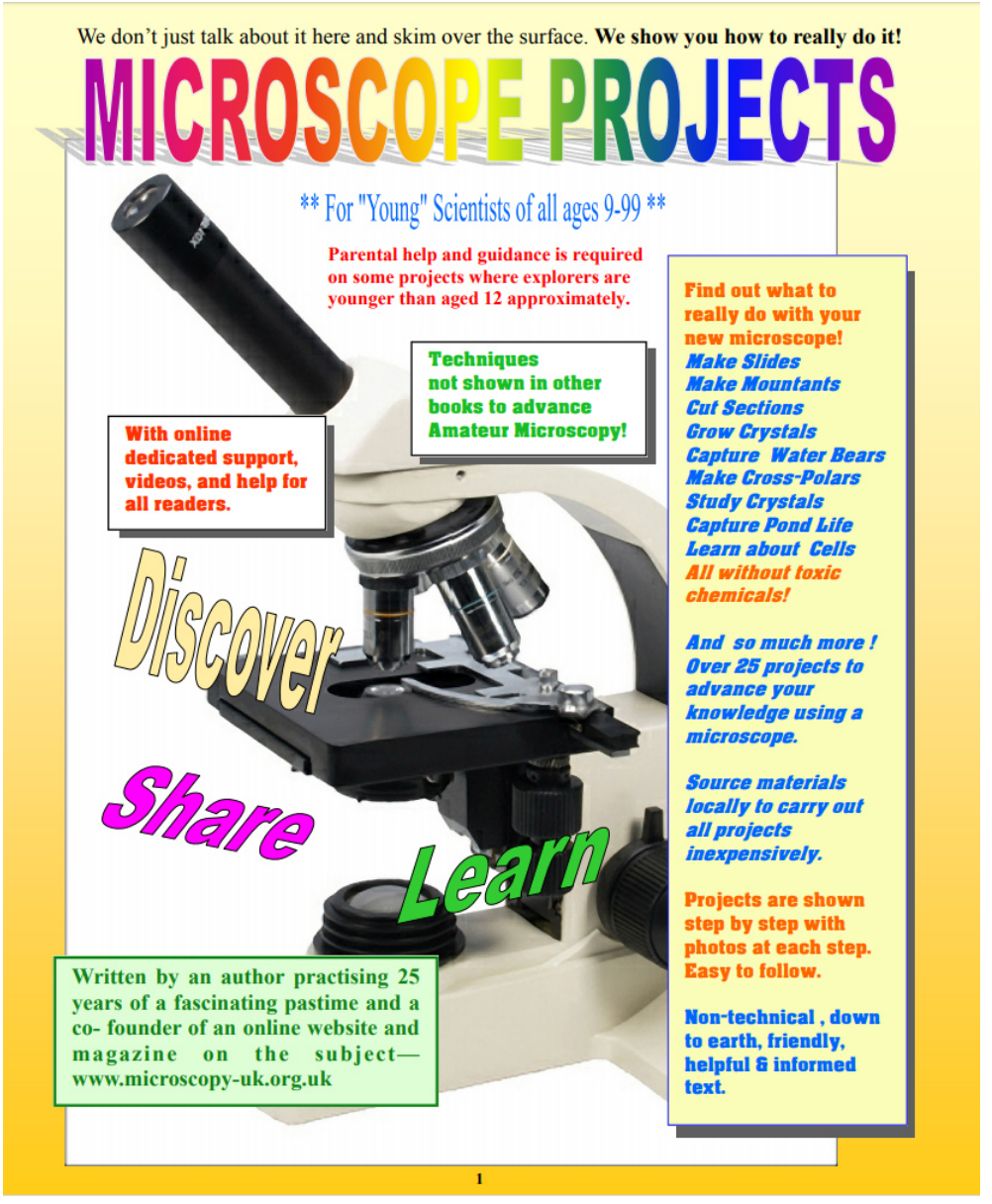
Making Your First Slide

This project has been extracted from one of the books in our book shop that I wrote to help give people new to Microscopy some interesting and informative projects to get them up and running. It is a good example of the projects in the book which I carried out myslf to ensure what I wrote was valid.

There are lots of books around. Look on Amazon and you witll see whatI mean. Unfortunately, most are old and although valid back in the day, with the changes on safety and the banning of certain chemicals, are not very helpful now.

My book uses everything easy to obtain and no unsafe use of voliatile chemicals. In fact most of the items and liquids required can be found ascommon items in one's household.



Over 25 projects with easy to understand step-by-step guides, with me photographing each and every stage ofthe work.

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HOW HARD TO DO = 5/10

PROJECT 3: part 1

Looking at cheek cells & making your first semi-permanent slide

Required: Fructose sugar (also called 'Fruit Su gar'), slides, coverslips, tweezers, small jar, paintbrush, pva glue, nail varnis h, enamel paint, Iodine or Methylene blue, small labels, a cotton bud, a saucer. Optional : plant hairs, pollen, spores.

