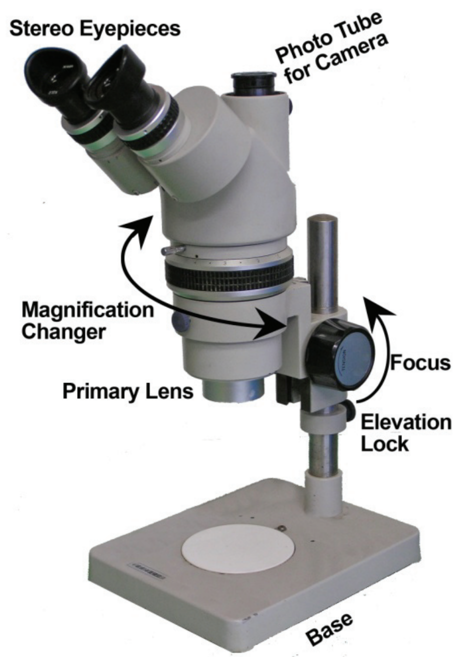
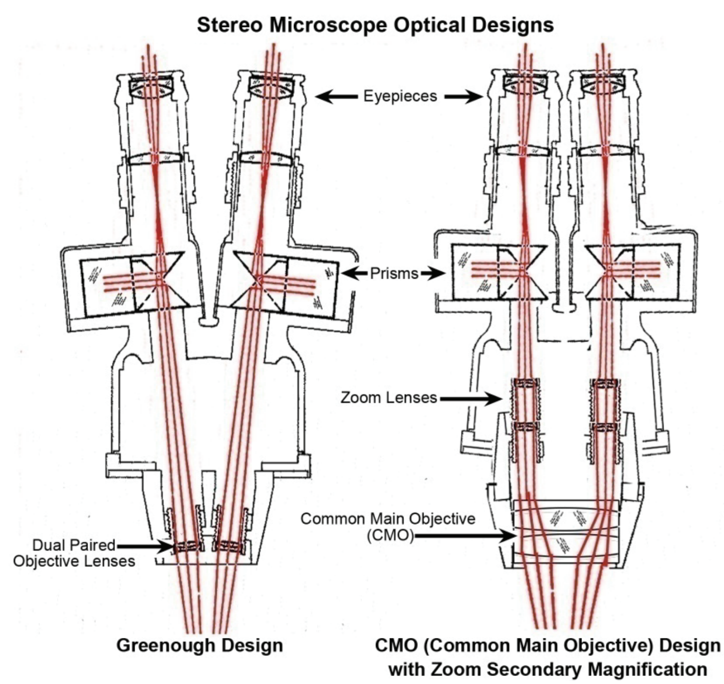
Appendix VIII. Stereo Microscopy

Introduction

The stereo microscope, also known as a dissecting microscope or operating scope is an entirely separate design from the more familiar compound microscope, and serves a different purpose. It produces a three-dimensional visualization of the sample being examined through the use of two separate optical paths so that the left and right eyes receive slightly different viewing angles which the brain interprets as a three dimensional image. The stereo microscope should not be confused with the compound microscope which is also equipped with binocular eyepieces. Both eyes see the same image in the compound microscope so the visual image is no different from that obtained with a single monocular eyepiece. In this type of microscope the binocular eyepieces provide greater viewing comfort rather than three dimensional imaging.



The design of the stereo microscope provides great working distance and depth of field which are essential qualities for this type of microscope. Working distance and depth of field are inversely correlated with resolution. The greater the depth of field and working distance the lower the resolution (the distance at which two adjacent points can be visually resolved as separate). The stereo microscope's useful magnification is up to 100X which is approximately the same magnification of the 10X scanning objective in a compound microscope. In practice, the majority of stereo microscope observations are made between 4X and 40X.



Optical Design and Magnification Total magnification in the stereo microscope is achieved by a primary magnification which is further increased by a secondary magnification. The primary magnification of the microscope is determined by a primary objective lens which can be either a single lens called a Common Main Objective (CMO) or a paired set of objective lenses often called a Greenough design. The main advantage of the CMO design is the infinity focus which produces two collimated light paths with parallel axes between the objective and the eyepieces that allow the easy insertion of various optical and accessory components such as camera ports and drawing tubes. This gives the greatest versatility and makes it the preferred choice for research applications. Some models have interchangeable primary lenses giving a choice of several different ranges of magnifications. The Greenough design is often smaller, less expensive, rugged, and simple to use and maintain. They are a popular choice for simpler workhorse applications which don't require a large variety of accessories.

The secondary magnification is achieved in one of three ways depending on the optical components. The simplest is fixed magnification which has a single set secondary magnification. This means that the microscope is limited to one final magnification. This is most often seen in small and highly portable scopes intended for field use; some of which can fit in a pocket. The next type is a system of selectable set magnifications controlled by a rotating drum or ring. The most complex system is zoom magnification, capable of a continuously variable magnification across a set range. Zoom systems can also have interchangeable primary lenses or use auxiliary objectives that increase total magnification by a set factor. Also, total magnification in both fixed and zoom systems can be varied by changing eyepieces.

