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Equipping a Home Biology Laboratory

Other than a microscope and accessories, it doesn't take much special equipment to learn about biology. You'll need some general lab equipment, chemicals, and so on, but much of what you need can be improvised or substituted for by items inexpensively available from the drugstore or hardware store. If you keep a close eye on your budget, you can complete most or all of the lab sessions in this book for surprisingly little money.

You'll have decisions to make that balance cost versus time versus quality. For example, many lab sessions call for prepared microscope slides. If low cost is your top priority, you can prepare many of those slides yourself for a few cents each in materials, but at the expense of significant time and effort and possibly lower quality. Conversely, if you want top quality and cost is a low priority relative to your time, you can purchase very high quality prepared slides, although those may cost $5 to $20 or more apiece. Or you can compromise by purchasing inexpensive prepared slides for a buck or two apiece. Their quality won't be as good as that of the expensive prepared slides, but they'll probably be good enough for your purposes, and buying them will certainly save you a lot of time and effort.

If you're pursuing biology as a hobby, your budget may range from next-to-nothing to essentially unlimited. Many golfers get along just fine with a $250 set of clubs, but there's no shortage of golf enthusiasts with $1,500 drivers and $5,000 iron sets in their bags. And you'll often find that a kid with a $250 set of clubs outplays a guy lugging around $10,000 worth of clubs. (As a teenager, Barbara was a scratch golfer, and regularly embarrassed middle-aged rich guys.)

DIY biology enthusiasts are no different. If you attend a DIY Bio meet-up, you'll find kids who've accomplished amazing things on next-to-no budget gathered heads-down with doctors and lawyers and executives who've spent $100,000 or more to turn their garages into serious biotechnology labs. It's not about how much equipment you have; it's what you do with the equipment you do have.

That said, lack of equipment *can* limit what you can accomplish. Or, more precisely, lack of functionality. If you need to spin down a plasmid mini-prep, for example, there's no alternative: you need an ultracentrifuge. If you have $15,000 burning a hole in your pocket, great. Go buy a commercial ultracentrifuge. We couldn't afford that, so we built our own functional equivalent for less than $150. And for what we need to do, it's actually just as good as that $15,000 commercial unit.

In this chapter and throughout the book, we've tried to focus on getting a lot done on as small a

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budget as possible. That doesn't mean you should never use commercial products when there's a cheaper alternative. For example, gel electrophoresis is used to separate and purify DNA, proteins, and other biologically-important molecules. If you're a home schooler, you'll need a gel electrophoresis apparatus to complete one or two lab sessions and then just move on to the next lab sessions. You probably want your gel electrophoresis apparatus to be as inexpensive as possible, so we'll show you how to build a usable apparatus for $10 (about $9 of which is for 9V batteries). But if you're a DIY Bio enthusiast, you'll probably be using gel electrophoresis frequently. You'd soon tire of replacing expensive 9V batteries every few runs, so it makes sense to spend $300 or so on a commercial gel electrophoresis tank and power supply that minimizes the running costs.

If you're on a tight budget, you may need to skip some of the lab sessions or at least some parts of some lab sessions, but try to make that a last resort. We'll try to point out as we go along where you can improvise and substitute to get the job done. We also recognize that it can be very expensive to buy many different items piecemeal. For example, you may need only one gram of a particular chemical for one of the lab sessions in this book, but the minimum you can buy from a science supplies vendor is, say, a 30 gram bottle for $5. That wouldn't be too bad if you needed only that one chemical, but since you need many different chemicals the cost adds up fast.

Accordingly, we've put together a customized kit that includes many items that are difficult to find, hard to substitute for, or expensive to purchase piecemeal. In order to avoid retail markups and keep the cost to you as low as possible, the kits are available only direct from our own company, The Home Scientist, LLC (*www.thehomescientist.com*). We can ship the kits to all 50 states, but shipping regulations make it impossible for us to ship them to other countries. Sorry.

Microscopes and Accessories

The one piece of equipment most closely associated with biology is, of course, the microscope, and rightly so. Biology as a modern science would not exist without the microscope, and good microscopes are essential day-to-day tools for most biologists.

Choosing a suitable microscope is a non-trivial task, so we devote a significant amount of space in this section to explaining the things you need to know to choose the right microscope for you. Before we get into that, though, we'll offer some advice about how to go about acquiring the microscope you decide best fits your needs.

For most people, buying a suitable microscope is a major purchase. You don't want to pay more than you need to, but neither do you want to paint yourself into a corner by buying too little microscope.

Microscopes range in price from $25 toys to professional models from German and Japanese manufacturers that cost from $3,000 or $4,000 to $25,000 or more. If you can afford a top-tier microscope, great. Buy a suitable model from Leitz, Zeiss, Fujinon, or one of the other German or Japanese microscope makers. Your credit card will be smoking, but you'll have one of the finest optical instruments on the planet, and one that will last a lifetime.

Most of us aren't that lucky, but fortunately there are affordable alternatives. The best Chinese microscopes offer 90% of the optical and mechanical quality of the top-tier models at 20% of the price. We'll make specific recommendations by brand and model later in this chapter, but for now be aware that although the best of the Chinese microscopes are very, very good, most Chinese microscopes are of very poor quality. It's impossible to tell the difference just by looking at the microscopes or comparing prices, so the key to getting a good one is to buy from a reputable vendor.

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The first thing to decide is whether you want to keep the microscope indefinitely or use it only for a short period, such as a school year. Once you decide that, you can decide whether to buy a new microscope, buy a used microscope, or rent the microscope.

Buy a new microscope

If you intend to keep the microscope, buying new is usually the best option. You'll pay more than you would for the same model used, but you'll get exactly the microscope you want with exactly the options you want. You'll also get a warranty, which for most better models is a limited lifetime warranty. (Don't overvalue the warranty; if treated properly, good microscopes almost never need to be repaired, other than trivial failures like bulbs and fuses.)

Buy a used microscope

A good microscope that has been well cared for is as good now as the day it was made. Unfortunately, the converse is also true: a bad microscope will never be anything but a bad microscope, so you have to be very careful buying used.

Pricing for used microscopes is all over the map. Inexpensive no-name microscopes have essentially no resale value. House-brand models from Home Science Tools and similar vendors may on average sell for 33% to 50% of the current selling price for the same model new, but we've seen prices listed for such scopes that range from 10% to 100% or more. The best Chinese scopes, such as the midrange and better National Optical models, may sell for 70% to 80% of their current new selling price. Current top-tier models may sell for 90% or more of their current new selling price. In fact, some models are so popular that you may have to join a waiting list to get one, and these may actually sell for more used than their current list prices. Discontinued older top-tier models may sell for 80% or more the current selling price of the equivalent replacement model.

The advantage of buying a used microscope locally is that you can actually see and touch it before buying. The disadvantages are that the selection is likely to be limited, and you'll have to negotiate the price with the seller. If you want to buy locally, check Craigslist and your local homeschool group. The advantages of buying a used microscope on-line from a reputable vendor are that you'll get what you pay for (although never more than you paid for, as can happen buying locally), that the selection will be much better, and that the vendor will do at least some minimum screening and usually provide at least a short warranty.

Rent a microscope

If you need the microscope for only a short period, renting is another option. The advantages to renting are that it requires the least cash outlay and you can select among many models. The disadvantage is that you may have to pay as much as 50% of the current selling price of the scope to have the use of it for only a year or less. The rental vendor will charge your credit card for the price of the scope initially—often at list price—and then refund your money less the rental fee once you return the microscope. Also, some rental vendors are very picky about the condition of the returned scope, so you may end up paying a higher rental fee than you expected to cover “damage” such as minor scratches.

On balance, for those who intend to keep the microscope indefinitely, we recommend buying a new or used model from an on-line vendor, depending on your comfort level with buying used. If you need the microscope for a limited time, such as a school year (or several school years), buy a new or used model from an on-line vendor. Make sure it's a respected brand name—National Optical, Swift, Motic, and Leica are the best brand names in mid-priced scopes—and then resell it to a local home schooler when you no longer need it. (Never sell the microscope back to an on-line vendor. You'll get only a small fraction of what you would by selling it locally.)

All of that said, let's take a detailed look at what you need to know to make an informed purchase.

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Microscope Types

Broadly speaking, three types of microscopes are useful for studying biology. (Well, there's also the electron microscope, but few home scientists can afford one of those.)

Compound microscope

A *compound microscope* is what most people think of as a microscope. It's used to view small specimens, usually by transmitted light, at three or four medium to high magnifications, typically 40X, 100X, 400X, and sometimes 1000X.

Stereo microscope

A *stereo microscope*, also called a *dissecting microscope*, operates at low magnification, typically 10X to 40X. Some models offer only one magnification, others two, and zoom models offer continuously variable magnifications. Stereo microscopes are used to view medium to large specimens, usually by reflected light.

Portable microscope

A *portable microscope*, also called a *field microscope*, is small enough to carry in your pocket on field trips. Some models offer fixed magnification, often 30X, while others provide zoom magnifications to 100X or more. Most models include a battery-powered LED illuminator and use reflected light only. Some models make provision for using standard slides to examine specimens by transmitted light as well.

In the following sections, we'll take a closer look at each type.

Compound Microscope

A *compound microscope*, shown in Figure C-1, is what most people think of as a microscope. You use it to view small specimens, usually by transmitted light, at three or four medium to high magnifications, typically 40X, 100X, 400X, and sometimes 1000X.

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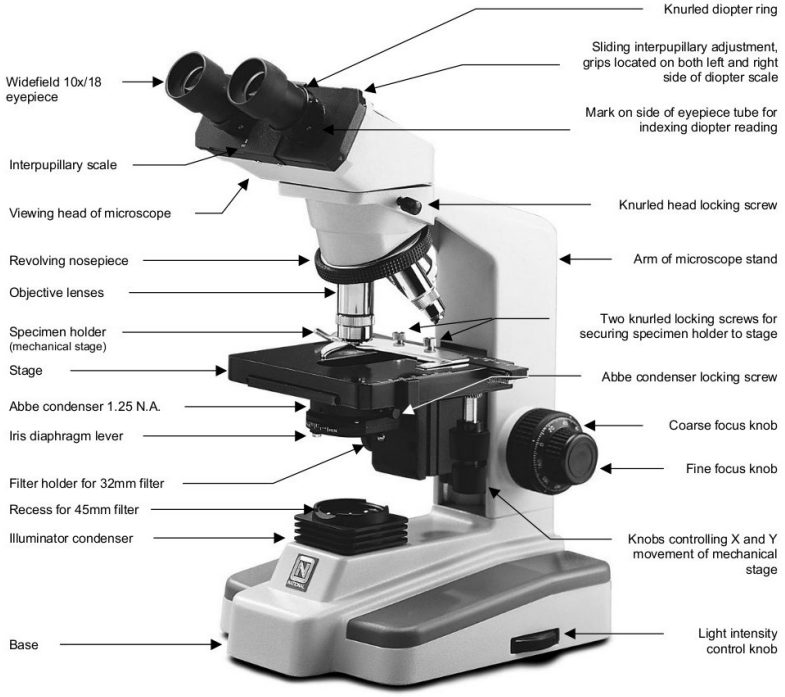

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Figure C-1. A typical compound microscope (image courtesy National Optical & Scientific Instruments, Inc.)

The following sections describe some factors to consider when you choose a compound microscope:

Head style

Compound microscopes are available in the four head styles shown in Figure C-2.

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Figure C-2. Monocular, dual-head, binocular, and trinocular head styles, left to right (images courtesy National Optical & Scientific Instruments, Inc.)

Monocular

A *monocular head*, shown on the left in Figure C-2, provides only one eyepiece. This is the least expensive of the four head styles, and is suitable for general use.

Dual-head

A *dual head*, shown left-center in Figure C-2, provides two eyepieces, one vertical and one angled. The second eyepiece allows two people to view a specimen simultaneously, for example a teacher and a student. A dual head is also very convenient if you want to mount a still or video camera to image specimens. Dual head models typically cost $50 to $100 more than comparable monocular models.

Binocular

A *binocular head* , shown right-center in Figure C-2, provides two eyepieces to allow viewing specimens with both eyes. One eyepiece is individually focusable to allow the instrument to be set up for one person's vision. The advantage of a binocular head is that it's less tiring to use over long periods and may allow seeing more detail in specimens. The disadvantage is that the focusable eyepiece must be adjusted each time a different person wants to use the scope. Binocular models typically cost $150 to $250 more than comparable monocular models.

Trinocular

A *trinocular head*, shown on the right in Figure C-2, provides two eyepieces for binocular viewing and a separate single eyepiece for viewing by a second person or for mounting a camera. Trinocular models typically cost $300 to $400 more than comparable monocular models.

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At any particular price point, a monocular-head model offers the maximum bang for the buck. You'll get better optical and mechanical quality with the monocular head than with any of the multiple-head models.

Regardless of head style, most better models allow the head to be rotated through 360° to whatever viewing position you prefer. The left image in Figure C-2 shows the traditional viewing position, with the support arm between the user and the stage. The other three images show the reversed viewing position, with the stage between the user and the support arm. Most people prefer the latter position, which makes it easier to manipulate slides, change objectives, and so on.

Illumination type and power source

The next issue to consider is the illumination type and power source.

Early microscopes and some inexpensive current models have no built-in illuminator. Instead, they use a mirror to direct daylight or artificial light up through the stage and into the objective lens. Because any mirror small enough to fit under the microscope stage gathers insufficient light to provide bright images at high magnifications, such scopes are limited to use at low and medium magnifications unless they are equipped with an accessory illuminator. Most microscopes include built-in illuminators of one of the following types, roughly in order of increasing desirability:

Tungsten illumination
: *Tungsten illumination* is the least expensive type, and the most common on low-end microscopes. Tungsten illuminators use standard incandescent light bulbs. They are relatively bright, but they produce a yellowish light and considerable heat. In particular, as the light is dimmed, it shifts further toward orange. This warm color balance can obscure the true colors of specimens. The heat produced by the incandescent bulb may kill live specimens and quickly dries out temporary wet mounts made with water. Lamp life is relatively short.

Fluorescent illumination
: *Fluorescent illumination* costs a bit more than tungsten, and was quite popular before the advent of LED illuminators. Fluorescent illuminators provide bright light that appears white to the human eye, but is actually made up of several different discrete colors that are mixed to appear white. Accordingly, color rendition can differ significantly from the true color rendition provided by daylight. Fluorescent bulbs emit much less heat than incandescent bulbs, and so are well suited to observing live specimens. Some fluorescent illuminators are battery-powered, but most use AC power. Lamp life is relatively long.

LED illumination
: *LED illumination* costs about the same as fluorescent illumination. LED illuminators have become very popular, largely replacing fluorescent illuminators. LED illuminators have the same color-rendition problems as fluorescent illuminators, but are otherwise ideal for many purposes. LED illuminators draw very little power and emit essentially no heat. Their low power draw means they're the best choice for a battery-powered microscope, and are ideally suited for portable microscopes that can be used in the field. Lamp life is essentially unlimited.

Quartz-halogen illumination
: *Quartz-halogen illumination* is the most expensive type, and the one preferred by most experienced microscopists. Quartz-halogen provides the brilliant white light needed for work at high magnification that reveals the true colors of specimens. Unfortunately, quartz-halogen lamps also produce more heat than any other type of illuminator. Their high power draw means they are AC-only. Lamp life is relatively short.

Choose quartz-halogen if it is available for the microscope model you purchase. Otherwise, choose LED. Fluorescent illumination is obsolescent, and tungsten is appropriate only for an

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entry-level scope.

