Introduction to the Audio-visual laboratory:

Please familiarize yourself with the layout of the lab and where you can find the resources to enable you to carryout the work as described in the study guide.

This sound file is provided as a source of information and a guide, or prompt, to carry out the work contained in each program. It you follow the sound you should complete all the work with reasonable efficiency. At the end of the weeks work you may find it useful to run through the sound again, just listening.

Essential study in the AV lab consists of work in the carrel where you are now sitting, guided by this sound file and viewing the demonstration material. This material will be examined in some way be it in the quizzes or the semester examination.

When you are studying the anatomy of plants try to always consider how that the part you are looking at is related the rest of the plant - build a picture of the Whole plant in terms of structure and function. It is important to keep in mind that the plant you are studying in the laboratory is a part of a complex system of interacting organisms in nature, agriculture or horticulture, and that plants from different places may vary morphologically and anatomically in response to their environment.

One last point, the figures and plates in your study guide are supplemented with high-resolution online images and diagrams. So for each anatomy module the online resources include a sound file (as .mp3 and readable .pdf), a series of high-resolution diagrams and micrographs and your virtual slidebox. Having said that, the best learning resources are the slides that we ask you to examine using the microscope, particularly the ones you make yourself with the fresh material provided. The skills we will examine at the end of semester are those associated with sectioning, staining, communicating your findings with annotated images and the interpretation of cell and tissue patterns.

The whole plant – introduction to Plant Anatomy

As you progress through this course, we want you to develop a clear understanding of the differences and similarities between the two main groups of the Angiosperms (that is the flowering plants), these are the Monocots and Eudicots. A few archaic Angiosperm plants are neither monocots nor eudicots, but in the past were included with eudicots in a group known as dicots. You may still see the term dicot used in place of eudicot.

1. The primary plant body: the shoot apex

Primary growth is growth deriving directly from divisions in the root or stem apex

Secondary growth, which is growth in girth, not length, results from meristems that begin activity only later. Secondary meristems occupy a cylindrical position within stems or roots. After divisions in the apical meristem plant cells grow in size and differentiate in different ways.

You need to be able to distinguish monocots from eudicots in terms of their morphology and structure of the various parts of the Plant body. Monocotyledons are plants such as grasses, oats, barley etc. and Eudicotyledon plants range from many of the flowers in your gardens to trees such as Eucalypts. The figure in your notes shows a diagrammatic representation of the anatomy and morphology of a eudicot.

Cell division is most clearly seen as the laying down of a new wall across a cell, dividing it into 2. Then during and after elongation cellulose and other wall substances may be laid down to thicken the cell wall, and after elongation has ceased lignin may be deposited in walls of some cells. Together with cellulose this gives various types of secondary thickening (not to be confused with secondary growth). During all this time connections are maintained between cells with plasmodesmata. Cells tend to occur in groups functioning together or differentiating together. These tissues are summarised in the table in your notes.

Now read the section on “The primary plant body: the shoot apex” in your study guide and look at the demonstration of germinating and developing seedlings. Note that whilst for eudicots you can see their cotyledons, whether they stay below the ground (e.g. Pea) or are raised above it (e.g. Casuarina or Cucumber), in monocots most of the stored nutrient for seedling development is in endosperm and the cotyledon can only be seen when the embryo is viewed under a microscope. When you have completed these tasks return to this sound file.

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In Seedlings the roots take up inorganic nutrients and water and the cotyledons or endosperm provide organic nutrients for seedling development. Eventually the shoot that develops harvests light and uses its energy to fix carbon and manufacture other organic products. In later programs we will consider roots and transport within the plant. But this week we will examine the shoot apex, introduce you to the tissue systems of plants.

Look at the longitudinal section of *Vicia faba* shoot apex. This is the French Bean plant and it is a Eudicot. Examine it under the compound microscope. You may be able to distinguish cells differentiating into vascular strands - xylem may have small areas of wall thickening visible as oval shapes or spirals. You will also be able to see bulging leaf primordia towards the apex and more mature leaves arising further down and covering over the apex. Thus the apex of this plant is protected by its leaves.

