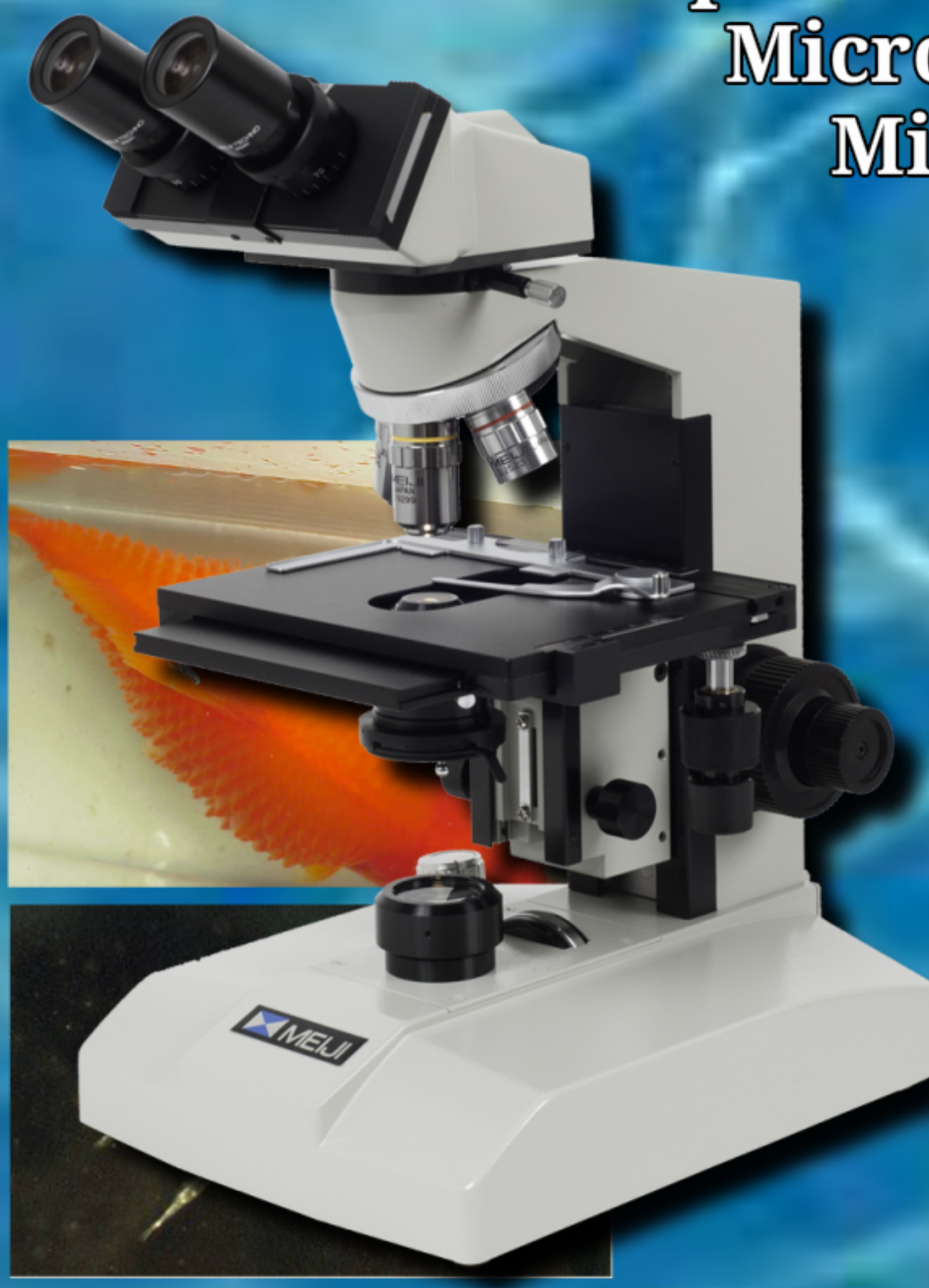
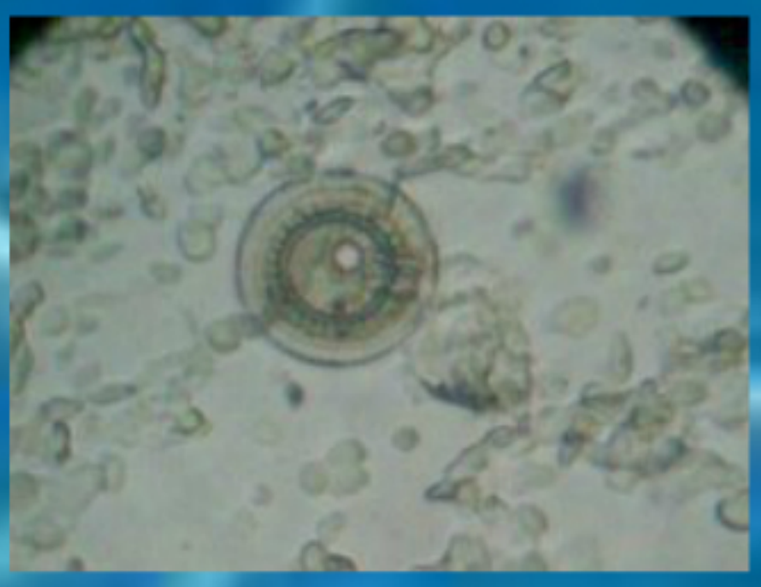
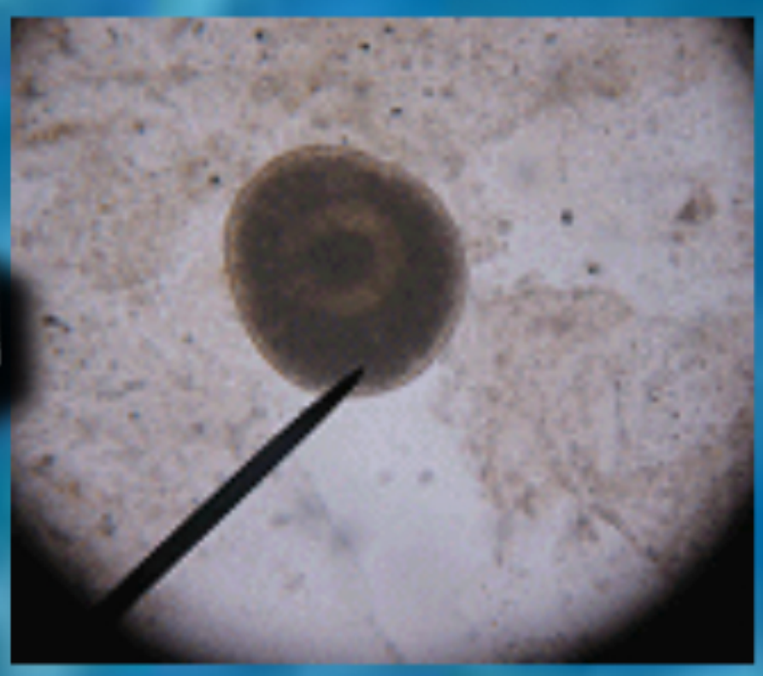
Wet Lab Preparation