Illumination methods

The next thing to consider is which illumination method or methods the microscope supports.

Brightfield illumination

Brightfield illumination is supported by all compound microscopes, and is the only method available with most. With brightfield illumination, you view specimens by transmitted light that passes directly through the specimen. Scattered light reduces contrast and detail, and is minimized by restricting the diameter of the light cone passing through the specimen to match the field of view of the objective lens being used to view it.

The advantage of brightfield illumination is that it provides very bright, clear images. The drawback is that many types of specimens have so little inherent contrast that it's very difficult to discriminate detail in the specimens. Staining specimens provides color contrast that reveals these otherwise-hidden details, but staining can be time-consuming.

Darkfield illumination

Darkfield illumination is the exact opposite of brightfield illumination. Rather than viewing the specimen by transmitted light and blocking as much scattered light as possible, you block transmitted light and view the specimen only by scattered light. This is accomplished by using an opaque circular mask below the specimen that prevents light from passing through the specimen. By adjusting the condenser diaphragm, you produce a bright ring surrounding the specimen that illuminates the specimen from just outside the field of view.

At first glance, the image provided by darkfield illumination appears to be a simple negative of the image provided by brightfield illumination, but that is not the case. Some things are visible with darkfield illumination that are invisible with brightfield. For example, live microorganisms in a drop of water may be invisible with brightfield because their transparency and refractive index is so close to that of water. With darkfield, those microorganisms are revealed brightly lit against a dark background.

Unfortunately, darkfield illumination also has several drawbacks. First, as you might expect, the image can be quite dim, particularly at high magnification, and images of even colorful specimens present in essentially monochrome. Second, darkfield demands extraordinary cleanliness and care in sample preparation. Every extraneous spec of dust shows as a blazing spot of light in the field. Specimens must be extremely thin and even, much more so than for brightfield. For best results, you must use top-quality slides and coverslips, and the best slides for use with darkfield are thinner (about 0.7 to 1.0mm) and more fragile than those typically used with brightfield illumination.

Some microscopes include darkfield stops as standard equipment, or offer a darkfield kit as an inexpensive option. Even if your microscope does not, you can still use darkfield as long as your microscope has a filter holder beneath the sub-stage condenser lens. One way is to use darkfield stops or a darkfield kit supplied by a different vendor. (The size of the stop should be matched to the objective lens, ranging from 20mm for a 4X objective down to 8mm with a 100X objective.) Another is simply to use an opaque circle (such as a peel-and-stick dot) pasted to the center of a colorless or colored glass filter placed between the lamp and the bottom of the sub-stage condenser.

Phase-contrast illumination

Phase-contrast illumination is a complex illumination method in which small phase shifts in the light passing through a transparent specimen are converted to visible amplitude (contrast) differences. Phase-contrast illumination reveals the low-contrast details that would otherwise be invisible without staining. This has two huge advantages. First, it eliminates the time spent

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preparing and staining specimens, which can be significant. Second, it allows live microorganisms to be observed in their natural environment, without the possible damage caused by fixing and staining them. (There are some stains, called *vital stains*, that can be used on living cells, but the choices are quite limited.)

Many professional-grade microscopes include phase-contrast illumination as a standard feature, and it is available as an option on some other models. Unfortunately, it's an expensive option. A phase-contrast kit for a standard microscope typically boosts the price of the microscope by $800 to $3,000.

Köhler illumination, devised by August Köhler in 1893, is a type of brightfield illumination that is extremely even and provides the highest possible contrast. Unfortunately, using Köhler illumination requires physical features that are not present on many microscopes, including a positionable lamp and a focusable lamp condenser.

Fortunately, the alternative, called *critical illumination* or *standard brightfield illumination*, is perfectly usable for most visual work. In fact, many experienced microscopists prefer critical illumination to Köhler illumination for visual work at high magnification. The extreme evenness of Köhler illumination is important for professional quality results if you are shooting images through the microscope, but otherwise critical illumination works fine.

Nosepiece and objective lenses

The next features to think about are the nosepiece and objective lenses, which are one factor in determining the magnifications that are available with a particular microscope.

The *nosepiece*, also called the *turret*, is a rotating assembly that holds 3, 4, or 5 *objective lenses*. By rotating the nosepiece, you can bring a different objective lens (usually just called an *objective*) into position and change the magnification you use to view the specimen. Inexpensive microscopes use friction-bearing nosepieces; better models use ball-bearing nosepieces with positive click-stop detents. Figure C-4 shows a typical nosepiece with three objectives visible.

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Figure C-4. A typical microscope nosepiece with objective lenses

The nosepiece may be mounted in the forward position (tilted away from the support arm) or

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reverse position. If you use the scope in the forward viewing position (with the support arm between you and the stage), having the nosepiece mounted in the forward position makes it a bit easier to change objectives. If you use the reverse viewing position, it's easier to use a nosepiece mounted in the reverse position.

Objective lenses are usually color-coded to make it obvious which one is currently being used. The standard color codes are red (4X), yellow (10X), green (20X), light blue (40X or 60X), and white (100X). Not all manufacturers follow this standard.

Most inexpensive microscopes—those that sell for less than $300 or so—provide only three objective lenses, 4X, 10X, and 40X. Used with a 10X eyepiece, such microscopes provide 40X, 100X, and 400X magnification. Although such microscopes may be suitable for most first-year biology lab work, we think it's a bad idea to buy one unless you are absolutely certain it will suffice for the work you intend to do, now and in the future. The problem is that these 400X microscopes do not provide the higher magnifications needed to observe bacteria and other tiny specimens, which is required for second-year biology and other more advanced work.

Microscopes of the quality needed for advanced biology studies and beyond include a fourth, 100X, objective lens, which provides 1000X magnification with a 10X eyepiece. Some models have a five-position turret. The fifth position may be empty, or it may include a fifth objective, often 60X.

The quality of the objective lenses is as important as their number and magnification. Microscope objective lenses differ in two major respects, color correction and flatness of field.

Color correction

The level of *color correction* is specified as either *achromatic* or *apochromatic*. Achromatic lenses are corrected for chromatic aberration at two specific wavelengths of light, usually red and green. An achromat brings those two wavelengths to the same focus, with other wavelengths very slightly out of focus. An apochromat is corrected for three specific wavelengths of light--usually red, green, and blue--and brings those three wavelengths to the same focus, providing slightly sharper images than an achromat. Apochromatic objectives are extremely expensive, some costing more than $10,000, and are found only on professional-grade microscopes. Any microscope affordable for a home lab uses achromatic objectives.

Used microscopes, even very old models, can be excellent bargains. As a teenager in the 60's, Robert used a WWI-era Zeiss microscope that was superb, both optically and mechanically. But you have to be careful and know what you're doing.

Some old microscopes have apochromatic objectives, but those objectives were optimized for photomicrography with black-and-white film rather than visual use. Their correction is superb in the blue, violet, and ultraviolet range, but when used for visual observing their image quality is actually inferior to a modern achromat.

Flatness of field

Standard objectives have limited correction for spherical aberration, which means that only the central 60% to 70% of the field of view is in acceptably sharp focus. Semi-plan objectives have additional correction that extends the sharp focus area to the central 75% to 90% of the field of view. Plan objectives extend the area of sharp focus to 90% or more of the field. This additional correction for flatness of field is completely independent of color correction. You can, for example, buy semi-plan apochromatic objectives and plan achromat objectives.

Finally, some vendors offer optional upgrades to superior lens coatings, often under such names as Super High Contrast or something similar. These superior coatings don't improve color correction or flatness of field, but they increase image contrast noticeably and are worth having.

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For most home lab use, ordinary achromatic objectives provide perfectly acceptable images and are the least expensive choice. Our own microscope, a Model 161 dual-head unit shown left center in Figure C-2, has the upgraded ASC objectives, which we purchased for their higher contrast and superior visual image quality.

All but toy microscopes are *parfocal* and *parcentered*. Parfocal means that all objectives have the same focus. When you focus a specimen at 40X, for example, and then change to 100X, the specimen remains focused. (You may have to touch up the focus with the fine-focus knob, but the focus should be very close to start with.) Parcentered means that if you have an object centered in the field of view with one objective and you change to a different objective, the object remains centered in the field of view. Professional-grade microscopes provide adjustments for both parfocality and parcentrality, but student- and hobbyist-grade microscopes are set at the factory and cannot be adjusted by the user. That means it's important to check these settings as soon as you open the box of your new microscope.

To check parfocality, place a flat specimen (a thin-section or smear slide is good, if you have one; otherwise any flat specimen) on the stage and focus critically on it at the lowest magnification. Then change to your next highest magnification and check the focus. It should be in focus or nearly so, requiring at most a partial turn of the fine-focus knob to bring it into critical focus. Change to your next higher magnification and again check the focus. Again, it should require at most a small tweak with the fine-focus knob to bring the specimen into sharp focus.

To check parcentrality, center an object in the field of view at the lowest magnification and then switch objectives to the next-higher magnification. The object should remain centered, or nearly so. Repeat until you are viewing the object at your highest magnification. Because it's easier to judge whether an object is centered at high magnification, center the object at your highest magnification and then work your way down to lower magnifications. If the object remains centered (or nearly so), your parcentrality is acceptable. If the position of the object in the field of view shifts dramatically when you change objectives, the parcentrality is off. The only solution is to return the microscope for a replacement.

Eyepieces (Oculars)

The *eyepiece* (or *ocular*) is the second factor that determines the overall magnifications available with a particular microscope. The eyepiece magnifies and focuses the image provided by the objective lens and presents it to your eye. Multiplying the objective magnification by the ocular magnification yields the overall magnification. For example, using the 100X objective with a 10X eyepiece provides 1000X overall magnification.

Magnifications are specified linearly rather than areally. For example, if you use 1000X magnification, a square object that is actually 1 µm (micrometer, one millionth of a meter or one thousandth of a millimeter) on a side appears to be 1000 µm (or 1 mm) on a side. The image of that object at 1000X magnification therefore covers 1,000,000 times as much area as the actual object.

Standard microscope ocular barrels are either 23.2 mm (usually abbreviated to 23 mm) or 30 mm in diameter, which means it's easy to exchange oculars if you need a different magnification range. The standard ocular magnification factor is 10X, but 15X oculars are readily available to increase the range of magnifications available to you. Avoid zoom oculars, all but the most expensive of which which produce inferior images.

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Most standard oculars are unobstructed, but some have a standard or optional pointer or reticle (grid or graduated scale). A pointer is primarily useful in a teaching or collaborative environment, where one person can place the pointer on an object of interest so that the other person can identify it unambiguously. A graduated reticle is useful for measuring the size of objects in the field of view, and a grid reticle is useful for counting large numbers of small objects in the field of view.

You might think you can achieve any magnification you want simply by combining different eyepieces and objective lenses. For example, you could combine a 25X eyepiece with a 100X objective to give you 2,500X magnification. Why, with a 30X eyepiece, you can get to 3,000X magnification, and with a 50X eyepiece to 5,000X!

Well, you can, but you won't actually see any more detail than you would using that objective with a 10X or 12.5X eyepiece. The image scale will be larger at 2,500X to 5,000X, of course, but the image will also be much dimmer and fuzzier. You will see no more detail than you can see at 1,000X or 1,250X and probably less. That's why such high magnifications are called *empty magnification*.

Unfortunately, the laws of optics mean there is an upper limit to the useful magnification for any particular objective, even if it is of the best possible optical quality. Straying above that limit is possible, but the image quality will be degraded.

As a rule of thumb, determine *maximum useful magnification* by multiplying the numeric aperture (NA) of the objective by 1,000. For example, a 100X objective with an NA of 1.25 has a maximum useful magnification of (1.25 X 1,000) = 1,250X.

Focuser

The next consideration is the focuser, which may sound trivial to those who've never used a microscope. It's anything but. A microscope with a smooth focuser is a joy to use, and makes it easy to achieve the best possible focus to reveal the maximum amount of detail. A microscope with a poor focuser is almost unusable.

Microscopes use one of two methods for focusing. Most older models and some current models keep the stage in fixed position and move the head up and down to achieve focus. Most current models and some older models reverse this, keeping the head in a fixed position and moving the stage up and down to achieve focus. Either method works fine for general use, but if you plan to mount a camera on a dual-head or trinocular model the fixed-stage method may be problematic because the weight of the camera makes it more difficult to focus and may cause focus drift.

Inexpensive microscopes have one focus knob that changes focus at intermediate rate, which makes it difficult or impossible to achieve critical focus. Midrange models have separate coarse-focus and fine-focus knobs. More expensive models usually have a coaxial focus knob, often one one each side of the microscope, with the coarse focus on the inner knob and fine focus on the outer knob, as shown in Figure C-5. Coarse focus tension should be adjustable.

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Figure C-5. Coaxial focusing knob, with coarse focus (inner knob) and fine focus

You use the coarse-focus knob to bring the specimen into reasonably close focus, and then use the fine-focus knob to tweak the focus slightly to achieve the sharpest possible focus. If you are viewing a three-dimensional object, particularly at higher magnifications, you'll find that you can't bring the entire depth of the object into focus at the same time. You use the fine-focus knob to adjust focus slightly as you're viewing the object to view different "slices" of it in depth.

Many coaxial focus knobs, including the one in Figure C-5, provide a graduated scale. One obvious use for this scale is in a collaborative situation. One person can focus critically, note the scale setting, and then turn over the microscope to the second person, who refocuses as necessary. When the first person returns to the eyepiece, merely resetting the fine-focus knob to the original setting puts the specimen back into critical focus. A less obvious use of the graduated scale is to determine relative depths of parts of a specimen. By setting a baseline focus on one level of the specimen and then noting how much change in scale units is needed to refocus on parts of the specimen at different depths, you can get a relative idea of the differences in depth of different parts of the specimen.

Mechanical Stage

Inexpensive microscopes use a pair of clips to secure the microscope slide to the stage. Although (barely) usable at low magnifications, this method becomes increasingly difficult as you increase magnification. The problem is that a very small movement of the microscope slide translates into a huge movement in the field of view. At low magnification, the smallest movement you can make manually shifts the object significantly within the field of view. At high magnification, the smallest movement you can make manually may move the object completely out of the field of view. If you're viewing a living, moving object (such as a paramecium), it can be almost impossible to keep the object in the field of view.

The solution to this problem is a mechanical stage, shown in Figure C-6. With a mechanical stage, you clamp the slide into an assembly that provides rack-and-pinion geared movements. Turning the control knobs, shown in Figure C-7, moves the slide continuously along the X-axis (left or right) and the Y-axis (toward or away from you) in extremely small small increments.