Putting a specimen on a slide and adding a drop of water plus coverslip is a good way for observing subjects under the microscope. But the water dries out, so you can't keep the slide to look at again. Here, we are going to explore how to make your own slides, which should enable you to look at them again and again for a few years. We need something to put on the slide and I thought, as we are looking at cells, we would use one of the cells from your own body. Excited? In fact, we are going to store all the information about you on that slide because just one of your cells contains all the coded information to build a completely new you. Your Genetic Code.

We will use a common and risk-free liquid which you will make as a 'mountant' for the cells. This will suspend them in a solution and help keep them from deteriorating, as well as refracting light to increase contrast to show the transparent cells more readily.

If you do not wish to learn more about genetic code, skip the text below!

DNA, Genes, Your Code and You .

In computers, code exists as electric charges in super-microscopic switches arranged in blocks. A memory card or USB memory stick can contain billons or trillions of switches. A computer program or phone App. is an arrangement of instructions coded onto these switches.

Living things store code in their cells as an instruction set (program) on how to replicate the cell. All cells in your body die off after a certain period, but before they do, they are replaced by the old cell producing a new one. Instructions on how to do this are coded onto an arrangement on a molecule composed of two chains that coil around each other to form a double helix [A]. This code is responsible for the development, functioning, growth and reproduction of all known organisms and many viruses. DNA and ribonucleic acid (RNA) are nucleic acids, alongside proteins, lipids and complex carbohydrates (polysaccharides). Nuclei c acids are one of the four major types of macromolecules that are essential for all known forms of life. DNA stands for Deoxyribonucleic acid.

[A]- DNA—Double Helix

Codes in the genes can be patterns inherited from your parents and can be used to see if you might be susceptible to specific biological conditions (like eye or hair colour) or biological weakness like a weak heart.

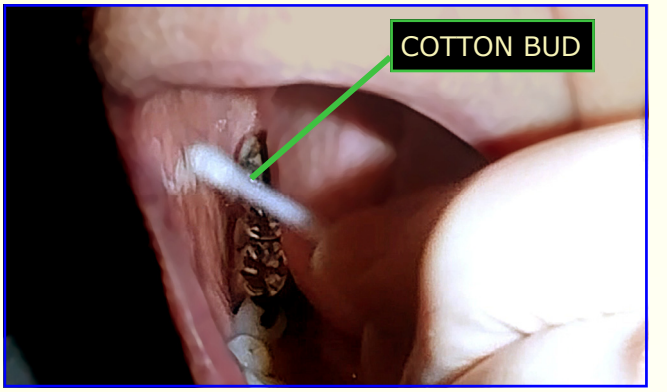
Your genetic make-up can be determined from just a single cell. Often, people take a swab of their cheek cells, which are easily and painlessly detached from inside their mouths, and sent off to companies that send back reports about ancestral history or signs of biological weaknesses. You are going to look at one of your cheek cells under the microscope—a cell containing your genetic make-up. You won't see genes though, just a bit of you .

Code can be constructed in lots of different ways. Our alphabet, for example, where the 26 letters can be used in many different arrangements to form words. Those words can be arranged to form whole sentences like a list of instructions. All of the genetic material in an organism is called The Genome . In humans, the genome contains more than three billion "letters" of DNA. The letters GATC stand for the nucleotide bases: guanine, adenine, thymine and cytosine, which are re ad by the cell when genes are active. They are molecules.

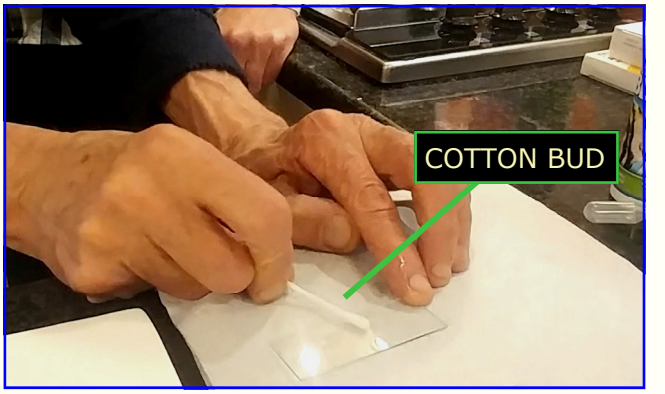
Let The Project Begin—PART 1

We are going to collect cheek cells—*Eukaryote cells,* from your mouth, stain them, and put them under the microscope. Then we will do that again, but this time, we are going to mount the cells on a semi-permanent slide, which you can keep for awhile to look at or show your friends. This type of slide will not last many years as the solution we use eventually breaks down the staining of the cell, making it less easy to see details.

Step 1: Swab Mouth.
Take a cotton bud and wipe it against your cheek inside your mouth. *(Cotton buds are readily available in supermarkets and chemists*). Get it quite wet with saliva covered cheek tissue by spinning the cotton bud as you stroke your cheek several times. Cheek cells are continuously being shed and replaced so the bud will wipe away a few dozen cells without harm.

