Microscope Advisory

The following is based on my experience, my knowledge from studying and communicating with other microscopists. Others may have different opinions, experience and knowledge.

First of all an overview of the microscopes suited to the examination of compost, soil and liquid biological consortia (compost tea, compost extracts, EM, etc.).

There are several types of light microscopes regarding the way the light is utilized to observe the specimen. There are incident light microscopes which reflect the light off of the specimen and back up to the eye. This set up is often expensive and really not necessary unless one wishes to identify bacteria or enter into the realm of research beyond telling protozoa from bacteria, etc. There are dissecting or stereo microscopes which also use reflected light but are of a lower magnification and are generally utilized for observing larger organisms like insects and leaf surfaces. For the purpose of observing a broad range of microorganisms, transmitted light microscopes are probably most appropriate. This means that light passes through the specimen up the objective (magnifying lens) through the observation tube and eyepiece(s) to the eye(s). There are upright microscopes and inverted microscopes which utilize this method. The inverted scopes have the objectives positioned under the specimen with the light above and the image is conveyed to the eye by mirrors and prisms. The advantage to the inverted scope is that one is able to observe a deeper liquid contained in a dish (petri dish) rather than just a drop on a slide. These are used for cell manipulation, cloning and egg fertilization. I like mine because it allows for a more extended observation of microorganisms before the sample dries out.

The upright transmitted microscope is most suited to our purpose of observing samples of compost, soil and compost tea, etc.

<u>Lighting and Condenser;</u> It is important to get a microscope with a light source, not just a mirror (unless that's all you can get). The light is directed so as to shine up towards the specimen and objectives usually via a series of mirrors and prisms. The light shines through the condenser which is just below the stage where the specimen sits, through the specimen, through the eyepiece and conveys the image to the eye. A microscope suited to our task should have an iris diaphragm in the condenser to restrict the amount of light (numerical aperture) and also manipulate contrast. It is preferable for there to also be an iris on the field light (light below the condenser). This is used to ensure that only the area which the objective is seeing is illuminated and this changes from objective to objective. It is also essential that the light intensity is adjustable. Most scope transformers have this feature.

The lighting technique where both the field light and the condenser have an iris is known as Kohler illumination. It is also critical that the condenser have the capability of being centered for Kohler illumination. Sometimes the field light is also

centerable (American Optical series 10) and in the high end research scopes the lamp itself can be manipulated. This illumination method is named after August Kohler, a German, who devised it, so that he might take better microphotographs back in 1893. Most of the genius applied to the development of optics and microscopes is attributable to Germans. August went on to work with another German of notoriety, Ernst Abbe the co-founder of Zeiss microscopes among other things. It is very wise to choose a microscope with Kohler illumination, especially if you are using bright field observation.

Most microscopes of any minor worth come with an Abbe 1.25 NA (numerical aperture) condenser. It is like saying my car came with wheels. This is the bottom of the line condenser, except for fixed light toy type microscopes. It works fine and is entirely sufficient. As you can probably determine the Abbe condenser was developed by Ernst Abbe. His mathematics concluded that you should use a condenser with the NA slightly less than or equal to the NA of the highest NA objective you are using. Many microscopes come with a 100X oil immersion objective with an NA of 1.25. Most 40X objectives have an NA range of 50 to 95 so the condenser iris can be adjusted to match. The lower objectives have a lower NA. Abbe went on after developing this simple two lens condenser to make condensers with more lenses and much greater correction of optical aberrations. I won't get into it here because your eyes will glaze over and I'll get lost. Suffice it to say that a step up from the Abbe 1.25 NA is the achromatic or aplanatic aspheric (or spheric) condensers which generally are found with an NA of 0.90 or 0.95. There are achromatic-aplanatic condensers which range up to 1.40 NA but we are talking serious money for them and the objectives that match. The condenser in my big Leitz Orthoplan is achromatic 0.90 NA and if my memory serves me right the condensers in the Leicas at the Corvallis SFI lab are achromatic-aplanatic 0.90 NA (drool). To learn all you could possibly wish to know about optics go to http://microscope.fsu.edu/primer/anatomy/objectives.html

The only reason why I bring up the condenser differences is so you won't be scared off by a microscope for sale which has an aspheric (or spheric or aspherical) or achromatic condenser with a 0.90 NA. It will probably be superior to an Abbe 1.25 NA. Most likely if you are buying a lower end new bright field microscope it will have an Abbe 1.25 NA condenser anyway. If you buy a used Leitz, Zeiss or American Optical it may not.