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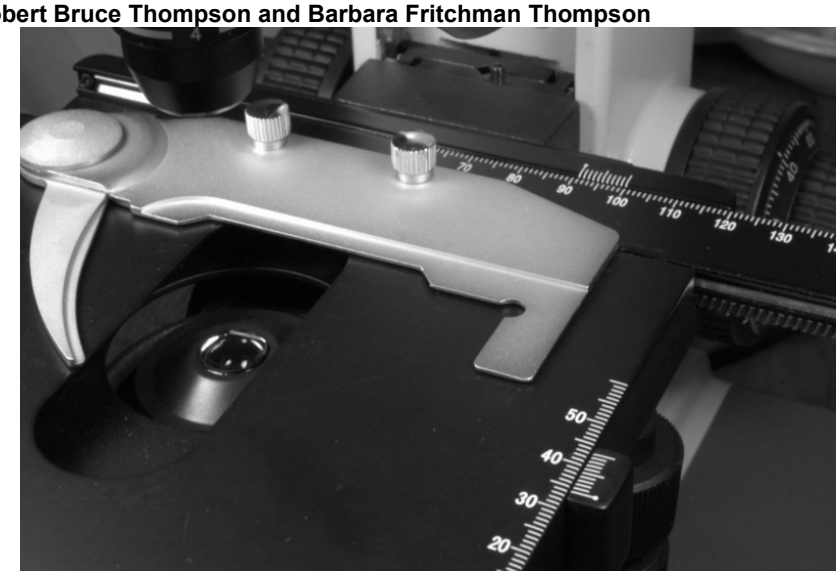

Figure C-6. A typical mechanical stage (note the verniers on the X and Y axes and the top lens of the Abbe condenser below the stage)

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Figure C-7. Knobs control the X- and Y-axis movements of the mechanical stage

With a mechanical stage, centering an object in the field of view becomes trivially easy, as does keeping a moving object in the field of view. Because the mechanical stage provides X-axis and Y-axis verniers, it's easy to return to a specific location on the slide even after you've moved it completely outside the field of view. We wouldn't even consider using a microscope without a mechanical stage. Life is too short.

Substage condenser, diaphragm, and filter holder

Despite the fact that they're located below the stage (and therefore below the specimen), the *substage condenser* (the top lens of which is visible in Figure C-6) and the *diaphragm* and filter holder, shown in Figure C-8, have a significant effect on image quality.

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Figure C-8. The diaphragm control lever (note the swing-out filter holder to the lower left)

The condenser sits between the diaphragm and the stage, focusing light from the illuminator onto the specimen to provide a bright, sharp image. Toy microscopes have no condenser. Somewhat better microscopes use a simple fixed-focus condenser, usually rated at 0.65 NA (Numerical Aperture, where the NA of the condenser must be at least as high as the NA of the objective lenses it is to be used with. A 0.65 NA condenser can be used with at most a 40X objective. Oil-immersion 100X objectives with a 1.25 NA rating require a 1.25 NA condenser.) Midrange microscopes use a focusable Abbe condenser, usually of 0.65 NA and usually with a spiral focusing arrangement. Better models provide a rack-and-pinion focusable Abbe condenser with a 1.25 NA for use with any objective up to a 100X oil-immersion objective.

The diaphragm is used to control the diameter of the light cone where it intersects the specimen being viewed. Ideally, you want the diameter of the light cone to be the same size as the field of view of the objective lens you're using. At low magnification, where the field of view is relatively large, you want a larger light cone; at higher magnification, where the field of view becomes correspondingly smaller, you want a smaller light cone. If the light cone is smaller than the field of view, the field is not completely illuminated. If the light cone is larger than the field of view, "waste" light from outside the field of view reduces contrast and image quality.

Toy microscopes have no diaphragm. Basic models have a disc diaphragm, which is simply a metal disc with several (usually five or six) holes of different diameter that can be rotated into position. Disc diaphragms provide only compromise settings, but are generally quite usable. Better microscopes have iris diaphragms, which can be set continuously to provide any size of aperture, from a pinhole to wide open.

Many sources, including some that should know better, state that the diaphragm should be used to control image brightness. Wrong. Adjusting the diaphragm does dim or brighten the image, of course, but that is not its primary purpose. To control image brightness, use the illuminator switch to dim or brighten the lamp.

The diaphragm controls the diameter of the light cone from the condenser, which affects the contrast of the image and how evenly the specimen is illuminated. There is no set rule as to what diaphragm setting to use at a particular magnification. The optimum diaphragm setting depends on the nature and transparency of the specimen, the magnification you are using, and your own preferences.

As a starting point for general work (using the entire field of view), match the light cone approximately to the field of view of the objective lens you're using. If the outer edge of the field is noticeably dimmer than the center, the diaphragm aperture you're using is too small. There are times when it's worth using a very small aperture and accepting darkening at the edge of the field to get a better view of the center of the field.

As a rule of thumb, adjust the diaphragm for optimum contrast and the illumination dimmer switch for optimum brightness.

Most microscopes provide a swing-out filter holder immediately beneath the diaphragm, and often include a few round glass filters—often blue, yellow, and clear. Filters serve two purposes. First, they can be used to enhance minor color contrasts in a specimen. For example, if your specimen is mostly colorless but with some very pale yellow structural detail, using a blue filter darkens the pale yellow, making detail more visible. Second, using filtered light can help you wring out that last bit of detail if you're using achromatic objectives. Remember that achromats don't bring all colors of light to the same point of focus, so when you view a specimen with full-spectrum light, there is some blurring. Using a color filter effectively makes the illumination monochromatic, which means that even an achromatic objective can bring it to sharp focus.

Polarizing microscopes are specialized instruments. They're used mostly by geologists, but have many applications in biology. Few home biologists can justify spending several hundred dollars or more for a dedicated polarizing microscope. The good news is, you don't have to.

For a few dollars, you can buy two pieces of polarizing film from Edmund Scientific or another lab supplies vendor. (We use a set of filters mounted in 35mm slide holders.) Place one of the polarizing films flat on the filter holder and hold the other between the eyepiece and your eye. Rotate that film to observe the specimen by polarized light. No, it doesn't do everything that a real polarizing microscope does, but it does a lot and you can't beat the price.

The Final Decision

So, with all of that said, which compound microscope should you get? Obviously, that depends on both your needs and your budget, but we can offer some advice to help you make a good decision.

Entry Level 400X microscope (~ $240 street price)

If you need an entry-level 400X scope, we recommend the National Optical Model 131-CLED with the optional mechanical stage. This scope can serve a student from elementary school through high school, excepting advanced biology. The optics and mechanicals are good to very good. The only major missing feature is the 100X oil-immersion objective, which is needed for cell biology studies in high school advanced biology courses.

Basic 1000X microscope (~ $350 street price)

If you need a basic 1000X scope, we recommend the National Optical Model 134-CLED. This scope is excellent for hobby use, and is the only scope a student will need from middle school or junior high school through high school advanced biology. This scope is essentially a Model 131 upgraded to include a 100X oil immersion objective, a focusable 1.25 NA Abbe condenser, an iris diaphragm, and a standard mechanical stage.

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Mainstream microscope (~ $460 to $1,600+ street price, depending on model and options)

If you need a mainstream microscope, we recommend one of the National Optical 160-series models, the Model 160 (monocular), Model 161 (dual-head), Model 162 (binocular), or Model 163 (trinocular). The only major feature missing from these 160-series scopes is support for Köhler illumination. The model 165 (binocular) and 166 (binocular plus a camera port) add support for Köhler illumination, as well as a five-position turret and a Siedentopf head on the binocular models.

Achromatic objectives are standard on the 160, 161, 162, and 163, with upgrades to ASC (Achromatic Super Contrast) or plan objectives. ASC objectives are standard on the 165 and 166 models, with plan objectives optional. Phase-contrast objectives are available as a factory option on the 162, 163, 165, and 166 models.

Any of these 160-series microscopes is a superb choice for hobby use, and is the only scope a student will need from middle school or junior high school all the way through university and graduate school, particularly if you opt for phase-contrast support. The optics and mechanicals are excellent, and the feature list is impressive. Even people who use professional-grade microscopes every day are invariably surprised by the level of mechanical and optical quality the 160-series microscopes provide at this price point.

Does National Optical pay you to say this stuff? Nope. Nor do we own stock in them, nor have any other financial interest. They've never sent us so much as a free mouse pad. We use National Optical scopes, but we paid for them with our own money. Our only connection with National Optical is as satisfied customers.

We recommend National Optical models because they provide the quality control that is lacking in most Chinese microscopes. Chinese factories are famous for huge variations in product quality. The same factory can turn out both truly excellent microscopes and visually identical models that are fit only as boat anchors, and all on the same day. National Optical rides close herd on its Chinese factories, making sure they meet NO quality standards, and hand-inspecting every scope before it's shipped to the customer.

National Optical microscopes are widely distributed, as you'll find if you do a quick Google search for the model or models you're interested in. National Optical also imports microscopes under the Motic and Swift Optical brand names, both of which also offer excellent bang for the buck.

Finally educational microscopes sold under the Leica brand name are available from many vendors. These microscopes are actually made in China (in the same factory that makes National Optical and Swift models, we're told) but are backed by the Leica name. Leica models are somewhat more expensive than NO or Swift models with comparable features, but the Leica name undoubtedly adds to their resale value.

Stereo Microscope

A *stereo microscope*, shown in Figure C-9, is also called a *dissecting microscope* or an *inspection microscope*). It uses two eyepieces, each with its own objective lens, to provide a 3D image of the specimen.

These microscopes are used to examine relatively large solid objects at low magnification, usually by reflected rather than transmitted light. Most stereo microscopes provide a top illuminator that directs light downward onto the specimen. Better models often also provide a bottom illuminator that allows specimens to be viewed by transmitted light.

Stereo microscopes operate at low magnification, usually in the 10X to 40X range. Some models

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have fixed magnification, usually 10X, 15X, 20X, or 30X. Other models offer a choice of two magnifications, often 10X, 15X, or 20X and 30X or 40X. Zoom models offer continuously variable magnification.

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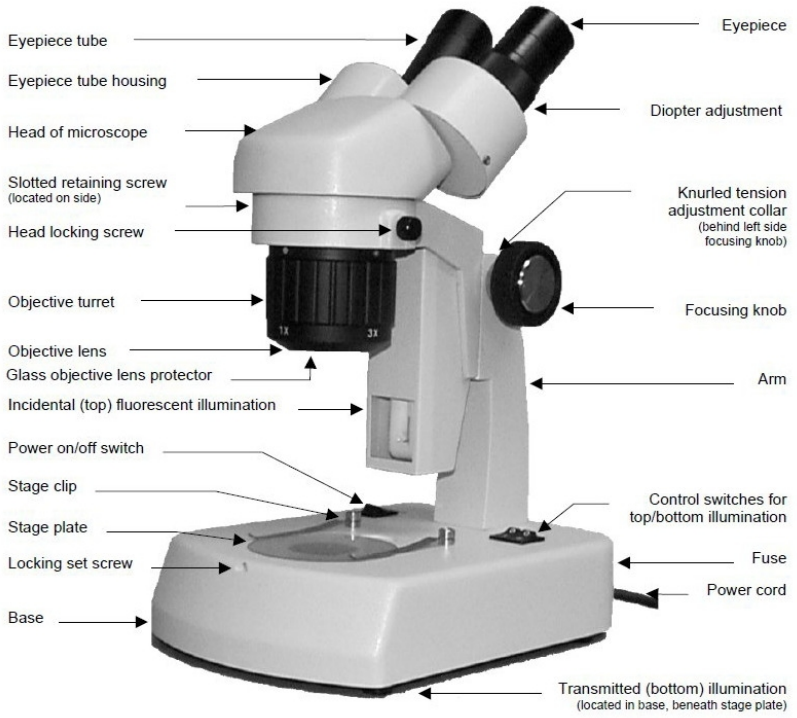

Figure C-9. A typical stereo microscope (image courtesy National Optical & Scientific Instruments, Inc.)

A stereo microscope is very helpful for dissections because it leaves both hands free for using the dissection instruments. It's also useful for examining relatively large specimens such as leaves. As helpful as it can be, we don't consider a stereo microscope to be essential for most biology lab work. Buy one if you can afford it, but don't skimp on the compound microscope. It's better to buy a good compound microscope and no stereo microscope than to buy inexpensive models of each. If you don't have a stereo microscope, you can often substitute a magnifier or pocket microscope, or in some cases simply use your compound microscope at its lowest magnification.

The best (and most expensive) stereo microscopes are made by German and Japanese companies such as Leitz, Zeiss, Fujinon, and Nikon. Affordable high-quality models are available from National Optical, Motic, and Swift. For an entry-level stereo microscope, we recommend the NO 400TBL-10-2, which is available for $175 or so in 20X, 30X, and 40X models. For a dual-magnification model, we recommend the NO 409/410/411, which is available for $270 or so in 10X/20X, 10X/30X, and 20X/40X models. (Note that all of these magnifications assume you are using the standard 10X eyepieces. You can buy extra eyepiece pairs in 5X, 15X, or 20X to expand your available range of magnifications.)

Portable Microscopes and Magnifiers

As useful as standard microscopes are when you're working in your lab, you'll probably also want a portable microscope like the one shown in Figure C-10 to take along on field trips. You can pay

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anything from $10 or $15 up to $500 or more for a portable scope. Having lost more than one of these to accidents and dunkings, we prefer the cheap ones.

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Figure C-10. A typical portable microscope (image courtesy United Scientific)

It's easy to think of these $15 microscopes as children's toys—and in fact they are excellent gifts for children—but they're also serious scientific instruments. A typical $15 model might provide 30X fixed magnification, battery-powered illumination, and little more. Somewhat more expensive models might offer higher magnification, sometimes 100X or more, white LED illumination, and provision for using standard microscope slides. Some models offer zoom magnification, and may even be convertible to a tiny telescope. The best models—those that cost $100 to $500 or more—have optical and mechanical quality similar to that of a desktop microscope that sells for about the same price.

Particularly if you don't have a stereo microscope, you'll probably want at least one magnifier. These range from standard magnifying glasses that are sold for a few bucks at WalMart to the high-quality magnifiers intended for scientific and industrial use that are sold by Edmund Optics and similar vendors. Our favorite midrange magnifier is the 5X Viewcraft Lupe, which sells for $20 or so. It has excellent optics for the price, and its clear acrylic base means you don't ordinarily need a separate illuminator. It also has built-in millimeter scale, which is quite useful for measuring specimens. We use it often in the lab and in the field. In fact, we used it with the Live View feature of our DSLR to focus many of the images we shot through our microscope for this book.

Microscope Accessories

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Even the best microscope is useless without some essential accessories. It's easy to go overboard with microscope accessories, so we'll try to limit our advice to the must-have items. Okay, maybe we'll talk about some nice-to-have items as well.

Cleaning Equipment

It's impossible to do good work with a dirty microscope. Microscopes are dust magnets, and of course under magnification a grain of dust looks like a boulder.

Your first line of defense is a dust cover, which should always be in place unless you are actually using the microscope. Most microscopes come with a fitted vinyl dust cover. If yours did not, either buy a suitable one or substitute a plastic wastebasket liner. A soft bristle brush and canned air are excellent for getting rid of any dust that still manages to accumulate.

You'll also need a supply of lens cleaning fluid and lens cleaning tissues. Don't substitute household items for either of those. Window cleaner or eyeglass cleaner may contain chemicals that may damage or smear your lenses, and facial tissue or toilet paper leaves fibers on the lenses and may scratch them as well.

Follow the microscope manufacturer's instructions for cleaning. Don't over-clean. In ordinary use, your objective lenses—other than your oil-immersion objective—may not need to be cleaned from one year to the next, and unnecessary cleaning risks wear or damage to lens coatings. The eyepiece lens may accumulate skin oils transferred by your eyelashes. Clean it as often as necessary. Clean the oil-immersion objective each time you use it.

Immersion oil

Most objectives of 40X or lower magnification are designed to be used with only air separating the bottom of the objective lens from the top of the coverslip. *Oil-immersion objectives*, including the 100X objectives common on high school and university grade microscopes, are designed to be used with a drop of oil bridging the gap between the objective lens and the coverslip.

> A typical microscope includes 4X, 10X, and 40X objectives designed to be used dry and a 100X oil-immersion objective. In casual writing or conversation, the first two objectives are often called “low” and “medium”. To avoid ambiguity between the 40X and 100X objectives, the former is usually called “*high and dry*” or “*high-dry*”.