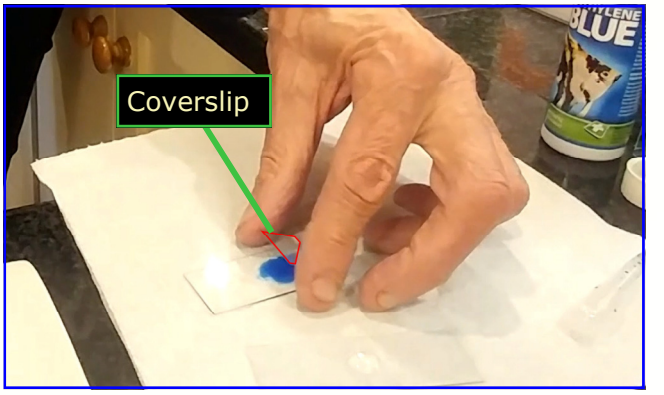


Step 2: Rub the swab onto a glass slide.
Put a drop of water onto a slide with the pipette and rub the cotton bud into the water. The cotton bud may absorb some of the water, so put another drop or two of water onto the cotton bud where it meets the slide—helping to wash some invisible cheek cells onto the glass. I used two swabs as I'm going to prepare two slides: one stained Methylene Blue, the other—Iodine.



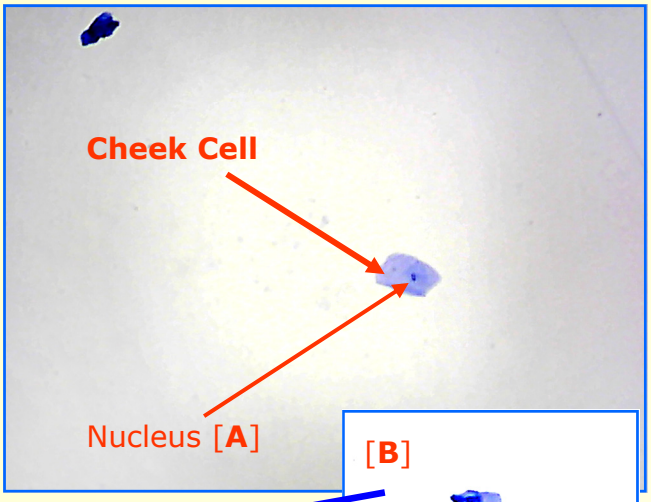
Step 3: Stain with Methylene Blue.
Use the pipette and add a tiny drop of Methylene Blue to the water. Stir and mix lightly with the pipette. If you are using Iodine, do the same with another pipette. I think Methylene Blue gives a better result, more pleasing to look at than Iodine. Make sure there is enough water on the slide to cover the area of the coverglass and to ensure no air bubbles are left in the sample.

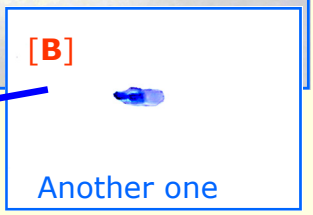
Step 4: Place a cover slide onto the droplet.
Avoid unwanted air under the slide making tiny bubbles form: you place the coverslip down, one edge on the slide, holding it lightly between your finger and thumb, and then you tilt it forward to 45 degrees over the sample. Let it go and it will drop down onto the water, pushing any air out to one side. Use a piece of kitchen towel offered to the edges to blot up the spilled water.

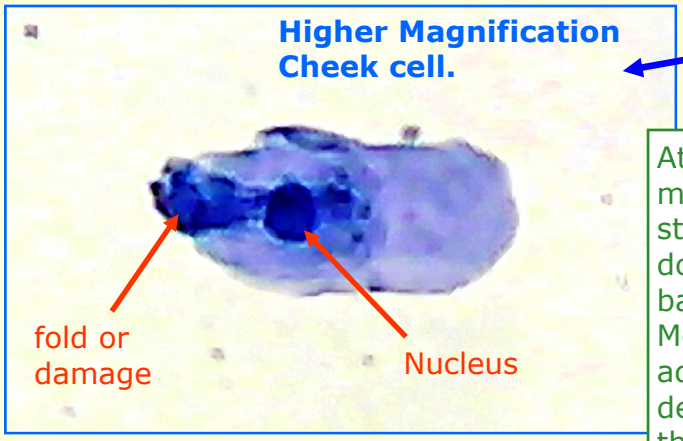


Looking at your results.
I will show you the kind of results you get using either Methylene Blue as a stain or Iodine. Remember to use the lowest magnification of your microscope initially to search around the slide. This way, you can quickly identify something worth seeing at a higher power of magnification.

Looking at your results.
You should see lots of tiny irregular shaped cheek cells. They might be spread quite far apart due to adding water. Many of them have folded over or become damaged, but you should see some flat perfect ones to look at, like my one here —[**Right**]. Inside the lighter blue (or brown if you used Iodine) you will notice the darker stained Nucleus—[**A**]. I found a better contrasted one to look at more closely—[**B**], the small inserted picture on the right. Let's look at that one with higher magnification.