Next examine the apex of the water plant, *Myriophyllum*, also a Eudicot. Compare this with the figure in your manual of this slide. In this species the nodes and internodes are very clear, the internodes increasing in length successively back from the tip. You will also notice that in the stem there are gas spaces present, this is a common

feature of water plants and you will see more of this later in. semester. Return to the sound when you have the slide in front of you.

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Myriophyllum is a convenient plant to examine shoot development because of its regular pattern of development. The apical dome is clearly visible and a succession of leaf primordia appear as bulges on the axis. The mature leaves cover and shield the apex but not to the extent that occurs with terrestrial plants such as *Vicia faba* where the apex needs protection from desiccation.

Leaf primordia originate with periclinal divisions in the outer cortex (that is along the circumference of the stem). As the leaf develops, its outer surface begins anticlinal divisions which increase its diameter. The leaf then takes shape due to the activity of the apical and peripheral meristems.

Shoot elongation between nodes takes place by cell elongation. The mechanism of this will be dealt with in lectures. In *Myriophyllum* the increase in length of the internode is made more obvious by the increase in size of the gas cavities that occupy the periphery of the internodal ground tissues.

Another feature of the shoot apex that is shown in both *Vicia* and *Myriophylum* is the presence of small axillary buds that under the appropriate conditions will grow out to become lateral branches. Figure 1 in your manual shows two types of these – one is a branch with leaves, the other is a branch with flowers. In the shoot, all laterals, whether leaves or branches, have a superficial origin. That is, their vascular tissues arise from the periphery of the vascular cylinder of the stem. This contrasts with branching in roots which arises from near the centre of the tissues, we will deal with this in the primary roots program. The shoot tip therefore shows all of the features of the mature shoot but they are compressed and in miniature.

To emphasize this point, look at the mature structure of a cabbage on the demonstration bench. In cabbage (*Brassica oleracea)* the leaves have matured but they have not unrolled, and neither have the internodes expanded. They expand only at a later stage when the plant goes to seed, that is when it produces a flowering stalk in the second year of growth. The plant hormone gibberellic acid when applied to the plant causes this elongation of the stem as occurs at flowering.

Not all shoots grow exclusively by expansion of cells produced by the apical meristem. In monocots there are frequently continuously dividing meristems at the bases of leaves and the bases of internodes. These are called intercalary meristems and their divisions give rise to the cells above them, which expand and differentiate into new leaf and node tissue. It is for this reason that grasses continue to grow after having been eaten by cattle or mowed in the garden lawn even when the leaf apical meristem has been cut off. The figure of a monocot shoot meristem in the manual shows the position of intercalary meristems in monocots. Examine this diagram and then return to the sound.

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You may have noticed that the leaf primordia (the little buds) do not arise at random, but in a regular arrangement, and this. arrangement determines the form of the shoot. This pattern of leaf primordia frequently follows a spiral. The pattern is known as phyllotaxy.

Shoots are made up of leaves and stems or internodes, as you have seen in the development of the shoot apex. However, just as you might expect, some plants have adapted to a different way of harvesting light and use photosynthetic sterns called cladodes or modified petioles called phyllodes.

2. Tissue systems in plants

This part of the program is on structure of vascular, ground and dermal tissues. Read the notes on Tissue systems in plants and then return to the sound file.

2.1. Ground tissue

Now we shall examine ground tissues. These can be regarded as the tissues through which the conducting tissues xylem and phloem are threaded. In the primary body of a plant, ground tissues are generally the most abundant. It is only after secondary growth starts that wood and bark (secondary growth in xylem and phloem) become the larger part of the plant

There are three types of ground tissues.

Parenchyma is thin walled and of simple unspecialised structure. The other two are collenchyma and sclerenchyma, which are thick walled mechanically supporting tissues that we touched on in the shot apex and leaves program.

The term 'parenchyma' is used for the individual cell, and also for parenchyma tissue as a whole. Although parenchyma is unspecialised anatomically, it is highly specialised biochemically and ultrastructurally. Parenchyma can be specialised for photosynthesis and if you want a word for this type of parenchyma it can be called 'chlorenchyma'. In other tissue, parenchyma can serve various functions. For instance in submerged water plants it may form a very spongy tissue with large air, spaces between cell which give buoyancy to the plant - this is called aerenchyma. Generally, however, parenchyma serves the role of the cells in which the biochemical conversions of the plant go on.