Avoid using an oil-immersion objective without oil, because image quality is noticeably poorer if only air separates the objective lens from the coverslip. There are two exceptions to this dictum. First, at times you will need to change magnifications back and forth repeatedly, for example, to locate objects at low magnification and then zoom in on them at high magnification. Second, oil-immersion works best with permanently-mounted coverslips. With temporary wet mounts, the coverslip is free to slide around on a thin layer of water or other mountant. With a drop of oil linking the loose coverslip to the objective lens, the coverslip tends to stick to the oil and objective lens, so only the slide itself moves. In either of these situations, it may be necessary to use the oil-immersion objective without oil, accepting lower image quality.

Don't use just any oil. An oil-immersion objective is optimized for oil with a refractive index of about 1.54, which is the RI of commercial oil-immersion oil. Commercial oil-immersion oil is also formulated to avoid damaging the objective (specifically, the adhesives used to glue the lens elements) and to be non-gumming. For all these reasons, you should use only commercial oil-immersion oil with your microscope, ideally *Cargille Type A immersion oil*. It's usually sold in small containers, often 5 to 10 mL, because you use only a drop at a time. Even a small bottle lasts a long time.

Okay, we admit it. We've sometimes found ourselves fresh-out of Cargille Type A oil and substituted another oil. In a pinch, we've raided the kitchen for ordinary olive oil (RI ~1.47) and found it an acceptable substitute.

That said, if you use a substitute oil and your objective lens falls off, don't blame us.

NEVER allow immersion oil to remain on the lens after you finish a session. Even the best oil will eventually gum up your objective lens, making it difficult or impossible to clean thoroughly. Worse still, that oil can eventually penetrate the lens mounting and get between the individual lenses in the objective. If that happens, the only realistic alternative is to replace the objective.

After you finish your session, clean the oil-immersion objective with an approved lens-cleaning solution and dry it with lens-cleaning tissues. Follow the precise cleaning instructions provided in your microscope manual. If you were using a prepared slide, don't forget to clean the slide as well.

Photomicrography Equipment

Photomicrography is the process of recording images through a microscope. (Microphotography, conversely, is the process of making very tiny photographs, such as the microdots formerly used by spies.) Using a camera to record the specimens you observe with your microscope is a very useful adjunct to narrative descriptions and sketches of your observations.

The ideal setup for photomicrography is a microscope with a built-in digital camera, many of which can record high-resolution video in addition to still images. Such a setup vastly simplifies getting good images, particularly with respect to focusing and exposure. With many models, you can view the live image on a computer display as you record it. The downside is cost. Even the least expensive built-in cameras, despite their relatively low image quality, add hundreds of dollars to the cost of the microscope. Better models add thousands. And, of course, because the camera is intimately tied to the microscope, it's not easily upgradeable and failures can be very expensive and time-consuming to have repaired.

The second option is an add-on camera specifically designed to be used with a microscope. These cameras are installed in place of the vertical eyepiece on a dual-head or trinocular microscope, although some can be used with binocular models by installing the camera in place of one of the eyepieces. The least expensive models cost less than $100, but typically offer only 640X480 resolution. Better models cost several hundred to several thousand dollars

If you have a digital SLR, you already have the core of an excellent photomicrography setup. A typical DSLR, even an entry-level model, has better resolution and more capabilities than even most high-end dedicated microscope cameras. For example, few dedicated microscope cameras have resolutions greater than 8 megapixels, support the superior RAW image format, or have auto-bracket or extended dynamic range (XDR) features.

To use a point-and-shoot camera with your microscope, check the manufacturer's website for a microscope adapter. For a DSLR, either purchase the dedicated microscope adapter made by the camera manufacturer or mount the camera to the microscope with a T-ring and a generic adapter. We used the Edmund Optics Microscope Adapter (#41100) and a Pentax K-r DSLR with a K-mount T-ring adapter to shoot many of the photomicrographs in this book.

Even if you don't have a dual-head microscope or a camera adapter, it's possible with patience and trial-and-error to shoot usable photomicrographs merely by setting a point-and-shoot digital

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camera to macro mode and holding it up to the microscope eyepiece. If you attempt this method, you may get better results if you use a short length of cardboard or plastic tube between the eyepiece and the camera lens to block extraneous light and make alignment easier.

Slide Preparation Equipment and Supplies

Even if you intend to use mostly purchased prepared slides in your studies, you'll still need the basic equipment and supplies required to make your own slides. If you're a student, you need to learn how to make slides as a basic lab skill. If you're involved in DIY bio or doing other research of your own, you may be making a lot of slides, so you might as well start from day one with all of the equipment and supplies you need to make your life easier.

The amount of time and effort needed to make a slide varies widely. You can make a simple stained smear mount of bacteria or other microorganisms in a few minutes. Histology tissue specimens take much longer. If you need to dehydrate a specimen, embed it in paraffin, section it, and stain it, you may be looking at an hour or more of actual work, possibly spread over several days. But no matter what type of slides you're making, having the right equipment minimizes the amount of time and work required.

Slides

Microscope slides are rectangular pieces of thin, flat, transparent glass or plastic that are used to mount and view small specimens with a compound microscope. The standard and by far most common size is 1x3" (25x75mm), although slides for specialized purposes can also be purchased in 2x4", 4x4", and other sizes. Standard glass slides are nominally 1.1mm thick, with acceptable variation of ± 0.1mm. (The older standard was 3/64" thickness, which translates to about 1.19mm, so 1.2mm slides are quite common.)

> Plastic microscope slides and coverslips are readily available, but that doesn't mean you should use them. They eliminate the danger of getting glass slivers in your skin, but that's about the best that can be said about them. Plastic is perfect for elementary school students, but completely unsuitable for any serious work. Even the best plastic slides and coverslips are optically inferior to glass.

You might expect something as simple as a microscope slide to be a fungible commodity. That's far from true, however. A good slide must meet numerous requirements. It must be flat and of even thickness, without any bubbles, streaks, unevenness, or other flaws in the glass. The glass itself must be colorless and homogeneous. Sharp edges must be polished smooth, and the slide must be pre-cleaned of all dust or other contaminants. The cheapest slides fail on some or all of these counts. Fortunately, even high-quality slides—most of which are also made in China or India—cost only a few dollars per box of 72.

> We prefer to use slides that have one end frosted for labeling with a pencil. With plain glass slides, the alternative is to use sticky labels or a glass marking pen.

In addition to a good supply of flat slides, you'll probably want at least a few *well slides*. These are considerably more expensive than flat slides, but they're useful for tasks like observing live microorganisms in a drop of pond water. They're also useful for making permanent whole mounts of specimens such as small insects. There are two types of well slides:

Shallow-well slides

Shallow-well slides, often called *depression slides*, are of standard thickness (~ 1.2mm) and have one or more shallow circular depressions. To use them, you simply place a drop or two of your pond water or other specimen in the well and cover it with a coverslip. Sooner or

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later—sooner if you're using quartz-halogen illumination—the water drop will evaporate. You can delay that by using a dab of glycerin or petroleum jelly to make a seal between the slide and the coverslip.

Deep-well slides

Deep-well slides, often called *cavity slides*, are about three times thicker than standard slides and have one or more deep cylindrical cavities. These slides are ideal for confining small live insects (such as ants) and similar tasks. The wells in these slides are deep enough to allow using the *hanging-drop method* for observing the motility of protozoa, as described in chapter 1. If you're going to have only one type of well slide, go for the deep-well version. You can substitute it for a shallow-well slide, but not the converse.

If you don't have a well slide handy when you need one, make your own. Use super glue (or even petroleum jelly) to affix a small rubber or plastic washer to a flat slide. Use a standard coverslip.

Never buy flat microscope slides by the dozen. First, most slides that are sold by the dozen are very poor quality. Second, you'll pay a significant premium versus buying them in standard 72-slide (half gross) or 144-slide (gross) boxes. Third, you can easily use up a dozen slides in one session.

Slides have one other important use that's completely unrelated to microscopy. They're perfect for making home-made *thin-layer chromatography (TLC) plates*, which have numerous uses in a biology lab.

Commercial TLC plates are readily available, but expensive. TLC plates the size of a microscope slide sell for a buck or two each, and you usually have to buy them in boxes of 50 or 100. With just some microscope slides, a few inexpensive chemicals, and some basic labware, you can make up your own TLC plates for about a dime each. And most of that is in the cost of the slide, which can be recycled over and over to make new TLC plates.

Making the plates is easy, but a bit messy. Robert has a video on his YouTube channel that illustrates the process:

http://www.youtube.com/watch?v=pNDQkM3jasA

With a supply of homemade TLC plates and a Coplin jar (described later in this section), you're prepared to do TLC. With a bit of care, your results should be about as good as if you used expensive commercial TLC plates.

Coverslips

A microscope *coverslip*, also called a *coverglass*, is a square, rectangular, or circular piece of extremely thin, flat, transparent glass or plastic that is used to cover a specimen that has been mounted to a slide. In addition to protecting the specimen (and the objective lens from contacting the specimen), coverslips actually function as part of the optical train—microscope objectives are designed on the assumption that a coverslip will be positioned between them and the specimen—so you should never observe a specimen without a coverslip in place.

The truth is that we frequently observe slides without coverslips, particularly for quick looks at bacteria smears. Yes, we give up some image quality by not using a coverslip, but sometimes the time saved is worth the trade-off.

Coverslips are available in four thicknesses, denominated #0 (0.083 to 0.13mm), #1 (0.13 to

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0.16mm), #1.5 (0.16 to 0.19mm), and #2 (0.19 to 0.25mm). #0 coverslips are specialty items that are not stocked by most vendors. #1 and #2 coverslips are widely available, but #1.5 coverslips are harder to find. Fortunately, #1 coverslips are acceptable for nearly any purpose.

Some texts recommend using only #1 coverslips, and there's actually a good argument in favor of that. If your objectives are designed for use with #1.5 (or even #2) coverslips, #1 coverslips cause very little image degradation.

The advantage of using #1 coverslips may become apparent the first time you use your oil-immersion objective. At that high magnification, depth of focus is nearly nil. If you're using a #2 or even a #1.5 coverslip, you may find that you cannot focus sharply on the specimen—particularly a specimen that has depth—without ramming the objective into the coverslip. For that reason, we use #1 coverslips by default, particularly for permanent mounts, even though our objective lenses are designed for #1.5 slips.

Many microscope objectives are labeled with the recommended coverslip thickness or range of thicknesses. High-end objectives may be adjustable to optimize them for a range of thicknesses, typically something like 0.11mm to 0.23mm. Less expensive objectives are not adjustable, but are designed to use coverslips of one particular thickness, typically 0.17mm, or about the middle of the acceptable variation for #1.5 coverslips.

Most objective lenses are labeled with the coverslip thickness they're designed to use. For example, Figure C-4 shows the objective lenses of a typical microscope. That “0.17” label visible on the objective lenses indicates that these objectives are designed to use a 0.17mm coverslip.

Using a coverslip that is thinner or thicker than the objective is designed for introduces chromatic aberration and reduces the quality of the image. For objectives with an NA of 0.4 or less, coverslip thickness is not a major issue. With 60X to 100X oil-immersion objectives, coverslip thickness becomes more important.

Handle with care

Manipulate coverslips with forceps to protect the coverslip from skin oils and other contamination and to protect your fingers against glass shards. We actually handle coverslips with thin latex or nitrile gloves, but until you're used to manipulating them it's safer to use forceps.

Coverslips are available in a variety of sizes and shapes. The most common and least expensive for any given quality level are square, and are readily available in sizes from 18mm to 24mm. (Larger sizes allow larger specimens, but conversely they also give you more area to search, particularly if you are using high magnification.) Rectangular coverslips, such as 22x40mm, are less common but still readily available. These are useful for mounting very large or multiple specimens on a single slide.

Although it appears odd to most people at first glance, there's nothing wrong with using two or three coverslips to mount multiple specimens on one slide. Multiple mounts on one slide can make some tasks a lot faster, because you can quickly flip back and forth between specimens without changing slides.

Round coverslips are much less common now than they were a few decades ago. Before about 1970, many slides were prepared using Canada balsam (essentially, highly refined pine tree sap) as a mounting fluid. Even exposed to air, Canada balsam remains tacky for some time, so the final step in slide preparation was to place the slide on a turntable and rotate the slide while

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trimming off any excess Canada balsam that had squooshed out from under the cover slip. That's easy to do if the coverslip is circular, but impossible if it's rectangular or square.

Although Canada balsam is still available (and still preferred by some microscopists), it has been largely replaced by Permount and similar synthetic mounting fluids that dry without tackiness. Consequently, trimming excess mountant is no longer necessary (although it still makes the final slide look neater), and round coverslips are going the way of the dodo. It doesn't help that round coverslips typically sell for several times the price of square ones of similar size and quality.

Slides and coverslips are inexpensive enough that we often use them once and discard them, but many people do recycle them. If you want to do that, swirl them in sudsy tap water, rinse them with distilled water, and then use a final rinse of pure acetone. Place slides in a slide-storage box to drain and dry. Place coverslips on a clean sheet of lint-free paper or cloth to dry.

Some microscopists store slides and coverslips in jars of acetone or absolute alcohol, which keeps them clean and sterile. When you remove one from the jar, the liquid evaporates rapidly, leaving you with a clean, dry slide or coverslip.

Butane lighter or alcohol lamp

A disposable *butane lighter*, *alcohol lamp*, or other flame source is needed to *heat-fix* bacterial smear slides. Heat-fixing kills any live bacteria present and causes them to stick to the microscope slide. (Otherwise, they'd be washed away during staining or mounting.) To heat-fix a slide, allow the smear to dry and then pass the slide—bacteria side up—several times through the naked flame. If you don't heat it long enough, the bacteria won't adhere. If you heat it too long, the bacteria explode or char. You'll soon get a feel for it. A butane lighter is convenient for heat-fixing one slide at a time. If you're making many smear slides, an alcohol lamp is better.

Forceps and slide tongs

Forceps are what non-scientists call tweezers. They're available in many sizes and styles in plastic or metal. For manipulating cover slips, we prefer the plastic variety. *Slide tongs* are similar to test tube tongs, but designed to grip a standard size microscope slide. They're used to hold a slide during heat fixing or to manipulate the slide during staining procedures. They can also be used to manipulate coverslips.

Mountants

A *mountant* or *mounting fluid* is a liquid that is introduced between the slide and the coverslip. An ideal mountant is clear, colorless, and (for best image quality) has a refractive index very close to that of the crown glass used in coverslips (RI = ~1.54). If it is to be used to make permanent mounts, it should dry without shrinking, cracking, or bubbling. If it is to be used to make temporary mounts, it should not evaporate quickly. In any case, it must not rupture, fade, or otherwise damage specimens.

The most common mountant by far among amateur biologists is ordinary distilled water. Although its refractive index, about 1.33, is lower than ideal, it's much better than having an air gap (RI = 1.00) between the slide and coverslip.

If distilled water is unsuitable, you can substitute *glycerin*, also called *glycerol* in straight or diluted form. Glycerin evaporates much slower than water, days to weeks versus minutes to hours.

If you're observing live protozoa (such as paramecia), you'll need some way to slow them down. In water, a paramecium can travel 10 or more body lengths per second, so just keeping it in the

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field of view can be challenging. The best solution to this problem is to mount the slide with an aqueous solution of *methyl cellulose*, which is much more viscous than water.