By Dr. Erik Johnson

Biopsies and Parasites
Microscope Set up
Microscope Use
Injections
Anesthesia







Wet Lab

Water testing

Environmental Assessment

Fish Exam

Set Up Microscope

Collect Skin Biopsy

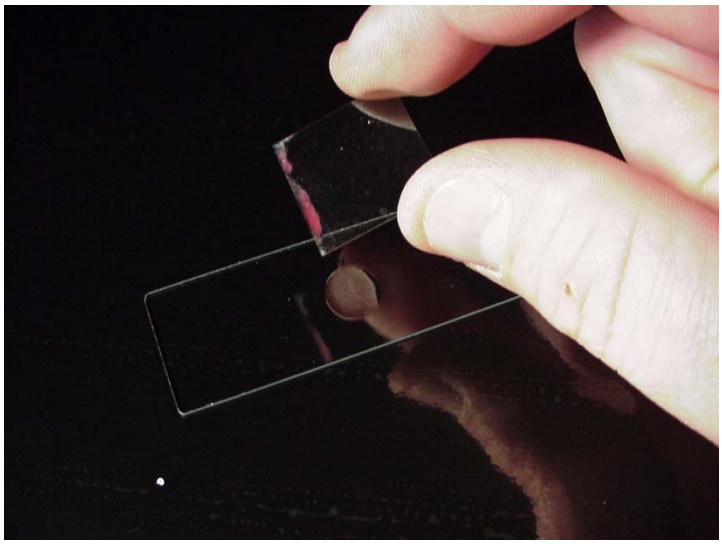
Collect Gill Biopsy

Use Microscope

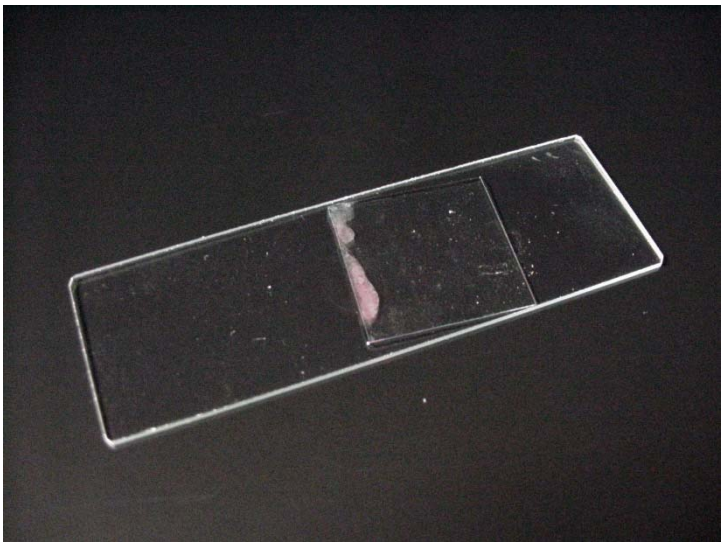
Anesthetize Fish (Oil of Cloves)

Collect Blood Sample

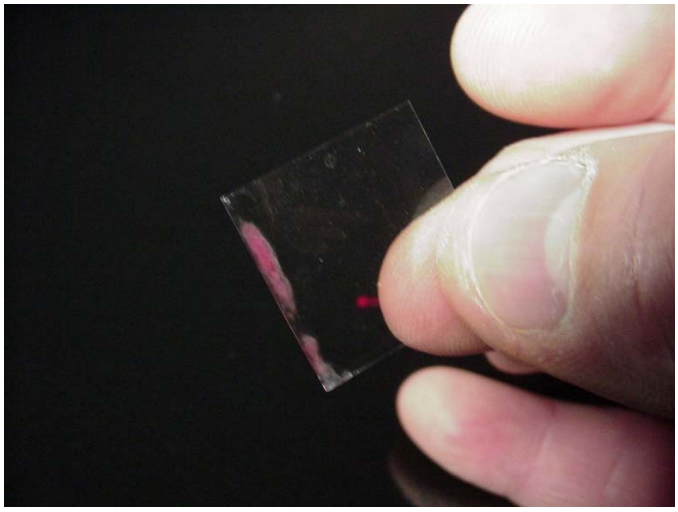
Best places to biopsy a Koi or goldfish. I can biopsy all three areas at one time, just a “scrape, scrape scrape” and collect mucus from all thee spots in a larger and large blob.



The coverslip with the scraping of gill tissue is about to be dropped on the drop of water on the slide.
The drop of water needs to be of modest size or the parasites will have ‘freedom’ to swim away from the mucus in the prep, or the prep will just overflow the slide when you drop the coverslip.



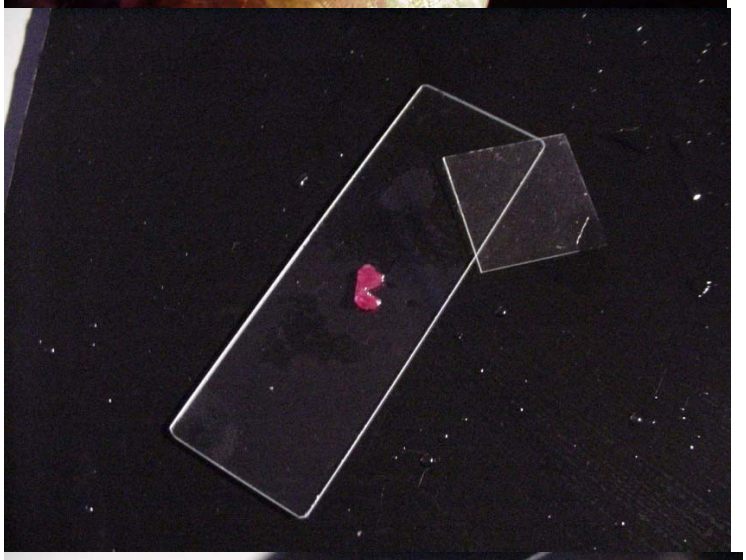
The coverslip is on the drop of water on the slide, ready to look at, under the miscroscope.



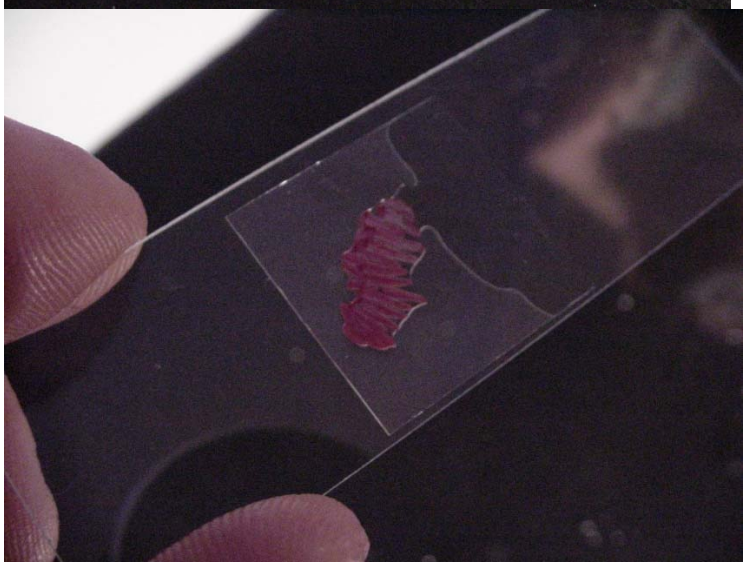
A close up of the coverslip with the freshly scraped gill tissue

Using a coverslip under the gill cover to “scrape” a bit of gill tissue. You have to be kind of aggressive with this. But some people prefer it to snipping gill tissue.
If you break a glass slide under the gill cover it’s okay. Put the fish back into the water and it will cough out the shards with no damage.
Amazing, huh?