Finally, parenchyma also serves the purpose of being a storage depot for various substances solids like starch, proteins, fats, and also of soluble sugars, mineral nutrients and organic acids in vacuoles.

Examine the parenchyma of potato, looking particularly at the starch grains. You will come across many other examples of parenchyma including starch sheath, in stems.

Now we shall look at collenchyma. Collenchyma has a non-lignified thickened secondary wall and its cells are living at maturity, while sclerenchyma has lignified thickened secondary walls and it is empty of protoplasm at maturity. The characteristics of collenchyma are summarised in the notes.

Celery is a good example of massive development of collenchyma. The celery we eat is a thick petiole. The leaves are small in proportion compared with, say, a leaf of *Eucalyptus*. Pull off some of the celery 'strings' which from ridges or ribs along the outside of the stalk. These are collenchyma.

Now follow the directions in the manual to make a fresh section of the celery petiole (don't attempt to section an individual string) and examine the collenchyma in TS.

Keep this slide as you will look at it again in the section on xylem. Note the characteristic shape with the uneven thickenings, the greatest thickening being in the corners of the cell. After you have examined collenchyma in TS and compared the figures in your manual, examine the macerated tissue provided. Some collenchyma cells are very long and thin, almost like sc1erenchyma fibres.

Collenchyma is an unusual tissue, as the cell wal1s thicken while the cel1s are still expanding. The walls are also slightly elastic but more markedly plastic. A slight force will extend the cell and it will return to its original shape, but a large force will cause the wall to stretch and remain at its new length. Thus collenchyma can provide mechanical support in a growing and expanding stem or leaf. As you will see in subsequent programs, collenchyma occurs in stems and leaves but not in roots. A second general feature that you will see is that as in the celery petiole, collenchyma is distributed round the outside of stems and petioles where its effect will be greatest.

Now let us turn to sclerenchyma. There are two types of sclerenchyma, sclerids and fibres. The f**ibres** are found universally and we shall consider them first. Their characteristics are listed in the notes. Fibres are long narrow cells, which can be over 1 cm in length in some species. The meristematic cell from which they differentiate is much smaller than this, and during their growth the tip of the cell grows intrusively between other cells.

Look at the macerated tissue of *Phormium tenax* the N.Z. flax. Stain it, with toluidine blue. Compare with the TS shown on the demonstration bench. As well as recognising the shape, you will now be able to recognise the colour that fibres stain with toluidine blue and safranin. Look once more at your slide of collenchyma stained with toluidine blue and compare with the slide of fibres from N.Z. flax. Notice particularly the difference in colour.

Sclerenchyma differentiates after growth in length has ceased. The cell walls are thickened with cellulose etc. and lignin is subsequently deposited, beginning in the middle lamella. At maturity the protoplast disintegrates unlike collenchyma, which is a living cell.

Sclerenchyma fibres have great strength. They are elastic, not plastic and sclerenchyma tissue, weight for weight, is about as strong as a metal rod. Like collenchyma, sclerenchyma is distributed round the outside of stems where its strength will be most effective.

Sclereids are individual cells with thickened lignified walls, but of a multitude of irregular shapes, not generally fibrous. They frequently occur in water plants and in hard leathery leaves and also make up the hard tissue in seeds and fruits. Examine the sclerides from pear as described in the notes. Then examine the demonstration of different types sclereids.

2.2. Vascular tissue

Now we shall examine xylem tissue and then phloem tissue.

Xylem is involved in transport of water and mainly nutrients up from the root to the shoot; phloem is involved in transport of nutrients, particularly sugars, from leaves to roots and growing points of the shoot. the distribution of primary xylem and phloem (that derived from apical meristems) is shown in Figure 1.

In the root, xylem and phloem tend to form a central core of alternating strands; while in the shoot, xylem and phloem occur together in strands called vascular bundles, generally near the periphery of stems, and as a network in leaves. Figure 1 shows how leaf vascular bundles in leaves (that is leaf veins) are branches of vascular bundles in stems. It is essential to appreciate that xylem and phloem form a continuous system for conducting nutrients throughout the plant, connecting particularly the two nutrient gathering tissues-mature roots and mature leaves - with all the growing points.

