MUSHROOM MICROSCOPY

SOME GENERAL INFORMATION / TIPS

(PC & DJS - updated September 2022)

- **LESS IS MORE!!** The smaller the amount of tissue under your cover slip the better will be the clarity and detail you'll see. This may sound like a contradiction in terms: surely the chances of finding something are greater if you have a larger sample in which to look for it? Not so with mycology! The cells you're viewing are so tiny and will be present even in the minutest speck of tissue. Too much material is very likely to obscure the detail you're after.
- **The key to successful microscopy is in the skill of preparing of your slide!** The more microscopy you do, the more you'll realise how true this is. Ideally prepare your slide using a dissecting scope: relying on your naked eye, however good your eyesight, is already limiting your chances of making a really good preparation. You'll need forceps and a sharp razor blade / scalpel to extract a tiny sample from your fruit body to place in the centre of a slide, slicing it up even further if necessary. Add just a drop of your chosen wetting agent, cover with a cover slip and tap gently (with the rubber end of a pencil), then you're ready to go. Detailed instructions follow:

CONTENTS

BASIDIOSPORES and when they need staining

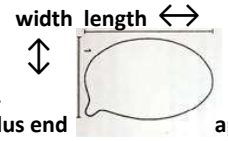
- **Hyaline spores** (those which are white or cream but react negatively to iodine) are barely visible under a compound scope and therefore need staining – usually with Congo Red.
- **Amyloid spores** are those which react positively to iodine, becoming either blue (weakly positive) or purple-black (strongly positive) when a drop of Melzers reagent (containing iodine) is added. This is clearest seen (with the naked eye) when added to a thick spore deposit (see below under Taking a sporeprint for more). Ornamented amyloid spores (as in *Russula, Lactarius, Melanoleuca*) must be viewed in Melzers to make their ornamentation visible.
- **Dextrinoid spores** also react positively to iodine, but become rusty brown - either weakly to strongly. In *Hebeloma* the degree of dextrinoid reaction is often critical to species identification therefore Melzers must be used.
- **Brown to black spores** need no staining under a compound scope and can be viewed using water or 10% ammonia.
- **Metachromatic spores** (in *Macrolepiota*) become reddish-purple when Cresyl Blue is added.

MEASURING SPORES. Immature spores are not necessarily typical in size therefore for real accuracy it's best to measure those from a sporeprint under x1000 magnification with oil immersion (see below under Oil immersion). Failing that, spores taken from the top of the stem (where they've dropped naturally) should be used (see below under Caulocystidia). Failing that, use a gill edge preparation and chose spores which have washed well away from the tissue and are well coloured / stained – immature spores tend to be paler. Using a gill prep. at x400 magnification will give you a rough idea of size - often enough to help with identification but not considered accurate enough for a reliable description.

- **How to measure.** There are different ways of measuring the size of spores and other cells in fungal tissue. Most common has been the traditional eyepiece graticule. This is a scale etched onto a glass disc placed within the eyepiece of the microscope, where it remains in focus as the image of the specimen on the slide is adjusted. By moving your slide using the mechanical stage whilst rotating the eyepiece, the length or breadth / width of the subject is lined up with the graticule scale and the number of divisions or tenths of a division is noted. This is then repeated with other selected cells. The results are then converted into microns (micrometres, µm). The conversion factor depends on what magnification is being used (e.g. x 100, x 400 or x 1000) and this needs to be calibrated for your particular microscope but then can be applied to future measurements. The calibration uses a slide etched with an exact scale in microns. In

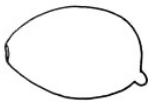
many microscopes, the eyepiece graticule is made to measure one micron at x 1000 but this should be checked to be sure. Other methods for measuring involve photographing an image and measuring the size of objects in it. The measurement can be done with computer programmes designed for the task, which also require calibration. Derek's approach is to use the direct measurement to provide initial, quick characters (e.g. of 10 spores) but to also photograph down an eyepiece (the one with the scale!) using a small portable camera. This can then be used later, e.g. for measuring a larger number of spores, if their size is a critical character in identification. This is also useful for measuring spores that are moving too quickly to be lined up against a scale. The spores can be measured by printing the image and copying the scale to measure like using a ruler.

- Chose spores which are lying flat to measure, i.e. with the apiculus (hilar appendage) in focus at the bottom of one end and the apex in focus at the opposite end – as shown here.



- Spore size in any one species will vary - sometimes by up to 4µm in length, less so in width, so a selection should be measured – ideally selecting a minimum of 10 spores, some larger, some smaller, to give an average.
- Q value, sometimes used in descriptions, is spore length divided by width. It can be useful in comparing species with similar length spores but where their width is critical. (A Q value of 2 or more indicates a spore having a width at least half that of its length; a Q value of less than 2 indicates a spore having a width less than half its length.)
- Spores can have a smooth surface or be ornamented with spines, warts or nodules - sometimes this is easy to see, sometimes needing x1000 to see. Some genera have only smooth spores, some only ornamented, some may have both according to species.
- **Measuring ornamented spores.** For spores with spines, warts or low ornamentation: ***Exclude*** the ornamentation from your measurement. For nodulose or lumpy spores (as in *Inocybe*, *Entoloma*): ***Include*** all extrusions in your measurement. Imagine your spore encased in a rectangle then measure the longest and widest points.

