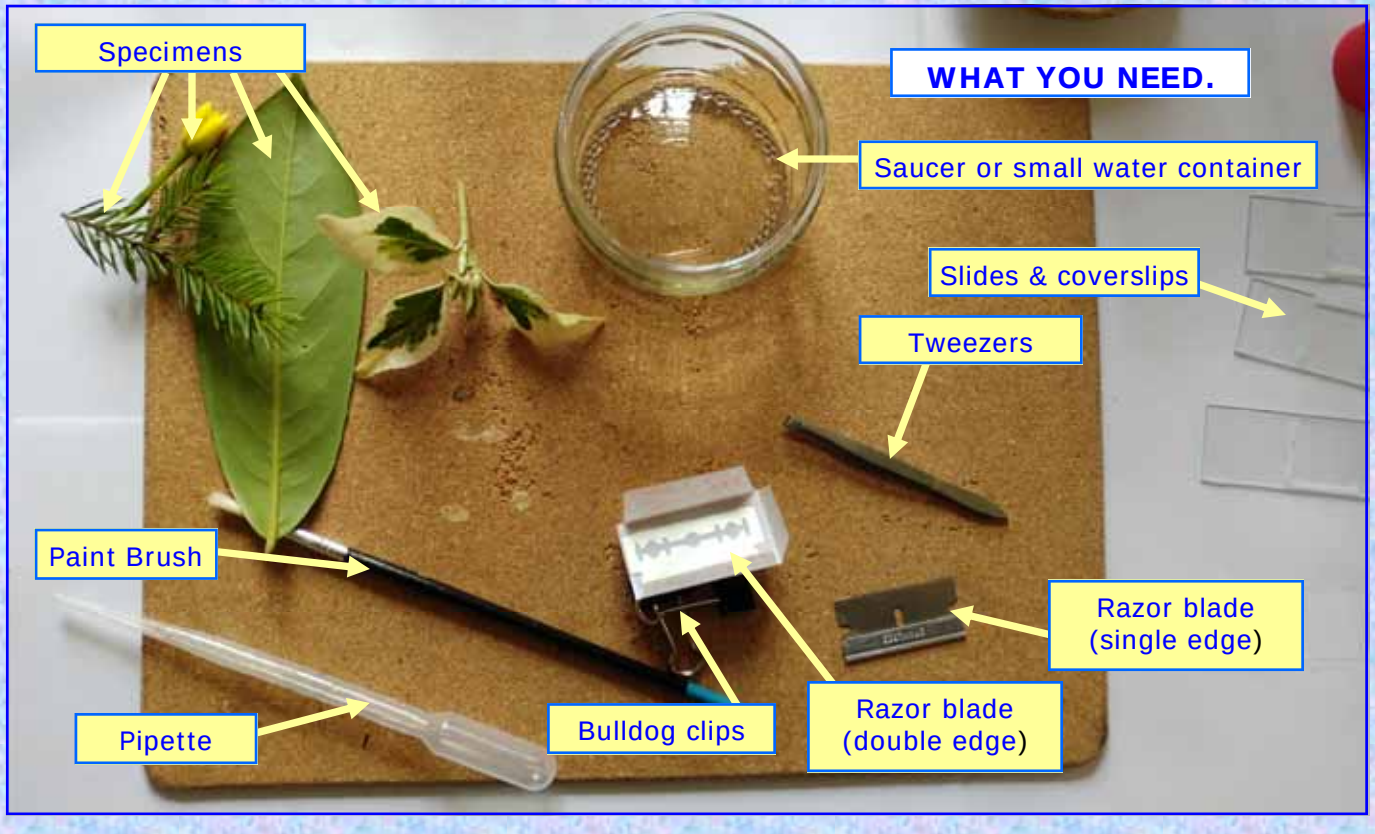
Project 15:
How to make thin slices of specimens. And mount them.
[For 18+ age group only!]

There are several methods you'll see on the internet and older books on hobby microscopy stating how you can make thin sections (mostly of plant stems and leaves) by hand. I have tried most and some others suggested to me by colleagues and friends. Most of them do not work very well, indeed, if at all. The problem is that thin sections means just that—*thin.* They have to be transparent, which is extremely difficult to do without wax-embedding and cutting the tiny wax block using a rocking microtome—a device purposely created for that task. Trying to make thin sections by hand involves using a very sharp razor blade, cut-throat razor, or other refined cutting edge. No child should be allowed to utilise such blades, even with parental oversight. One slip and you can lose a finger or worse!