> Methyl cellulose is a *physical immobilizer*, which simply means that protists have a hard time swimming through this viscous fluid. (Glycerin is another immobilizer in this class.) A *chemical immobilizer* slows down protists by fatiguing them. Two common chemical immobilizers are a 3% solution of copper(II) acetate and a 1% solution of copper(II) sulfate.
>
> If you have the kit for this book and you want to try a chemical immobilizer, mix a few drops of Barfoed's reagent—which is 6% copper(II) acetate—with the same amount of distilled water. Or simply use the Barfoed's reagent without dilution, but make sure there's a least a couple drops of water in your wet mount and use only a small drop of Barfoed's.

Finally, if you want to make permanent slides, you'll need a permanent mountant such as *Permount* or *Canada balsam*. (We think Permount is better in every way than Canada balsam, but some traditionalists continue to prefer the latter.) These mountants dry clear and transparent, with little or no color, essentially cementing the coverslip to the slide. You can substitute colorless nail polish, such as Sally Hansen's Hard As Nails. We have slides five years old or older that were mounted with Sally Hansen's Hard As Nails and they are as good now as when they were made.

Biological Stains

Biological stains are natural or synthetic dyes that selectively bind to different parts of cell structures. For example, one stain may bind strongly to the cell wall, coloring it intensely, while leaving the nucleus untouched. Another stain may do the converse.

Most people don't realize how important stains are to biologists. The first problem microscopists faced was the fact that most of the things they wanted to look at were opaque. They solved that problem by cutting very thin sections with microtomes. But that left another serious problem. Most of those thin sections were essentially transparent, with almost no contrast between different parts of the cell structure. Back in 1858, someone had the cunning idea of using *carmine*, a dye obtained from female cochineal beetles, to stain sections. Sure enough, carmine was differentially absorbed by different parts of the cell structure, increasing contrast immensely.

> For school work or if you're just experimenting with staining, it's cheaper and easier to buy small amounts of prepared stains individually or in kits rather than making them up yourself. If you use stains in larger volumes, as some DIY biology hobbyists do, making up the stains yourself from raw chemicals is both cheaper and more flexible. Instructions for making up various stains are readily available on-line.

Ironically, despite the fact that biologists have tried literally tens of thousands of different stains in the intervening 150 years, the most widely used stains today are the first two stains that came into common use after carmine. They're *hematoxylin*—which for some reason many people (including some biologists) pronounce he-muh-TOX-uh-linn rather than the correct he-MAT-oh-ZYE-linn—a dye derived from logwood, and *eosin*, one of the first synthetic dyes. They were first used in about 1865 in the so-called *H&E staining protocol*, and are still used that way today. In fact, H&E is probably the most commonly used staining protocol even today, with the possible exception of the *Gram staining protocol*.

> Although there is considerable overlap, biological stains are broadly grouped into *bacteriology stains*, used to stain bacteria, and *histology stains*, used to stain tissue sections from plants and animals. Most bacteria are so tiny that the goal of

staining them is sometimes simply to make them visible against the background clutter. Histology stains, conversely, are used to selectively stain different cell components to reveal internal cell structures. The Gram staining protocol is one example of a bacteriology staining protocol; the H&E staining protocol is an example of a histology staining protocol.

There are literally scores of biological stains and staining protocols in common use, and hundreds in occasional use, although most home biologists can get by with just a few stains. Even with our tendency to accumulate neat stuff, we have “only” 31 different stains at our microscopy station. We consider two stains to be essential for any microscopist:

Methylene blue

Methylene blue is a general primary stain used for nuclear material and other acidic cell components. It's one of the most widely used primary stains, and is effective on animal, plant, bacteria, and blood specimens. Various types of methylene blue stains are available commercially, from simple aqueous solutions of the dye to versions that incorporate sodium hydroxide or potassium hydroxide to versions that use various alcohols and other organic solvents instead of water. For routine use, any of them suffice. You can substitute methylene blue in most staining protocols that call for carmine or Janus green B.

Eosin Y (1% aqueous)

Eosin Y, usually supplied as an aqueous solution, is a general primary stain for cytoplasm material, which it stains pink or red. It's also one of the two stains used in the popular H&E (hematoxylin & eosin) staining protocol. Eosin Y may be substituted in most staining protocols that call for congo red or neutral red.

For some staining protocols you can substitute erythrosin B, also known as FD&C Red #3. Results are generally inferior to those obtained with eosin Y, but are still quite usable. The red food coloring dye sold in grocery stores is usually a mixture of erythrosin B and Allura Red AC (FD&C Red #40). We've used this mixture in diluted form with reasonably good results.

Your next priority should be the following three stains, which are needed for the bacteriology Gram staining protocol.

Hucker's crystal violet

Hucker's crystal violet is the primary stain in the Gram staining protocol. Gram-positive bacteria retain this stain after decolorizing, while Gram-negative bacteria do not. A 1% crystal violet solution is sold in drugstores, usually under the name gentian violet. This solution is usable for Gram staining, but inferior to Hucker's crystal violet.

Gram's iodine

Gram's iodine functions as a mordant in the Gram staining protocol, binding the crystal violet stain to Gram-positive bacteria, but it is also useful as a primary stain in its own right. Gram's iodine stains the starch in plant cells dark blue or black and the glycogen in animal cells red. You can substitute drugstore 2% iodine tincture diluted one part tincture to five parts distilled water

Safranin O

Safranin O is a red counterstain usually used for Gram staining, and is also used as a primary stain for cartilage, mast-cell granules, and mucin. You can substitute eosin for most purposes, including Gram counterstaining, although safranin O is preferable for the Gram protocol.

A *primary stain* is used to differentiate the structural element that is of primary interest. A *secondary stain* (or *counterstain*) is one of contrasting color to the

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primary stain that is used to provide additional color contrast by staining other structural elements. For example, in the H&E protocol, hematoxylin is the primary stain, which stains nuclei (and a few other structures) blue. Eosin is the counterstain, and stains other structures red, pink, or orange. A stain may be used as a primary stain in one staining protocol and as a counterstain in another.

With just these stains, you're equipped to use the most important staining protocols. If you have room on the shelf for a few more, we'd choose *toluidine blue* (for staining nuclear material during mitosis and also as a fast alternative to H&E staining), *Sudan III* (for staining lipids), and *Wright's blood stain* (for staining, uh, blood). If you intend to explore histology in any depth, you'll need *hematoxylin* to use the H&E staining protocol. (You can substitute methylene blue for hematoxylin in the H&E protocol, although the differentiation is usually inferior to that obtained with hematoxylin.)

If you want to try staining without purchasing special biological stains, you can try various stains that are readily available locally. You can view an excellent article at *www.crscientific.com/microscope-stain.html* about using standard food coloring dyes as biological stains.

In addition to the stains, you'll also need various other chemicals that are used for *destaining*, also called *decolorizing* or *clearing*, which is the procedure used to remove excess stain from a specimen. Common destaining agents include distilled water, dilute hydrochloric acid, ethanol, isopropanol, and acetone. Although all of these are available in very pure form, called *histology grade*, we've used ordinary lab grade, USP grade (from the drugstore), and even technical grade (from the hardware store) destaining chemicals without any problems.

Vital stains, also called *supra stains* or *supravital stains*, are those that can be used to stain living organisms without harming them. Many common stains can be used at high dilutions as vital stains, including methylene blue and eosin Y. When used for staining living organisms, the stains are highly diluted, usually in the range of 1:5,000 to 1:500,000 (versus typically 1:100 to 1:1,000 for non-vital staining).

??? Production: If we're short on space, we can drop the following sidebar. I just thought it was a really neat story. RBT

Serendipity or Urban Legend?

The Gram staining protocol was devised by Danish physician Hans Christian Gram in 1884. It involves first staining a specimen with a solution of crystal violet (also called Gentian violet), which stains all of the bacteria purple. An iodine-iodide solution is applied next to fix the violet stain. Rinsing with alcohol removes the stain from some but not all bacteria. The specimen is then counterstained with safranin O, dried, and viewed. Those bacteria that retain the crystal violet are stained blue or violet, and are called Gram-positive bacteria; those that did not retain the crystal violet are stained pink or red by the safranin O counterstain, and are called Gram-negative bacteria.

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Gram staining was a revolutionary technique for bacteriologists and epidemiologists worldwide. Its value was that it allowed scientists to quickly discriminate between some pathogenic (disease-causing) bacteria and other harmless bacteria that appeared visually similar to the pathogens. Even today, Gram staining is one of the two most widely-used staining protocols and is routinely used for quick screening.

After Gram introduced his eponymous staining protocol, other scientists immediately began tweaking it, trying slightly different formulations and concentrations. Over the next 45 years, dozens of variants were announced, but it turned out that none of them had any great advantage over the original protocol. One thing all of these variants had in common was using aniline, then called analin oil, as a component of the violet stain. Eventually, a more-or-less standard Gram staining protocol that incorporated the best of the variations came into common use.

That changed in 1929, when Yale undergraduate bacteriology student Thomas Hucker introduced a variant that immediately became the standard and has been used universally ever since. It all started when Hucker's professor asked him to prepare a paper to present at a biology conference. Hucker asked his professor for topic suggestions, and the professor recommended that Hucker contact hospitals and university labs to ask them about their specific methods for using Gram staining. Hucker intended to compare and contrast these protocol variants in his paper.

Replies soon began to arrive in the mail, but Hucker was disappointed to find that every organization he contacted was using essentially identical protocols. With one exception. Dartmouth College reported that instead of anilin oil they were using ammonium oxalate in the crystal violet stain. Hucker tried this ammonium oxalate variant, and found that it indeed yielded much better results than the methods using anilin oil. He wrote up his paper, sent a copy to the Dartmouth bacteriology department, and thanked them for their advice.

A few days later, Dartmouth called Hucker and told him that he must be mistaken. They were using the same protocol, with analin oil, that everyone else had reported using. They'd never heard of using ammonium oxalate. Hucker was puzzled, but he knew what he'd seen, so he presented the paper at the conference, to great acclaim.

After returning from the conference, Hucker decided to find out what was going on. He boarded a train and visited Dartmouth. He met the head of the bacteriology department, who once again denied having any idea what Hucker was talking about. He'd been on vacation when Hucker's original letter arrived, and denied having even seen the letter, let alone responded to it. Deciding to get to the bottom of this puzzle, the bacteriology chairman started walking around the building, collaring people and asking if they had any idea what had happened.

He finally got his answer in the chemistry department, which was situated next door to his own department. Apparently, one of the chemists had gotten Hucker's original query letter and had taken it upon himself to respond. He wandered next door to the bacteriology department and noticed that a bottle of the stain had “A.O.” listed among the contents on the label. Of course, that was shorthand for “anilin oil”, but the chemist for some reason decided that “A.O.” must be an abbreviation for ammonium oxalate. He so informed Hucker by return mail, and the rest is history.

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As pretty as this story is, we wonder if it's really true. A biologist might conceivably abbreviate analin oil as “A.O.” but no chemist would assume that “A.O.” was an abbreviation for ammonium oxalate. “NH4 Ox” perhaps, or even “Amm Ox”. But not “A.O.”. On the other hand, if it didn't happen that way, how did it happen? Whether the story is true or not doesn't really matter. What matters is that, by pure accident, Hucker discovered a much superior Gram staining protocol, which has been in universal use since 1929.

Coplin staining jar

Although it's not essential for some staining tasks, a *Coplin staining jar*, shown in Figure C-11, is a handy item to have around. These jars are available in glass or polypropylene and have built-in grooves to support microscope slides vertically, usually ten slides in five back-to-back pairs. To stain a slide, you simply fill the jar a bit more than halfway with the staining solution and slide the slide into one of the grooves.

A Coplin jar is particularly useful in two situations. First, if you have many slides to be stained with the same stain, it's much more convenient to batch-stain them than to stain each slide individually. Second, although some stains operate very quickly—a few seconds to a minute or two—some other stains must be left in contact with the specimen much longer, sometimes as long as several days. We keep half a dozen Coplin jars on hand for just these reasons.

Insert graphic here. Use file hbl-0C-11.jpg

Figure C-11. A Coplin staining jar (image courtesy United Scientific)

A Coplin staining jar is also a perfect chromatography jar for developing the homemade TLC

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plates described earlier in this section.

Slide storage

Wet mounts are temporary. You make the mount, observe the specimen, and then discard or recycle the slide and coverslip. But if you intend to make permanent mounts, you'll need an organized way to store the slides.

The most popular solution is a slide storage box. These are available in wood or plastic from most lab supplies vendors, often in many colors. Boxes typically store 10, 12, 25, 50, or 100 slides, each in its own numbered partitioned. A label area provides a numbered line for each slide.

Slide storage books or wallets are a bit harder to find, but some microscopists (including us) prefer them. Each page stores (typically) a half-dozen to a dozen slides, in one or two columns, with room for detailed labels rather than just one-line descriptions. Although these books are more expensive and occupy more space than slide boxes, the slide labels are much more visible and it's much easier to remove and replace the slides.

Whichever method you use, if possible store slides flat rather than vertically. Back in the Bad Olde Days when we made permanent mounts with Canada balsam, slides stored vertically for months to years were sometimes ruined when the coverslip detached or simply slid down the slide. Almost as bad is when your nicely-centered specimen floats down to the bottom edge of the coverslip under the force of gravity. That's not supposed to happen with modern mountants like Permount, but we'd rather not find out the hard way.

If you have imaging equipment, you may not need to store slides at all. One slide may require a dozen or more images to show all of the features of interest on that slide, but you can store hundreds to tens of thousands of high-resolution images on something as small and durable as a USB flash drive. Just make sure to back it up.

Culturing Equipment and Supplies

Culturing is the procedure used to grow microorganisms in a controlled environment. Many microorganisms reproduce very quickly. Under ideal conditions, some can double their numbers every 20 minutes or less. In other words, if a single microorganism is present to begin with, after 20 minutes there are two, after 40 minutes there are four, after 60 minutes there are eight, and so on. That may not sound impressive until you realize that after 10 hours (30 doubling periods) have elapsed, that single microorganism has become more than a billion microorganisms.

All of that assumes that the microorganisms have space to grow and food to feed them. That's the purpose of culturing. Culturing provides surface area (or volume) and the nutrients necessary for growth. We'll use culturing to produce large numbers of microorganisms for various lab sessions in this book. But culturing isn't limited to lab procedures. Wine and beer making uses culturing on a large scale, as does cheese making, biofuel production, and many other endeavors. In fact, you've probably unintentionally cultured bacteria yourself. If you've ever opened a bottle of milk and found it'd soured, you were holding a bottle of a bacteria culture.

Autoclave

If you're working with microorganisms, it's important to be able to kill them reliably. For example, if you've cultured an unknown bacterium, it's irresponsible to toss it out with the household garbage unless you're certain no live bacteria remain.

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There are many ways to kill microorganisms, including chemical action, flame, dry or wet heat, ultraviolet light, and ionizing radiation such as X-rays or gamma rays. In a home biology lab, the first three methods are the most practical.

> To *sterilize* something means to kill all microorganisms present. To *disinfect* or to *sanitize* something means to kill almost all of them. (That's why we call that goopy stuff hand sanitizer rather than hand sterilizer.)

Chemical disinfectants, such as chlorine laundry bleach or Lysol, may kill 99.999% or more of the live microorganisms present, but they are not 100% effective. For many purposes, that's good enough. For example, if you need to dispose of a live bacteria culture growing in a Petri dish, the traditional method is to soak the dish overnight in a 1:4 solution of chlorine bleach and water. That kills very nearly 100% of the live bacteria present—not to mention viruses, protists, molds, fungi, and any other living things—at which point there is no longer a biohazard and the culture can be discarded with the household trash or flushed down the drain.

