

Discovering Plant Tissues in a New Dimension

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In botanical laboratory courses students observe different tissues using thin sections of various organs. Visualization of whole plant organs is limited by the low clarity of the tissues, and thus requiring a clearing procedure to improve the visualization. This lab exercise uses a new clearing agent (Visikol™) and allows students to easily observe and study whole mount plant organs in three dimensions. This exercise helps to understand and reason about the relationship between structure and function of plant tissues. This hands-on experience motivates and engages students in biology classes, and excites them to learn more about the world of botany.

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Introduction

Traditionally, students engaged in learning practical botanical microscopy in laboratory courses observe different tissues using thin sections of various organs such as roots, stems, leaves, etc. These thin sections of tissue provide a great deal of information, yet students experience difficulty in imagining the same issues in a 3-dimensional orientation. Visualization of a whole plant organ is limited by the low clarity of the tissues, and thus requiring a clearing procedure to improve the visualization. Although there are many clearing solutions, the most commonly used is acidified chloral hydrate (Lersten, 1967; Herr 1993). However, chloral hydrate is a Federally Regulated narcotic, and requires a special permit to purchase or use, placing this technique out of reach for routine teaching. Recently a new simple clearing method for clearing plant tissues was

described (Villani et al., 2013). Here, we have designed a lab exercise that uses a new clearing agent, Visikol™, to clear plant organ and tissues that allows students to observe and reason about the relationship between structure and function of plant tissues. This simple lab exercise introduces students to basic leaf anatomy and primary root structures and allows them to navigate in three dimensions through different organs, to understand the internal structure of tissues and relate it to the function in the plant. In addition, students will observe that root tissues arise from apical meristems in three dimensions and follow differentiation of different type of cells, tissues and tissues systems. This lab exercise is presented as is supplementary to the traditional botanical microscopy labs that learning is based on studying plant tissues in 2-dimensional space using thin section of fresh or preserved organs. This simple hands-on experience helps to motivate and engage students in biology classes, and excite them about the world of botany.

Student Outline

Exercise Activity 1

Submerge three leaves in 10 mL Visikol™ until they become transparent (time will depend on the thickness of the tissues). Students will make a wet mount of leaf and will be instructed to observe the internal anatomy of the leaf and identify different tissue systems (epidermal, ground and vascular tissue systems) of the leaf. Compare and discuss the structure of each tissue in the leaf and relate it to its function in the plant.

1. Obtain a leaf from an aromatic plant (mint, basil or oregano) already cleared in Visikol™ for 12-24 hours.
2. Place one leaf with the top side facing up on the microscope glass slide, add two drops of Visikol and make a wet mount.
3. Add cover slip by placing one edge at one side of the drop of the liquid and then slowly lower the other edge of cover slip on the specimen trying to avoid the formation of air bubbles. Note: if air bubbles are trapped in the mount, gently tap with a pencil on top of the cover to move the bubbles out.
4. First examine the leaf at low power. Examine the epidermis, observe the shape of the cells and if there is or not a space between them.
5. Locate a pair of guard cells (often a kidney-shaped) and a pore between them, also known as stoma (in plural, stomata). These cells are specialized to control the water movement. Are epidermal cells surrounding the stomata, are stomata sunken below the general surface? How is the distribution of stomata (are they scattered or they have a particular arrangement, like in row)?
6. Locate epidermal hairs or trichomes. Observe their shape, size and arrangement. How many cells are forming the trichome? What is a trichome? What are the functions of trichomes?
7. Observe a mushroom-shaped structure, sticking out of the surface, these structures are essential oil glands. Here some chemical compounds responsible of the characteristic aroma are produced and accumulated. There are two types, one small also known capitata glands and another larger one also known as peltate glands. How many cells are forming each gland? Observe the glands at different power. Do you notice any distribution pattern?
8. Examine the leaf from the upper (adaxial) to the lower (abaxial) part of the leaf using the fine focus knob.
9. First observe the compact layer of photosynthetic cells, this is the palisade parenchyma.
10. Continue using the fine focus knob and observe deeper layers. You will encounter cells arranged differently, this tissue is also known as spongy parenchyma. What is the shape of these cells? Are they tightly arranged or loosely arranged (do you see spaces between the cells)? How are the cell walls (thin, thick)?
11. The palisade parenchyma and spongy parenchyma constitute the mesophyll of ground tissue of the leaf. Can you see the chloroplasts in these cells? How are the cell wall, thick or thin?
12. Locate the midrib and vein system. These are specialized cells which conduct water and minerals (xylem) and transport substances synthesized in the leaf mesophyll to other tissues in the plant (phloem), they are grouped together in stands called vascular bundles. Observe thickening in the cell wall of the xylem. What shape do you see?
13. Observe the vascular system and note the diverse and elaborate patterns of veins and vein endings. This pattern is used for identification such as net like or parallel.
14. Continue using the fine focus knob and you will observe the lower epidermis. Locate the epidermal cells, guard cells, stomata and oil glands. Do you notice any difference between the structure of the upper and the lower epidermis? (For example are more or less guard cells and stomata, different distribution?)