Did I mention that the condenser must be capable of adjusting up and down? I am now if I didn't.

<u>The Phase Contrast Option;</u> You may wish to consider getting a microscope outfitted with a phase contrast condenser and objectives. The best sort of phase contrast condenser (generally speaking) to get is a Zernike style turret condenser. Phase contrast works by having rings inserted in the objectives and in the condenser which match up on the focal plain (sort of). This makes the light come (sort of) across the specimen providing a greater contrast to observe transparent organisms.

OR in the words of my friend Bobby Martin from Martin Microscopes;

"TRANSMITTED LIGHT PHASE CONTRAST **is an illumination technique which enhances the phase difference between the specimen and its surroundings. Specimens which have a refractive index similar to their surroundings can be invisible in brightfield, but are well defined in Phase Contrast. Phase Contrast requires a special set of objectives with phase rings placed in the back focal plane of the objective. A matching phase ring is placed in the condenser."**

Frits Zernike, another German of course, is the inventor or discoverer of phase contrast microscopy. He won a Nobel Prize in 1953 for his discovery. The condenser named for him generally consists of several phase rings in a turret which are turned in to match the objective being used. There is also a setting for brightfield and usually for darkfield which we won't explore right now. The phase objectives work equally well in the brightfield setting. Phase contrast has its advantage in seeing the details like flagella (little tails on microorganisms) more easily. I find it much easier on the eyes and it reduces 'eye floaties' visibility. The drawbacks are that phase contrast is best used with a thin sample and so brightfield is superior for looking at thick samples like compost mixed with water, etc. Generally I examine compost samples with brightfield and look at compost tea with phase contrast. Color may also be distorted in phase contrast. If you can afford phase contrast, I recommend it. You will also have brightfield anyway. There is also differential interference contrast (DIC) which is similar and probably better but it is very expensive.

Going back to the lamp, get the brightest you can afford at the time. There is such a tremendous difference in resolution from 20 watts to 30 watts and beyond that is gold. My Leitz has a 100 watt halogen lamp for transmitted viewing and 250 watts for fluorescent microscopy.

<u>Mechanical Stage;</u>
Progressing upwards from the condenser is the stage. You must get a mechanical stage. This is a stage which has a clamp type device for holding the slide (specimen) and it can be moved around under the objective via controls operated by two adjacent knobs. They move the specimen from side to side and back and forth (XY axis). This allows you to systematically move from field to field across your specimen and also allows you to follow a moving microorganism. If you do not get a mechanical stage you will kick yourself afterwards.

<u>Objectives;</u> For this work I usually end up using my 20X objective most because it gives me a wider field of view but still enough magnification to discern types of organisms. When I wish to get greater detail I turn in the 40X objective. The 4X objective is great for scanning over a sample, especially a sample of compost which may have larger organisms of interest like nematodes. The 10X objective is likewise useful for compost samples and for a wider field of view of compost tea to ascertain approximate density of microorganisms. You will find yourself rarely using a 100X oil immersion objective so don't worry about it but take it if it comes with the scope.

Before we move on with discussing objectives I must address another issue in the historical advancement of microscope optics. Microscopes were manufactured with a specific tube length so as to correlate with the magnification of the objectives. The industry standard was 160 mm except for Leitz which used 170 mm and 160 mm. This length was engraved on the objectives. 160 mm and 170 mm objectives are interchangeable, however they are not parfocal to each other. All this means is that if you are using one and then turn in the other you will need to re-focus. When these 'tube length' microscopes had other devices (polarizers, magnifiers, etc.) inserted into the light path an additional adjustment lens was necessary. Another German company, Reichert, developed infinity corrected optics and objectives in the 1930s which, as the name implies, allows the infinite insertion of devices without additional lenses. Reichert merged with American Optical and Spencer. Therefore many older American Optical Spencer microscopes have infinity optics. You can tell an infinity corrected objective by the infinity symbol (sideways 8) engraved into it. Infinity optics did not catch on fully until well into the 1980s but today virtually all high end research grade microscopes utilize it. This does not mean anything is wrong with tube length microscopes. My Leitz microscopes are 170 mm tube length. It just means you cannot mix up the objectives. An infinity marked objective must be used with an infinity corrected scope. There are brand new microscopes manufactured using both methods but the infinity corrected scopes tend to be much more expensive. One advantage to purchasing a used American Optical (Spencer) with infinity optics is that they are inexpensive and the optics are top notch.