The problem is, some bacteria form spores, which are resistant to chemical disinfectants, including bleach. If you're disposing of the material, spores aren't usually a problem. There are zillions of spores surrounding you, on every surface in your lab and floating on dust motes in the air. Spores become a problem only when you're making up culture plates or culture tubes, which must be sterile. If any spores are present in the culture media or on the plates or tubes, they'll germinate and contaminate the colonies you're attempting to grow.

Flame kills spores quickly, and is commonly used to sterilize inoculating loops and the mouths of culture tubes when you're doing a transfer. But flame can't be used to sterilize the culture media itself. That's where the third method comes in: heat.

Most spores are very resistant to moderately high temperatures. Boiling water or steam at 100 °C kills most spores, eventually, but it may take literally hours to days to do so. Fortunately, boosting the temperature slightly and using steam—121 °C steam is the standard used for sterilization in biology labs—reduces the time needed to a few minutes.

An *autoclave* sterilizes contaminated materials by exposing them to high temperatures, usually in the presence of pressurized steam. Autoclaving can be done dry, but that requires higher temperatures and longer treatment, and can be used only for glassware and other temperature-resistant materials. Steam autoclaving can be used to sterilize autoclavable plastic items, which would melt at the temperature needed for dry autoclaving.

Commercial autoclaves cost hundreds to thousands of dollars. Fortunately, you don't need a commercial autoclave. A pressure cooker from WalMart or Target works just as well. If your pressure cooker doesn't come with a rack, also purchase an oven rack that fits inside the pressure cooker. You'll need that to keep containers from directly contacting the hot bottom of hte pressure cooker.

> If you buy a pressure cooker to use as an autoclave, be aware of the specifications. For more than a hundred years, standard pressure cookers have operated at 15 PSI. At that pressure, water boils at 121 °C (250 °F), which is hot enough to kill all microorganisms, including bacteria spores, very quickly.
>
> Some current pressure cookers operate at lower pressures, typically about 10 PSI. At that pressure, water boils at only 113 °C (235 °F). That temperature will still kill spores, but it takes much longer. If you already have such a pressure cooker, simply quadruple the time specified for autoclaving

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Incubator

An *incubator* is a device used to maintain a specific environment for culturing. Many environmental factors can affect the efficiency and effectiveness of culture growth, including humidity, level and type of light present, carbon dioxide concentration, and so on. But for most culturing the single most important factor is temperature.

Many microorganisms have evolved to have strong preferences as to their environment. For example, human pathogens (disease-causing microorganisms) evolved in human hosts, so they grow best at normal human body temperature, 37 °C. (That's why you develop a fever when you have an infection; your body is doing its best to make the infecting bacteria unhappy by boosting the temperature of their environment.)

Some microorganisms flourish across a wide range of temperatures—often growing faster as the temperature increases, but only to some maximum value—but many grow best only within a narrow temperature range. An incubator allows you to maintain the temperature best suited to growing whatever you're culturing.

> Although there are exceptions, many human pathogens grow relatively poorly at room temperature. Until you become proficient at handling potentially dangerous microorganisms, culturing at room temperature provides some safety margin. (You should still exercise all precautions; treat any culture as though it might contain bubonic plague.) The only real downside to room-temperature culturing is that it takes much longer. A culture that might flourish overnight at 37 °C may take several days to reach a similar stage at room temperature.
>
> The same caution applies to choice of culturing medium. Until you are proficient, avoid using media such as blood agar that encourage growth of pathogens.

You can purchase commercial incubators from lab supply vendors or on eBay, but that's overkill for most home lab tasks. For a few dollars, you can make your own incubator from a disposable foam cooler, an inexpensive thermometer, an incandescent light bulb, a plastic picnic plate or dish, and an oven rack or kitchen cupboard rack. If you want to get fancy, you can add a small fan to circulate the air and a dimmer switch to control the brightness (and heat output) of the light bulb.

Depending on the size of the foam cooler and the ambient temperature, you might need anything from a Christmas-tree bulb to perhaps a 25-watt bulb to maintain an internal temperature of 37 °C (the most common temperature for incubation of bacteria). To maintain humidity, place a dish of water beneath the rack.

Culturing Containers

You can grow cultures in any imaginable container. Microorganisms, after all, have no problems growing in the wild. However, for best results, you'll probably want to keep at least a few special culturing containers on hand. There are three common types of culturing containers, each used for slightly different purposes: Petri dishes, culture tubes, and flasks.

Petri dishes

A *Petri dish* is a flat-dish with a matching cover of slightly larger diameter. Petri dishes are available in glass and plastic, in a variety of diameters and depths. Some Petri dishes are divided into two, three, or four isolated compartments, which allows you to grow more than one culture in a single Petri dish.

Petri dishes are used with gelling culturing media, such as agar. Typically, you would prepare a

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warm liquid agar solution, fill the Petri dish to a depth of a few millimeters, and place it in the autoclave to sterilize it. Once the dish, with its sterile contents, cools down, the agar solidifies into a gel that resembles Jello. The dish is then carefully inoculated with the microorganism by streaking or flooding, and placed in the incubator.

Petri dishes, shown in Figure C-12, are useful for growing pure colonies—separate groups of specific microorganisms that will subsequently be cultured in broth to produce large numbers of a single microorganism—and for such tasks as testing antibiotic sensitivity with treated disks. Petri dishes are fragile and bulky relative to the alternatives. We keep a few on hand for when they're really needed, but otherwise we use culture tubes or flasks.

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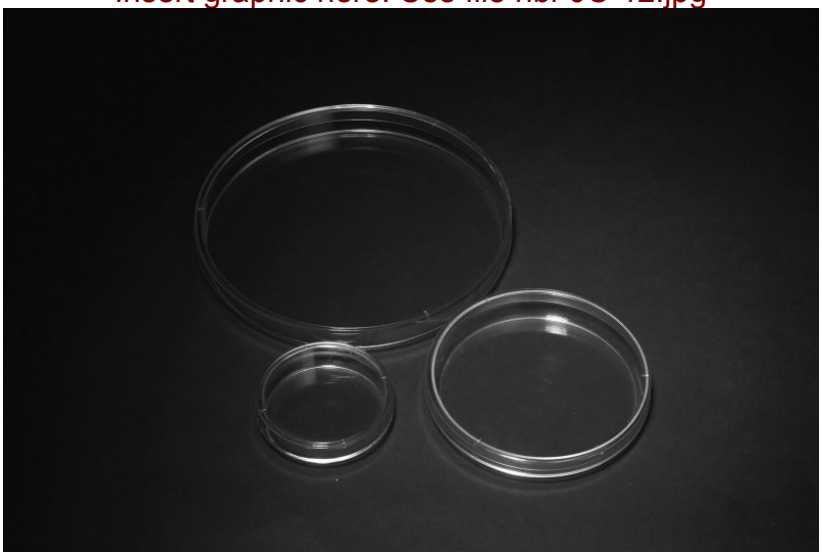

Figure C-12. Petri dishes (image courtesy United Scientific)

Many people prefer plastic Petri dishes, which are sold for a few dollars in sleeves of 10 to 25. Each dish is individually wrapped and sterile. Unfortunately, these dishes are sterilized at the factory using radiation but cannot be re-sterilized at home. (The plastic melts at the temperature needed to sterilize them.) After use, the dish is put in a bleach bath to kill any microorganisms present and then discarded. If you culture infrequently, plastic Petri dishes are a good choice. They're convenient, require no sterilization, and are reasonably economical.

If you culture frequently, you'll want at least a small supply of glass Petri dishes. These must be sterilized before each use, but can be reused repeatedly. Brand-name Petri dishes, such as Pyrex and Kimax, are very expensive. Depending on the size and type, one Petri dish may cost $10 or more. Similar no-name models made in China or India sell for a fifth to a tenth the price, and are perfectly usable. Incidentally, there's no need for borosilicate glass in Petri dishes, which are not subjected to thermal shock. Soda-lime glass is fine, and cheaper.

Culture tubes

A *culture tube* is essentially just a test tube with some means of capping it. Bespoke culture tubes are available in a huge range of sizes, shapes, materials, graduations, screw-top and plug closures, and so on. But simple test tubes with cotton-ball plugs work fine for a home lab, and are very inexpensive.

Culture tubes may be used with solid (gel) culturing media or with liquid (broth) culturing media. In either case, the tubes are filled with non-sterile culturing media, plugged with cotton balls, racked, and then placed in the autoclave for sterilization. For broth media, the culture tubes are allowed to

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cool and then used immediately or placed in the refrigerator for short term storage. For gel media, the culture tube is removed from the autoclave while still warm, and placed at an angle to cool. The agar hardens in place, increasing the available surface area, and producing a *slant tube*. We use culture tubes for about 95% of our culturing.

You'll also need a *wet/dry tube rack* that fits your culture tubes. These racks are made of polypropylene plastic, which can be autoclaved at 121 °C without melting. They're also dense enough that they won't float in a water bath. (If you don't have a rack, you can simply stand the tubes in a glass or polypropylene beaker while they're in the pressure cooker.)

Culture flasks

A *culture flask* is essentially a large-volume culture tube that is used only with broth media. Traditionally, standard Erlenmeyer flasks are used for this purpose. They are filled with culturing broth, stoppered with cotton balls, and sterilized. Like culture tubes, they are best used as soon as possible, but they can be refrigerated for several days without harm.

Culture flasks are used to produce large populations of one microorganism. Unlike gel media, where the microorganisms grow almost exclusively on the surface of the gel, broth media allows growth to occur throughout the media, greatly increasing the number of microorganisms produced and reducing the time needed to do it.

You might use broth culturing, for example, to produce a large sample of bacteria for DNA analysis rather than using PCR to replicate DNA from a smaller sample. Alternatively, you may be less interested in the bacteria themselves than in something they produce. The first example of large-scale broth culturing for this purpose was the early production of penicillin, which took place in industrial-scale culturing vats. Many antibiotics are produced the same way today.

For home lab work, the most convenient culturing flasks are 125 mL, 150 mL, or 250 mL Erlenmeyer flasks, which are readily available inexpensively. If you have the choice, buy the narrow-mouth versions, which are more easily stoppered, rather than wide-mouth versions. You can often get a significant discount by buying a case of 12 or 24.

Culturing Media

Some microorganisms aren't picky about what they eat. They'll grow on almost anything that is moist and has some food value to them. Other microorganisms are very selective about the media they'll grow on.

Culturing broth is simply a liquid that contains nutrients. Microorganisms require carbohydrates (sugars or starch), nitrogen, and trace nutrients. For culturing many bacteria species, diluted chicken or beef broth (strained or dipped to remove oils and grease) with some added table sugar works well.

Culturing gel is made up from agar, a material derived from algae. Like gelatin, agar is mixed with hot water and then cooled to form a gel. (No, you can't use gelatin for culturing; bacteria eat it and turn the gel into a runny mess.) Agar itself is merely an inert substrate. With the exception of a few marine bacteria, few microorganisms actually feed on agar. To provide food for the microorganisms, you add various nutrients to the agar mixture as you're making it up.

Food-grade agar is sold in supermarkets (often in the vegetarian section) for a few dollars an ounce. *Culturing-grade agar* is sold by lab supply vendors, and is more expensive. *Agarose* is refined from agar by removing agaropectin to leave pure agarose. It is used for gel electrophoresis and may cost a dollar a gram in small

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quantities. Is there really a difference, or can you use food-grade agar for culturing and electrophoresis? Well, it depends...

All agar is derived from algae, but most food-grade agar is produced from the algae *Gracilaria lichenoides*, while culturing-grade agar is produced from a different algae, *Gelidium sesquipedale*. Culturing-grade agar gels at about 35 °C versus about 42 °C for food-grade agar, but the important difference is in matrix strength. Gels formed with culturing-grade agar are about twice as rigid as those formed with food-grade agar, which means culturing-grade agar can be used in concentrations as low as 0.5% (0.5 grams/100 mL) and still form a usable gel. Food-grade agar must be used at twice or more the concentration to form a gel with similar qualities. That said, we have used food-grade agar successfully in the past and will continue to do so.

Agarose is recommended for gel electrophoresis because the agaropectin present in food- and culturing-grade agar restrains the movement of fragments through the gel, reducing the sharpness of the separation. If you're doing serious gel electrophoresis, no question, use agarose whatever the price. But if you're simply doing gel electrophoresis as a learning experience, food-grade agar is perfectly acceptable.

It's less expensive to make up your own culturing broths and gels, but many biologists prefer to use commercial products. These are available from Carolina Biological Supply and other vendors as dehydrated powders that contain a mixture of agar (if it's intended to produce a gel rather than a broth) and nutrients. You make them up just as you would plain broth or agar, but without adding any additional nutrients.

If you get seriously involved with culturing bacteria and other microorganisms, you'll probably need many different types of agar gels and nutrient broths. For example, at one time or another we've used standard nutrient agar, plate-count agar, potato-dextrose agar, beef-extract agar, beer agar, Luria agar, tryptic-soy agar, McConkey agar, tri-sugar iron (TSI) agar, brain-heart infusion (BHI) agar, Sabouraud dextrose agar, malt-extract agar, and probably half a dozen or more others we've forgotten.

Rather than purchasing these as agar powder products or even powdered nutrients—many of which have limited shelf lives—it's cheaper and more efficient just to stock the necessary individual nutrients such as peptone, dextrose, and so on, and make up the various types of agar or nutrient broths as needed.

Pre-made agar gel of various types is available in sterile bottles, which may require refrigeration before use. To use the pre-made gel, you place the bottle in a hot water bath to remelt the gel and then pour it into sterile containers. Pre-made agar gels are convenient but expensive.

Culturing Accessories

In addition to the major items already described, you'll need a few accessories. Most of those—a beaker and hotplate or kitchen stove burner (or a microwave oven) for making up agar, graduated cylinder for measuring liquids, and so—are items found in most homes or are general labware, but there are a couple of special items.

An *inoculating loop* (or *inoculating needle*) is a short piece of nichrome wire in a metal, wood, or plastic handle. It's flame-sterilized before each use, and is used to streak culture plates or tubes or to transfer material to culturing broth. You can make your own inoculating loop by embedding a sewing needle in a wooden dowel. Some people prefer disposable plastic

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inoculating loops/needles, which are sold inexpensively in individually-wrapped sterile packages.

To minimize contamination by airborne microorganisms while you are inoculating culture containers, keep one plastic *spray bottle* filled with water and a second filled with Lysol or a similar disinfectant handy. Before you inoculate your Petri dishes or culture tubes, dampen the work surface slightly with Lysol spray and spray the air in the work area liberally with water. The water mist causes dust in the air to clump and settle out, carrying bacteria and other airborne microorganisms with it.

Finally, keep a *disposal container* on hand. We use a plastic pail with a tight-fitting lid. Fill it with a solution of one part 5.25% chlorine laundry bleach to four parts water. When you finish using a Petri plate, culture tube, disposable inoculating needle or whatever, immerse it in the bleach solution, making sure that no air bubbles prevent the solution from reaching all surfaces of the item. Allow the items to remain in the bleach bath overnight or longer, and then remove them for disposal or for washing and reuse. The bleach bath doesn't need to be replaced often, but it will eventually accumulate agar scum and other dead but unsightly contaminants.

Histology Equipment and Supplies

Histology is the study of the microscopic structure of the cells and tissues of plants and animals, which are prepared and examined in the form of thin slices, called *sections*. Histologists typically use the following steps to prepare a specimen:

Fixing
: Living specimens, once dead, begin to decay. *Fixing* is the process of killing the specimen, if it is not already dead, and preserving it either by chemical means or by freezing. The goal of fixation is to prevent decay and to preserve insofar as is possible the original structure of the cells and tissues. Fixing is sometimes combined with the following step by using the first, dilute, ethanol bath as the fixative.

