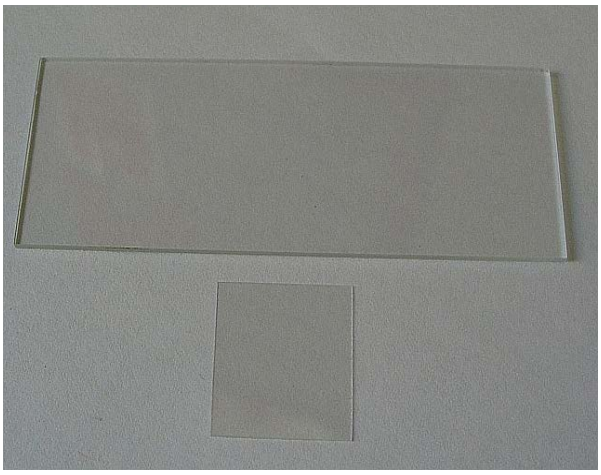
Microscope slides

A microscope slide is a thin flat piece of glass, typically 75 by 25 mm (3 by 1 inches) and about 1 mm thick, used to hold objects for examination under a microscope.
Typically the object is placed or secured ("mounted") on the slide, and then both are inserted together in the microscope for viewing. This arrangement allows several slide-mounted objects to be quickly inserted and removed from the microscope, labeled, transported, and stored.
Microscope slides are often used together with a cover slip or cover glass, a smaller and thinner sheet of glass that is placed over the specimen. Slides are held in place on the microscope's stage by slide clips.



A set of standard 75 by 25 mm microscope slides.

The white area can be written on to label the slide.



A microscope slide (top)
and a cover slip (bottom)

Microscope slides are usually made of optical quality glass, such as soda lime glass or borosilicate glass, but specialty plastics are also used. Fused quartz slides are often used when ultraviolet transparency is important, e.g. in fluorescence microscopy.

Preparation of slides

Slides preparation is an important part in many areas of biological, medical, veterinary and forensic sciences and you will often be required to prepare different kinds of slides. Specimens may be smears of fluids, thin sections or whole mounts of all or part of an organ or organisms. In all cases the material is mounted on a glass slide prior to its examination.

Mounting

The mounting of specimens on microscope slides is often critical for successful viewing. This issue has been given much attention in the last two centuries and is a well-developed area with many specialized and sometimes quite sophisticated techniques.

Dry mount

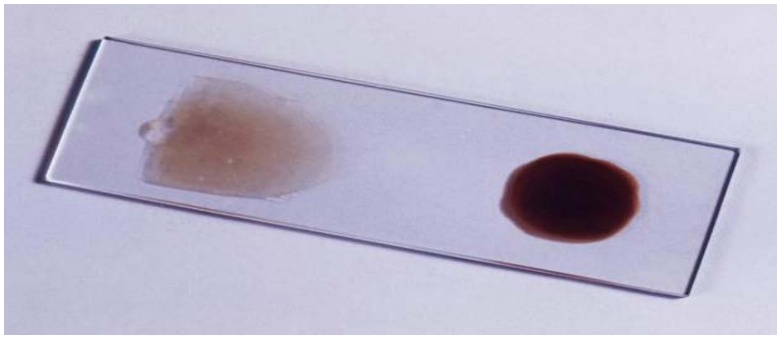
In a dry mount, is the simplest kind of mounting, the object is merely placed on the slide. A cover slip may be placed on top to protect the specimen and the microscope's objective and to keep the specimen still and pressed flat. This mounting can be successfully used for viewing specimens like pollen, feathers, hairs, etc. It is also used to examine particles caught in transparent membrane filters (e.g., in analysis of airborne dust).

Wet mount or temporary mount

These preparations may be needed for matter of minutes or hours only. They are mounted in water or glycerol or other fluid. After examination they are discarded.

The material under examination can be fixed and stained or even examined in a living state. In a wet mount, the specimen is placed in a drop of water or other liquid held between the slide and the cover slip by surface tension. This method is commonly used, here is an example, to view microscopic organisms that grow in pond water or other liquid media, especially when studying their movement and behavior. Care must be taken to exclude air bubbles that would interfere with the viewing and hamper the organisms' movements.

for example, protozoa such as amoeba. In this case a harmless aqueous stain such as 1% methylene blue can be used – it would then be known as a “vital stain”. If it is necessary to keep the slide for a matter of hours it is possible to reduce evaporative losses from the edges of the cover-glass by painting a ring of gum or molten wax or nail polish around its edge.



Blood smears for pathological examination, an example of wet mount.