First let us examine xylem.

In this programme we shall examine general cellular characteristics. In the next program we shall examine the distribution of primary xylem, in stems and roots and then we shall examine secondary xylem. Xylem is involved in bulk water flow up the plant. This has been recognised for 100 years.

Other components of xylem - tracheids, parenchyma, and fibres - are listed in Table 3, but first we shall look at the water conducting xylem vessels in celery petiole. Remember that xylem vessels are made up of a number of cells whose connecting end walls have broken down. Each cell is a vessel element, the whole tube is a vessel.

Now examine celery xylem in TS *Apium (celery)*. In your section you retained from your examination of collenchyma. Note lignified xylem cell walls stain red with safranin in the permanent mount and blue-green with toluidine (in contrast to the collenchyma which is purple). Then return to the tape.

Now prepare macerated tissue of celery as described in the notes and compare with the figures provided. If your preparation is not as clear, tap the cover glass to separate the cells more, or tease them apart further, and when you have decided what types of cell are present, and their abundancy, enter your findings in the table provided. When you have done this return to the sound file.

Observe the permanent mount of *Cucurbita* which has a TS and LS present. Compare this to the celery petiole. We will return to *Cucurbita* a little later.

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Next we will examine the xylem developing in the apex of a shoot. Examine the slide of *Vicia* locating the apical dome, the leaf primordia on either side of the stem. Then examine the slide of *Cucurbita* under the compound microscope at low and then high power. The section will show lengths of xylem, which happen to have been cut, but you will probably not be able to trace a single vessel for a long distance. Look carefully and see if you can distinguish annular and spirally thickened vessels. Enter your results for *Vicia*, celery and *Cucurbita* in the table provided in your study guide.

Read the notes on "Ontogeny of Vessels" and look at the figures in this section of your study guide. Note particularly the spiral (or helical) thickening inside the cell wall. This secondary wall is composed of cellulose, which is laid down first, and lignin, which is subsequently deposited within the wall, between the cellulose fibrils. The second thing to note is the disintegration of the end wall between two cells, leaving two adjacent vessels elements joined by a very low resistance pathway - that is the perforation plate, which is just a hole. Both the laying down of the secondary cell wall and the breakdown of the end wall depend on the activity of the cytoplasm. After this stage has been reached the cytoplasm also disintegrates and adjacent cells absorb its molecules, and this leaves the empty vessel through which water can flow.

Now examining the slide of the root tip of maize, and, by following the staining for xylem, see what a file of xylem vessel elements look like as it matures.

You have already seen different types of secondary wall thickening in the macerated xylem of celery and the TS of *Cucurbita*.

Now we shall consider the way in which these types of thickening are related to the stages of growth and differentiation of the stem or root. To find how types of xylem are related to stages of growth one follows a procedure, which in principle is very simple. A shoot or a root apex can have positions on it marked with Indian ink or positions distinguishable by leaf primordia. If one then examines it at time intervals (most simply recording by photography) the growth rate at various points behind the apex can be determined. Sections can then be cut at intervals and the types of xylem vessel developed at various stages of growth can be identified. From studies of this sort a major distinction has become clear. During extension growth xylem is differentiating, and some matures and becomes functional. The functional, mature xylem during elongation has only rings and helices of secondary wall thickenings. This first formed xylem is called proto xylem.

In protoxylem the wall thickenings are in rings (Annular thickenings) or in spirals or Helices, and this confers a special property on the vessels, which is that they can be stretched apart as the elastic primary wall is stretched between the rings or between the gyres of the helices. This means that a dead vessel, which is conducting water and salts can expand and remain functional as the tissue it is in expands.

There are three further important points to be noticed about protoxylem - these are, first, that the proto xylem vessels are smaller than later-formed vessels; second, that the first-formed protoxylem vessels are smaller and have annular thickening, while the later fonner proto xylem are a little larger and have helical thickening. Finally, during the elongation the protoxylem vessels may break leaving simple holes in the tissue, termed lacunae. The later formed xylem is called “metaxylem”. In these tissues the vessels have walls covered more completely with lignified secondary wall, and they can therefore only be functional in non-growing tissue (even if they do begin differentiating during expansion). Thickenings in metaxylem vessels are scalariform (ladder-like), reticulate (net-like) or complete with pits, which are specialised small openings (refer to the online images).