As an example, the Leica CME recommended by SFI is not infinity corrected. It is a 160 mm tube length but it is a good quality microscope and upgradeable to phase contrast and Kohler illumination. The upgrade to Kohler is straight forward. Unfortunately the phase contrast kit designed for use with this microscope is a slider type, which works but is more awkward and not as versatile as the Zernike style turret. I do not know how much more it costs for these upgrades. The scope comes with a 20 watt tungsten lamp. I guess I got off topic a bit here but thought I might as well discuss this scope while I was thinking of it.

When you are selecting objectives, it is a good idea to select ones that are 'plan' or 'semi-plan' which means they convey a flat and wide field of view. This applies to infinity and finite 'tube length' objectives. This is usually indicated on the objective casing (may say PL., Pl., Plan, S. Plan). There are many levels of correction available for objectives, most of which are beyond the scope of this little advisory and cost moocho money. If you wish to investigate, once again, go to http://microscope.fsu.edu/primer/anatomy/objectives.html Also it is a good idea to get one or more long working distance (indicated by LWD) objectives. If given a choice or limited by choice, in my opinion the 20X LWD is most practical. With a LWD objective you can work with lumpy thick samples without fear of getting 'it' on your lens. You should also know whether the objectives require a cover slip. Some objectives work with or without a coverslip (both).

<u>Eyepieces;</u> Most microscopes come with widefield 10X eyepieces and these are absolutely adequate. I believe all new eyepieces compensate for eye glass wearers but this is usually indicated by inscription of a little pair of glasses on the eyepiece. You can experiment later if you wish with higher magnification eyepieces but the resolution usually suffers.

<u>The Head;</u> It is much more comfortable using a binocular observation head but certainly if all you can get is a monocular that will do the job. If you are considering photography or computer/TV interface a trinocular head is superior. I have a trinocular head on all of my scopes, except the stereo (dissecting) microscope.

<u>Focus;</u> Some older scopes come only with one focus action. Shy away from these. You want one with coarse and fine focus.

Hopefully I have covered the basics. If you have questions please ask. Also please remember that all that I have written is based on my experience and what I have picked up from reading and communicating with other microscopists. There is always room for potential inaccuracies and opposing opinion.

<u>Makes;</u>

Leitz/Leica; (German, Canadian, US made)
Without question my favorite brand of microscope is Leitz. It has been the workhorse of laboratory research for many years. The optics are superb. Leitz evolved into Leica which name was actually incorporated to reflect the company's manufacture of cameras. They opened a factory sometime around WWII in Ontario, Canada where most of the 'used' microscopes present in North America were manufactured. This factory had a worldwide reputation of very high standards and their scopes and cameras are still in demand. This factory now produces military optics and I believe Leicas are produced in the US and Germany. If you can afford to buy a high end Leica that is the way to go. You can get a good used one for $10,000 to $15,000. There are some old Leitz's around which range in price from $500 to $6000 or more. I paid $4500 for my Leitz Orthoplan which was an incredible buy because of the accessories included and then I spent another $4000 collecting more accessories (objectives, filters, condensers). Some of the good Leitz models include, Orthoplan, Dialux, Ortholux, SM Lux, SM. You can find these microscopes on ebay but this is not the best place for a novice unless you have someone helping you and/or unless you have reason to trust the seller and ask all the necessary questions. If you are buying a used or new microscope, the ideal situation is to buy from a recommended local dealer who guarantees his products and will work with you to understand your needs and the operation of the scope. This may be more expensive but it may save future pain. If the dealer has several types of scopes, take in a sample of compost or ACT and get him to demonstrate the differences (i.e. phase or brightfield, Kohler or not).

Zeiss; (German)

From everything I've heard Zeiss also manufactures an excellent microscope. They are still cranking out new ones but there are quality ones available used. I have never tried one so I cannot speak from experience. The one potential problem I have heard of is that older Zeiss objectives have a tendency to delaminate. New Zeiss scopes are expensive similar to Leica.

Olympus and Nikon also make very good quality high end scopes if money is of no concern. They are made in Japan (still I think).