The best method I've seen and tried was pioneered by the late Walter Dioni, a major contributor to Micscape Magazine. This is the one I will demonstrate to you. I have actually made slides from scratch using this method, so I know for certain it's possible, despite it being a time-consuming hit-and-miss affair. Anything else you see about using cotton reels to make microtomes are far less effective. You only have to try doing what is said to realise the difficulties involved. Anyway. Lets give it a try and see what the issues are and what kind of results we get.



A few additional items are not shown:

PVA Glue,
Iodine or Methylene Blue Dye,
Electrical Insulation Tape,
Glycerine of Fructose Mountant.

Most of these items can be purchased locally or online. The specimens were just cuttings taken from a Laurel bush, a pine tree, an unnamed bush, and a small flower.
All from my garden. I never used the Laurel leaf in the end. I started with a pine leaf

1) Making the sectioning tool (a)

Prepare some well slides using the pva glue. Paint the pva on two or three times to build up the walls in case our slices are slightly thicker than they should be.

Being extra careful, take the double edged razor blade and fold it in the middle. Press down on the folded top until it snaps into two equal sized pieces. This is the first step in making a cutting device (a sectioning tool). Cut a small strip of insulation tape ready to use.

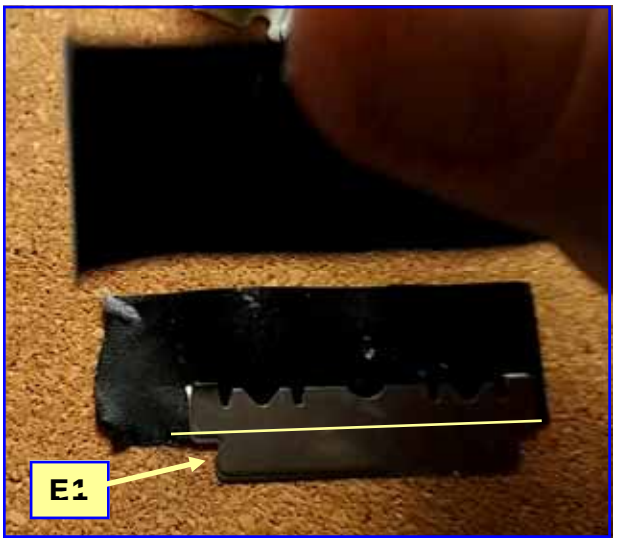
2) Making the sectioning tool (b)

The trick is we have to put the two blades together, one on top of the other, perfectly aligned. If the blade's snapped edges have curled slightly, ensure they face outward away from the two surfaces you are putting together.

Use tweezers, to avoid any chance of nicking your fingers on the sharp edges. You must align the cutting edges perfectly. Be patient. When you are certain they are perfect, hold them with a finger and pick up your cut piece of tape.

3) Making the sectioning tool (c)

Slip the piece of insulation tape, sticky-side up, under the blades. You can see mine in place, but the blades have popped out of alignment. We'll fix that in a moment. Cut another piece of tape the same size. Now, align the blades and carefully bring the new piece of tape, sticky side down, into contact with the other piece of tape and the blades. This will fix them tentatively into their alignment. Ensure enough cutting edge pokes out from the joined tape—**[E1]**, below the yellow line I put in the image.



4) Making the sectioning tool (d)

The placement of the tape is critical—[**right**]. We need enough cutting edge poking out to go fully through whatever we are going to cut (section). But we also need an overlap quite wide at the top—the non cutting side. In a moment, you'll see why, and its importance.