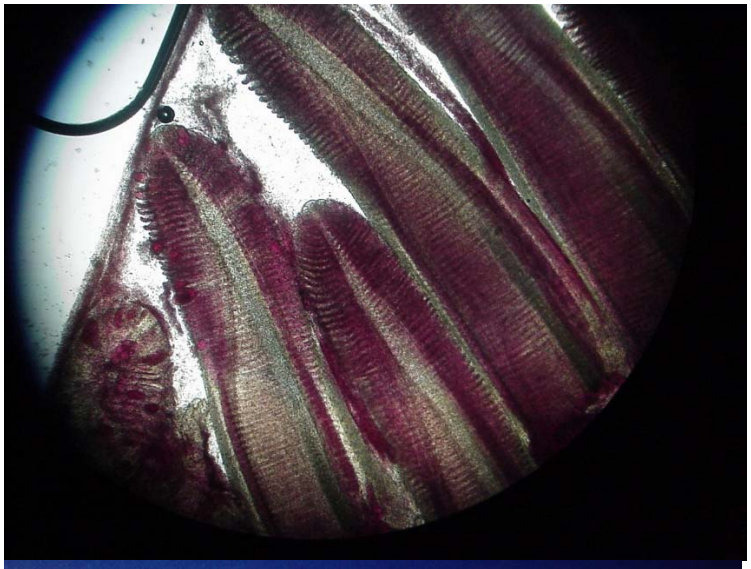
Instead of a gill “scrape” some people snip a piece of gill tissue.
If I am about to make pretty images of gill tissue, like OI want to show a client how the gills are “happy” or clubbed and mucus covered, I have to snip.
Otherwise, for parasites I scrape.



A gill SNIP about to be covered with the coverslip. It’s hard to get the slip to lay down nice and flat because the gill tissue on a snip is “big”.

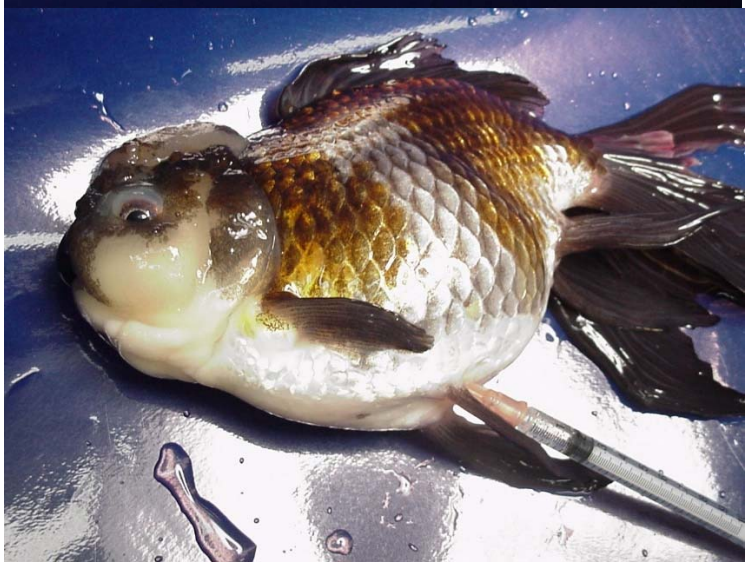


Coverslip on the gill tissue. Tamp it down a LITTLE bit and put another drop of water in the side of the coverslip to “hydrate” the sample.



What a nice “gill snip” tends to look like. These gills are screwed up, The filaments are sticking together and that “beige” color is distressed tissue.

The “gear” you need for an injection or a blood draw from a fish.
These “tuberculin” syringes are available in some states without a prescription. 666MetPeds and most online pharmacies will fill “prescriptions” for these online, even when handwritten on napkins with crayon, with the doctor’s name “Dr. Doolittle” on “Anystreet USA”



BEST of all injection sites is the base of the PELVIC fin. There’s hardly anything in the vicinity and there’s a soft spot under the pelvic fin you can get through pretty easily even in BIG koi.

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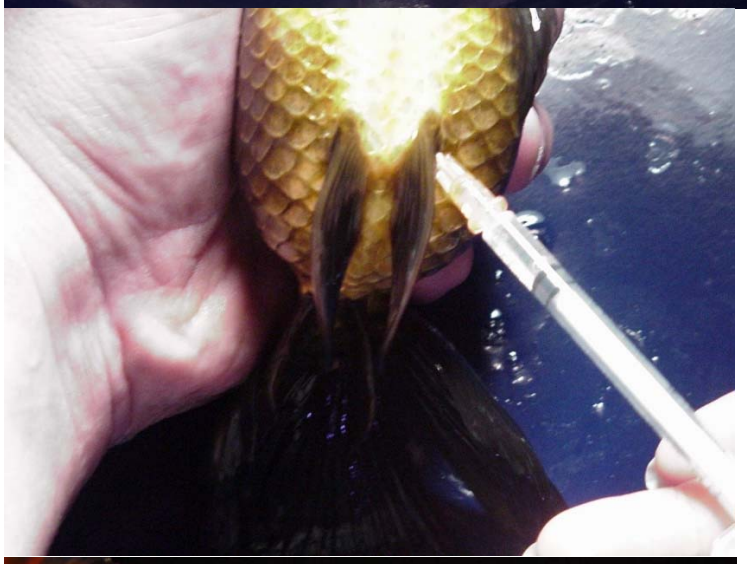


BEST of all injection sites is the base of the PELVIC fin. There's hardly anything in the vicinity and there's a soft spot under the pelvic fin you can get through pretty easily even in BIG koi.