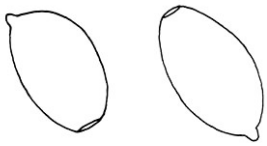
Germ pores. These are the small flat openings at the apex end of some spores and their presence / absence / position can be a key character. If present the germ pore may be either central or eccentric. A central germ pore is one that is aligned with the central axis through the length of the spore. An eccentric germ pore is one that is displaced to the side of that axis. Spores can be flattened – ie narrower when viewed in one position when rotated around the central axis and wider when rotated by 90 degrees, referred to as side view and face view respectively - or they can be symmetrical around that axis. The apiculus is almost always eccentric with respect to the central axis, so its position indicates which view is seen.



Examples:

A flattened spore with an eccentric germ pore visible in side view (left) or through the spore in face view (right).

A spore that is not flattened and with a central germ pore visible in both side and face view.

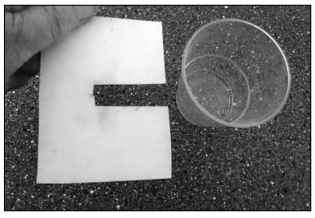


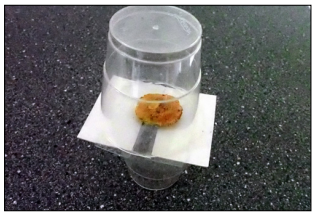
Loosening perispores. In *Hebeloma* (and occasionally in other genera) the spores in some species have an outer gelatinous coating which 'comes away'. As this layer is not iodine positive – unlike the rest of the spore – it remains colourless in Melzers and can be seen around the rim of maturing spores as an irregular layer as it gradually detaches.

HOW TO TAKE A SPORE PRINT

Method 1: Separate the cap and stem at the apex then lay the cap gills down on a slide (two slides if it's big / several caps on one slide if they're tiny). Cover with a suitable container to keep out drafts (this is vital) and leave for several hours / overnight, depending on whether you need a thick deposit to assess its colour or for testing with Melzers for amyloidity / dextrinoidity, or a thinner deposit sufficient for microscopic examination of mature spores.

Method 2: This is a better system if you want to leave the specimen whole rather than removing the stem. You'll need two plastic mugs or similar, two slides and a piece of plastic coated card (eg from a fruit juice carton) with a slot cut out as shown. Place the card on one mug, then your two slides on top, then your whole mushroom with stem pointing downwards into the mug and gills resting on the





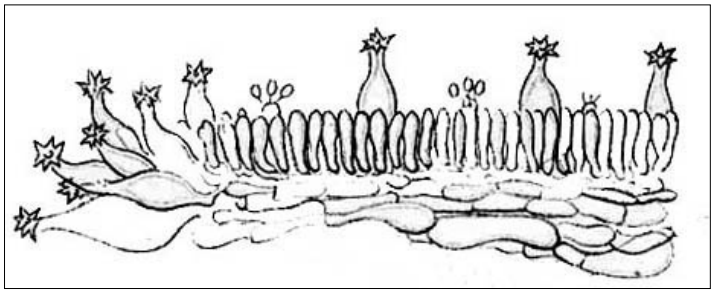
two slides now pushed close together around the stem. Cover with the second mug. (Using the slides alone with no card underneath often ends in disaster when the whole arrangement gets knocked inadvertently!)

- Have a selection of cards to hand with different sized slots cut out to accommodate different stem widths.
- **TIP:** For both methods cover your cap with a piece of damp kitchen paper to encourage the dropping of spores especially in warm dry conditions. Top up the dampness if it dries out. Even so, sometimes this trick will fail to produce results.
- **NB: MUSHROOMS WILL RARELY DROP SPORES IF THEY'VE BEEN CHILLED IN THE FRIDGE!!**
- It's good practice to set up a sporeprint of any interesting specimens as soon as you can after collection. If you've collected several specimens, use one to drop spores and the rest can be placed in the fridge till you're ready to work on them.
- **Spore print colour.** To assess the colour you need a thick deposit. Let the print dry off naturally after removing the cap, then check for and remove any extraneous debris which may have dropped with the spores. Now take a razor blade and scrape the spore mass into a concentrated pile on your slide, cover this with a second slide which can be fixed in place with some sticky tape. The true colour of the spores can now be assessed, moreover if your collection turns out to be important the print can be retained indefinitely together with dried material and can also be used for DNA sequencing.