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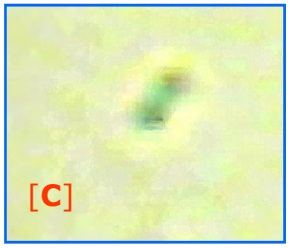
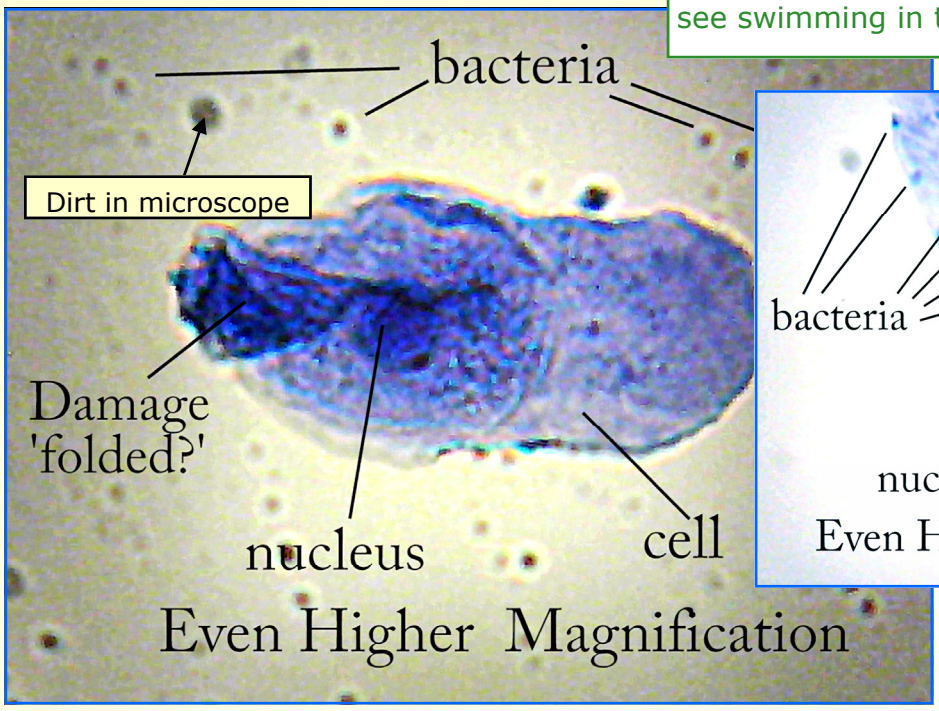
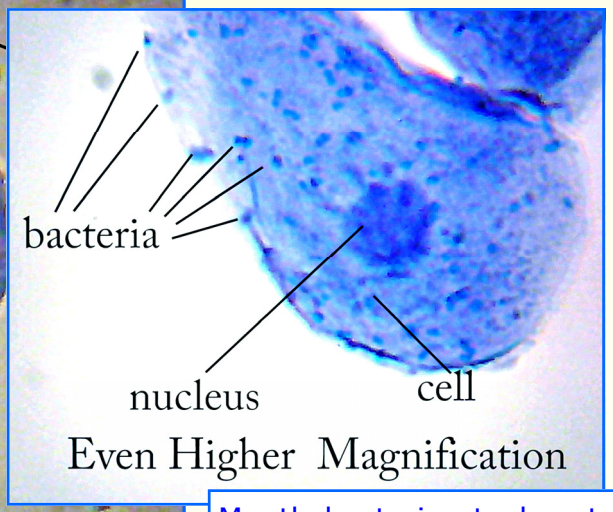
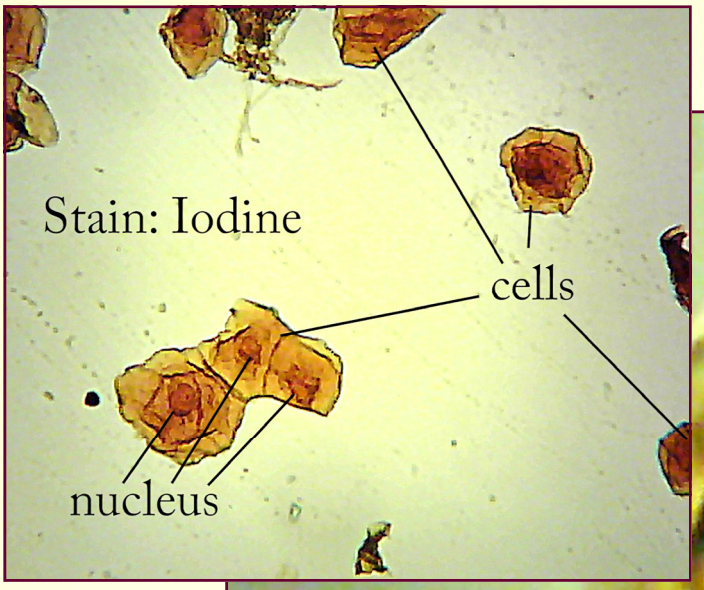
At higher powers of magnification, you may start seeing very tiny dots. These are often bacteria—(right & below). More interestingly, if you adjust the focus to look deeper or shallower into the sample, you might catch a few swimming bacteria like this one here—[C], which you can see swimming in the online support video.