Illumination

The most common form of illumination for a stereo microscope is reflected illumination rather than transmitted illumination. That means the light is reflected from the surface of an object rather than transmitted through an object like a compound microscope. This allows the examination of specimens that are opaque or too thick to allow light to pass through them. Stereo microscopes can use transmitted light illumination as well if equipped with a light source beneath a transparent stage or inset in the base; however the transmitted illumination is not focused through a condenser like a compound microscope. Optional illuminators are also available for reflected or transmitted dark field microscopy.

Primary Applications of the Stereo Microscope

The stereo microscope is most often used to study solid specimens or to carry out tasks such as dissection, or sorting that require a close-up view. The long working distance and the fact that specimens can be examined without any preparation or processing makes this a uniquely versatile instrument for field use. Aquatic organisms can be easily examined live in a suitable container of water.

Different models are available ranging from large sophisticated research models often equipped with cameras to small pocket size models for on-site field examination. A recent innovation has been a new class of research stereo microscopes designed especially for fluorescent imaging requiring special light sources and exceptionally high light gathering ability. A digital camera is used which combines high image quality with the ability to image weak light signals. These microscopes are very large and extremely expensive but they have opened a whole new way of studying the often weak fluorescent material with a much larger field size and on opaque materials that would be impossible on a compound fluorescent microscope.

Choosing a Stereo Microscope

The choice of the best model for a particular application is determined by the following criteria:

The physical environment in which it will be used. Larger research grade microscopes are best used in a laboratory or on larger research vessels with electrical power for cameras and light sources, and climate control to protect more delicate optics and electronics. Smaller simpler and more robust models are suitable for use where environmental factors are less ideal. Pocket sized and preferably water proof models are best for small boats and on-foot research.

Whether imaging is required. Stereo microscopes for imaging require higher quality optics and a separate trinocular port for the camera. Ultra high resolution and low light level cameras are best used in a laboratory. The new relatively inexpensive CMOS cameras with a USB2 interface are ideal for portable use because they can be plugged into any notebook computer with basic software installed.

The primary intended use. Microscopes intended to cover a wide and varying combination of research applications should have the greatest range of magnifications and light sources. Here the CMO design should be considered first. Microscopes intended for surveying or sorting specimens can have a more modest range or in many cases a fixed magnification. The simpler Greennough design might be a better choice for this work.

The type of stand required. The standard short desk stand can accommodate specimen and container sizes over typically a 6” to 12” range. Optional taller stands are often available where a greater range is required. Most versatile is a stand and arm combination which allows a large range of vertical and horizontal movement which can be used for applications such as close-up examination of marine animals too large to place and move under a standard stand.

Budget and appropriate quality for the intended use. Stereo microscopes can cost anywhere from up to $20,000 for the most sophisticated research models to a few hundred dollars for the most basic models. Besides budget restrictions, it is inappropriate to get a very expensive microscope for a destructive environment such as exposure to salt spray since any microscope will have a short service life. The very least expensive microscope adequate to perform the required work is a better choice with frequent replacement as required.

With reasonable care stereo microscopes have long service lives and highly serviceable older models can be found at bargain prices by a careful shopper. A search on the internet will located microscope dealers that carry used equipment inventories. The AO Cycloptic, the first modern commercial stereo scope, is a robust instrument, ideal for a field station or research vessel use and can be found at very modest prices. A quick survey on EBay produced 536 stereo microscopes for sale. The majorities were new student grade scopes of questionable quality but there were also older models, some of which were top of the line in their day and now offer sophisticated performance at bargain basement prices.

Care and Maintenance of a Stereo Microscope

A well designed stereo microscope requires surprisingly little maintenance. Most problems can be prevented by some simple, common sense, proactive preventative steps. Bear in mind that cleaning optics is inherently destructive over a long period of time so preventing optical contamination is better than cleaning it off. One of the most useful microscope accessories that is too often unused is the simple dust cover. A microscope should always be covered when not in use. Special consideration should be given to the type of cover where ever there is the possibility of water, chemical or blowing sand affecting the scope.

Common dust is usually not of concern and if excessive enough to be bothersome is easily removed with a source of air, either commercial canned air, or an ear syringe. The most common type of contamination that requires prompt and thorough cleaning is finger prints. The oils in a finger print can actually etch the optical coatings on the lens. Eye makeup such as mascara can be a chronic problem in the contamination of the eyepieces. The best solution is to discourage the use of eye makeup by personnel using microscopes. Salt spray needs to be removed by the careful use of fresh water cleaning using damp clothes, never liquids that could get into the scope.

Proper cleaning of optics

1. Have proper materials on hand including good quality lens paper, a source of air and lens cleaner.
2. Always first use air to blow off the optical surface to remove any grit that could scratch the optics during cleaning.
3. Never touch an optical surface with any dry material. Always moisten the cleaning cloth or tissue with lens cleaner or use your breath to fog the lens.
4. Suitable cleaning materials include lens tissue, microcloth, or a well laundered clean handkerchief.
5. Clean in a circular motion without applying excessive force. Make several passes using a clean surface each time.
6. The use of solvents should be carefully restricted to lens contamination such as oil or mounting media that actually requires it. Never apply any solvent directly to a lens but always apply it to lens paper of a cotton swab. Shake off excess liquid before applying to the lens. Materials like oil will require the use of multiple swabs or papers as they must be discarded after each pass. Check all safety instructions for any solvent and make sure you have adequate ventilation, and personal protection as required.