Prepared mount or permanent mount

If the slide is to be kept for long-term reference, for a matter of days or even years, it must be made as a permanent preparation. For pathological and biological research, the specimen usually undergoes a complex histological preparation that may involve cutting it into very thin sections with a microtome, fixing it to prevent decay, removing any water contained in it, staining specific parts of it, clearing to render it transparent, and impregnating or infiltrating it with some transparent solid substance. As part of this process the specimen usually ends up firmly attached to the slide.

Strew mount

Strew mounting describes the production of palynological microscope slides by suspending a concentrated sample in distilled water, placing the samples on a slide, and allowing the water to evaporate.

Mounting media

The mounting medium is the solution in which the specimen is embedded, generally under a cover glass. Simple liquids like water or glycerol can be considered mounting media, though the term generally refers to compounds that harden into a permanent mount. Popular mounting media include Permount, glycerol jelly, and Hoyer's mounting medium. Properties of a good mounting medium include having a refractive index close to that of glass (1.518),
non-reactivity with the specimen, stability over time without crystallizing, darkening, or changing refractive index, solubility in the medium the specimen was prepared in (either aqueous or non-polar, such as xylene or toluene), and not causing the specimen stain to fade or leach.

Examples of mounting media

Aqueous

Popularly used in immunofluorescent cytochemistry where the fluorescence cannot be archived. The temporary storage must be done in a dark moist chamber. Common examples are:

1. Glycerol with antiquench
2. Gelatin
3. Mount™
4. Vectashield
5. Prolong Gold

Non-Aqueous

Used when a permanent mount is required

1. Canada balsam
2. DPX (Distrene 80 - a commercial polystyrene, a Plasticizer e.g. dibutyl phthalate and Xylene)
3. DPX new (with Xylene but free of carcinogenic Dibutyl phthalate)
4. Entellan™ (with Toluene)
5. Entellan™ new

6. Neo-Mount™ (compatible with aliphatic Neo-Clear® but not compatible with aromatic solvents like Xylene)

The making of a permanent stained preparation mounted in Canada balsam involves five process:

(i) Fixation
(ii) Staining
(iii) Dehydration,
(iv) Clearing, and
(v) Mounting

Fixation and staining have already been mentioned in the earlier Lecture and the purpose of dehydration, clearing and permanent mounting is outlined below.

1. Dehydration

The purpose of dehydration, i.e. the removal of water, is to allow complete infiltration of tissues with Canada balsam. Unless all traces of water are removed, infiltration is incomplete, the tissues appear opaque and bacterial decay ultimately sets in.

If carried out too rapidly, dehydration causes distortion and shrinkage, especially of delicate tissues, by setting up violent diffusion currents. It should therefore be done gradually and sufficient time allowed for the complete extraction of water. Dehydration is commonly effected by the passage of the stained specimen or slide through successively stronger solutions of ethanol (ethyl alcohol) ending with immersion in absolute alcohol 100% ethanol

CAUTION: Alcohol is a highly flammable material.

2. Clearing

The purpose of clearing is to remove all traces of alcohol, thus allowing the tissues to be infiltrated with the Canada balsam or other mountant. Examples of clearing agentes are1, 2- dimethylbenzene (xylene) for small soft tissues; clove oil and cedar wood oil for thick tissues. Incomplete dehydration is to absolute alcohol. Toluene is another good clearing agent but it is a bit costly.

3. Mounting

After the tissues have been cleared, they are mounted in a semi-fluid 1, 2-dimethylbenzene (xylene) balsam mixture. The 1,2- dimethylbenzene (xylene) subsequently evaporates and the balsam hardens. However, there are some disadvantages of using this mountant. Xylene is inflammable and toxic; the drying process is prolonged and this mountant discolours with time. It is advantageous to use DPX because it dries up easily.

Labelling of slides

Slides act as a permanent record of tissues, organs and specimens. They may be of pathological origin, e.g. hospital patients, prepared “in house”, etc. In all cases it is essential that the slide is properly identified by adequate labelling.

Labels should therefore carry the following information:

1. The name of the organism – if the whole organism is mounted then the slide can be marked WM=whole mount or E=entire
2. The part of the organism used, e.g. liver, root.
3. The type of preparation, e.g. smear; squash; TS = transverse section; VS = vertical section; LS = longitudinal section.

Storage of preparation slides

Prepared permanent slides are valuable. They are widely used by educational establishments in the teaching of histology (the study of tissues of plants and animals) and cytology (the study of cells). In some cases the preparation may be uniquely valuable. It could for example be an important reference slide or a slide of the diseased tissues of a hospital patient.

Microscope slides with prepared,stained, and labeled tissue specimens in a standard 20-slide folder.