- **Examining spores from a sporeprint**. A print taken overnight on a slide will often produce a thick deposit – useful for colour or to test with Melzers but too thick to use as it is under the compound. In this case take a scraping of spores from your print with a razor blade and tap it onto a new slide. If you have a thin deposit, ie after a few hours you can see there are spores visible on your slide, you can use the slide direct for viewing with a scope. Whichever of these methods you use, first use a dissecting scope to check for any extraneous hairs / debris on your slide and carefully remove with forceps / a small paint brush, otherwise your cover slip won't sit flat enough. Now add your drop of liquid, your cover slip, tap it down gently then blot with kitchen paper to remove any excess. It is now ready to view.

- **BEWARE OF ALIEN SPORES**! When collecting in the field take care to place individual collections into separate covered containers. Mushrooms placed loose in a basket will continue to drop their spores and air currents will inevitably spread them about. Hence when you come to examine a gill later you may well find a myriad of different spores contaminating your prep and won't know which belong to your specimen. If your specimen was growing adjacent to other species in the field this may well happen naturally anyway! The benefit of using spores from a print is that this hazard is to a large degree eliminated.

CYSTIDIA



A gill section diagram (of a typical *Inocybe* species) showing cheilocystidia - on the gill edge - at the left hand end, gill trama (hyphae) along the bottom, and along the top (on the gill face) a few pleurocystidia and basidia with spores attached amongst a mass of basidioles.

Cystidia are sterile cells found on the gill of basidiomycetes and, like the spores, are amazingly varied in size and shape. Their function is not fully understood but to the mycologist they are extremely useful for identification purposes. There are various different sorts of cystidia.

CHEILOCYSTIDIA

These are sterile cells found on the sharp outer edge of a gill. In some genera they are absent; in a few genera though present they are not considered useful for identification purposes; in many genera, however, they are vital in identification.

- **How to prepare a slide to view cheilocystidia**. 1. – Remove a complete gill from your fruit body using forceps / a razor blade and (preferably using a dissecting scope) lay it flat on a slide. 2. – With a sharp razor blade slice off the *thinnest possible* piece of outer edge and discard the remainder of the gill. 3. – Carefully add your drop of liquid, then your cover slip, if possible keeping an eye on which part of your sliver is the outer edge: it is the outer edge, not the cut edge, where your cheilos will be. 4. – Tamp it down gently and view with the compound at low magnification to start with to locate your sample, then move up to x 400. (Remember, your view will be turned up-side-down by a compound scope.)
- **Staining your prep**. Any gill edge is made up of a palisade of cells; as they are usually hyaline (colourless) they often need to be stained with Congo Red in order to be seen clearly. Mixed in with cheilocystidia will be basidia – some with spores attached, basidioles - immature basidia, possibly paracystidia, hyphal tissue. The best way to stain is to add your drop of Congo Red and leave to soak in for a minute or so, then add a drop or two of 10% ammonia to dilute the stain and with a pin carefully move your piece of gill tissue to the side of the liquid. Blot up most of the liquid (taking care not to suck up the gill tissue as well!) then add a tiny bit more ammonia and gently lower your cover slip. Tamp down just once to start with and view, then tamp again to separate out the cells – the ammonia helps with this. Go gently to start with: if you're too heavy-handed your sample will disintegrate! Repeat until you can make out where your cheilos are if present, searching along the gill edge whilst focussing up and down.

- Make a note of their shape and size, whether their walls are thin / thick / coloured, any ornamentation etc.
- NB: If you can see only the top part of the cystidia protruding beyond the mass of cells, you either have too much tissue in your prep or need to tamp it down more. To see all details of shape and make measurements you need cystidia which are fully exposed and ideally you'll have a few floating free amongst the spores which will have washed off your gill edge.

PARACYSTIDIA

These are clavate to pyriform thin-walled sterile cells found on the gill edge in some genera (e.g. *Inocybe* and *Psathyrella*). They form clusters between the cheilos and can be anything up to about half the cheilos in height. They play little part in species identification unless their presence / absence is significant and mentioned in a key.

PLEUROCYSTIDIA

These are sterile cells found on both flat surfaces of a gill (the gill face) and their presence / absence is equally important in identification as the cheilos. Some genera which have cheilos always have pleuros as well; some genera have cheilos but never have pleuros as well; some genera vary according to species having both types / just cheilos / neither.

NB: No genera have pleuros but no cheilos, therefore if your gill edge examination shows that cheilos are absent there's no need to check the gill face for pleuros as well – you can safely assume they are absent too.