Dehydration
: During the *dehydration* phase, water is removed from the specimen because water interferes with later processing steps. This is usually done by immersing the specimen in a series of ethanol baths of increasing concentration, typically 30%, 50%, 70%, 95% and 100% (anhydrous). The ethanol draws water out of the specimen, gradually in the early baths to prevent damage. The final bath of anhydrous ethanol removes nearly all of the remaining water.

Clearing
: During the *clearing* phase, the specimen is immersed in an organic solvent, usually xylene or toluene, which removes the alcohol from the specimen.

Infiltration
: During the *infiltration* phase, the dried and cleared specimen is soaked in a bath of the liquid embedding material—usually molten paraffin—which drives off any remaining clearing agent and replaces it with the embedding material.

Embedding
: The *embedding* phase consists of transferring the infiltrated specimen to a mold, which is then filled with liquid embedding material. Once that embedding material solidifies, the block containing the embedded specimen is removed from the mold and trimmed to remove excess embedding material.

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Sectioning

During *sectioning*, the embedded specimen is placed in a microtome, which is used to cut very thin sections of the specimen. Depending on how the specimen is oriented in the embedding material and the microtome, these sections can be longitudinal (lengthwise) sections, cross-sections, or tangential sections.

Slide preparation

The thin section is transferred to a microscope slide, which is warmed to melt the embedding material and cause the section to adhere to the slide. The specimen is then stained and mounted in the usual manner.

As you may imagine, preparing histology specimens can be complicated and time-consuming, and not only in terms of physical preparation. Obtaining suitable specimens can itself be time-consuming, challenging, and expensive.

Accordingly, some people decide histology isn't worth the effort and instead use prepared slides exclusively. At anything from $2 to $50 apiece, good prepared slides aren't inexpensive—the cheapest ones aren't worth having—but a well-chosen set of high quality prepared slides can often be resold for most of what it cost after you have finished using them. For learning purposes, we think the best compromise is often to make your own bacteriology slides and some of your histology slides, but purchase prepared histology slides for specimens that would be too difficult, time-consuming, or expensive to prepare yourself.

If you do decide to prepare your own histology slides, you'll need some specialized equipment and materials.

Microtome

A *microtome* is a device for cutting very thin sections of material. Professional microtomes cost hundreds to thousands of dollars, but manual versions like the one shown in Figure C-13 are suitable for a home lab and are available for $50 or less. Although there are work-arounds—we have used a paraffin-filled drinking straw and a razor blade to cut cross-sections—there's really no getting around it: if you want to make histology (tissue section) slides, you really need a microtome. If your focus is on microorganisms, you don't.

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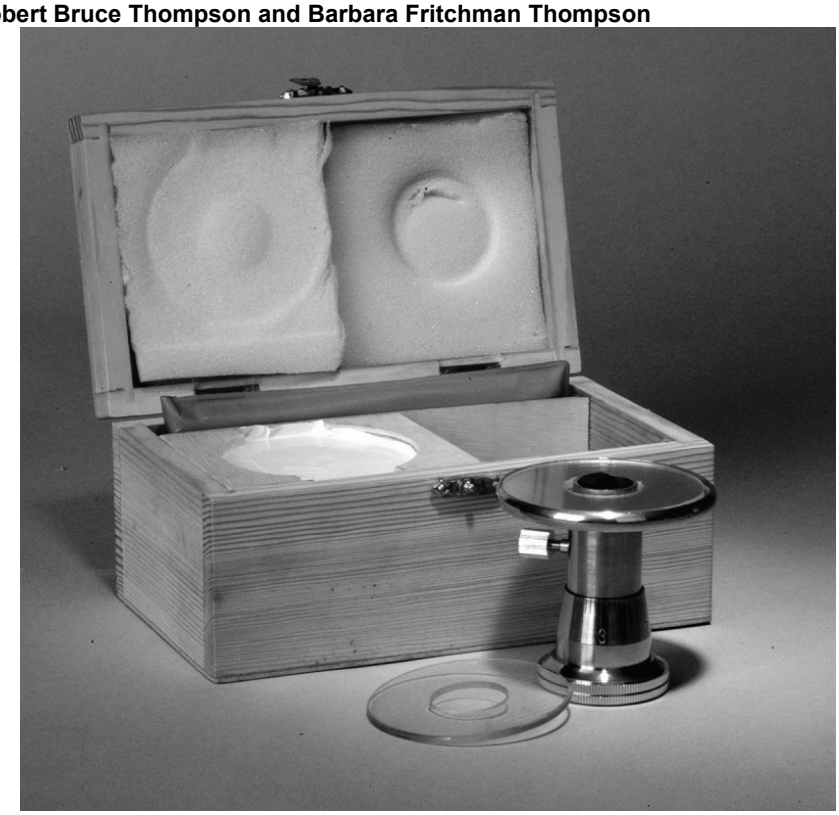

Figure C-13. A manual microtome (image courtesy United Scientific)

??? Production: this probably should be set off in some kind of "Quick Projects" box. For the time being, I've formatted it as a Sidebar. If we want to add a graphic, there's an example at http://www.microscope-microscope.org/activities/school/microtome.htm. RBT

Build a $1 Microtome

Buy a real microtome if you plan to do much sectioning, but if you'd just like to play around with sectioning, you can build a usable microtome for less than a buck with just a few minutes' work. You'll need the following items:

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- A large, empty wooden or hard plastic thread spool. These are becoming hard to find, as thread is sold mostly on plastic foam spools nowadays. You can find wooden spools at flea markets or ask your parents or grandparents if they have a wooden thread spool they're willing to contribute to the cause.
- Several flat metal washers, ideally with a diameter close to that of the thread spool and the diameter of the bolt, with a central hole slightly larger than the bore of the thread spool.
- A bolt, with fine (UNF/NF) or extra-fine (UNEF) threads if possible, that's about the same diameter as the thread spool bore. It should slide easily in and out of the thread spool bore with as little slack as possible, and should have a threaded length a bit longer than the thread spool bore. Take your spool to a hardware store and find a bolt that fits.
- A flat nut to fit the bolt.
- Some epoxy cement or super glue.
- A scalpel or single-edge razor blade.
- A candle.

To make the microtome, proceed as follows:

1. Glue a stack of washers together concentrically. The assembled stack should be 0.5 cm to 1 cm thick.
2. Apply glue to one side of the washer stack.
3. Slide the bolt into the thread spool bore until it protrudes from the other side of the bore.
4. Slide the washer stack down over the bolt and press the glued side of the washer stack against the top surface of the thread spool until it adheres, with the hole in the washer centered over the thread spool bore. Remove the bolt from the thread spool bore.
5. Carefully apply glue to one surface of the nut, making sure to keep it out of the threads.
6. Center the hole in the nut over the thread spool bore and press the nut against the bottom surface of the thread spool until it adheres.
7. Allow the glue to set, fixing the nut to the bottom of the thread spool and the washer stack to the top.

The procedure for using the $1 microtome is a bit different than the one for using a commercial microtome, because the $1 microtome has no specimen clamp. To use the $1 microtome, take the following steps:

1. Thread the bolt into the nut until the tip of the bolt is just above the bottom surface of the washer stack, leaving a well deep enough to contain the specimen.
2. Drip hot paraffin wax onto the tip of the bolt and, using forceps, immediately press the prepared specimen into the wax before the wax solidifies completely. Keep the specimen centered in the well.

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3.Drip additional wax into the well until the well is full and the wax is slightly above the surface of the washer stack. Allow the wax to cool and set.

4.Turn the bolt to force wax up through the washer until the top of the specimen is slightly above the surface of the washer stack.

5.Position the scalpel blade or single-edge razor blade against the surface of the washer stack at a very slight angle (as nearly parallel to the washer surface as possible).

6.Press the edge of the blade through the wax to trim it off flush to the surface of the washer. Use straight pressure; do not attempt to saw through the wax. Discard this wax plug. The wax should now be flush with the top surface of the washer.

7.Turn the bolt a fraction of a turn to force more wax (and embedded specimen) above the surface of the washer stack. (Your goal is to produce slices that are so thin they are transparent. You'll soon learn by trial and error how much to turn the bolt to produce sections of the desired thickness.)

8.Use the scalpel to slice the thin section and transfer it to a slide. Examine the specimen at 40X to determine if it is suitable. If not, continue cutting sections until you have one that shows the detail you want. Once you have a suitable specimen, pass the slide (specimen side up) through a flame to melt the wax slightly and adhere the specimen to the slide. You can then observe the specimen normally.

Dehydration baths

If you're processing one or a few small specimens, you can use a stoppered test tube as a *dehydration bath*. Partially fill it in sequence with the different concentrations of ethanol you plan to use. For multiple or larger specimens, substitute a series of jars of the appropriate size, each containing one of the ethanol concentrations. Allow each specimen to soak for the indicated time in each concentration, drain it, and transfer it to the jar with the next higher concentration.

To avoid handling damage to specimens, we use “teabags” made from lengths of string and a piece of muslin or cheesecloth. Place the specimen in the center of the cloth, lift the corners, and tie off the bag to secure the specimen. Suspend the bag in each jar, using the lid to keep the string out of the liquid. To change jars, just lift the teabag, allow it to drain, and transfer it to the next jar.

Embedding blocks

An *embedding block*, shown in Figure C-14, is simply a mold that is used to hold the infiltrated specimen while it is covered with embedding material in liquid form. Some embedding blocks are made of soft material and are designed to be sectioned right along with the specimen. Others are made of harder material, and can be re-used repeatedly. With the latter type, after the embedding material solidifies, the portion containing the specimen is popped free of the block or pried out with a needle and then sectioned.

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Figure C-14. Embedding blocks (image courtesy United Scientific)

General Laboratory Equipment

In addition to equipment used solely or primarily for biology, you'll need some general laboratory equipment.

Balance

A *balance* is not essential for most tasks, but is useful for making up solutions accurately, weighing specimens, and so on. An inexpensive battery-powered electronic balance with 100 gram capacity and 0.01 gram (centigram) resolution is sufficient, and can be used both in the lab and in the field. Better still is a dual-range balance that offers centigram resolution to 100 grams or so and decigram (0.1 gram) resolution to 500 grams or so.

Beakers

A *beaker* is a cylindrical container used for making up solutions, holding hot or cold water or ice baths, and so on. Beakers are available in glass and polypropylene, either of which can be used for most tasks, although the polypropylene models cannot be heated with direct flame. You'll want a range of sizes from at least 50 mL to 250 mL. If you plan to heat solutions in a microwave oven, polypropylene is fine. If you'll use a stove burner or hotplate, have at least one borosilicate glass beaker on hand.

Centrifuge tubes

Although they're intended for use in a centrifuge, standard *centrifuge tubes*, shown in Figure C-15, are also useful for many other tasks around the lab, including storing solutions and specimens. Buy the polypropylene versions with screw caps, which are autoclavable. We keep a supply of 15 mL and 50 mL centrifuge tubes on hand. The conical end versions are usable for most purposes. Self-standing versions, which have a skirt around the conical end, stand upright on a flat surface. The 50 mL self-standing versions are also excellent containers for developing chromatography paper strips.

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Figure C-15. 50 mL standard centrifuge tubes (image courtesy United Scientific)

Smaller *microcentrifuge tubes*, shown in Figure C-16, usually called *Eppendorf tubes* or *Eppie tubes*, are available in sizes from 0.25 mL to 1.5 mL or more. They're intended for use in micro-ultracentrifuges like the Dremelfuge described later in this chapter, and are also useful for storing small solid or liquid specimens. Buy the polypropylene versions with snap caps, which are autoclavable.

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Figure C-16. 1.5 mL microcentrifuge tubes (image courtesy United Scientific)

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Flasks

An *Erlenmeyer flask*, also called a *conical flask*, is essentially a conical beaker with a narrow neck. Erlenmeyer flasks are useful as culture vessels, gas generating bottles, and for temporary storage of solutions and liquid specimens. For general use, keep at least one 125 mL or 250 mL Erlenmeyer flask with solid, one-hole, and two-hole stoppers to fit it. If you're doing large scale broth culturing, buy as many in whatever size or sizes as you need.

A *volumetric flask* is used to make up solutions to accurately known volumes. It has a long, narrow neck with an index line to indicate the nominal volume. In use, the solution is make up to slightly less than the final volume, transferred to the flask, and the flask is then filled to the index line. The most useful sizes in a home biology lab are 25 mL, 100 mL, and 500 mL. For most purposes you can substitute a graduated cylinder without much loss of accuracy.

Graduated cylinders

A *graduated cylinder* is a tall, narrow container with graduation lines to indicate the volume it contains. Polypropylene graduated cylinders are suitable for most purposes other than measuring strong organic solvents, and are considerably less expensive than glass models. You'll want at least one 10 mL and one 100 mL graduated cylinder.

Polyethylene pipettes

Polyethylene pipettes, also called *beral pipettes* or *disposable pipettes*, are essentially one-piece plastic eyedroppers. They're available in various capacities from 1 mL to 5 mL or more. Buy models with graduated stems. We prefer the 3 mL models with stems graduated to 1 mL by 0.25 mL, which can be interpolated to 0.05 mL or less. These pipettes are usually sold in 10-packs for a dollar or so, or in bags of 100 for $5 to $8. Although they're referred to as “disposable”, in fact they can be used over and over simply by rinsing them with distilled water after each use.

Graduated pipettes

Graduated pipettes, also called *Mohr pipettes* or *serological pipettes*, are used to measure small volumes of liquids with very high accuracy. The most useful sizes in a home biology lab are 1.0 mL x 0.01 mL and 10.0 mL x 0.1 mL. You should never pipette by mouth, so you'll also need a *pipette bulb* or a *pipette pump*. For most purposes, you can substitute a polyethylene pipette, either by using its graduations or by calibrating your polyethylene pipettes with your balance and then counting drops.

Hotplate and wire gauze

A *hotplate* (or kitchen stover burner) is useful for heating solutions, making up agar culturing medium, and so on. Use a *ceramic wire gauze* to protect the bottom of the beaker or flask from the hotplate or burner surface. For most purposes, you can substitute a microwave oven.

pH meter and pH test paper

A *pH meter* is used to measure pH with high accuracy. Professional-grade pH meters are accurate to 0.001 pH units or better, but cost hundreds of dollars. Models accurate to 0.1 pH unit are available for $25 from Cynmar and other retailers. Models that sell for $50 to $100 may be accurate to 0.01 pH units, and may include a digital thermometer function that is accurate to 0.1 °C or better. For times when you just need a quick, rough indication of pH, use *pH test paper*.

Reaction plate

A *reaction plate* is a small rectangular plastic plate with an array of cylindrical wells, usually 2x3, 3x4, 4x6, or 8x12. Reaction plates are often used for testing one sample against many reagents or testing many samples against one reagent, while using only small volumes of both the samples and reagents. They're also useful for making up small amounts of various concentrations of chemicals, organizing small specimens, and so on. Sterile plates can be

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used for culturing many samples simultaneously in small volumes. The 24-well and 96-well versions are the most useful in a home biology lab.

Thermometer

You'll need at least one *thermometer*, unless your pH meter also measures temperature. For general lab use the least expensive alternative is one or more glass-tube thermometers. Ideally, you should have one thermometer for general use with a range of -20 °C to 110 °C and resolution of 1 or 2 °C, and a second thermometer with a narrower range, at least 0 °C to 50 °C, but better resolution.