Exercise Activity 2

Students will make a wet mount of 10-day old *Arabidopsis* plantlet and will be instructed to observe the complete plant body, the primary root and primary shoot. Students will observe that growth in roots is arising from the apical meristems in three dimensions.

1. Obtain a 10-day-old *Arabidopsis* plantlet cleared with Visikol™ for 12-24 hours.
2. Prepare a wet mount of the plantlet and add a cover slip.
3. Locate the root tip and find the root cap. The cap is between the soft apical meristem and the hard soil particles. What is the function of the root cap?

4. Look carefully, just above the root cap, and you will identify the division zone, the source of new cells for the root growth. Using the fine focus knob and you will locate the apical meristem, protoderm (the young epidermis), procambium (young vascular system) and ground meristem (cells between protoderm and procambium). What is the size of these cells?
5. At lower power, in addition to the division zone in the root, identify the cell elongation zone (just above the division zone). Do you notice any difference in these cells?
6. Continue observing and you distinguish a zone rich in root hairs. This is maturation zone in the root. What is the characteristic shape of cells in this zone? What is the function of the root hairs? Are the cell walls thick or thin? How many cells are in a root hair?
7. Observe the vascular tissues, where are they located? Are they in the center of the root or in the surface?
8. Examine the leaves with lower and high power from the upper to the lower part. Can you identify the tissues? Does *Arabidopsis* have trichomes? What is the shape? Why would plants have or not have trichomes? What could an advantage of trichomes provide to a plant?
9. Locate the shoot meristem, and observe the cell size and shape.

Further Questions for Students

- A. Do you see any difference (shape, size) between the superficial layer of cells and the deepest ones in the leaves? Can you relate the structure you see with the function it has? Can you suggest another shape or structure for that function?
- B. How many layers of tissues you can to see form one side of the leaf to the other? Would you expect to find many more layers? What would be the limiting factor?
- C. In the leaf you have observed, did you see the trichomes on both upper and lower surfaces? What is the function of the trichomes? Plants from which environments would you expect to find them?
- D. What is the purpose of having oils glands and trichomes on the surface in the leaf?
- E. Why do you think the meristem in the root (or shoot) is located in deeper tissues and not on the surface?
- F. Do you expect to find oil glands in the epidermis of the root? Why?

Materials for Preparation of the Plant Material

- Visikol™ (Visikol Inc., New Brunswick, New Jersey, USA, www.visikol.com)
- Whole fresh leaves. It is suggested leaves of an aromatic (basil, mint, etc.) and no aromatic plant (*Arabidopsis thaliana*) for comparison of different structures that can be found in the epidermis.
- Fresh young *Arabidopsis sp.* plantlets. If enough seedling are available it is suggested different ages (1-10 days-old).

Materials for the Lab Exercise:

- Leaves (aromatic plant) and 10- days old *Arabidopsis* plantlets cleared 12-24 hours in Visikol™.
- Stainless steel forceps
- Microscope glass slides and cover slips
- Gloves
- Microscope
- Camera with imaging software (if available)

Notes for the Instructor

We have selected two lab exercises, one that introduces to leaf structure, and the second one that introduces root structure. Keeping in mind that leaves are the site of photosynthesis, transpiration, it is crucial that students consider the surface of light harvesting and gas exchange, permeability of the epidermis to gases (presence of stomata), intercellular spaces, and the distribution of the vascular tissues.

Whole fresh leaves (oregano, basil or mint are suggested because of the essential oil glands and

trichomes) were submerged in Visikol™ until they became transparent, then the whole leaf was mounted on a microscope slide with two drops of Visikol™ and a cover slip was added. Using the fine focus knob, different layers of leaf tissues were easily identified such as epidermis, with stomata, oil glands, trichomes, underlying palisade cells, vascular tissues etc.

The instructor can point out the importance of the epidermis as a continuous and protective layer, formed by epidermal cell, stomata and modified epidermal cells. Hairs or trichomes can be glandular (oil glands) or non-glandular (covering) hairs. There is a great diversity of forms and these hairs may be used for

identification. The function of hairs is for protection, in some species is related to water conservation in the leaf, others have anti-herbivore function. For example, aromatic plants synthesize and accumulate essential oils in glands on the surface of the leaves. Two different essential oil glands, capitate and peltate oil glands could be easily distinguished (Fig. 1A). Essential oils are synthesized to protect the plant against different organisms and thus exhibit many biological activities such as antimicrobial, antifungal and antiviral activities (Koroch et al., 2007). The presence, location and size of the oil glands in the epidermis can be discussed. Using the fine focus knob, different layers of leaf basil tissues were easily identified such as epidermis, with stomata, oil glands, underlying palisade cells, vascular tissues etc. (Fig. 1D-I). These observations may lead to further discussions of the importance of having a complete three dimension image of the localization of each specialized cell in the leaves and thus understanding the morphology of the leaf.

Another example can be illustrated using 10-days old *Arabidopsis* plantlets. Here, with *Arabidopsis* students will observe branched covering trichomes, that are non-secreting epidermal cells (Fig 1B). Cleared root tips can be used to point out the zones of root development, cell division, elongation zone and maturation zone with root hairs as extensions of epidermal cells (Fig 1C). Students can use the coarse and fine adjustment knob to investigate the meristematic tissue without cutting the tissues (Fig. 1 J-K).