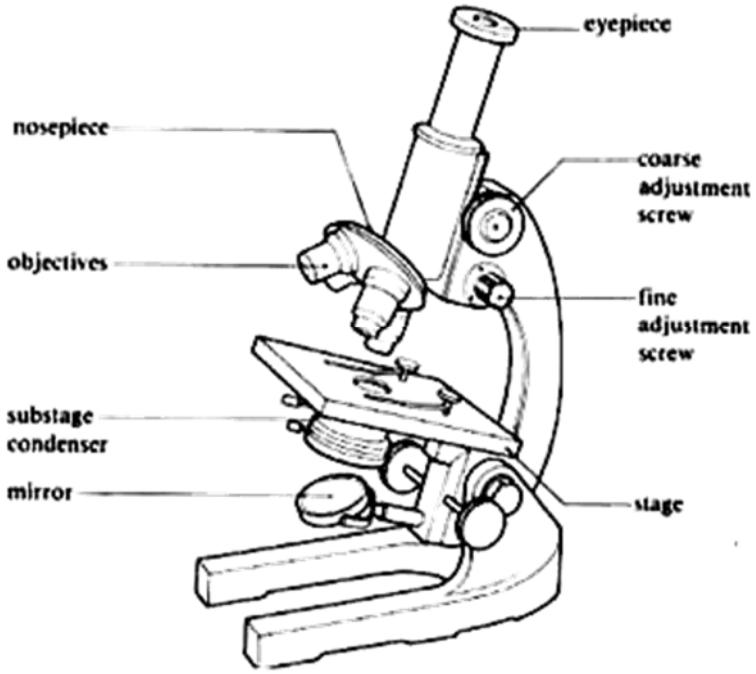
An incognito injection site "intra-muscularly" low on the side of the caudal peduncle so if you remove a scale or leave a "keloid" (scar) it doesn't show on judging.

A better intra-muscular shot site. Better chance of missing a bone or the spine. Thicker scales, and you'll usually remove one. If the fish is "up" for this, they flex pretty hard. It must hurt more.
Which is why I prefer to inject through a plastic bag into the pelvic fin "pit" or "soft spot".

The Microscope

An essential piece of equipment

Without a microscope it is simply impossible to tell the difference between a water quality and a parasite problem. The microscope should be considered the most basic of tools in fish disease diagnosis - **indeed, an accurate, full diagnosis of the disease and its cause just isn't possible without a microscope!**!



A modern monocular microscope

Why is it so important?

Unusual behaviour such as heavy breathing, rubbing, flashing, lethargy is often taken as a sign of parasite disease - yet the same behaviour can be due to water quality problems, internal organ disease or many other causes. Without a microscopic examination of both the skin and gills it is simply impossible to tell the difference and any treatment undertaken is based on nothing more than guesswork! While simply taking a guess as to the cause and treatment required ***may*** work it is just as likely to make matters worse. So microscopy is a vital and basic step in diagnosing and treating fish disease.

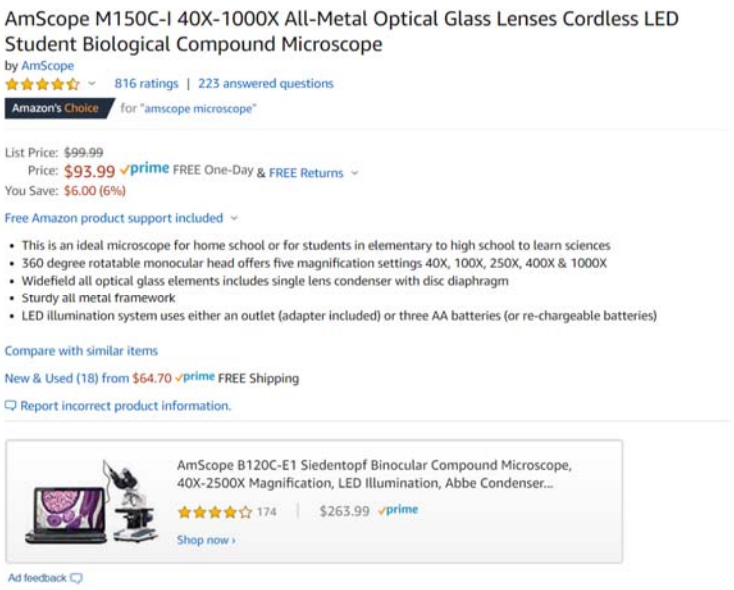
A modern microscope has consolidated many of these things into “one” part. For example, instead of an “iris diaphragm” many microscopes have dimmable lights and a “disc diaphragm”

I exhibit a contemporary microscope I like. It’s got a rechargeable battery with glass optics and a steel durable body. It’s a monocular and that’s fine because nowadays using a USB camera is the way to go.

Buying a microscope

They come in a wide range of types and prices, costing less than a hundred to several thousand pounds - although fish keepers generally do not require an expensive model. There is also a market for good second-hand 'scopes.

It really depends on what you want to use it for. Most of the common parasites such as flukes, white-spot and Trichodina can be easily identified with the cheaper models, but a better quality model is required for critical examinations of cell structures and some small parasites. Generally speaking as prices increase you are paying for better engineering, illumination and optics



Models for fish keepers

There are two basic styles of microscope available. The cheaper **monocular** model has a single viewing tube (as above) - which is fine for occasional use. The **binocular** models enable you to view with both eyes, giving a better field of view. If you want to take photos or video - then you will really need a **trinocular** model with a dedicated phototube.

What I use is a decent quality microscope (for portability I use a Swift FM31 Field scope) with a USB camera on it. That is SO much easier than perching over the scope.

Two other considerations which can make a considerable difference are illumination and the stage.

Illumination

The better the lighting - the clearer the image. Most of the cheap models have an understage mirror which reflects light to illuminate the slide. This can pose problems in dull conditions or lead to contortions with table lamps to try and improve the illumination of the specimen being studied. By far the best option is a fixed or plug-in understage light system, which gives a consistent amount of light. A basic plug-in system can be purchased for under £50 and, in my opinion, is money well spent. More expensive microscopes have built-in halogen lamps with brightness control

Stage

The stage is the part of the microscope where the slides are placed for viewing. As you will appreciate, only a small part of the slide can be seen at any time and the slide needs to be moved to see other parts. Incidentally, the view that can be seen at any time is called the **field of view,** which reduces at higher magnifications.

On the cheaper models the slide is held by clips and has to be moved manually - but this cannot be done smoothly and it is virtually impossible to return to a given position on the slide.

All but the most basic of models have a mechanical stage fitted with vernier screws that allows you to move the slide smoothly and examine it in a methodical pattern. Again, this usually adds less than £50 to the basic cost and is invaluable when scanning the slide for parasites.

There are different qualities of mechanical stage- and some of the more robust stages can be quite expensive.

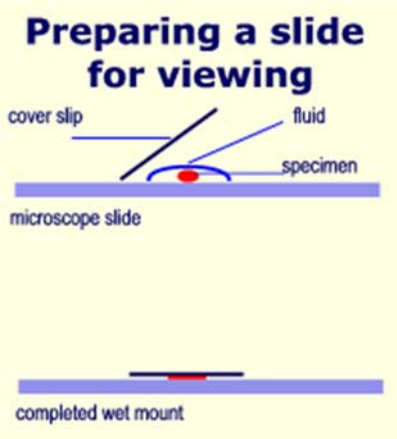
Specimen needs to be as thin as possible

Before any specimen can be examined under the microscope, a slide has to be prepared. No matter how good your microscope is, the final image can only be as good as the slide you are viewing, so proper preparation is important.

A normal compound microscope works by passing light up through the viewed specimen, so it is important that the sample or specimen is a thin as possible. This means working with relatively small amounts of algae, mucus, sediment or whatever. This is particularly important when viewing mucus sample as some of the smaller, transparent parasites might not be seen if the preparation is too thick.

Slide and cover glass

Ideally a new slide and cover glass should be used for each preparation, but in practice slides and slips will be re-used many times. In which case it is important that they are clean and free of smears (cleaning with alcohol will help remove smears - propan-2-ol from a chemist or drug store).



