Soil Learning Center

Microscope Intensive Course

Lesson 1: Introduction to the Microscope

Hello. This Dr. Elaine Ingham. We're going to be talking about putting the microscope together using the steps that you have to go through every time you start the microscope. Any time you've moved the microscope, you're going to have to go back through all of this because if you move your microscope, then things can come out of alignment. So you're going to have to redo that. Even every morning, it would be a good idea, every time you turn the microscope on, to go back through and do all of these steps.

So when you're looking at the microscope here, you'll notice that it's got a nice cover on it. You always want to leave your microscope covered so you protect it from dust and dirt. The thing that will destroy your microscope faster than anything else, other than dropping it, would be leaving it uncovered. You get dust and dirt and grains of sand or something into the gears, into all of the moving parts. Then cleaning the same becomes real difficult to do and it gets dirty. You're going to have to take it a microscope person to clean it way more often than you normally would have to do. Microscope people always recommend that you get the microscope cleaned every year. Well, if you're keeping this in a pretty clean environment, away from most dirt and dust flying through the air, if you're always careful about leaving the cover, you might be able to get away with two or three or maybe even five years between cleaning.

How do you know when your microscope needs to be cleaned? Well, when you start seeing lots of little dots on the lenses, where when you move your microscope slide, you see those little dots stay in place. It's not part of your sample. It's on the lenses of your microscope. So that's a time to get it cleaned, when you can't get rid of the dust and the dirt, the particles are inside. You do not ever want to be sticking pipette or a cleaning instrument, cotton swab, down into the inner parts of your microscope because there are anti-dust coatings on all of those lenses, and if you wipe those, now you've got streaks instead of little dots and dust. You've got big streaks and then you have to go to the microscope person to clean it. It's going to cost you money because he's going to have to take those lenses out, reapply the anti-dust coating, put it back in, and now you're talking hundreds of dollars for them to do that. So we want to leave the inside parts of the microscope alone. In order to increase the time where you got to take the microscope in to get it cleaned, leave the cover on it when at all possible. Now, the cover is just a plastic cover, real fancy like this from the microscope manufacturer. If you lose that, you could use a plastic bag or brown bag, anything to cover and protect your microscope.

So now we're looking at microscope. I want to go through all of the different parts of the microscope. As we approach this process, you want to think of it in three ways. The first step is to take your eyeballs and focus them on your sample, and your sample is going to be sitting here on the stage. We then want to take our light and focus it on the sample. Then we're going to make certain that this eyepieces are focused on that sample. Now, both of your eyes are at the same plane of focus, not have one eye at one plane of focus and the other eye at the other plane, you will end up with headaches if you don't fix that. We always suggest that you get a binocular microscope because if you close one eye and are looking through just a monocular, a one eyepiece to be looking through, you'll end up with, again, terrible headaches because as you got one eye closed and you're focusing on something with just the one single eyepiece, your brain is trying to get both eyes into the same plain of focus, and you can't do it when one is closed, there's nothing for it to see through, and you'll end up with incredible headaches. So you want to get binocular microscopes. The last step then is to make certain that these eyes, both of your eyes, are in the same plane of focus.

So a quick go through all the parts, and then we'll go back and talk about the processes of focusing the different parts of microscope. First of all, we have eyepieces. So when you took your microscope out of the container it was shipped in, these come as separate items in that container, and you have to slip them into the open orifices. Now, they always come with a closure on the microscope. I'm going to just quickly get up and go grab an example of that closure. So when the microscope came, they always protect the inside of the microscope by having these little caps. If you ever take your eyepieces off, immediately get a cap or if you're switching out and you're going to put a camera

on here, as rapidly as you possibly can, you want to get this opening into the inside of your microscope close back up. So you don't leave anything open to the environment for dust and dirt to get inside. So save these closures and make sure that you can always protect the inside of your microscope.