These exercises can be easily performed with any plant material students can bring to class. In advanced classes, students can be engaged in conducting comparative studies of structures and organ formation using either a wider variety of plants (for which students can directly sow and then observe over time) for which this lab practicum can be performed or use a series of plantlets of different ages of *Arabidopsis* (e.g 0 to 10-day-old).

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the authors and is patent pending, filed by Rutgers University. Rutgers has granted an exclusive license to Phytosys LLC (co-owned by the authors) to commercially market Visikol™.

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About the Authors

Adolfina Koroch received her Ph.D. in Biological Sciences from the National University of Cordoba, Argentina. Her areas of expertise include plant biology with a focus on medicinal and aromatic plants. In 2001, she came to the US as visiting scientist at Rutgers, The State University of New Jersey to conduct research on the *in vitro* culture, molecular biology and chemistry of bioactive components of native US medicinal plants. She is currently Associate Professor at the Science department at Borough of Manhattan Community College- CUNY, where she teaches introductory biology courses and plant biology.

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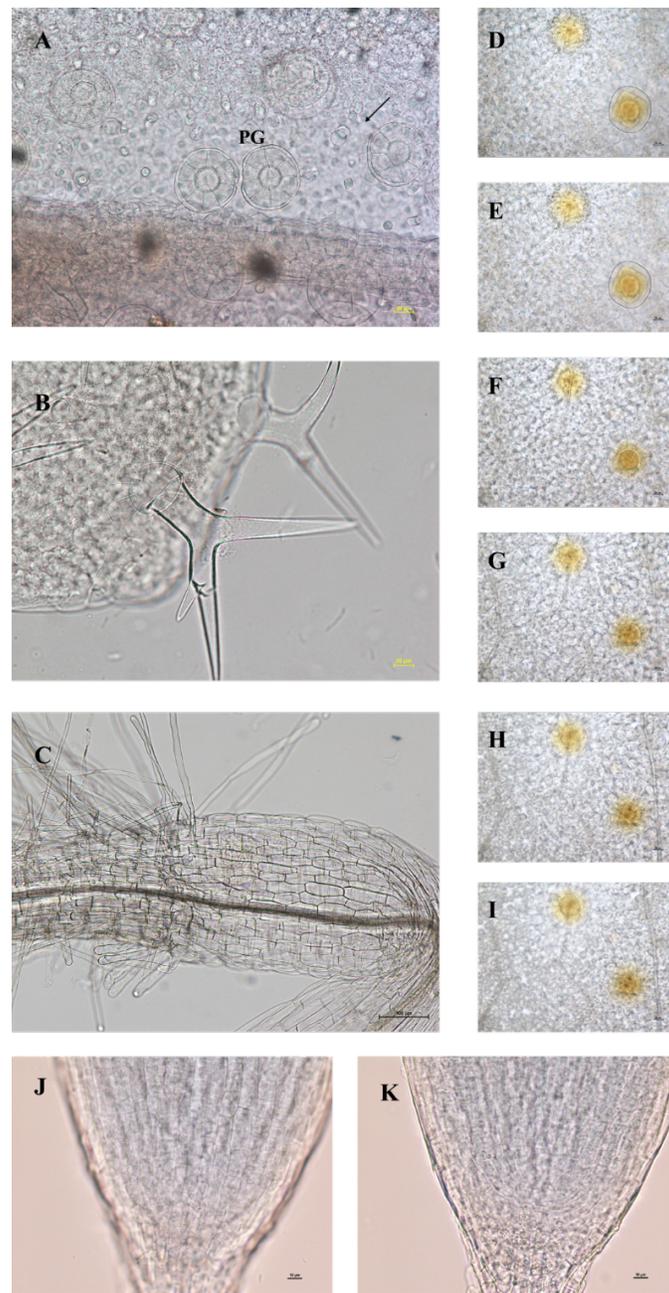


Figure 1. Samples of light micrographs of fresh, whole mounted botanical specimens. (A) Spearmint leaf. Epidermis showing abundant peltate gland (PG) with eight-celled apical disc and stalk cell in the center and capitate glands (arrow); (B) *Arabidopsis* leaf, epidermis, covering branched trichomes; (C) *Arabidopsis* root tip, maturation zone abundant in root hairs; (D-I) Basil leaf series micrographs from upper side to lower side of the leaf showing epidermis, mesophyll cells and vascular tissues (J, K) *Arabidopsis* root tip close up. Superficial view epidermis (J), and deeper layers of division zone showing the new source of cell for root growth (K).

chemistry, analytical chemistry, pharmaceutical lead development, synthetic chemistry, histotechnology, and biological chemistry. While at Rutgers, Tom led the development of Visikol as a tool for researchers studying plant histology and pathology, and worked to distribute Visikol to researchers over 150 universities and institutions. He currently is finishing his graduate thesis while acting as the research Project Coordinator at the New Use Agriculture and Natural Plant Products program where he has mentored dozens of graduate and

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