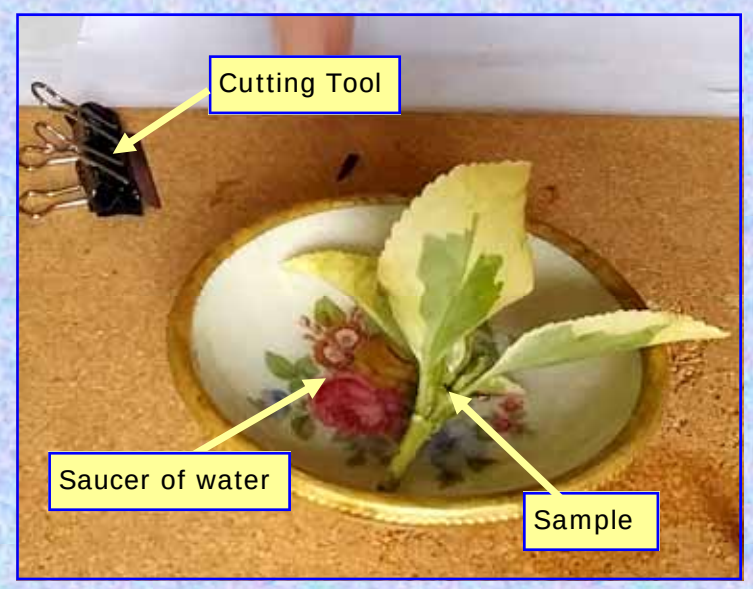
Above: the tape stuck together and the gap left for the cutting edge to poke out.

I turned the blade over here—[left], so you can see the edges of the two blades (marked with yellow lines). The gap between them is due to me opening the blades a bit, where the tape is acting as a hinge. We will close these up shortly.

5) Making the sectioning tool (e)

If you have a large bulldog clip, put it over the blades. I have two smaller ones purchased from the stationary section of a local supermarket. The tape needs to be folded over to take up all the space between the non-cutting edges and the clip or clips. If a space is left, when you use the device and press the blade down to cut through a specimen, the blades will slip upwards into the space, and deny sufficient cutting edge depth to go through the specimen. This is one of the reasons for the tape. There is another reason too.

This first attempt, I'm about to show you, was not the best method. The general principle is here but I found it difficult to see exactly what I was doing. I changed out the water container for a shallow saucer. It helped a bit. In the end, I did something slightly different. But let's see this method first. The idea is to place the specimen you are going to section into the water. By doing this, any tiny, almost transparent slices, get swept off the blade and into the water without being damaged. It helps keep the blade free. Some slices disappear up between the two blades. They might stay trapped there or get swept as you slice again.



6) Cutting sections

Put the sample end in the water. If you wish, use the single edge razor to trim the sample, in this case the stem. Take the sectioning tool we just made, hold the specimen down firmly and flatly in the water, and bring the sectioning tool down firmly and resolutely over and over again along the stem. Tiny sections, some good, some not so good, will start appearing in the water. Pause a moment, and check the outer edges of the blades. Use a wet paint brush to gently wipe any sections on the blade into the water. Make sure when you cut, you bring the blade edge into contact with the bottom of saucer to ensure you went right through.

7) Better?

I decided it might be best to put some drops of water on a glass slide and use that as my cutting surface. Here is me sectioning a pine leaf. Same principle. Hold the leaf into the water, slice down, once or twice, pause, but this time—look for the slices on the slide and use a paint brush to move them over to the saucer of water. Then start again. Of course, some of the slices will be thick, where you moved the cutting tool along the specimen, effectively leaving a length between this slice and the last one. If you look closely at the slices in the water, they are obvious.

When you have a few slices in the saucer, remove the clips from the cutting tool. Take a wet paintbrush, and gently ease the blades apart (the hinge idea—the other reason for the tape) and wipe out any sections trapped inside into the water in the saucer. *These are likely to be your thinnest sections**—[F1].***

So, how did we do?

We need to mount some slices to find out.

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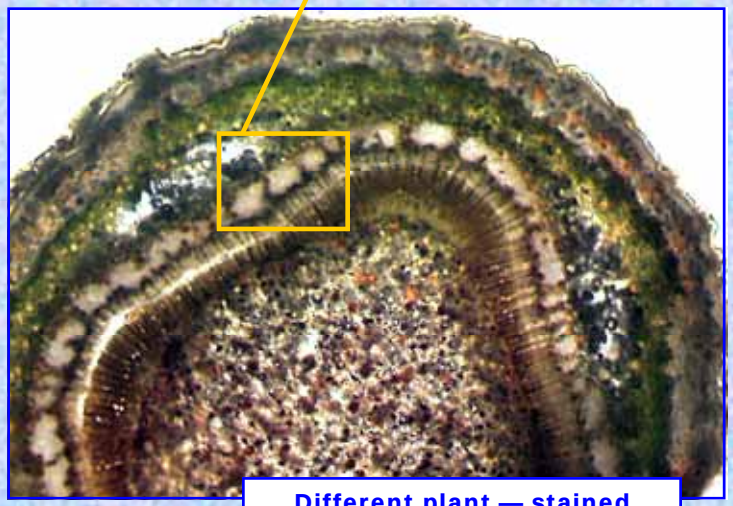
8) Mounting the slices