You may like to read about pits in your text book, we will encounter them again when we study Secondary growth. Metaxylem vessels are larger than protoxylem vessels. There is often a perceptible jump in diameter between protoxylem and metaxylem vessels, which constitutes a means of distinguishing between them in TS (as well as the differences in wall thickenings which also distinguish them in LS).

Now here is a question to consider. Do you think that a functional protoxylem vessel with helical thickening formed during elongation can start to add extra wall layers between the gyres in order to become a scalariform vessel later on?

Make sure you are clear on the answer to this question when you do the self-test quiz.

Now examine the slides of *Cucurbita* as described in your notes, complete the table on types of secondary wall thickening in xylem and do the self-test quiz. Then return to the sound file.

Xylem tissue is made up of four types of cell - refer back to Table 2, read the notes on Xylem Parenchyma and xylem tracheids in your notes and refer to the figures provided.

Now examine the pattern of leaf veins or venation, in *Acalypha* the pattern of venation can be seen, particularly clearly because the cells sheathing the veins or vascular bundles are coloured red with anthocyanin, a plant pigment. Hold the leaves against the light and note the main vein or midrib as a continuation of the petiole. Other large veins depart at intervals, and between these is a network or reticulum of smaller veins. Reticulate venation is typical of Eudicots.

Now look at the monocot leaves and you will see the parallel venation typical of monocots.

The veins are vascular bundles containing xylem and phloem tissues that transport water inorganic and organic substances about the plant

Xylem fibres are similar to the sclerenchyma fibres studies earlier - we will look at Xylem fibres again in the secondary growth program as they are more common in secondary tissue. Do the self-test quiz before returning to sound file.

Now let us consider Phloem. Phloem transport will be dealt with in lectures. Our knowledge of the structure of phloem is much less complete than that of xylem. Phloem is a complex tissue, as listed in your notes. Phloem parenchyma and fibres are like parenchyma and fibres in other tissues, but note that a fibre cap is frequently found outside the phloem tissue in stems and some leaves. Look at the figure provided to see how phloem is made up. The real puzzle is the structure and the mechanism of transport of the contents of sieve tubes, which are the elongated cells involved in long distance transport. At maturity sieve tubes have little obvious internal structure, and adjacent sieve tubes through many small pores in what is called a sieve plate are connected Now spend some time examining phloem and sieve tubes.

Follow the instructions on phloem of *Cucurbita*, use the prepared slide and plus the macerated stem tissue provided. Refer to figures in your notes and online. Then return to the tape.

The main problem with studying sieve tubes is their extreme fragility. Even the mildest treatment causes extremely rapid formation of artefacts. These are of 2 sorts - first the content of the sieve tube disorganise and clump together to give what are called slime plugs. In snap-frozen, thin, tissue sections slime plugs are absent, and there is much discussion of what mature intact protoplasmic structure of a sieve tube is. The second type of artefact is the very rapid deposition of callose (not to be confused with cellulose) on the sieve plate and within the sieve pores. Again callose deposition is not seen in snap frozen preparations, although it is found in sieve tubes from dormant plants. Identification of phloem in tissue sections is often by association, in stems and roots we know the relative position of phloem to xylem, and in stems we also often see phloem caps usually made up of fibres. There are special stains (e.g. aniline blue) which stain callose but we need to use a florescence microscope to see it.

Read the notes starting with Development of Sieve Tubes and Companion cells.

2.3. Dermal tissue

The outermost layer of cells in all aerial plant parts is slightly different from cells inside it and is called the epidermis. Following the directions in your Study Guide prepare a small piece of *Acalypha* leaf and the figures in your manual, identify the non-chlorophyllous epidermal cells and stomatal guard cells. When you have identified the cells return to the sound.