- **How to prepare a slide to view pleurocystidia**. 1. – Start off with steps 1 and 2 as for cheilos above BUT this time, having sliced off your thin piece of edge, *retain the remainder of the gill and discard the edge piece*. (This will ensure that the cystidia you find are pleuros and not cheilos). 2. – Turn the gill lengthways on the slide, place a finger on one end to steady it and with a sharp razor blade cut it roughly in half, at right angles to your first cut which removed the piece of edge. Discard the severed half, retaining the section under your finger. 3. – On this section place your finger almost up to your latest cut and, using your nail to support the blade, slice off several extremely thin parallel slivers of gill – the thinner the better. Discard all but your slivers. 4. – With a pin tease the slivers gently apart then add Congo Red or ammonia and proceed as for 'Staining your prep' above.
- You should see gill trama (made up of hyphae) running along the centre of each sliver, and along either edge of your section a pallisade of basidioles and basidia with spores attached (or floating loose). If pleuros are present they will be protruding beyond the basidia along either edge, usually similar to the cheilos in shape, occasionally plentiful but more often quite scarce – sometimes only one or two on each sliver! Careful searching is therefore needed, especially if you need to know if they're present or absent to follow a key (e.g. in *Psathyrella* or *Mycena*).
- With practice, to save time you can combine the two procedures described above (for cheilos *and* pleuros), retaining your gill edge sliver *and* your gill face slivers in one prep, but to avoid error make a visual note which is which. It's worth watching with care using your dissecting scope as you add your drop of liquid because your slivers will move position at this point!

CHRYSOCYSTIDIA

These are cystidia which have inner contents which stains yellow when viewed using KOH or ammonia and tend to be shaped rather like the head of a cobra. They occur in *Hypholoma, Stropharia* and *Pholiota.* In these genera ascertaining whether this particular type of cystidia are present on the gill edge, the gill face, both these surfaces or neither, can be critical in species identification.

- **TIP**. Using Patent Blue in place of KOH or ammonia in your slide prep gives the best results, making these particular cystidia very easy to see: they stain bright blue and stand out from the rest of the gill tissue.

CAULOCYSTIDIA (in *Coprinellus* commonly called setules)

These are sterile cells, similar in size and shape to the hymenial cystidia (those on the gill), but located on the surface of the stem (the stipitipellis). They are entirely absent in some genera but in others they can often be of key importance. With a x10 handlens or stereo dissecting scope they can sometimes be seen as a white fine dusting on the stem surface (described as pruinose) usually under the apex but sometimes lower down than this or even covering the whole stem.

- If details of caulocystidia are required in a key it is best practice - certainly always safest - to prepare a slide and view with a compound scope.
- **How to prepare a slide to view caulocystidia**. 1. – With a sharp razor blade make two small nicks in your stem, one just under the apex and one an inch or so below. 2. – Now with a pin carefully raise a few surface fibres (the fewer the better) where you made your lower nick, then with fine forceps take hold of the raised fibres and carefully peel a thin strip from the surface between your two nicks. 3. – Lay your strip on a slide (having first moistened the appropriate bit with your finger), aiming to keep the outer stem surface flat and facing upwards. 4. – Have a cover slip ready in one hand, and with the other gently add your drop of liquid then cover immediately. (This is to avoid your sample curling up!) 5. – Tamp down and view – only low magnification will be needed, x400 at most.
- If your specimen is tiny it may be impossible to peel fibres from the stem, in which case cut the whole stem into sections to view.
- Caulos often occur in clusters and usually reduce in number down the stem. You may need to ascertain (exactly) how far down the stem they occur. For this repeat the above procedure making your two nicks progressively further down the stem.

- To avoid error when you peel off your fibres, make a mental note which end of your sample was higher on the stem.
- You may encounter other cells on the stem surface: cauloparacystidia (thin-walled balloon-shaped cells) may be present, mixed with true caulos; septate thin-walled cylindrical 'hairs' (hyphae) may also protrude from the surface. Only cells resembling the gill cystidia in shape are true caulos).
- NB: Spores found on your stem preparation slide will be mature (having dropped naturally) and are therefore a good source for measurement in the absence of a sporeprint.

BASIDIA

These are the fertile spore-bearing cells found all over a gill and have 4 (occasionally only 2) sterigmata extending at their apex, to which the developing spore is attached by the apiculus (until discharged). Basidia generally play a less important role in identification than cystidia but there are instances when you need to observe them with care.

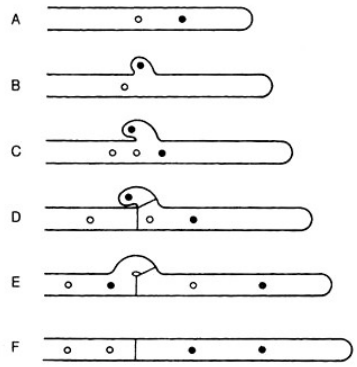
- A few species consistently have 2-spored basidia (having only 2 sterigmata), this being a diagnostic character in their identification. (These species may also have a few 3- or even 1-spored basidia.) Some species may have a mix of 4- and 2-spored basidia.
- In *Entoloma* it is often necessary to ascertain not only whether basidia are 2- or 4-spored but also whether they have clamps at their base – present in some species, absent in others. (See below under Clamps for more on this.)
- Following the procedure above for finding cheilos and pleuros will also give good views of basidia though you may need to tamp down on the cover slip more to separate the cells out further. Check out several basidia, focusing up and down with care, before deciding if they are 4- or 2-spored. **Beware**: It is *very* easy to mistake a 4-spored basidium for 2-spored when only two of the four sterigmata may be visible and lying in focus.
- **TIP**: In brown/dark-spored genera there is an alternative and simpler way to find out if basidia are 4- or 2-spored: Lay a single gill flat on a slide and *view dry with no coverslip*, first at low power then possibly up to x100 or 400, but take care not to contaminate your objective by letting it come into contact with the gill. By focussing up and down you will be looking down on hundreds of spores still attached to the sterigmata of the basidia. If basidia are 4-spored the spores will be clustered in sets of 4 forming a mass of tiny squares; if 2-spored no such pattern will be visible and spores will appear randomly placed. This method can be very useful as a quick check in *Conocybe, Galerina, Coprini*; if you suspect you're seeing 2-spored basidia this way, this is best then confirmed using the compound in the conventional way.