So eyepieces. You'll notice on your eyepieces, there's going to be information on there that says WF, means wide-field. 10x, so that tells you what's the magnification factor here. We always use 10x because these are high quality eyepieces, fairly inexpensive when you go to buy replacement parts but, still, it does cost money. You'll notice sometimes that there are people who will sell 15x and 20x eyepieces. Not worth the money for that little bit of extra magnification because they do not also improve resolution. That's one of the critical things that we've got to worry about is our ability to resolve the sample. So we've got to collect as much light as possibly from our sample. Get as much light as possible to our eyeballs so we can distinguish the edges of those bacteria, the edges of your fungi just as sharp and as clear as you possibly can. So we need good resolution to see the little flagella on the flagellates to be able to distinguish that between the ciliates with hairs cilia all over their body. So 10x eyepieces are going to give you maximum magnification with the maximum resolution.

Now, you'll notice on a lot of microscopes where you have focusing knobs here. With this particular microscope, both of the eyepieces will focus. Most microscopes are not going to have the ability to do both. One of the eyepieces will be fixed, the other, you'll be able to focus. When we come to that last stop, that's going to be very important so that we can make certain both eyeballs are on the same plain of resolution. Now, you'll notice that you can pull these eyepieces apart back and forth just like a pair of binoculars. You can adjust them. Some microscopes, the eyepieces will move in this direction but in some way, you can set these eyepieces the right distance apart for your eyeballs. You don't really want to buy a microscope, binocular microscope that you can't do that because everybody's faces are a little bit different size. So you need that ability to move it apart and get them just the right distance. So one of the things we'll be doing when we go to use the microscope is looking here and adjusting these so that you see a single circle of light. That circle of light that you see through here is called a field. So the field that we're looking at, you want to see just one circle of light, not two kind of circles next to each other. You've got to adjust these so that you're seeing a single circle of light. Also, you want to be careful when you're this that your eyeballs are not right against the eyepieces. You want your eyes back and one of the things you're going to do is adjust your head different distances back and forth so that you get the right distance, the right distance apart on your eyepieces so you're seeing that one circle of light. It's going to take a little experimentation, a little trial and error on your part to be able to do that. So, eyepieces.

This is called the headpiece and you will typically find a screw, some kind of nut that you can loosen up a little bit so that headpiece will move from side to side because as I'm looking through here and I'm going "Whoa. Look at this thing. Come over here. Take a look at this," I do not want to move the microscope because if I move the microscope, I may hit the stage, I may tap or hit something so that we're not on the same field anymore, and now your organism is lost. So instead of turning the whole microscope, you just turn the head. So then somebody else can look and go "Whoa. That's incredible." Then you come back and you can continue on. So the headpiece.

Now, going down the microscope a little bit more, we're looking at the nosepiece here. So the nosepiece moves the objective lenses. You'll notice that we have a bunch of different length objective lenses here. So always the shortest one is going to be your least magnification. You can see on the side here, it's going to tell you what that magnification is. Lenses today always come with that information on the side. So exactly what kind of objective lens it is is also on there, width of that lens and the magnification is there as well. So we're looking at 4x. If we go on to the next one, this is a 10x objective. So we're going to be magnifying everything by a factor of 10 fold. As we go around we've got a 40x objective and we have 100x objective. We don't really need the 100x objective on our microscopes because we're not going to be looking at anything that requires us to expand 100 times. Remember that we've got the 10x eyepieces here. When you're looking at total magnification, it's going to be this number times this number. So if we're looking through our 4x objective times 10x eyepieces, total magnification is going to be 40x total magnification. We may go to our 10x objective. 10x magnification times 10x of magnification, a total magnification of 100. When we get to our 40x objective, 40 times 10, 400 total magnification. Get up to 100, 100 times 10 would give you 1000x. So we don't need that 100x objective. All of the organisms that we're looking at are going to be totally visible using that total magnification of 400x. Classical microbiologist organisms say you can't possibly see microorganisms, see bacteria at 40x magnification, total magnification but you got to think about the

methods that they were taught. When they're going to prepare a slide with the organisms in, say, a drop of water, and they're going to look at that, they're going to put one drop of their sample on their microscope slide, and then they're going to take their cover slip, and they're going to spread that drop of water out across the surface of that slide, just go back and forth until that liquid dries on the slide. What happens to the microorganisms as that drop of water dries out? If human beings happen to be sitting some place on the surface of the planet and they air dried, how much would your body shrink? That's what we've got to think about going on with these classical methods that as these organisms dry out, their bodies shrink down. We're going to have to be looking at them with a higher magnification than we would have had we looked at this drop of soil suspension or water, whatever it is, without drying it out.