Mouth bacteria stuck onto the surfaces of the cheek cell. They got stained too!

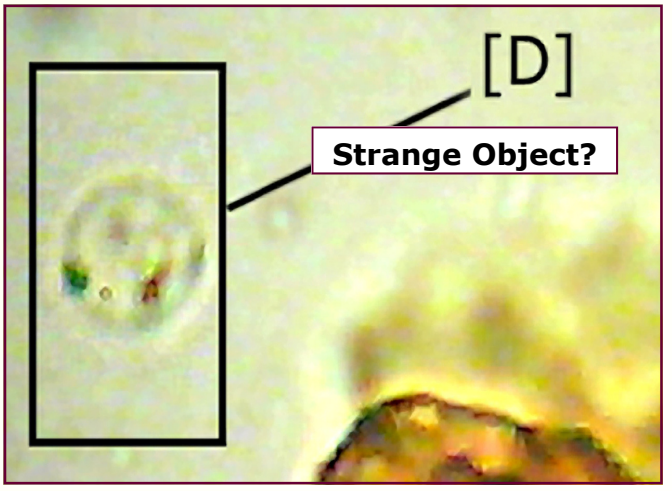


Cheek cells stained with Iodine.
Mid-level magnification—(**left**), and very high magnification—(**below**). Can you see the swimming bacteria, circled?

?
A MYSTERY

When I was recording a video of an Iodine stained cheek cell, I noticed an unusual object in the water sample floating past. You can see it more clearly in the online support video. I took a snap-shot of it here, so you can have a look at it too. What do you think it is? It's a transparent sphere with what looks like several bacteria stuck on the surface of the sphere—[**D**] below. At first I wondered if it was a single celled protozoa, but I doubted it. After a while, I think I reasoned out what this odd object is. Looking at things through a microscope requires the skills of a detective. Answer below but think about it first.

Stains
Different colour/chemical stains have varying effectiveness, depending on what you stain. Take a look at page 57 to determine which stains work best for the different things you wish to explore.

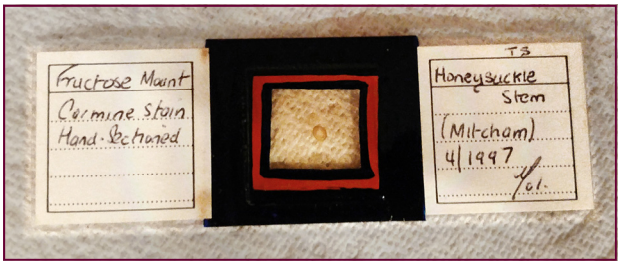


Mystery solved?
I believe the bubble itself is a tiny drop of saliva from inside my mouth and cheek. Such a liquid is more viscous than ordinary water, 'gooier', thicker, stickier, and several bacteria have become stuck in and onto it.
In part 2 of this project, we'll take some more cheek cells and try to mount them on semi-permanent slides for future viewing .

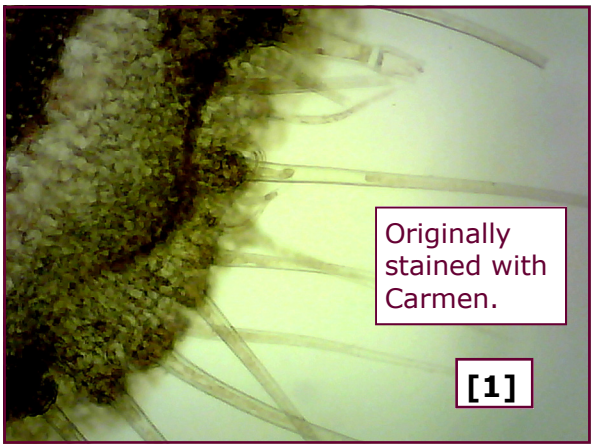
HOW HARD TO DO = 8/10

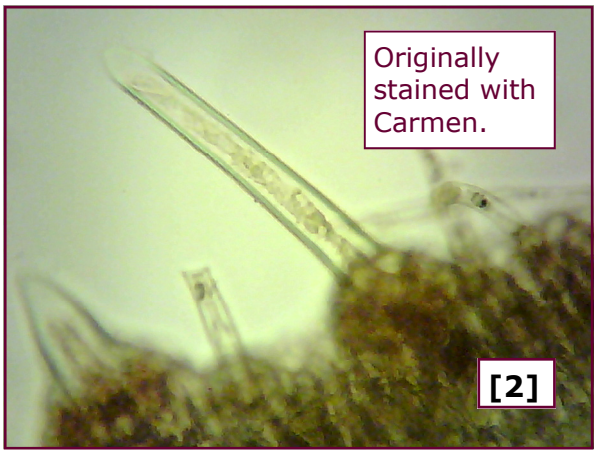
PROJECT 3: part 2
Slide Making

In this project, we are going to make real slides in which you can keep specimens to look at for several years. Here is one I made 23 years ago. The type of slide we are making is a type of aqueous mountant slide, where we use fruit sugar, fructose, as the mounting liquid. Making professional slides which last 50 to 100 years is a complex procedure involving washing specimens in chemicals, fixing them, and making wax blocks with the specimen in. These blocks are then sliced. The almost-transparent slides are placed onto glass slides, and the wax melted and cleaned away. A mounting solution is added and a cover slip fixed in place. Many of the chemicals used are now considered toxic and are not easily available except professionally. It's unfortunate, as I believe the toxicity and risk involved using tiny quantities, is very small and less than the risk of crossing a busy road. Another issue is the process is quite involved and I want to show you an easier way to make slides using non-toxic materials which, although not suitable for all types of specimens, will last several years. The slide I made was so long ago, that I didn't expect to see anything because using fructose as a mountant means the stain eventually disappears, making it difficult to see the specimen.