BASIDIOLES

These are immature basidia. They are hyaline, at this stage lacking sterigmata and also very plentiful – often packed in a palisade along both the gill edge and face. Not used for identification purposes, these thin-walled clavate cells can in some genera be easily confused with thin-walled cystidia.

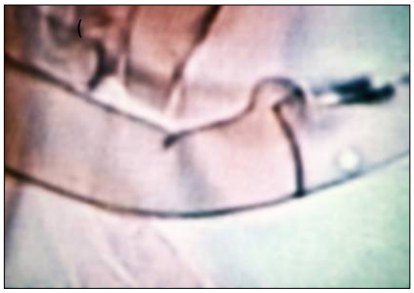
CLAMPS and SEPTA

Fungal cells develop and grow by elongating - sometimes branching - their cells, then dividing into two. The division is created by a dividing wall known as a septum. In a typical Basidiomycete, clamp connections are formed as part of a process in which two different but compatible nuclei in one cell are each copied and one of each type placed in both cells. The process is shown diagrammatically below and is essentially a branching that turns back on itself, carrying one of the nuclei and fusing back again as the septa are formed to divide the cell into two.



In the diagram left, the lowest example marked "F" shows the typical septum (plural septa); the example above it, marked "E", shows the typical clamp connection. Both are often seen in fungal hyphae when examining specimens under the microscope. The clamps are easily seen in some tissue, particularly if the tissue has cylindrical hyphae with lots of septa. More difficult is finding clamps at the base of basidia in genera like *Entoloma* or *Coprinellus*. In this case, the particular shape of the base of the basidium is an important guide, since the clamp can be thin-walled and may have collapsed in your prep. See below (A to C) for examples and further explanation.

(A) Typical clamp connection in a cylindrical hypha



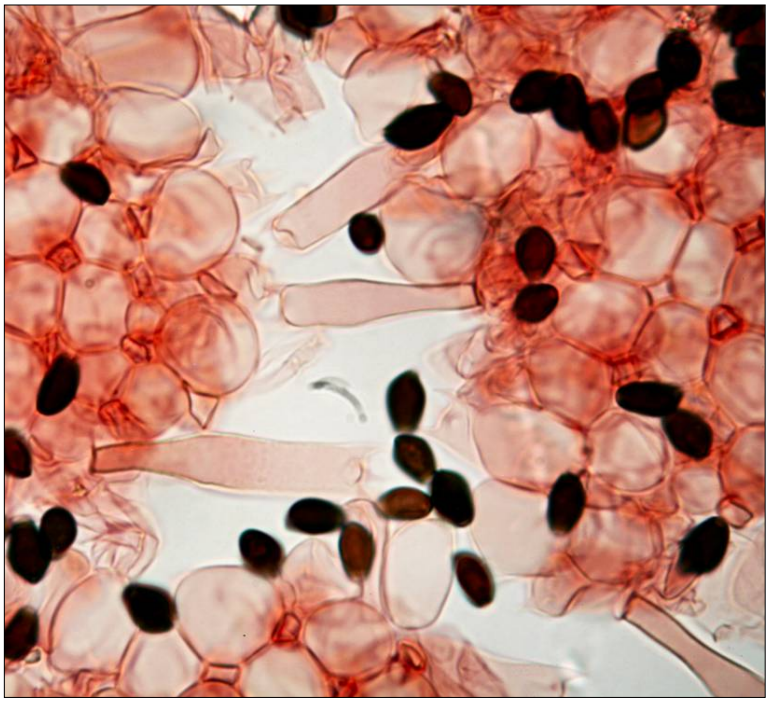
(B) Clamp connection at the base of a basidium