Once they have this slide, once they have this sample dried down, now they're going to go get their cigarette lighter and flick that bit and run this through that flame, maybe 10, 20, 50 times, whatever they feel is necessary to, again, shrink those organisms down even more so they adhere to the slide because now they're going to put stains on that, and let the stains sit on the surface of those organisms, and the organisms because of their morphology, because of their cell wall structure, because of their plasma membranes, something is going to absorb that stain, then they rinse off the excess stain and now we'll put on another stain to counter-stain, and then something else, and then something else. So those organisms have to be very friendly adhered to that slide.

But think of what happens when you run this slide and these organisms through a flame and dry them to crispness. You turn them into charcoal. So you're going to have to use higher magnification. Yes, if you prepare your sample in that fashion, then you are going to have to use 100x objectives but we don't dry our organisms down. We're going to take our sample, dilute it in a little bit of water, shake it up, put a drop of that in our microscope slide, move that sample so that it's just the size of your cover slip. We're just going to move that water back and forth, and then drop that cover slip onto that drop of your sample at whatever dilution you used so that now we're going to be looking at those organisms in situ without drying, without any other change than we added water. What soil microorganism is intolerant of water? They're not. So it's a very normal, very typical thing for them to have water coming through the soil. So we haven't really changed anything going on in the soil other than we've spread it out so we can look at it. So we can see our bacteria. Well, what you'll find out is that you'll be able to see your bacteria when you're using the 4x objective. It's hard to distinguish important characteristics. So we're going to use the 10x objective. Well, they get a little bit bigger but we're going to do all our work using that 40x objective because everything is large enough so that we can see all organisms and all the characteristics that we need. So everything we're going to do is going to be using 40x objective when we're actually counting, when we're actually measuring bacteria or fungi or protozoa. A lot of times with nematodes because they are so large, we can get away with using the 10x objective but you may have to bounce up to the 40x objective so that you can see salient points and determine whether their root feeders or bacterial feeders or fungal feeders. So you'll be moving around from objective to objective.

Now, notice that I'm very careful about talking about the 4x objective or the 40x objective. If you're not real careful, if you just start saying, "Well, we're going to use 40x," well, then what are you really talking about? If we're looking at 40x objective, that's one thing. What if we're talking total magnification of 40x? Well, then you're using the 4x objective because times 10, 40x. So be very careful when you're speaking or certainly whenever you're writing instructions for somebody else, make sure that you're very careful to talk about total magnification or you talk about what objective that you're going to use. So I will try to be very careful and make it perfectly clear what I'm doing, what I'm using. Every once in a while I will probably mess up.

So now we've got the objectives. You will always start with the 4x objective and typically when you leave your microscope at the end of the session, when you're cleaning everything up, when you're going to put the cover back on this, you want to make sure that you dial in the 4x objective and leave it in that position because the 4x objective is the shortest. You'll see that when we put our stage up to the highest point, that there's lots of space there. So if you're traveling and things are kind of bouncing around, you don't have any worries about that objective hitting any other part of the microscope. Now, notice that when we dial in that 10x, "Ooh, we're getting a little close here." You can see that as you're traveling and things are bouncing around, you might have "Well, you're probably safe but now let's look at that 40x objective." I probably don't even want to put it all the way in there because I might scratch my 40x objective. Certainly if you were going to go on and you were going to use the 100x objective, you would definitely cause some damage. So leave it where if you decide suddenly to come along and pick this up and go out to

the field with it, you're not going to be damaging your microscope objectives. When you look at this whole microscope, the most expensive part of this microscope are the objective lenses. The 100x objective is most of the cost. The 40x comes next. The 10x comes next. Your eyepieces and the rest of the body of this microscope is probably only 10% of the cost of the whole microscope.

So I do want to say just a note about different microscopes. You can see that this is one particular built, slightly more expensive microscope. This cost about $1,300. We found less expensive microscopes are every bit as good if maybe not, even a little bit better, than these really expensive microscopes for $300, $350 with camera. You can get just as good a microscope but all the parts, all the pieces are pretty much the same. No matter what make of microscope that you buy, they're going to have pretty much the same. So I'll try to point out where we might have slight differences between microscopes as I go through this but for the most part, they're pretty much the same. So no matter which one you buy, you want to pay attention to leaving that shortest microscope objective in place whenever you leave the microscope at the end of the day.