American Optical (Spencer); (American made)
These scopes are no longer made but as mentioned previously they were quality made microscopes and are available inexpensively used. This is one type of scope which is probably worth buying on ebay abiding by my prior guidelines. Good models are the Series 10, 110 and AO 20 (or H20). These scopes are all equipped with Kohler illumination, infinity corrected optics and can be had complete with objectives for $100 to $800 or more depending on luck and accessories. For brightfield observation they are as good or better than brand new scopes. These scopes also can be fitted with a phase contrast condenser but I have found it to not be as high a quality as Leitz phase contrast. There are also less expensive models which I am not acquainted with. For info about American Opticals go to;
http://www.xmission.com/~psneeley/Personal/Microscope.htm

New Microscopes on a Budget;

Meiji; (Japanese made)
If I were going to purchase a new microscope and I needed to watch my pennies I would choose a Meiji. This microscope combines excellent grade optics with a well made body which still uses brass workings (gears) when other similarly priced microscopes are using plastic (teflon) or aluminum. One specific microscope that I think has the best bang for the buck is the MT4000 series. This scope comes with plan and semi-plan infinity corrected objectives. It has Kohler illumination, a 30 watt halogen or LED lamp, a binocular or trinocular viewing head with 10X widefield eyepieces, a mechanical stage with nice low controls. The microscope has a lifetime warranty, excluding the electrics which is 90 days. This costs roughly $1850.00 with a trinocular viewing head and brightfield. If you want phase contrast this goes up to $2500.00 but if you have the money you should consider the Meiji MT4000's big brother the MT5000 series that comes with 30 mm eyetubes and highpoint eyepieces just like high end Leitz microscopes. This is a real bonus because it expands your field of view and eases the hardship on the eyes. The MT5000 series also comes with better objectives (10X, 20X, 40X, 100X) which are U plan providing a much flatter field of view. A brightfield model costs roughly $2350.00 with a trinocular viewing head. You can choose to go with phase contrast with a Zernike style turret phase contrast 1.25 NA condenser with stops for phase contrast for 10x, 20x, 40x, 100x with brightfield position and a darkfield stop for the 40x objective. The cost being roughly $3350.00. In my opinion the additional expenditure for the MT5000 series is well worth it if you have the money to spend.

The best price I have seen on this scope equipped with a trinocular viewing head is $3350.00 at;

http://www.milescoscientific.com

Jason may give you a discount if you tell him Tim sent you.

He also has a variety of other brands at; http://www.professionalmicroscopes.com

Lomo;
Lomo is a Russian made microscope which has a good reputation. The optics are supposed to be excellent but the workings are sometimes (according to hearsay) a little loose. They are a reasonably priced microscope with some unique features which may be worth considering. You can check them out here; http://www.lomoamerica.com/microscopesFrames.html

Motic & National;
These are Chinese made microscopes made by the same company. To the best of my knowledge Motic uses plastic (teflon) workings and National employs aluminum or plastic. National is a fairly cheaply made microscope and is cheaply priced. They are often advertised without the brand name (i.e. DC5-163) and are made to look attractive by having a built-in camera to interface with a computer. If you consider one of these scopes please do not get one with the built in camera. If the camera needs repair you need to ship the whole scope, cameras and software are continuously becoming obsolete. The scope referenced has a 20 watt lamp and no Kohler illumination. The optics are, so I've heard very decent so if you find a basic scope for a great deal, it may be worth grabbing. The Motics are a big step up from National but the same advice stands about the camera. If you want to use a camera get a trinocular head and separate camera. Motics are reasonably priced for what you get and the Chinese are apparently rapidly improving the quality of their microscopes and I have been told by some that there is nothing wrong with plastic (teflon) workings but I've got a wait and see attitude on that one. Motic carries a nice looking inverted phase contrast microscope I would consider if I had the available coin. Fortunately there is a very honest used microscope dealer in Florida named Ed Nightingale who carries Motics, Nationals and a realm of good used microscopes including Leitz, Zeiss and American Optical. They will give you the straight goods on new and used, this brand, that brand, parts, everything. You will find them here;
http://www.microscopesfromnightingale.com

Some other good used resources are;

http://www.labx.com scopes, etc. for sale by various sellers

http://www.martinmicroscope.com/index.html – Bobby Martin's site; scopes, Martin camera adapters, parts, knowledge

http://www.surplusshed.com – some funky stuff here and dirt cheap

In conclusion, ultimately you work with what you have. I know guys who build microscope parts on a lathe and scrap old projectors to build condensers. If you can only get a monocular microscope with a mirror, work with it and learn. I have bought a microscope with no tube to carry the light and no power source. I used a piece of chrome drain pipe to carry the light path and wired in a house current dimmer switch for the power supply. That scope now has better resolution than other ones like it with the factory parts.

If you do end up getting a brightfield microscope and start using it to look at live microorganisms contact me after you get started or take a course and I'll share a trick with you. Most people close the iris diaphragm on the condenser almost shut to provide contrast on the organisms. I use a way that is better most of the time leaving it wide open.

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