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The outer surface of all epidermal cells is covered with cuticle, which is a waxy waterproof substance based on cutin. You will be familiar with the fact that leaves cannot be wetted. This is due to the Cuticle. It is almost impermeable to both water and air and we must regard its presence as one of the major differences between algae and terrestrial plants. Without it terrestrial plants could not exist as they would dry out as fast as a wet piece of blotting paper hung in the open air.

Cuticle covers the whole surface except for the stomatal pores and may have a complicated and characteristic structure. The loss of water and uptake of C02 by leaves therefore takes place through stomatal pores whose aperture can be controlled by the plant.

You will have observed that a single stoma in *Acalypha* consists of two crescent shaped guard cells with an opening between them - the stomatal pore. Look at the image of Acalphya stomata. This shows the uneven thickening of the guard cell walls on which stomatal function depends. The walls of the guard cells around the pore are thicker and less extensible than the outer walls. When the turgor of the cells increases due to accumulation of solutes the guard cell volume increases and the cells curve outwards. Physical models confirm that this picture accounts for opening stomatal pores. Conversely, when water is lost the guard cell volume is reduced, their inner walls become less bent and the pore aperture decreases and can close completely.

The stomatal pore opens into a relatively large sub-stomatal cavity, which in turn is connected to all the intercellular spaces in the leaf through which gases diffuse. There is a good image of this online. Prepare an epidermal peel of *Tradescantia* and tear a section of *Ligustrum* (that is you rip the leaf) as described in your notes. When you have done this and are ready to view the preparations of *Ligustrum* and *Tradescantia* return to the audio file.

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Stomata in Eudicotyledons can vary greatly in size and density, they can also vary with the type of leaf - dorsiventral leaves such as *Acalypha* or *Ligustrum* have most or all stomata on the lower surface. In pendant leaves there may be many more on one or other surface, or there may be a similar number on both surfaces. Some Examples of stomatal density are given in in your manual. Compare your preparation of *Ligustrum* to the micrographs produced under the scanning electron microscope given in your manual before returning to the sound.

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Now examine the epidermis of *Tradescantia,* which is a monocot. Compare your preparation to the permanent mount provided in in your slide box then return to the sound.

Were the pores of your preparation of *Tradescantia* open or closed? Frequently, stripping the epidermis has the effect of closing the stomatal pore, but these often open if left in water or, even better, a dilute KCI solution under the light of the microscope lamp. In your study guide is an image of the stomata of *Commelia cyanea*, this is an Australian native species, which is related to *Tradescantia*.

The stomata of Grasses (Graminiae or Poaceae) and sedges (Cyperaceae) (but not of all monocots) are quite different to those of *Tradescantia* and *Commeliana*. The guard cells are long and narrow with very thick walls in the middle and bulbous ends. However, opening and closing follows the same principles as the other kind of stoma -the difference is just in the type of asymmetric thickening. Now read the description of these kinds of stomata in your manual. Have a look at the demonstration slides of the epidermis with stomata of *Zea Mays and other* grass stomata.

When your have completed this read the notes on cuticle, look at the demonstration slides (if you have time) and return to the audio file.

As we noted earlier, the epidermis is covered by a cuticle made of a waxy substance. Look at the online images of *Nymphaea*, the water lily and *Banksia serrata* with sudan staining which partitions into waxes and oils. You can see that the cuticle thickness can vary greatly. In *Nymphaea*, the cuticle is very thin, the plant lives in water and presumably would rarely be water stressed. Banksia on the other hand lives in dry soils with poor water retaining capacity and must minimise water loss. These environmental responses will be investigated further later in semester.

Now lets turn to trichomes. Trichomes may be simple hair-like structures of one or more cells or complex multicellular gland-like structures. The role of simple non-glandular trichomes may be to protect the leaf in some way or to increase the humidity immediately around the stomata. Glandular trichomes on different species tend to be characteristic of particular species and are used in taxonomy. They may expel very different substances, for example the digestive enzymes of carnivorous plants, toxins such as strychnine or salt from a mangrove leaf.

If you have time look at the demonstration of trichomes, then answer the self-test quiz, then return to the sound.

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Check your answers to the quiz with those at the end of this week's program. If you don't agree with the answers given discuss yours with a staff member in the lab or the tutorial.

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When you have done that you are finished. Please clear up your carrel and leave it ready for the next person to use.

Goodbye until next time