Continuing on down the microscope; his is the microscope stage. This is where we're going to put our slide. Now, when we put our slide on the microscope, it's going to sit on that stage, you'll notice that in all of your microscopes that there's always a lever, there is a holder. So you got to open it up, slide your microscope (slide) in there, and then put the lever back down so that that slide is firmly held. So as you're moving around, as you're doing things, that slide doesn't pop out and go zooming across the room, so making certain that it is held in there. Now, you'll notice that all of these microscopes have stage controls. You absolutely want to have this mechanical stage control. Some real inexpensive microscopes, they just give you two clips to hold that microscope slide in but you can't move it around. We are going to want to start at one field, move to the second field, third, fourth, fifth, move over at six, seven, eight, nine, 10, move over, 11, 12, 13, 14, 15, move over, 16, 17, 18, 19, 20. Those are the fields we're going to want to examine. It really makes life so much easier if you have the mechanical control. Trying to move this around, really difficult.

What if a ciliate or a flagellate goes zooming across your field screen, or even a bacterium, zoom, across your screen, across your field of view? You're going to want to follow it because you want to identify, is that a ciliate or is that a flagellate. So as I'm looking through here, I want to be able to follow it. If you're trying to move your microscope slide around with your fingers, you're going to drive yourself crazy. It's just not worth it. So make sure you have the mechanical control on your microscope. Okay. So now we are moving our slide around. We're getting to the different fields. So we have our eyeballs, we've got to go through that process of focusing our eyes on our sample. Now, I'm going to go through all of this again and actually do this.

Right now I want to go through the basics. So now I'm going to start on how do we get the lights focused on our sample because in order to increase, to improve resolution, to maximize resolution to the greatest degree possible, we've got to maximize the amount of light that actually gets to our sample. So focusing our way down, looking down further in the microscope, bottom up to the stage, here's our light source. Now, somewhere on your microscope is going to be a light switch. Please make sure that you have your microscope plugged in and make sure that you've got a protection, you've got some kind of transformer or you've got a surge protector of some kind that you plug into because surge in the electricity can blow your lights, and then you're going to have to replace your light.

Nowadays, a lot of people have LEDs and those LEDs should last the life of the microscope. You should not ever have to change that lamp. With this particular microscope, I do have to change the lamp. So when you want to change the lamp, you're going to have to tip your microscope and change that lamp by opening up the little door, putting a new lamp in. So you can see that that's not a lot of fun. I'd have to take that slide off my microscope in order to get to that lamp. If you have lamps that have to be changed, you might think about going to your microscope person and having them change it to an LED light or have extra lights that you can replace that bulb in the bottom so you always want to make sure you have two or three extra lights. You will reduce the amount of times you have to change the light bulb if you can hook it up to a surge protector.

So where on this microscope is the turn on switch? Well, this one happens to be at the back. Sometimes they're around the side. Sometimes they're around the bottom. Some of them, the turn on switch is going to be up on the arm. So you got to go exploring and finding it. So here is this one. How do you know that your microscope is actually plugged in? When you flip the ON switch, the light turns on. Now, be aware that you also have a switch

some place down here on your microscope you have a dimmer. So notice how as I open it up, close it down, there is just a diaphragm between the actual bulb and the outside of the microscope. So sometimes if somebody plays a trick on you and you turn your microscope on, you go "Oh, no. It's broken because there's no light coming out." Well, check the dimmer switch just in case that got bumped. Now, when we first start out looking through our microscope, it may be that the brightness is too high. So you might, at low magnifications, want to turn that light down a little bit so you don't blast your eyeballs as you first look through here. So we know that the microscope is in ON when we could the lights coming through.

Now, notice here right at first, even with the cover on my microscope, that I've got a lot of dust and dirt on the surface of that glass. So I want to take a nice piece of cloth, especially the kind of cloth they sell to clean eyeglasses, real fine gentle cloth that's not going to cause any scratches on the surface of the glass. Clean that excess dust off. If you're in a really dusty environment like we are here during the summer time in Oregon, there's going to be dust that accumulates on that lens while we're working on the microscope for several hours. So you may want to go back and recheck this every once in a while. Any bit of dust on there is going to reduce your resolution.