Please read the SAFETY AND DETAILS SUMMARY after the slide-making steps and before starting this project.





This is a hand-cut section of a Honeysuckle stem which I mounted in Fructose in 1997, twenty-three years ago this year. They show the hairs around the stem. [**1**]—lowest magnification. [**2**]—higher.

What you will need.

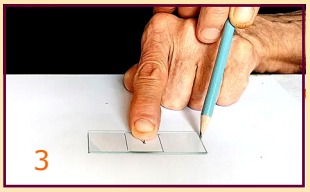
A small jar and fructose sugar to make the mounting medium. You buy fructose at a local supermarket or health shop. It's often called fruit sugar. Be wary not to get 'preserving sugar' which sits along side it on supermarket shelves. This is for making jam and marmalade and also contains pectin. You need a small jar, maybe one used for paste or spice, washed and dried. Make a mark on the side of the jar, half-way up, and put another line above it about half as far as the first line (see right). Fill the jar with Fructose to the first line and pack it down with a spoon and fill to the line with hot water. Leave for a while, and then add more water to your higher line. Stand on a radiator or somewhere warm for 24 hrs to see it clear. It's probably best to leave the jar in a warm place for several days so that any tiny air bubbles float up and escape the liquid. You won't see them, but they're there.

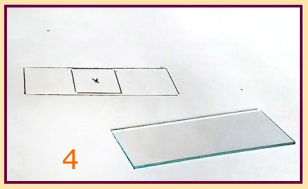
A few tips

1) It's best to use distilled water not tap water so it is completely free of minerals and fluoride. I actually used tap water here, (it's fluoride-free) so I ignored this!
2) Hot water, not boiling is best. Stir well, several times in the next few hours.
3) Place on a hot radiator or use a pan of very hot water, but not boiling, and stand the jar in that to dissolve the sugar. Make sure the heat is off.
4) Leave in this warm place until it is clear. A few days more if you want to be sure the liquid is free of air bubbles.

Step 1: Making a template.

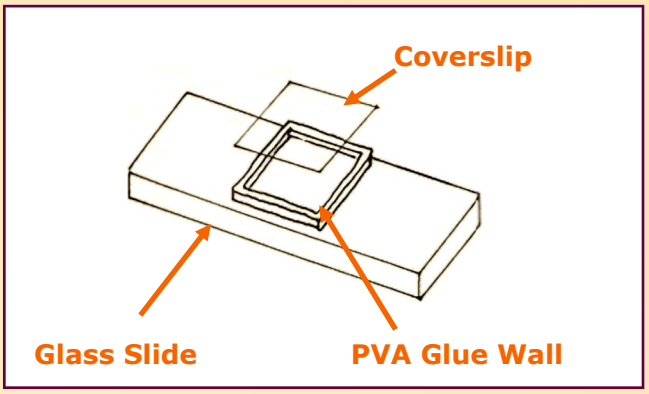
We are going to make slides which have tiny wells on them. Put a cover slip onto a piece of paper, and draw a line around it using a pencil or pen. Remove the slip and put a glass slide over your drawn square so the square is dead centre. Draw around the slide. Keep the pencil tip or pen tip as close as possible to the edges of the coverslip and the slide when drawing. Be precise.





Step 2: Before making the well slides.

The idea here is to make a thin wall on the slide to hold the liquid and specimen. The walls need not be high at all for our cheek cells but for other specimens, you need to make the wall high enough for the specimen to sit down in the mountant (fructose) without the coverslip sitting on it. So, the well must be deeper than any given specimen depth.

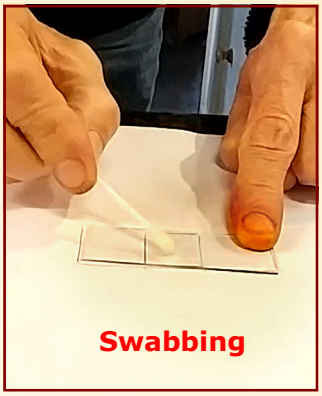


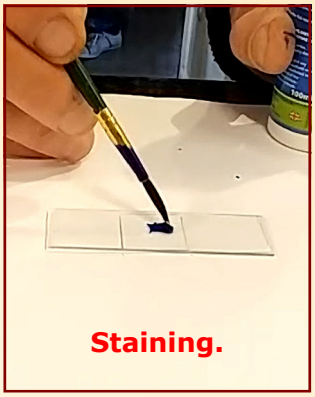
Step 3: Making the well slides.