1. The specimen is placed, together with one drop of pond water onto the centre of a clean slide. Do not use tap water or distilled water as these may kill any parasites present. If the specimen is thick, use a seeker needle to gently spread it as thin as possible.

2. A glass or plastic cover slip is then lowered gently on top making sure that no air bubbles are trapped. The best way to place a cover slip is to hold two opposite edges between first finger and thumb. Holding the slip at a 45º angle, place the bottom edge on the slide just to one side of the specimen and then slowly lower the cover slip until it is flat. You can use a seeker needle to help lower the slide by placing it under the cover glass and slowly lowering it into position

3. Once the cover slip is in position apply a small amount of pressure with a seeker needle to spread the sample under the slip and squeeze out any air. Don't apply too much pressure; just enough to spread the sample. Do not use your finger as a finger print may contaminate the cover glass.

4. Practice makes perfect - so try making slides of all sorts of things; blanket weed, algae, mulm etc.

A sequence of steps to take

There is a clear sequence of steps to take to achieve perfect viewing. These are detailed in the box below. A word of warning: Take care when adjusting the focus knobs that you do not advance the objective lens onto the slide! It is very easy to break the slide and possibly damage the objective.

When setting up the focus it is best to view from the side and lower the objective so that it is nearly, but not, touching the slide. Now adjustments can be made while viewing through the eye-piece and slowly winding the objective **UP** and **AWAY** from the slide - this way you avoid any potential damage to either the slide or microscope.

Getting things in focus

Once the specimen is in focus, fine adjustments in illumination and iris aperture can be made to improve viewing.

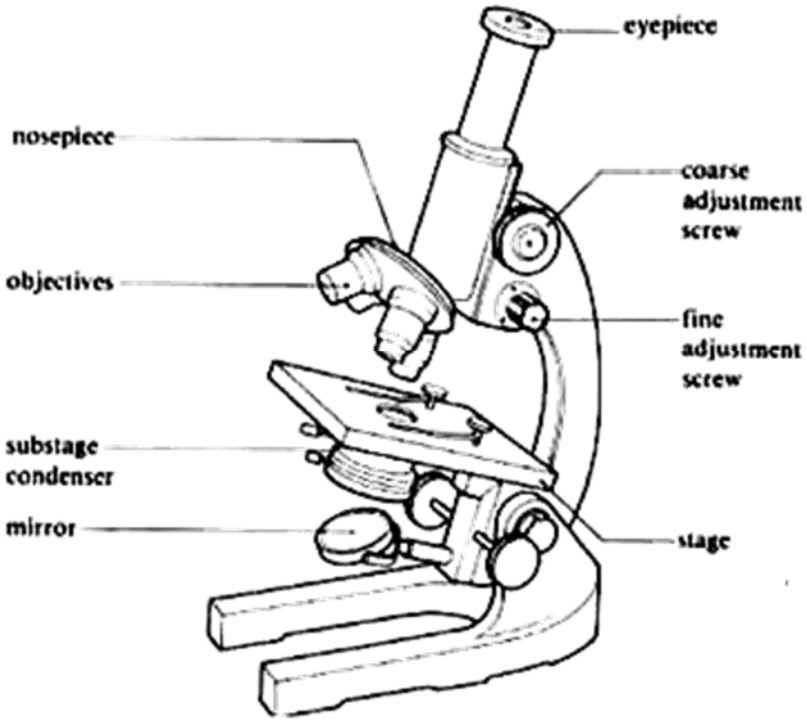
The slide should be scanned systematically, usually by finding the top corner of the cover glass and then moving the slide slowly across the stage to the adjacent corner. When the opposite side is reached the slide is moved up until a new field of view is visible and then moved slowly across to the other side. This is repeated until the bottom of the slide is reached.

Higher magnification are obtained by rotating the nosepiece turret and selecting another objective and then re-focusing.

Parasites are often transparent

Since many parasites are transparent to light it is often necessary to use various techniques to highlight them. The two most popular methods are phase contrast and darkfield. Both of these methods are outside the scope of these pages, but essentially they manipulate the light so that transparent objects are more readily visible. These specialist methods usually mean adding special condensers of objectives to your microscope. While these methods are useful they are not essential for fish disease diagnosis.

If there is a problem with viewing any specimens with an ordinary brightfield microscope it is possible to increase the contrast by racking down the condenser or closing up the iris aperture, although it does reduce resolution.



Scheme for setting up a simple monocular microscope

1. Make sure the 10x eyepiece is in place at the top of the draw tube
2. Raise the body tube a few inches above the stage - by looking from the side and turning the course focus knob
3. Rotate the nosepiece and click the lowest power objective into place above the stage (usually a 10x)
4. Adjust the illumination if using a mirror, turning the flat side of the mirror towards the light source so that light is reflected up towards the condenser
5. Rack the condenser up to within 2mm below the stage and adjust the iris diaphragm until it is half open
6. Place the specimen on the stage making sure that the cover glass is uppermost and secure it with either the stage clips or the mechanical stage arms
7. Adjust the angle of the mirror so that a spot of light appears on the slide directly below the objective lens
8. Looking from the side and using the course control knob, lower the objective until it is just above the slide
9. Look through the eyepiece. Adjust the mirror to give an even amount of illumination
10. Use the course control knob to slowly rack the objective upwards and look through the eyepiece until the specimen is in focus. (Tip) it is sometimes easier to focus on the edge of the cover slip to start with as this gives a nice clean edge when in focus - whereas mucus can sometimes be difficult "to find"
11. Use the fine focus to obtain the sharpest possible image
12. If the light is too bright either use a bulb with a lower wattage (if using a table lamp to illuminate the mirror) or adjust the iris diaphragm to reduce glare
13. Focus the light source onto the slide by slowly racking down the condenser - watch that this does not affect the mirror angle. Adjust the condenser and iris diaphragm to give optimum illumination. Ideally, once the condenser is set in the optimum position, there shouldn't be any need to keep altering it.

While this long list may seem daunting, it is because I have tried to cover every step. You will also note that much of it revolves around optimizing the light source if it is mirror based. With a fixed light source many of these steps can be ignored. After you have set up the microscope a few times it should become second nature.

General microscopy

For general microscope work, a magnification between 40X and 400X is usually sufficient and will allow for relatively easy identification of most common ectoparasites.

Setting up

The most common cause of disappointing results is poor setting up of the microscope before use. The performance of almost all microscopes can be improved if a little time is spent focusing before use. No matter what quality microscope you buy, it makes sense to get the best possible image it is capable of.

Neglected

An often neglected part of the microscope is the substage condenser, found on all but the most basic of instruments. The condenser focuses and concentrates the light uniformly onto the specimen. Most importantly, because it controls the size of the cone of light illuminating the stage. It also controls resolution - i.e. how sharp or fuzzy the image is.

Critical focusing

Ideal illumination is obtained by critical focusing which ensures that the specimen and light source are properly centred and focused, with just the right amount of light to give a clear, uniformly bright image.