Now, as we go up to the stage again, we've got another glass surface that we need to clean on the top of our condenser. So you want to dial in your 4x lens so you can actually get to that piece of glass, and you want to clean that. If necessary, you go up here, do your eyepieces and you clean them. Do not wear any makeup when you're going to be working on the microscope because makeup brushes off, it accumulates here. So leave the makeup off when you're going to be a scientist and do some work on the microscope. So we've cleaned the glass surfaces that we can easily reach, and that's pretty much the extent of the cleaning that we're capable of doing.

Now we're going to proceed below the stage so that we can focus our light on our sample. We've talked about the illumination as it's come on, we can see the light coming through. As we move up a little bit, you can see the condenser. Now, you control the condenser with this knob over at the side. You can see it just goes up and down. We want to focus that condenser, put it in the right place so that that light is focused on our sample. In order to do that, well, we'd have to have a slide on our microscope, already focused our eyeballs on that slide but then we're going to move this condenser up and down in order to focus this light on that sample. So what we need is a nice sharp edge of a piece of paper. So take a piece of paper like that or perhaps we could take one of our little paper towels. Typically using your right hand, we want to make sure that the edge of that towel or that piece of paper, whatever you got, is just as flat and tight up against to that glass as possible. So when you're using the microscope, typically it's going to be your right hand that you're going to get that piece of paper just as tight to that glass as you possibly can and now, with your left hand, you're going to be focusing with the condenser. The idea there is to find that edge so you may have to move the piece of paper back and forth until when you're looking through the microscope, you can find that piece of paper and you're going to focus up and down. So we will find the right place to put our condenser so that all the light is focused on our sample. Then you take the piece of paper, put it to one side, you're done with that. You've got your condenser set in the right place.

Now, on our condenser, notice that we also have this iris diaphragm. This goes left to right and you'll notice when it's all the way open, we're getting the maximum amount of light coming through the condenser to our sample but we're going to want to shadow the organisms in our sample. So we will use this iris diaphragm and start shutting it down so that we see those shadows. Now we can actually see those bacteria, the fungi, the protozoa. So we've got to remember to shadow. So how much are going to close this down depends on what you're seeing with your eyes. So that's got to be set when you're looking through. As you're looking at your sample, you'll be adjusting that iris diaphragm to try to see the smallest bits and pieces, the flagella, the cilia, all of the different structures inside the nematodes because shadowing is what allows us to see that contrast. So resolution versus contrast and this is how you're going to control that.

Okay. So we have our condenser set. We've maximized the amount of light. We've shadowed so that we can see the contrast. So we have just about everything done here. One more step. So now we've looked at most of the parts of the microscope. There's one last part we haven't really explored, and that's the focusing knobs. You'll notice the coarse focus is the larger dial here. The fine focus is the smaller dial. You'll notice when we move that coarse focus -- and you got knobs on both sides. I'm moving it back on the other side. You'll notice that as I'm moving that coarse focus knob, the stage is moving quite a bit. So when you rapidly want to move that stage around, you're going to use

the coarse focus. So typically we're going to use our 4x. We're going to focus using that coarse focus. We will then dial in our 10x objective but now we'll start using the fine focus that only moves the stage a little bit. You can't really see it here but when you're looking through your eyepieces, you'll see the effect of moving that fine focus once we get to the higher magnifications.

Okay. So now going on, once we have our eyeballs focused on our sample, once we have the light focused on our sample, here's the last step. We're going to go back to our eyepieces again. We've already got it set when we focused on our sample, we already have our eyeballs set to the right distance. Now we want to work on making certain that both of our eyeballs are at the same plane of focus. Most people's eyes aren't actually focused at the same plain of focus. So we need to do this adjustment. One of your eyepieces will be stationary. So typically if both of them focused like this, you want to choose one of them to be the stationary eyepiece. So let's choose the left. We're going to make certain we're focused on our sample, that our light is focused on our sample. Now we're going to close the eye that we can adjust. So whichever eye has the adjustable focusing knob, we're going to close this eye. Using our left eye in this case, we're going to use our fine focus and we are going to focus on something and memorize what that piece, that one little bit of material, what is the focus, what exactly does it look like using our left eye.