We will build our 'well' walls using layers of PVA glue. Put a glass slide on your template. Take a fine brush, dip it in the PVA, and paint a square on the glass just inside the small square you drew for the coverslip. Do this neatly. You only need a thin border of equal thickness and equal depth with no gaps. Let it dry. For deeper wells, repeat this process several times.

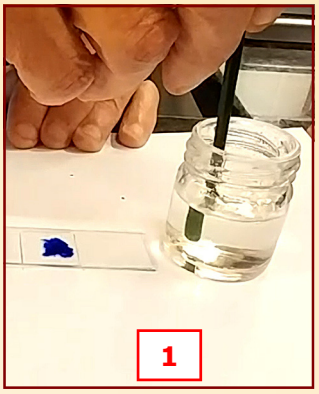
Step 4: Mounting the swabbed cheek cell.

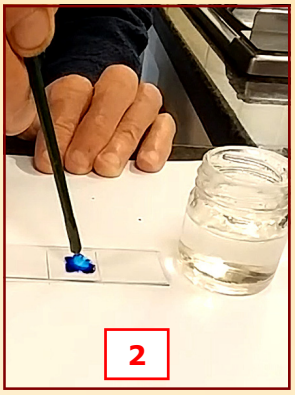
Dampen a cotton bud and wipe inside your cheek. Then wipe the bud onto the slide. Put a tiny drop of water onto the slide, where you wiped the bud. Use a brush to put a tiny drop of Methylene Blue into the mix and spread it lightly. Avoid making air bubbles by gently stirring with the tip of the brush. Leave for a minute or two for the stain to work well.


Swabbing

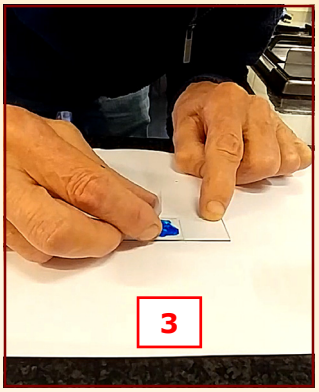

Staining.

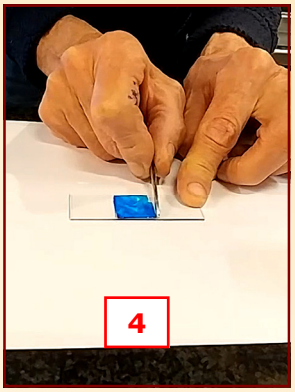
Step 5: Adding fructose mountant
Using the non-brush end of your clean paint brush, dip the tip *gently* into the jar of fructose—[1]. Remove the brush and lower it close to the stained solution on the slide. Let one or two drops drip down onto the water and stain—[**2**], then gently stir with the same end of your brush. Wash the brush, both ends afterwards so it doesn't get fouled up for later use.



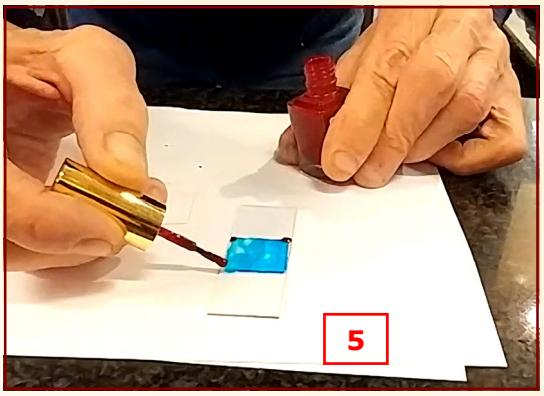


Step 6: Applying the cover slip
Breathe onto the coverslip to moisten it (helping to minimise any air getting trapped), then position and drop it onto the slide as previously learned—[**3**]. Make sure the coverslip is square to the slide, and positioned correctly over the well containing the mix of cheek cells. Use tweezers against the coverslip edge to gently slide the slip until square with the 'well' you made—[**4**].

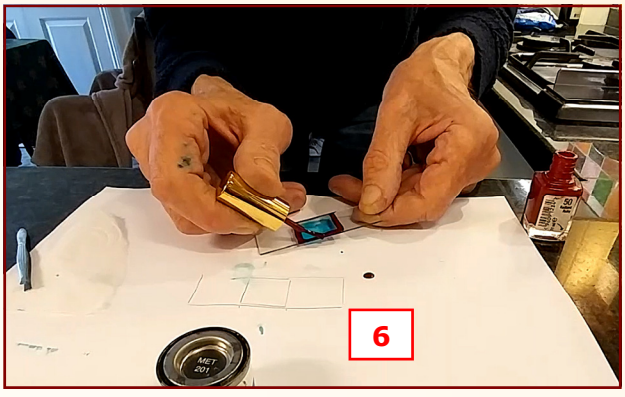




Step 7: Fixing the corners
Use kitchen towel or tissue to clean up any spillage, being careful not to dislodge the coverslip. Use a water filled brush either side of the slip to help wash away the stain. Leave the slide somewhere warm for an hour or two so the edges dry out a little. Then use nail varnish and apply a drop to each corner of the slip so that it goes from the corner and onto the slide—[**5**].