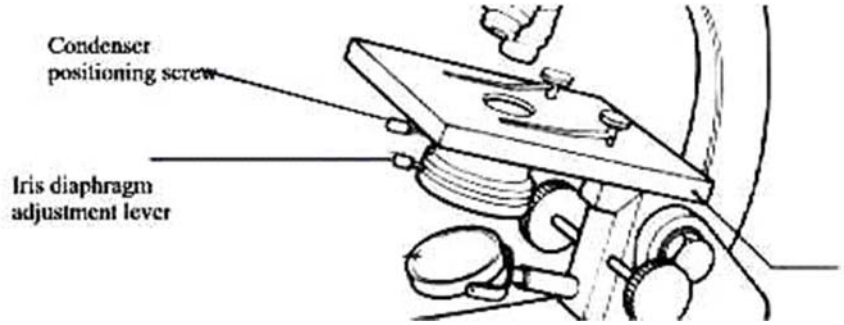
Setting up for critical focusing

Put a prepared slide on the stage and bring it into focus with the 10x or 20x objective

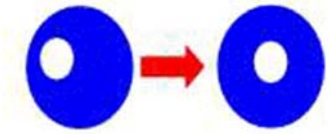
Next focus the condenser. How this is done depends on whether light source has a field diaphragm such as found with Köhler-type illumination. Köhler lighting systems have an iris or field diaphragm which controls the aperture of light going into the condenser. Although this form of illumination is gaining it popularity, it is would not normally be found on the average hobbyist microscope.

Focusing the condenser on a microscope without an iris diaphragm is carried out by removing the slide from the stage and placing a piece of thin card half-way across the light source aperture. Adjust the condenser - not the stage or objective - by racking it up or down until the card is in sharp focus when viewed through the eyepiece lens. This usually occurs just as the light interference halo turns from blue to red.

At this point the condenser is properly focused and should not need to be adjusted again. With Köhler lighting systems focusing is carried out in exactly the same way - only focusing on the leaves of the diaphragm rather than a piece of card.



Next, it is important that the condenser is centred or it will focus the light at some point to the side of the specimen. To centre the condenser close down the iris diaphragm and remove the eyepiece. Looking down the viewing tube you will see a small point of light (see diagrams). Make sure that the light appears in the centre of the tube by adjusting the condenser positioning screws. This takes a bit of fiddling about - but is simple once you are used to it.

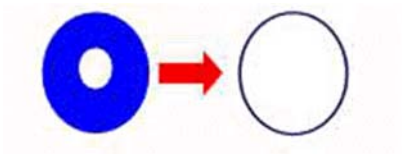


with the eyepiece removed, look down the viewing tube when you will see something like the diagram on the left - an off-centre light spot. Adjust the condenser centering screws until the light is in the middle

The iris diaphragm

The final step is to adjust the substage or iris diaphragm. This is done with a lever or screw found on the condenser which works in a similar fashion to that of the iris of a camera. As the iris is opened it allows more light into the condenser. The iris diaphragm is often used incorrectly to control light intensity. While this might seem logical, its proper use is to control the size of the cone of light entering the objective lens. The correct iris diaphragm setting varies with each objective. Consequently it needs to be re-adjusted every time you change magnifications

To set up the iris diaphragm look down the viewing tube without the eyepiece in place and slowly open up the iris diaphragm until the circle of light just about fills the viewing tube. Finally replace the eyepiece, re-focus and adjust the light intensity if your microscope has rheostat control.



Once the light spot is central, open up the condenser iris until the light just fills the field of view. Replace the eyepiece

Provided that your lens and slide are clean you should now get the best possible image.

Taking a skin scrape

Taking and preparing a skin scrape involves using a blunt scraper, such as a wooden spatula, to gently take a sample of mucus from either immediately behind the gill cover, alongside the dorsal fin or the base of the tail.

The scraper is held at approximately 45 degrees to the body and drawn backwards towards the tail in a smooth movement, lifting off a small amount of mucus from the sample site. The mucus sample is then smeared onto a clean microscope slide along with a drop of pond water. Never use tap water as any residual chlorine could kill any parasites that are present!

The sample is then covered with a cover-slip and examined under the microscope, usually low-power, for the presence and number of parasites. I would normally take at least two samples, from different sites, from each fish being examined.

Taking a skin scrape and gill biopsy

click on pictures to enlarge them

Taking a skin scrape using a wooden spatula to **gently** lift off a mucus sample from just behind the operculum

The mucus sample is put onto a glass microscope slide. A drop of pond water is added and a cover slip put on top

Taking a small gill biopsy using a fine pair of scissors

photos: Frank Prince-Iles

Be careful!

There are certain considerations if the examination is to yield useful results and avoid causing damage to the fish being examined. The most important concern is to avoid injury to the fish during what should be a simple, safe procedure.

If the fish does have parasites they will be found in the mucous layer (though some will also penetrate the epidermis, e.g. 'Ich'), so it is the mucous layer we need to sample for the wet-slide preparation. It is important to realise that damage caused to the epidermis while taking a mucus sample will be detrimental to the fish, so only gentle pressure with a blunt scraper should be applied - never use anything sharp that might damage the epidermis.

To sedate or not sedate - that is the question

Another consideration is whether the fish should be sedated while a scrape is taken. This is a subject that most books and magazines seem to avoid so I will put my neck on the block and give my thoughts on the subject.

In the first instance we want the fish to be still enough to do the scrape properly, while at the same time avoiding damage to the epidermis. With two people, one holding the fish and the other taking the scrape, it is possible to sample smaller fish and docile larger ones without the need for sedation. However, if there is likely to be a lot of flapping around, there is a real chance that mucus may be stripped off by the constant handling, thus giving an inaccurate result or, alternatively, a danger that the fish may be damaged.

On the other hand, if fish are routinely sedated prior to taking a scrape, there is the slight added risk that the fish could die from an overdose of anaesthetic. There is also the consideration as to whether the anaesthetic will affect the parasites and give a false result- although this has not been my experience if the scrape is taken and prepared quickly. In short, it seems to be a question of experience in deciding whether the procedure can be carried out effectively and safely without anaesthetic or whether the additional risk involved in sedating the fish is the lesser of two evils.

I must say that I have come across some rather inaccurate conclusions when people have tried to take scrapes from large, unsedated, lively fish! When dealing with larger koi on my own I invariably find MS222 anaesthetic a help. Your thoughts on this subject would be welcome!

The first few biopsies of the fish should be NON Anesthetized as we do not yet know via replicable studies whether certain parasites succumb, or are removed by certain anesthetics. I definitely prefer Oil of Cloves as a sedative because it is safe, cheap and easy. Chances of mortality in healthy fish is very low.

Gill sample

If a parasitic infestation of the gill is suspected it is possible to sample mucus from the gill. It goes without saying that this procedure is potentially dangerous and must be carried out with extreme care and only on sedated fish.

A mucus sample can be taken from the gills by gently inserting a cotton-bud under the operculum and rolling it over the gill filaments. Under no circumstances should any pressure be exerted that may damage this vital and delicate structure. The mucus is then spread onto the microscope slide. For accurate results it is important that the sample is prepared and examined as quickly as possible.