Once we've got that focused on and we've memorized what it looks like, now we're going to close our left eye but instead of using the fine focus knob, we're going to use this focus and move this eyepiece until that piece of material has precisely the same focus as you were seeing with your left eye or the eye with the stationary lens eyepiece. That way we're going to make sure that both of our eyes are at the same plane of focus and your brain is not constantly, all the time you're trying to use this microscope, adjust both eyeballs. So you're seeing the same plane of focus which ends you up with an incredible headache if you don't have both eyeballs set like that. So do that as the last thing that you need to do.

Normally, if you're the only person using this microscope, you leave these things set and you don't have to touch them again. So as long as nobody else has come in and adjusted anything on your microscope, the next time you use your microscope, you probably don't have to adjust your eyepieces again but let's say you start to use your microscope and you're going "Wow. I'm starting to get a headache," that should tell you these have gotten out of whack somehow and you need to go back and make certain both eyes are at the same plane of focus so the headache stops.

Our microscope slides. So typical, inexpensive microscope slides, you usually buy them in boxes like this. So you're getting quite a few microscope slides. Let me point out that you probably don't want to buy this particular variety of microscope slide. These, we bought. They were the cheapest ones. The scientific supply company and you just want to go online and find a scientific supply company close to you, probably and you're going to want to find microscope slides. Do not buy the 'el cheapo' ones from China because they come already dirty and you're going to have to clean them. What is it that's in their water in China? I would worry about that. So you can see why we have never opened this box because we learned that just because they were cheap did not mean they were going to be usable. So a little bit better. Don't buy things from China probably is take home message here but you're going to want to buy a box. If your microscope did not come with a box of microscope slides, then you're going to want to buy some. This is the size, nice and thin, that's the right size.

Now, you're going to want to buy some cover glasses as well. Cover glasses come in a variety of sizes. I would encourage you to get the 18 millimeter square or 18 millimeter by 18 millimeter cover glasses or cover slips. Notice that these are one and a half thickness. So these are 15 micrometers thick. So when you're dealing with these cover slips, they are very easy to break. They're fragile. I tend to clean all of my cover slips and my microscope slides between each sample. So I will reuse this microscope slide 100 times. I will reuse that cover slip as many times as I possibly can before I break it. But sooner or later when I'm cleaning up a sample, and I'll show you how to clean it up, sooner or later when I'm wiping it up, I'll get surprised by the phone rings or someone starts talking to me, and I will crash the cover slip and I have to throw it away. You might want a container where you're going to put all your broken glass because sooner or later you will probably break that slide. But if you can get 100 uses out of this before it breaks, before you have to go to a box and get another one, that's excellent. You're saving some money. So

starting out with our microscope slide, there's our cover slip already to put our drop of water, drop of our sample on that microscope slide. Then use the cover slip, drop the cover slip on top of that sample.

So now let's move to making our sample. You want to have a sample of some kind that you've found out and collected your material. Now, this happens to be have been sitting in this bag for quite some time so it's very dry. Sometimes some part of the year, your soil is going to be very dry when you collect it. So you're going to want to mix that sample. I usually mix the sample in the bag like this. Sampling, you want to understand how to sample from the area that you're concerned about. So we went out and we took at least three cores from the area that we were concerned with. This happened to be an area where the compaction was very close to the surface. We want to know what's going on at that surface of the soil, not down through the compaction layers. So think about what depth you're going to go to. So if there's a compaction layer within an inch of the surface of the oil and what you want to know about or where the roots, what's going on around that root system, you're taking three tiny little cores, one inch diameter down to that compacted zone.

What if you want to know about the compacted zone? Well then you would take that top layer off and then you would sample from the compaction zone below that. So think about what you really are asking the question about. So you want at least three cores from that area, maybe go to three different plants, sampling from within their root system. So you want to go halfway between the drip line of the plant and the stem. So halfway between so you know you'll be in that root zone, and then figure out what depth you're going to go to. Typically if there's no compaction that you're going to encounter, you would want a three-inch depth of the soil, one inch diameter. So one of the easiest things to use is an apple corer so that you can easily simply push that apple corer into the soil, pull up your sample, one sample goes in to the bag, and you collect them into the plastic baggy. You go to your second plant, take a core, put it in here, go to your third plant, take a core, put it in here. Now you have a representative sample. Well, wouldn't it be better to sample from five plants? Yes. The more sample you get, the more replication, the more likely you're going to get the average of that particular situation. Well, wouldn't 10 cores would be better? Sure. How about 100 cores? Okay. Now, it's a time versus how much are you improving the information that you're getting. You've got to make that decision. How many cores do you actually have to have?