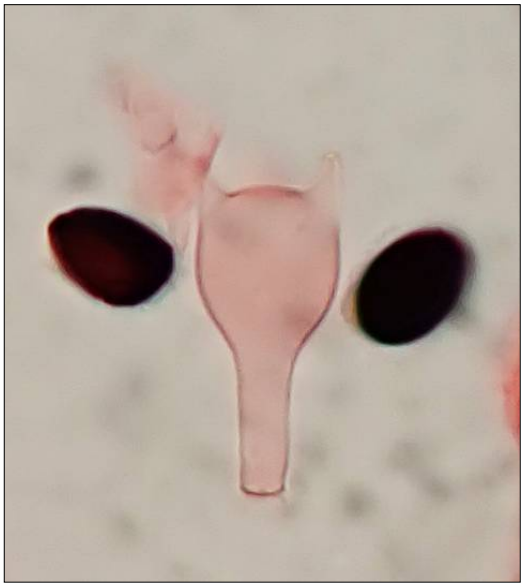
(C1&2) Clamp connections collapsed but discernable by the shape of the base of the basidium

How to study clamp connections. Clamps may be entirely absent / present in all tissue / present only in some part of the tissue, depending on species. Prepare you slide with as little material as possible. In some genera (e.g. *Coprinopsis* or *Crepidotus*) they are easily seen in all tissue if present, as in (A) above. In other cases, they are only reliably present at the base of basidia. It is important to stain the cell wall (e.g. with Congo Red) in such cases. Gently squashing a small fragment of stained tissue allows individual basidia to be seen and, (occasionally!) the clamps are clearly visible as in (B) above. More often than not they are difficult to see and become very thin-walled and collapse. In such cases, the shape at the base of the basidium reveals their presence thus:

- ***If clamps are absent*** the septum at the basidium base will be seen a simple straight line at right angles to the cell above it, as in examples (D 1&2) below.
- ***If clamps were present but have collapsed*** the septum at the basidium base will have a kink / slight dog-leg where the clamp was originally adjoined, as in examples (C1&2) above.

NB: To make the above assessment it is essential to locate basidia which are in full view with their bases exposed. You may have to experiment with several preparations using the minutest amount of material and be prepared to search around to locate several examples to confirm your assessment.





(D1&2) Basidia lacking clamp connections

- Finding clamps can be a challenge even for experienced mycologists! Once you've found one (or preferably several) you can be sure they're present, but the reverse doesn't necessarily apply: if you fail to find any it could be that they're absent but could also be that they're present but that you haven't succeeded in finding them! Diligent searching is nearly always required in order to be as sure as you can when you suspect that clamps are absent. Errors over this feature are a common cause of going astray in keys which require a decision to be made over the presence / absence of clamps.

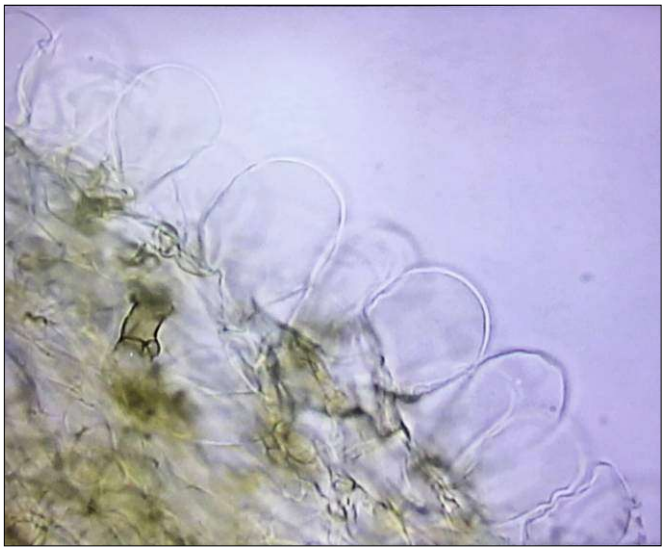
CAP CUTICLE (the pileipellis – not to be confused with the stipitipellis, the term used for the stem surface)

The surface layer of the cap of an agaric fungus can take many different forms and distinguishing these under the microscope is a fundamental part of characterising different genera or different groups of species within genera. The subject is a complex one, but introductory notes below are given as a starting point.

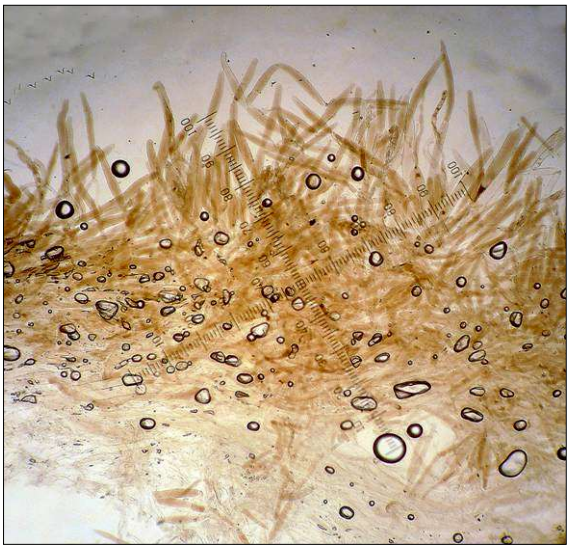
How to examine the cap cuticle under the microscope. Cut the cap in half (or cut a wedge-shaped segment). Lay it cuticle downwards on a slide, then with a sharp razor blade carefully cut a vertical slice as thin as possible excluding tissue other than the cuticle itself. Several slices can be made then select the thinnest. Alternatively, a slightly thicker slice can be cut then cut again at right angles to provide less broad slices. The aim is to have a thin section of the cap surface with as little of the underlying 'flesh' as possible still attached. This is then turned over, stained if necessary and studied. (E) right is an example of the cuticle surface cells found in some species of *Leccinum* stained in congo red. In this genus examining the cuticle structure is essential for reliable identification to species level: some species have a cuticle formed of distinct short linked cells as shown here, others have longer cylindrical cells which often break apart (disarticulate), and yet others have even longer flexuose cylindrical cells with fewer septa.