With care it is also possible to take a small sample of gill using fine scissors to take a small biopsy from the lamellae tips. I urge that this procedure is not carried out unless one is totally confident about the procedure as the tiniest slip could cause considerable damage to the fish.

How many is too many?

Generally one or two observed parasites per slide should not be a reason for concern, whereas ten or more per field of view would be. Usually, when there is a serious infestation it is quite obvious as the slide often seems alive with parasites!

There are two potential problems that may be encountered when examining a skin or gill scrape. All of the smaller parasites are virtually transparent and unless the slide is scanned slowly and methodically there is always the possibility that some parasites may be missed, especially if they are not moving. If there seems to be a problem it is worth lowering the microscope condenser to improve contrast or, better still, if the option exists, try viewing the slide under dark-field.

If the mucus scrape is particularly thick it may be necessary to view at different levels, starting from the bottom and working progressively to the top. Otherwise, there is the chance that smaller parasites may be missed because they are hidden in the mucus. This becomes more likely at higher magnifications.

Don't jump to conclusions

The second, more common, potential problem is simply jumping to conclusions. It is just too easy to spot the most obvious parasites, for example skin and gill flukes, and conclude that these are the problem. Even when you have spotted parasites it is vital that the whole slide is still methodically examined to make sure that nothing important is missed and so you build

up to the correct conclusion. My own record is: five different species of parasite on one slide - **but it took a full 15 minutes to find them all!**

If a fish is found to be heavily infested it is worth taking a scrape from one or two others - to determine whether there is a general problem in the pond or if this is an isolated instance. It is often the case that rather than the whole pond requiring treatment, just the one fish is ill and the parasite explosion has resulted as a secondary infection for that fish, not the whole collection.

Practice make perfect

Although the microscope is a fairly simple instrument to set up and use, interpreting what you see can sometimes be difficult. It really requires practice until such times as you know what is normal and what is not.

As a piece of advice I would suggest familiarizing yourself with healthy or non-urgent fish mucus samples - rather than waiting until a serious case arises. By getting used to taking and examining mucus samples you will be better prepared to make a judgment when a serious problem arises.

What to look for

I am assuming that initially the microscope is going to be used as as simple diagnostic aid to carry out procedures such as skin and gill examinations. It can of course be used for more advanced studies, such as plant and animal cell structure. You will probably have seen photos of slides on the site showing details of various fish organs such as gills. This is histology and involves special preparation and staining of the specimen.

At a basic level, microscopy would be used as part of a routine examination to check mainly for external parasites. This involves taking a skin or gill scrape / sample and making a simple wet mount as previously described.

Initially it is very easy to get confused by what you see on the slide - particularly if small non-parasitic aquatic animals happen to be sampled, leading to fears of some new, frightening parasite or disease!

Normal mucus

The first stage is to recognize what normal mucus looks like and disregard any "debris" or unimportant "introductions". These could be things like air bubbles trapped under the cover slide which appear as circular, dark-rimmed "grommets". Or perhaps normal cellular debris which appears as stationary, irregular-shaped - often dark patches. You are also likely to see trapped algae of all shapes and sizes. This is why it is important to get as much practice as possible before using your microscope in earnest.

Normal mucus in a wet mount at 100x magnification

click on thumbnail to enlarge the picture

Normal mucus which is seen a lumpy, transparent, light to grey substance. Also shown is a trapped air-bubble which is often mistaken for a strange parasite, and some typical cellular debris. Active parasites should be easily seen.

Five parasites

At the risk of oversimplifying what can be a complex issue, in the vast majority of cases (>90%), the usual findings would be a common parasite problem. In freshwater fish such as koi and goldfish these usually involve just five different types namely, flukes, Costia, Trichodina, Chilodonella or white-spot. So, simply being able to recognize these common beasties will make you a reasonably competent microscopist. There are, as already suggested, less common things you might

come across, but once you are familiar with these basics, it is fairly easy to focus on any abnormal findings and either look them up in a good book or ask for advice.

In most slide preparations the parasites will be "alive and kicking" and so they are easy to spot and recognize. However, it is important to scan the entire slide, slowly and methodically looking for parasites that are still. These can be more difficult to spot as all of these parasites are transparent and therefore tend to blend in with the mucus. However, with the exception of Costia, which is virtually impossible to spot unless it is moving, they can be seen once you have the experience and have "got your eye in".

This is why it is important to examine slides as soon as they are prepared as many parasites die if left for any period.

Recognizing parasites

There are many good books available which describe the common parasites. There are further details and photos on the relevant pages of this site. The movies of parasites will also give some idea of the way each parasite moves; an important diagnostic feature. I have included a short summary of each of the parasites below, but you will need to check the relevant pages for more detail;

- **White-spot:** Dark, slowly rotating circular parasites of varying sizes. Often with a lighter, horseshoe-shape visible. One of the larger parasites. Go to page

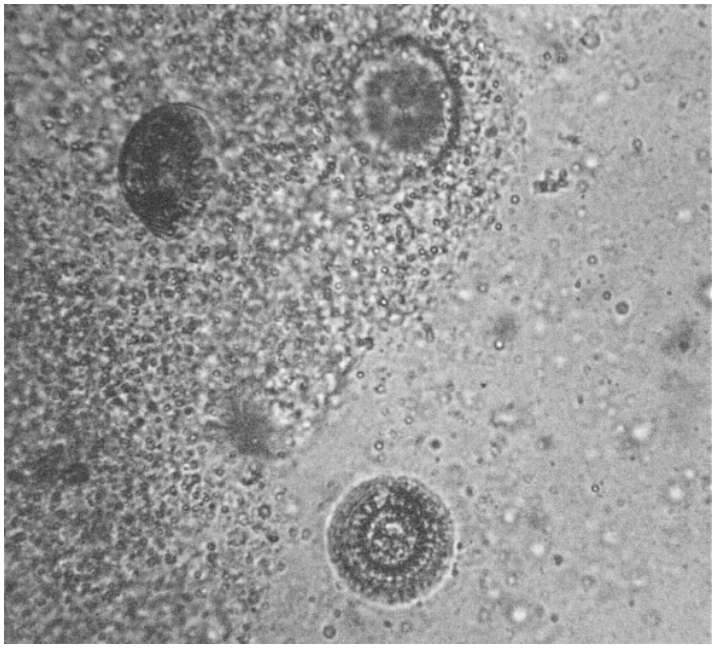
- **Flukes:** Long, worm-shaped animals that move in looping action. There are often clearly visible hooks at one end. Go to page

- **Trichodina**: Medium=sized round parasites with a series of inner, concentric rings. These zoom around like flying saucers. Go to page

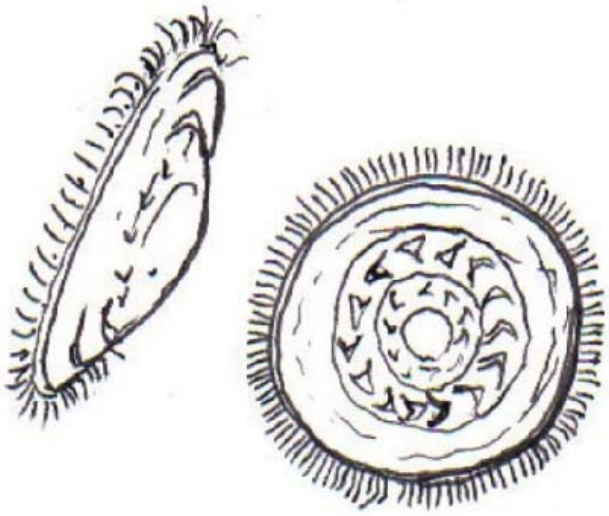
- **Costia:** The smallest parasite that is often easy to miss. A fast-moving parasite recognized by its flashing and twinkling as it moves in and out of focus Go to page

- **Chilodonella:** A medium-sized oval-shaped parasite that turns and glides. Go to page

^Above: Ich or white spot organisms appear as “round” “balls” with a horeshoe shaped nucleus.