So we've collected the cores, we've put them in our baggy, and now we've mixed everything inside our baggy up. Notice one of the things that's missing on this bag, is an explanation of where the sample is from. So you would want to take a marker, indelible marker, and mark on the bag where it was collected, the date it was collected, any pertinent information that you would want to know about that sample because by the time you get this back home, by the time you get this into the laboratory, you won't remember exactly what the sample was. So please get that magic marker and put the information on the bag. Put it on the outside. Sometimes people will send us samples where they've written the information on little pieces of paper and stuck the pieces of paper inside the bag with our sample. Well, what's going to happen to that piece of paper sitting inside here with moist soil? That paper is going to start decomposing. You're feeding the organisms in that sample with cellulose, with ink, with whatever materials they made that paper out of. You're adding that food and now you're changing the organisms that are in your sample. So you don't want to put anything, other than your sample, inside this bag. Can you reuse the bag over and over? No. You can't because we have now contaminated that whole inside of this bag with the sample. So if you clean this out, you can't sterilize the inside of this bag. So you're going to get a fresh sandwich size baggy or maybe a snack size baggy would be more efficient. You wouldn't use as much plastic. So, up to you to make decision what size you want to be making.

Okay. So now we're going to take our sample and we want to dilute it. We're going to first of all start off with one to five dilution of our sample. We want to get test tubes like this one where we have markings up the side where you can see the 1 milliliter mark right here, there's our 2 milliliters, 3, 4, 5, it goes all the way up to 15. It has a nice screw cap on the top. So once we get our sample measured in here, and then add our water. We can tighten that down so we can shake it. So where are you going to find these? Again, when you go to that supply company, a laboratory supply company, go to the internet, Google it, find your closest one, and start looking through what they sell. You want to find a nice little test tube like this with the demarcations on the side. Now, some people also like to, instead of a test tube like this, they like to go get the medicine spoons. Those work too it's just that most pharmacies don't give these away for free anymore. They're going to cost you money. Well, these aren't really expensive. You can get a whole bag of 100 or maybe 500 for $10 or $15. So it's not a massive cost. So the same thing here. You would put

your sampling up to the 1 mil mark, and then you would add 4 mils of water for total volume of your sample of 5 mils. So a one to five dilution because you've taken your 1 mil of sample into a total volume of 5 mils. So one to five.

What if you put your 1 gram or your 1 milliliter of sample volume in and then filled it up to 10 with water? Well then it would be 1 gram of your sample, 9 mils of water but total volume of 10. So it's a 1 to 10 dilution. So when we're trying to figure out dilution factors, it's going to be multiply everything by five if you fill it up to five more. Multiply everything by 10 if you fill it up to the 10 mark. Well, what if the organisms are still too concentrated when you look at that drop of water? Well, then you would take a mil of this, put it into another little container. So a mil of our 1 to 10 dilution, for example, put that mil in there and then fill it up to the five mark, 1 to 50 dilution. Well, what if we filled this up to the 10 mark? Then this would be a 1 to 10 dilution but we had already diluted it 1 to 10. So 1 to 10 times 1 to 10, 1 to 100. Our dilution factor would be 100. So we could do another 1 to 10 dilution. We could do another one and another one and another one out to the point where we dilute the organisms in our sample so we can easily count them. We are generally going to end up doing that with bacteria, especially in soils that aren't really healthy and have a massive concentration of bacteria in that soil. You're going to be diluting your soil so you can find out the right dilution factor so you can calculate your bacteria properly. With everything else, we only end up using the one to five dilution typically, or maybe a 1 to 10 dilution. So we're typically going to do our fungi and our protozoa and our nematodes at these one to five dilutions. That's typical, that's normal.

So I want to go through this process of showing you exactly how to make this…