(E) Typical cap cuticle cells found in *Leccinum*

Pluteus is another genus in which the cap cuticle is critical to species identification . Some species have a cuticle comprised of rounded cells pointing outwards, attached below by a cylindrical stem: this type of cuticle is known as a **hymeniderm**. (See F below though this example is from a different genus). Other species have a cuticle of long hyphae forming a layer of cells on the surface like strings of sausages: this is known as a **cutis** (See G below). These two forms – a cutis and a hymeniderm - are the two basic cuticle structures but there are many variations of both these forms, also other forms, all having somewhat complicated names. For further information the excellent glossary in Flora Agaricina Neerlandica vol 1 is recommended.



(F) A hymeniderm cap cuticle (*Parasola*) x1000



(G) A cutis cap cuticle of long cells (*Pluteus*) x100

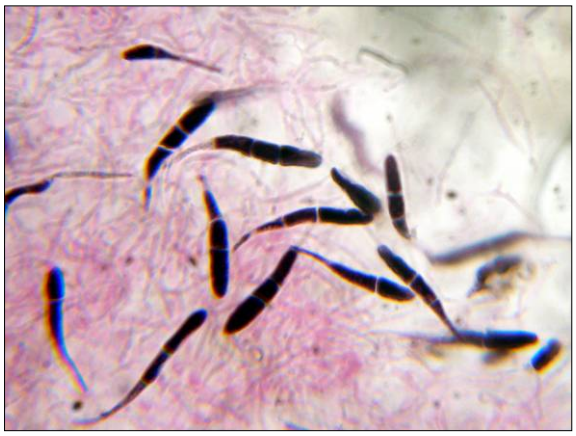
- **Taking a 'scalp'.** This is a short cut (literally!) to distinguishing between these two forms: Using a sharp razor blade, slice off a thin layer of the cap, placing it on a slide with the outer part upwards as it was on the cap. Once stained, the hymeniderm presents as an array of circular cells, while the cutis is composed of elongate cells. A similar distinction allows the genus *Galerina* - having a cutis - to be distinguished from the (often somewhat similar) genus *Conocybe* - having a hymeniderm. The genus *Parasola* (previously in *Coprinus*) with species like *P. plicatilis* also has a hymeniderm cap surface (F).

OTHER CELLS FOUND IN THE CAP CUTICLE

As well as being formed of hyphae, a cap cuticle may also contain cystidia (pileocystidia – known as dermatocystidia or dcys in *Russula* / setules in *Coprinellus*), also fine hairs – known as setae or crins, and may also have a fine membranous or grainy partial covering - known as veil. Depending on genus, these can all play a vital role and need microscopic examination.

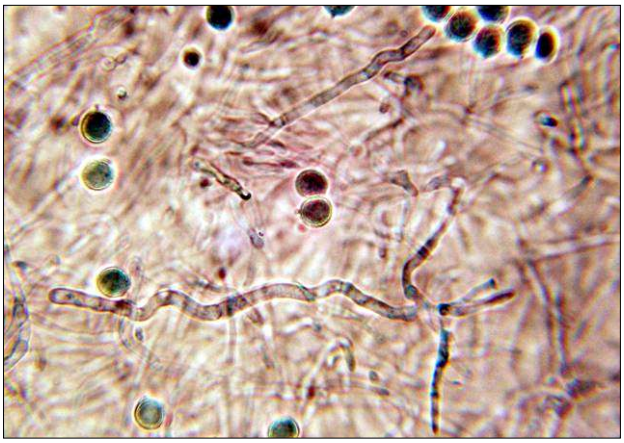
DERMATOCYSTIDIA and the genus *Russula*. In this genus the dcys are the only type of cystidia used for microscopic study. Together with spore colour and ornamentation, they play a vital role in species identification over and above features that can be assessed in the field. It requires a special technique to be able to view them using the stain Cresyl Blue.