Take the thinnest slices you can see in the saucer and using the paint brush, deposit them onto a clean slide. Put some drops of water on them, and a drop of stain. I used Iodine here. Leave them for a few minutes then add a drop of mountant—either the Glycerine based one or Fructose you made in an earlier project, or a purchased Aqueous mountant if you never made any. This is to start letting the mounting medium 'creep' into the specimens structure. (Remember to heat the mountant up first to 40C on a radiator or a low temperature in the oven. Not a microwave one!). After five or ten minutes, use the paintbrush to move the sections to a well slide. Apply a few drops of mountant making sure you drip it in carefully using the non-brush end of your brush. Make sure you put sufficient to fill the well. Breathe a few huffs onto a cover slip and put it down on the slide in a manner described in an earlier project. Avoid making air bubbles.

RESULTS

See text opposite page

Not stained



Different plant — stained

I took slices from several different plant samples. I can't recall which was which as I mounted them all onto a single slide. When writing a book, doing the projects, filming it all, time is limited. The results were mixed. About 30% were thin enough to reveal additional information under the microscope, the rest were too thick and confusing to look at. I think the method is quite useable and with practise and modifications can be improved immensely. Look at the images above. You have to understand that you can go out into your garden or a park, take a few cuttings and explore them in depth in a microscope and begin to learn how those plants are working. How many people could do that? You can become one of them. How can the sectioning device method be improved?

This image is a higher magnification photo through the microscope of the lower magnification one on the previous page, bottom right. The area indicated by the yellow square. A bit fuzzy? Blame budget microscope and cameras, and hand sectioning without toxic chemicals. But it still reveals a lot. At the start of this day, I just snipped a few bits of green stuff and tiny flowers in the garden, and now here I am seeing fluid supply tubes, different areas and cell arrangement in circling layers around a central core.

I'm beginning to glimpse mechanisms of energy storage, tougher outer protective skin. All by my own hand. You can do that!

Colour, structure, shape, position. All with something to tell. The microscopic world of life starting to show itself. Begin exploring too!

How can the method be improved?

This is an area of Amateur Microscopy which is slowly dying out, mostly because all the very informed and workable methods discovered by slide-makers back in the Victorian age who had a wealth of chemicals at their disposal, which in this era, we don't. I thought to focus on it somewhat to see if we can revive it.

Bright minds do bright things. I love the idea of problem solving. Walter Dioni who worked on this method, looked at ways to allow us all to mount slides, even though we are not in a professional industry with access to sophisticated machines, techniques, and access to even small quantities of powerful chemicals. He did well. I have watched wax embedding by amateurs, seen all the suggested methods for amateurs. The other amateur ones produce worse results than this one. I know. I tried them. I also know, it is not possible to produce perfect sections of organic material by hand alone. People have travelled this route for over 150 years,

These are the issues I find with the double razor blade method. And some ideas about improving it.

The gap

The two blades are bound together with tape because we might need to 'un-hinge' them to remove slices caught inside. This forces irregular thicknesses of the section up into a gap which flexes because the razors are very thin and move. We could glue the edges/sides together using pva, possibly inserting a spacer of a single dried layer of PVA glue just above and inside the two cutting edges. This would give a fine space for the sections to move into. It is easy afterwards to use the single blade razor to push in and separate the two blades and wet-brush out the tiny thin sections that went into the gap.

Most other methods used to section plant material rely on a supporting material. A piece of carrot for example in which one puts the leaf piece of plant stem; and then a surface which one slides a single blade over to take off the upmost piece of the specimen. Turning a screw or gently pushing the specimen through a bit more, offers another opportunity to take a slice etc.

Our double blade method does not require either a supporting 'carrot' or other material, nor a device based normally on bolts passing through cotton reels to work.

Two fine cutting edges (razor blades) arranged with a microscopic gap between their faces can stand the best chance of cutting through a specimen every time and deliver the same thinness of section.

This is a least time consuming method with the best chance of achieving any kind of useable result. Take it from me. If you want to section your findings without getting involved with time-proven complex methods. This is it.

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