Test tubes, brush, clamp, rack, and stoppers

In addition to serving as culturing tubes, *test tubes* are used in many routine lab procedures. We buy them by the case, usually 72 tubes, but they're not much more expensive by the dozen. Keep at least half a dozen on hand. We find the 16x100mm size most useful. You'll also need a *test tube brush* to fit the tubes, a *test tube clamp* for heating them, and a *test tube rack* to hold them. Buy a wet/dry rack, which you can also use for sterilizing them when you're using them as culturing tubes. Purchase at least a few rubber *stoppers* to fit the tubes, in solid and one-hole versions.

Wire gauze

A *wire gauze* is used to isolate a glass beaker or flask from exposure to direct flame or high temperature.

Major Equipment

There are several pieces of major (read “expensive”) equipment that we'll mention for completeness. Few homeschoolers will have the need (or budget) for these items, but all of them are important for DIY biology enthusiasts. In some cases, there are usable workarounds or inexpensive hacks that avoid the need for these items, often at the expense of producing less accurate results or requiring more time and effort to use. We use many these hacks ourselves, but if biology is your hobby and you can afford the real items, by all means buy them.

Spectrophotometer

A *spectrophotometer* measures the *transmittance* (or its inverse, *absorbance*) of light at various wavelengths, which may range from the ultraviolet through the visible spectrum and into the infrared. The simplest models, called *colorimeters*, measure transmission at only a few discrete wavelengths, usually in the visible spectrum. More expensive models measure transmission at very narrow intervals (often 2 nm or less) across a wide range of wavelengths. Alas, the least expensive spectrophotometers cost between $500 and $1,000 new, and even an inexpensive commercial colorimeter costs $200 or more. Used models are available on eBay for less, but there's often a good reason why they're being sold on eBay. (Hint: they're usually borked.)

Fortunately, the human eye is very good at discriminating differences in the color and intensity of light between two samples, so with a bit of extra time and effort it's often possible to get good data with no special equipment simply by visually comparing an unknown solution against a series of solutions of known concentrations. That's the procedure we'll use in this book, but if you own or can beg access to a real spectrophotometer you'll obtain more accurate data with less time and effort.

Cellphone Spectrophotometer

If you're willing to do a bit of hands-on hacking, check out the spectrophotometer project by Alexander Scheeline and Kathleen Kelley of the Department of Chemistry at the University of Illinois at Urbana-Champaign. Using only a few dollars' worth of readily-available parts and a cellphone camera or a point-and-shoot digital camera, they've put together an actual spectrophotometer, including the (free) software needed to calibrate it and capture data from the digital image files.

www.asdlib.org/onlineArticles/elabware/Scheeline_Kelly_Spectrophotometer/index.html

Ultracentrifuge

A *centrifuge* is a device that uses centrifugal force to separate suspensions and colloids, which remain homogeneous under ordinary gravity. (The Brownian motion of the solvent molecules is sufficient to keep the tiny solid particles suspended rather than precipitating out.) Tubes filled with the suspension to be separated are placed in the centrifuge head, which is then spun at high speed. The resulting centrifugal force causes the denser solid particles to be accelerated away from the center of rotation, where they accumulate in the bottom of the centrifuge tubes.

Hand-cranked manual centrifuges are available for less than $100, and entry-level motorized centrifuges for less than $250. Unfortunately, neither spins fast enough to be useful in a biology lab, where centrifuges are used to separate or purify DNA, proteins, microorganisms, and so on. Because these materials are both extremely tiny and not much denser than water, very high speeds (accelerations) are needed to precipitate them.

The solution is an *ultracentrifuge*. Standard centrifuges operate at a few hundred to a couple thousand RPM. Ultracentrifuges operate at 10,000 RPM or more and can achieve accelerations of 10,000 or more times the force of gravity. Alas, commercial ultracentrifuges cost several thousand dollars and up.

Fortunately, Irish DIY bio hacker extraordinaire Cathal Garvey developed a usable ultracentrifuge for those of us on a budget. His Dremelfuge is simply a micro-ultracentrifuge head. You supply the motive power (with a standard Dremel Moto-Tool or electric drill) and the shielding (Garvey uses a metal cooking pot). You can watch the Dremelfuge in operation at *www.youtube.com/watch?v=86WnXeTZO_Y* and purchase one at *www.shapeways.com/shops/labsfromfabs*. (Note that there are two models available, one for a Dremel Moto-Tool and one for a standard chuck drill.)

Garvey sells the DremelFuge as an ornamental item only, and warns that he's not responsible if you use it as an actual centrifuge. And with good reason. Centrifuge accidents are no joke. If the DremelFuge fails catastrophically, fragments will fly in all directions at Warp Factor 6, possibly causing serious injury or death.

If you decide to use the DremelFuge (we do), we strongly recommend you clamp it vertically—we use a Dremel 220-01 Workstation—with the power cord disconnected and the head in position. Cover the entire assembly with one of those 5-gallon plastic buckets sold at DIY supercenters. (We also wear safety goggles **and** a face shield.) Stand way back and sweet-talk someone you don't like into plugging in the power cord for you. After you've run it for the required time, disconnect the power, wait for it to spin down, and only then remove the bucket.

Seriously, this is no joke. Running the DremelFuge without taking these precautions is simply foolish.

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The DremelFuge accepts standard 1.5 mL plastic Eppendorf tubes, so you'll need a supply of those. Depending on what you're doing, the tubes may or may not be reusable. You may use two, four, or six tubes at once, and if you run the Dremelfuge at high speed you're likely to crack tubes occasionally, so order a reasonable number.

DNA Processing Equipment and Supplies

Although you can isolate DNA with equipment and materials found in most kitchens, doing really interesting things with DNA requires some special equipment and reagents. You can make some of the necessary equipment and reagents yourself, but for advanced work some must be purchased.

For serious DNA work, the first requirement is a DNA gel electrophoresis apparatus with power supply. Edvotek (*www.edvotek.com*) sells complete setups starting at $139 for the apparatus (shown in Figure C-17) and $145 for a power supply, which is the best price we've found. This is an entry-level DNA gel electrophoresis setup, but quite adequate for doing serious work. As supplied, the kit produces 7x7 cm gels. (You can buy an optional gel casting tray for 7x14 cm gels.) The power supply is fixed at 70 VDC, which is a good compromise for most purposes.

If you plan to do a lot of DNA work, you'll want a setup that allows you to run larger gels and possibly multiple gels simultaneously. You'll also want a variable-output power supply, ideally with a range of at least 50 to 200 VDC and sufficient amperage to run as many gels (or tanks) as you plan to run simultaneously. Such setups are available from Edvotek and other vendors.

For learning purposes, you don't need to buy a commercial DNA gel electrophoresis setup. We once bet someone that we could produce usable DNA gels with only items commonly found around a home, and do it all for less than $10. We won that bet, too. We used plain gelatin to make the gels, which we cast in a trimmed-down Gladware container with masking tape to form the casting dams. We made up the loading buffer and running buffer with table sugar, table salt, and baking soda, used food coloring as the marker dye, developed the gel in a flat Tupperware container with a stack of five 9V transistor batteries wired in series using aluminum foil for electrodes, and visualized our developed gel with some methylene blue from the aquarium supplies. It wasn't pretty but, hey, it actually worked.

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Figure C-17. Edvotek M6+ DNA gel electrophoresis setup (image courtesy Edvotek)

You'll also need some way to measure and transfer small volumes of liquids. For educational use, you can use inexpensive polyethylene pipettes, whose drop sizes range from 50 drops/mL (20 µL/drop) to 25 drops/mL (40 µL/drop). For serious work, you'll need a *micropipetter* like the one shown in Figure C-18. These are available in fixed-volume and variable-volume models, with variable-volume models starting at around $150. (Multi-channel models can fill eight or more wells in one operation, but cost several hundred dollars and up.)

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Figure C-18. Single-channel variable volume micropipette (image courtesy United Scientific)

If you need something better than a disposable pipette but don't want to spend $150 or more, consider using a $20 fixed-volume *minipipette* like the ones shown in Figure C-19. These are typically a bit less accurate than more expensive models, but more than good enough for most work. Whichever type you use, you'll need a supply of disposable tips to fit it.

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Figure C-19. Single-channel fixed volume minipipettes (image courtesy United Scientific)

For many tasks, you can simply extract as much DNA as you need to work with. For example, you can obtain sufficient DNA from one strawberry or carrot to run hundreds of gels, or you can run a broth culture to obtain large amounts of bacterial DNA. But sometimes you can obtain DNA only in amounts too tiny to analyze.

The solution is a procedure called *PCR* (*polymerase chain reaction*), which is used to amplify (replicate) tiny amounts of DNA into the larger amounts needed to analyze it by causing the DNA to replicate exact copies of itself. The procedure involves mixing the DNA sample with a solution that contains enzymes, amino acids, and buffers and then exposing the mixture to a series of precisely-controlled temperatures for specific durations. That series may require dozens to hundreds of repetitions, which is where a *PCR thermal cycler* comes in.

A thermal cycler provides either a receptacle for a standard 96-well plate or a grid—usually 3x3, 4x4, or 5x5—that accepts small microtubes. You place your DNA sample(s) in the well plate or microtubes, turn on the thermal cycler, and wait for it to complete however many cycles you've specified. When the cycles complete, each well or tube contains much more DNA than was originally present. That DNA can then be separated, purified, and subjected to analysis.

Commercial PCR thermal cyclers are extremely expensive. The least expensive model we found was the Edvotek model, at about $1,800. Fortunately, the DIY bio hacking community has come to the rescue. The OpenPCR initiative (*http://openpcr.org/*) offers a complete kit for $512, with the hard parts pre-assembled. We've so far avoided the need for PCR in our own work, but one of these is definitely on our wish list.

If you're very patient, you can actually do thermal cycling manually. All the equipment you'll need is some foam cups, a way to heat water, a thermometer, and a clock or watch with a second hand. You simply fill the cups with water at the required temperatures (keeping the temperatures at the necessary levels by adding hot water as needed) and move the Eppie tubes from each cup to the next after the required time has elapsed.

We actually did this once, just to be able to say we'd done it. We ran through two dozen iterations before we lost patience. Unfortunately, real-world PCR may require hundreds of iterations, and we're not that patient.

For some work, you'll need a freezer, but not just any freezer. Ordinary home freezers operate at

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about -20 °C, which isn't cold enough for some work. You can buy a *-80 °C freezer* from a lab supply vendor, but it'll cost a bundle. Fortunately, there's a cheap and practical alternative for a home lab. Obtain a small foam cooler and a supply of dry ice from the supermarket or an ice cream store. Place your specimens and some dry ice in the cooler, with the lid fitted loosely on top to allow gas to escape. Place the cooler in the regular freezer. The dry ice will keep the contents of the cooler at -80 °C for anything up to a week, and the dry ice can be replaced as necessary.

Finally, you'll need various supplies, including agarose for gels, loading and running buffers, enzymes, and so on. The supplies you need depend on what exactly you're doing. Get a catalog from Edvotek and visit the web site of Carolina Biological Supply (*www.carolina.com*).

Prepared Slides

Many of the lab sessions call for prepared slides, which you can make yourself or purchase individually or in sets. Cynmar (*www.cynmar.com*) and Nasco (*www.enasco.com*) carry a wide range of sets and individual slides at good prices. Carolina Biological Supply offers many slides that are not available from Cynmar or eNasco, but has generally higher prices. Home Science Tools offers a reasonable selection of slide sets and individual slides at prices that are generally between those of Cynmar/eNasco and CBS.

Before you purchase slides, check with your local home school group or co-op. Some have prepared slides available for members to borrow or rent inexpensively, and other members may have unneeded slide sets from prior years that they're willing to sell. Even if those slide sets don't include all of the slides you need and include some you don't need, you can use those sets as a starting point and add other individual slides as needed. And, of course, when you are finished using the slides you can sell them on to someone else in the group.

To decide which slides you need to buy, whether in sets or individually, we recommend scanning through the book to determine first which lab sessions you intend to do and which slides you want to have available for them. Then decide which slides you can prepare yourself and which would be better to purchase. For example, you probably don't want to purchase a prepared slide of squamous epithelial cells, because you can make your own in about 15 seconds by scraping the inside of your cheek with a toothpick, transferring the cells to a slide, and adding a drop of methylene blue stain. Similarly, it's easy to prepare your own slides of whole-mounted creatures from pond water, plant stems and leaves, and so on. Conversely, if the specimen itself is difficult to obtain (or hazardous) or if it requires complex histological preparation, sectioning, and staining, most people will understandably prefer to buy the prepared slide.

If you do intend to prepare some or all of your own slides, take scheduling into account, both in terms of availability of the specimens (which may vary by season) and in terms of the time and resources needed to prepare the slides. Rather than devote precious lab time to preparing slides, other than simple wet mounts of whole specimens, it's often best to prepare slides the day or the week before you need them for a session. Water-based wet mounts dry out quickly, but if you substitute glycerol as the mounting fluid the slide should remain usable for at least a week and possibly much longer. You can, of course, make permanent mounts using Permount or a similar commercial mounting fluid, or even colorless nail polish.

As an alternative to spending a lot of money on prepared slides, the customized kit for this book, available from The Home Scientist, LLC (*www.thehomescientist.com*), includes a disc that contains high-resolution color images of most of the microscopy images used in this book. You can view these on your computer and see more detail than in the printed images. We'll admit it up front: we're not expert photomicrographers and our work will probably never be published by

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Nature, but these images are a reasonably good substitute for using prepared slides. Not perfect, by any means, because no image can show you everything you can see while viewing an actual slide through an actual microscope, where you're able to move the specimen around, change magnifications to zoom in or zoom out on particular parts of the specimen, and so on. Finally, most specimens have depth, which means it's impossible to focus critically on all parts of the specimen at one time, particularly at higher magnifications.

Specimens

Many of the lab sessions require live or preserved specimens or live microorganism cultures. Many of these specimens, such as a human hair or pond water or tree leaves, are easy to obtain locally at little or no cost. Some, such as bacteria cultures, must be ordered from a science supplies vendor if you intend to complete those lab sessions. Wherever possible, we point out free alternatives to purchased specimens.

Here are the sources we recommend for specimens:

Carolina Biological Supply

CBS offers a huge variety of live and preserved specimens and live cultures, many of which are available to individuals and homeschoolers. (Sales of some potentially hazardous organisms are restricted to high schools or universities.) Their prices and shipping charges tend to be on the high side, but their quality is excellent. If you can't find something anywhere else, chances are good that CBS offers it. 800-334-5551 or *www.carolina.com*.

eNasco

eNasco is generally homeschool-friendly, and offers a wide selection of specimens. Some items are sold only to schools and businesses, although that's easy enough to get around. Although its selection is not as large as CBS's, eNasco generally has somewhat lower prices and their quality is generally excellent. eNasco is the first place we look for specimens. 800-558-9595 or *www.enasco.com*. eNasco also has an excellent FAQ on live materials at *www.enasco.com/science/page/livematerialFAQ*.

Dissection Specimens

We don't do any dissections in this book because we think there are better ways to spend precious lab time. If you want to make dissections part of your curriculum, you can purchase kits from Carolina Biological Supply, Cynmar, eNasco, and other vendors that include various specimens, dissecting instruments, and illustrated instructions.

As an alternative to doing actual dissections, you can purchase high-resolution color charts that cover dissecting various types of specimens from BioCam Charts (*www.biocam.com*). Many schools now use these charts exclusively, and not just to save money. Using these charts avoids the “yuck factor” that has killed a lot of students' interest in biology. No surprise, if one's first experience with biology is cutting up something dead, smelly, squishy, and generally disgusting. They don't call it gross anatomy for nothing.