Step 8: Seal the cover slip
Leave the slide somewhere warm for 15 minutes or more so the varnish dries, locking the slip to the glass. Paint the edge of the cover slip with nail varnish, ensuring the slip and glass slide are both covered at each edge to seal the coverslip. Work fast so the new varnish does not 'un-stick' the corners, which it might do as the additional varnish solvent works on the four dots—[**6**].



Step 8: Optional—paint with lacquer
Another option is you can paint over the varnish with lacquer or enamel paint (red or black is best) to strengthen the seal. It's worth doing if you want to keep the slide for longer than a few months.

Purchased from Amazon but also sold in Airfix or Revell model shops. Tiny can. Other enamels will do as well.

SAFETY AND DETAILS SUMMARY

Getting Results With Fructose Mounting
Staining and mounting with Fructose is probably not easy to master straight away. It takes practice to get good results. You don't really need to use a mountant on cheek cells as it's easy to just obtain them, stain them, and see them straight away. If you stained or just simply mounted something you can see with your eye, it's easier. I wanted you to discover how to stain specimens, use fructose as a mounting medium, and seal a slide. If you fail to get the result you wanted, try again. Once you master the technique, you'll be able to use it to preserve other things you find to look at again in the future. *Put a small label on slides you make & wish to keep, date it and title it.*

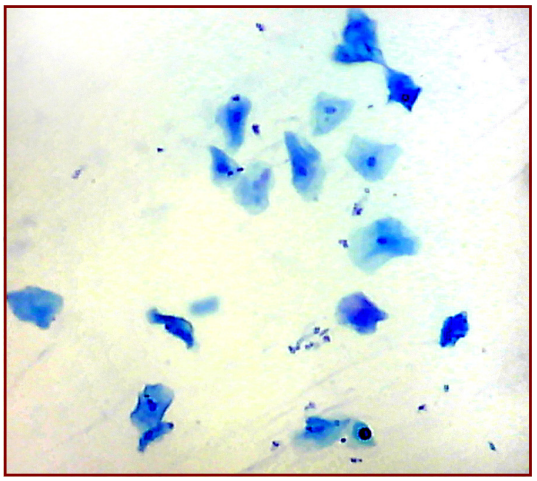
Fructose can be overly syrupy or over-diluted. With some specimens, it works well to mix some fructose with a little water to dilute it and leave the specimen in that (in a small dish, maybe) for a few hours before moving the specimen onto a slide and mounting it with Fructose. This will help to ensure air and water move out of the specimen and some diluted fructose moves into it—keeping the specimen's structure intact and allowing the non-diluted fructose to creep into the structure. One of the issues with mounting anything (putting a specimen into a more fixed state) is air. It can get mixed into a liquid solution and can visually impair viewing by causing air bubbles to obscure what you are looking at. In most circumstances, if you are very careful, it is avoidable.

Safety, Stains, And Using Methylene Blue.
Methylene blue is a safe stain even if consumed in small doses. But it can cause toxicity in high doses. Just don't drink it! Most chemicals are toxic if you drink or eat them in high enough quantities, even water—actually, but using them in a prescribed way, as I have described is fine!
Use disposable gloves to avoid staining your skin and work on an area with paper towels beneath to ensure you don't accidently stain other things. Methylene Blue is used to stain (dye) cheek cells because it stains DNA & RNA more readily than cytoplasm. The cell nucleus contains the DNA and RNA so it stains much darker than the matter in the surrounding cell cytoplasm. It also stains bacteria which are the little dots you see in your samples. It is also an effective stain on dead cells. Take a look at page 57 to find out what stains are best to use for different subjects.

Coverslips
Thin sheets of glass like coverslips break easier than you think. Always work with them over a kitchen towel. If you break one, the tiny fragments of glass will likely fall onto the paper which you can carefully fold up, put in an envelope and deposit in the trash. I work with my hands when using them, but it is advised, especially if you are young, to handle them with tweezers so if anything goes wrong, you do not risk getting tiny slithers of glass into your hands. If you need to clean one, lay it on a flat surface, press it down and clean it with water and mop up with kitchen towel.

Looking At Things Under A Microscope
When observing microscopic subjects and objects, you have to put a different kind of thinking into place. When looking at macro objects in the everyday world, it's relatively simple (most of the time) to interpret what we see. When looking at things in a micro world, one we are unfamiliar with, it requires practice and building up knowledge. Often, you are looking at a thin section cut from a larger microscopic subject—it's a bit like looking at Google Earth and trying to work out what the 'flattish' objects are.

Results
Here is the result at low power. I expected them to appear sharper. Maybe I should have stained them for longer, or perhaps the solution is too weak. You can increase the solution by adding a



little more fructose to your jar and leaving it in a warm place (on a radiator, for example) until it clears again. Practise makes perfect!

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Microscopy UK Front Page
Micscape Magazine
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Published in the December 2022 edition of
Micscape Magazine.

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