Method: 1. – Use a fresh cap and with a sharp razor blade make three small cuts forming three sides of a square with the uncut side nearest the cap centre. With fine forceps lift the cut side nearest the cap edge and gently peel the cuticle towards the centre until it breaks. This broken edge, being the thinnest part of your piece of cuticle, is the part to use and ideally should have no flesh tissue attached underneath and be almost translucent. If you still have white tissue adhering to the underside, lay your fragment flat on a slide with the inner flesh side uppermost and carefully scrape away any flesh with a razor blade as best you can. NB: Old or dried caps will often not peel satisfactorily. 2. – Take a slide, dampen the centre with your finger, then lay your sample – this time outer surface uppermost – flat on the dampened slide. Remove any bits of adhering debris and trim with a razor blade, leaving a nice flat small fragment of cuticle. 3. – Add a drop of Cresyl Blue, making sure all of the sample is wetted, leave for a minute or so, then add a cover slip, tamp down and view. 4. – Focussing up and down at x100, then at x400, you should see a mass of thin hyphal strands and amongst them (if present) some fatter cells which may be septate: these are the dermatocystidia (dcys for short). Not all species have them and if present they can be numerous or sparse, short or long, with anything from no septa to many, according to species. Example (H) has been stained not with Cresyl Blue but with



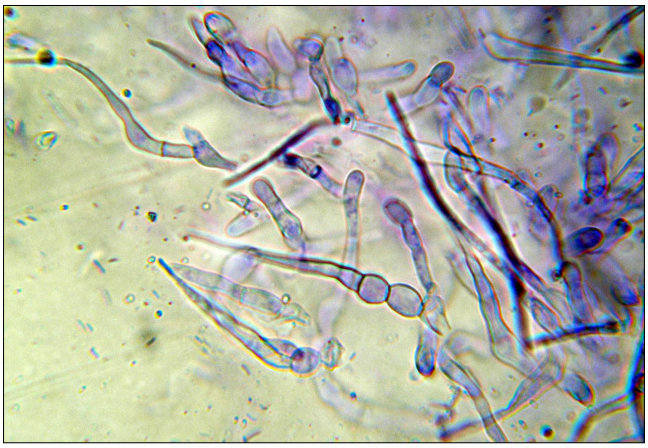
(H) Typical septate 'tadpole'-like dcys found in *Russula nobilis,* stained here with Sulphovanillin

Sulphovanillin – now not recommended for general use due to the hazardous nature of Sulphuric Acid but with the added advantage of staining the dcys black in some species, making them very easy to see, hence this photo. Note the mass of hyphae, stained pink in the background. For comparison example (J) shows dcys of a very different shape and size.



(J) Long vermiform dcys of *Russula melliolens* amongst the hyphal mass, stained with Cresyl Blue. (The dark blobs are spores - unusually smooth in this particular species.)

In some species of *Russula* it is the hyphae in the cuticle surface which are useful in identification rather than the dcys (which may or may not be present as well), and the same method described above using either Cresyl Blue or Congo Red will reveal them. The hyphal end cells of several species are remarkably elongated with pointed tips and are preceded by short swollen globose cells. This type of hyphae (seen in example K) occur in three common species: *R. vesca, heterophylla* and *violeipes.*



(K) The characteristic cuticle hyphae found in *Russula violeipes* stained with Cresyl Blue

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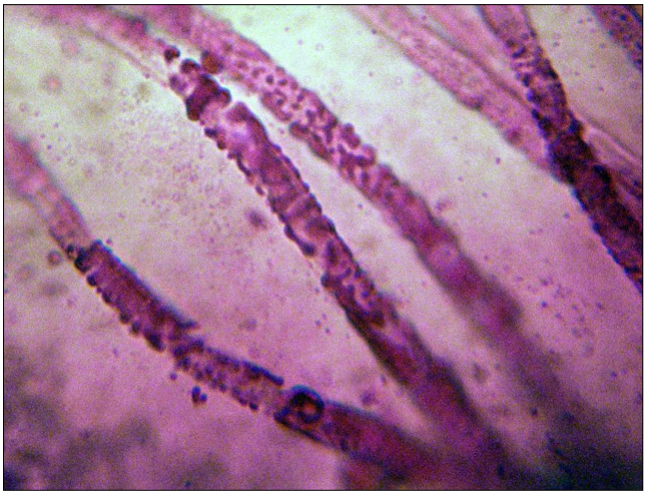
FUCHSINOPHILE HYPHAE

These are cuticle hyphae which occur in some species of *Russula* and have a surface roughened by granules, best seen when stained with Carbol Fuchsin, hence their name. However, to avoid having to handle the hazardous Hydrochloric Acid - needed for the staining process using Carbol Fuchsin - using Cresyl Blue is usually sufficient though not as effective as shown here in example L. Common species having fuschinophile hyphae and lacking dcys are *Russula pseudointegra, risigallina, claroflava* and *caerulea.*



(L) The fuchsinophile hyphae found in the cap cuticle of *Russula pseudointegra* showing the granules, stained here with Carbol Fuchsin.

For advice using oil immersion at x 1000 go to 'Getting started with a microscope', also available here on the BFG Microscopy pages.