^Above: Trichodina is the round “flying saucer” organism, it’s very mobile and sometimes hard to isolate under the microscope.



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^Above: Flukes appear as “stretchy worms” they are VERY common. Their treatment is any one of the many “Praziquantel” compounds but they often respond to serial treatments with Formalin Malachite.

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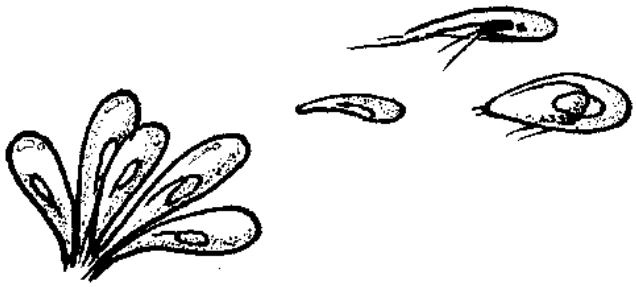
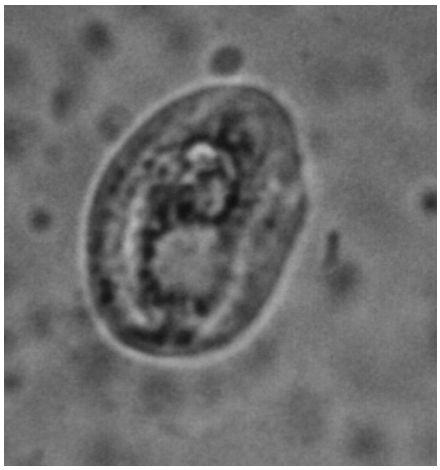
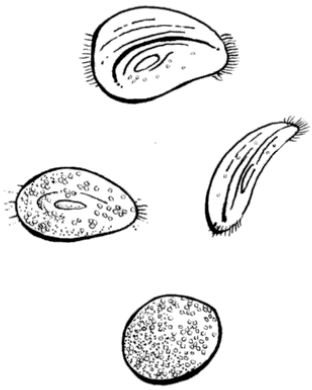
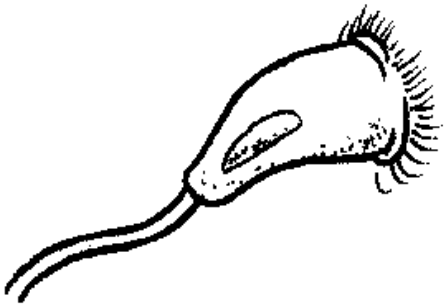






^Above: Childonella looks like an “ear” and has an organelle in the middle, that’s it’s stomach. It throws up the stomach, rubs it around on the fish and sucks it back in with slime - which is what it likes to “eat”.

Epistylis - borrowed from Kjskikoi International web site



^Above: Epistylis looks like fungus to the unassisted eye, is common in catfish and pond raised Koi and is pretty easy to clear in ornamental ponds with a good water change, pond clean out and some salt.

Oil of Cloves

Oil of Cloves as supplied with these instructions is a concentrated clove oil. It is ONE FIFTH as strong as dentists Eugenol®. These instructions apply to the Oil of Cloves you purchased from our business and not Oil of Cloves "in general" – If you are not entirely comfortable with these instructions and you are not willing to assume 100% of the liability for the use of this compound, do not use it. You may return it to us and you will be entitled to a full refund of price paid.

What It Is: Oil of Cloves is a concentrated Clove Oil. It smells strongly and will stain fabrics and can irritate your eyes, mouth and nose. It is non-toxic. Its most common use is toothaches, where a small plug of cotton can be soaked in Oil of Cloves and put in a dry socket and pain will subside. Oil of Cloves is also used in aromatherapy. It smells nice.

How Supplied: A 1-ounce bottle of Clove Oil will last a very long time.

Preparation: Please consider that any handling and anesthesia of a fish represents a stressor. Stress challenges the immune system of the fish. Handling and sedation should be 'worth it'. A large container of water is prepared using the water the fish is coming from as long as it is clean and fresh. If the water in the fishes' main facility is sub optimal or green with phytoplankton (algae) then make up fresh water of the same temperature, properly dechlorinated, with a pinch of Ph buffer or baking soda in it. Aerate the vessel of water, and arrange some means of covering the vessel of water. The fish go through an agitation phase during sedation and will spasm a little bit, both splashing, and potentially exiting the vessel.

Dosing: Oil of Cloves will be used at a rate of FIVE drops per ONE gallon of water that you've placed in the anesthesia vessel. So if the fish were in a two-gallon container, you would use ten drops of Clove Oil.

Dissolution: The Clove Oil would be dropped into a small Ziploc® bag with some water. Shake the sealed bag vigorously to turn the Clove Oil into a whitish cloudy emulsion with the water. Pour this emulsion into the vessel with the fish to be sedated. After about three to seven minutes the fish will start to twitch and spasm, rolling onto its side, splashing you. After a few short minutes it will calm and start breathing more and more rarely. Before it stops breathing completely, you should perform whatever process you had in mind; an injection, a biopsy, removal of some small defect. If you want the fish to stay asleep for a while, you should use a syringe or small tubing to pump or push treated Clove Oil water over the gills. When and if the gill excursions become 'rare' then you should start piping clean untreated water over the gills. When the fish becomes too lively again, resume using the Clove Oil treated water. And so on, back and forth. Close monitoring is essential to success.

If left alone in Clove Oil treated water, the fish will go 'deeper and deeper' until it fails. If left alone in Clove Oil treated water, the fish will stop breathing, and if left in for fifteen or more minutes, the fish would still be unlikely to die. I've seen fish treated with 600% of the normal dose, for hours, to be returned to clean water under pure oxygen and then have 90+ percent of them live! Clove Oil is VERY forgiving.

Waking a fish. It's not critical to 'walk' a fish around the tank. All you do is replace the fish in nice clean water it's used to. Make sure the recovery tank is WELL AERATED and make sure the fish cannot harm itself running into things or getting sucked onto the pump intake while it wakes. It will swim spastically for 15-20 minutes after waking. It can get wedged between rocks. Some people wake their fish in a plastic bag under pure oxygen in clean dechlorinated water. The longer a fish was asleep, the longer it will take to start to wake, and to wake completely.