

Microscopy with Ultraviolet Surface Excitation in Life Science Education

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1. Abstract

This project investigates how to integrate microscopy with ultraviolet surface excitation (MUSE) into a high school science classroom in order to enhance life science education as well as student interest and engagement. MUSE utilizes the unique property of ultraviolet light at wavelengths between 250 and 285 nm to propagate about ten μm into tissues, thus illuminating only the top cell layer. This allows for imaging of the cellular organization and microstructure without having to cut the sample into very thin sections. This capability has the potential to eliminate the need for pre-made slides, and be far more fascinating and informative for students. Laboratory experiments have been developed to directly expose students to plant and animal microanatomy with a personalized learning approach to producing high-quality microscope images. These exercises enable students to utilize autofluorescence and stained tissue samples to identify various microstructures relating to the Next Generation Science living environment core standards.

2. Introduction

Microscopy with ultraviolet surface excitation (MUSE) is based on the salient property of UV light at wavelengths between 250 and 285 nm to propagate only into the top 10 μm of a tissue specimen, illuminating only the top cell layer.¹⁻³ The resulting fluorescence images, arising from either the native tissue fluorophores or extrinsic contrast agents, are also localized within this narrow range. The MUSE technology was originally developed to examine defects on high-power laser optics, but has since been adapted to biological studies. This microscopy system enables one, with proper selection of imaging optics, to acquire high-quality images without implementing any additional optical sectioning method (such as confocal imaging) or physical sectioning of the specimen into very thin layers. In addition, UV light can photoexcite a wide range of common fluorescing stains, which subsequently emit light typically in the visible spectrum.^{2,3} MUSE imaging relies on the visible structural differentiation caused by either the nonuniform cellular distribution of naturally occurring biomolecules or the use of fluorescing stains to highlight different cellular compartments. This allows one to image the cellular organization and

microstructure without laborious effort (fix, dehydrate, embed in wax, cut, and stain) to produce a thin stained section, which can take anywhere from many hours to several days before the slides become available for viewing under the microscope.

Tissues produce autofluorescence when exposed to any form of photoexcitation, but UV light causes a relatively larger amount of autofluorescence, typically dominated by the emission by tryptophan.¹ The autofluorescence provides immediate visualization of structural differentiation within the sample, which enables one to identify various microstructures without staining the sample. MUSE can also be used in combination with fluorescent stains designed to target specific structures within tissues, allowing for easier structural identification. These stains can be excited by 250 to 285 nm of UV light (typically via transition of ground-state electrons to the second singlet excited state, $S_0 \rightarrow S_2$) and emit light in the visible spectrum (from radiative transition from the first singlet excited state, $S_1 \rightarrow S_0$).

In this work, tissue samples were stained using Hoechst 33342 and Eosin Y, which stain the nuclei and cytoplasm, respectively, and are safe for classroom handling and use.⁴ Premixed powders in a gelatin capsule have been made with 20 g of Eosin Y and 5 g of Hoechst 33342, and the capsule is readily soluble in 100 mL of de-ionized water, resulting in a stain solution that is plenty for a single class. A rapid and simple staining process has been developed so that any individual student can safely complete each step. The process is as follows: a 10-s phosphate-buffered saline (PBS) rinse, 20 s in isopropanol, a 10-s PBS rinse, 60 s in a stain solution, and a 10-s PBS rinse.⁴ The isopropanol is not required for the stain, but it helps to enhance the nuclear stain. It is possible to use staining solutions other than Hoechst 33342 and Eosin Y to achieve the desired results, but these possibilities require further research and development.

The goal of this work was to develop a curriculum involving MUSE that can be adapted to life science education in order to inspire students to learn more about the natural world. To do this, laboratory exercises for a high school science class were created to enhance student interest with a personalized learning approach. High school life science education is responsible for shaping the scientists of the future

by capturing and sustaining students' interest in the natural world. As part of this work, laboratory experiments have been developed to directly expose students to plant and animal microanatomy. These exercises involve imaging tissue samples and using native fluorophores (autofluorescence) or fluorescent stains to identify various microstructures relating to the high school life science curriculum. The experiments are designed to teach students about scientific practices while reinforcing their knowledge of the life science core standards. This work suggests that implementation of MUSE technology in an educational setting has the potential to increase student engagement while complementing the Next Generation science living environment core standards currently used in the United States.

3. Laboratory Exercises

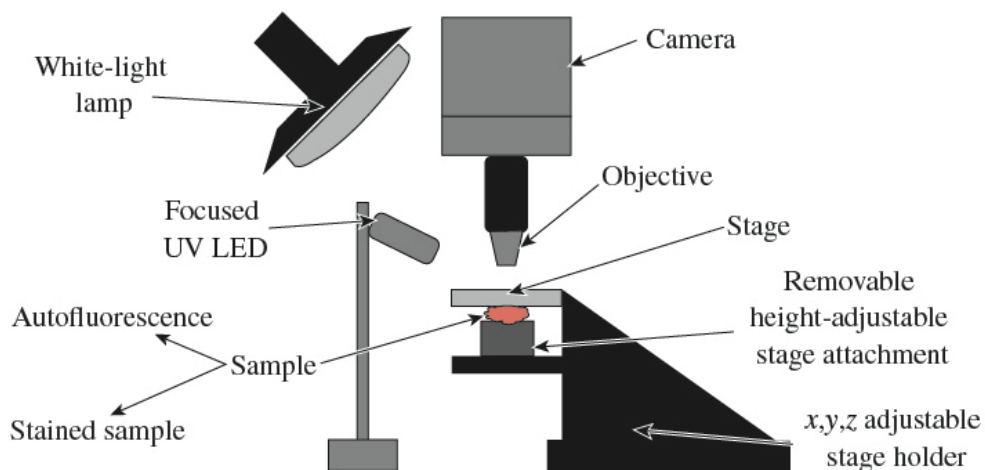


Figure 1: Schematic diagram of the MUSE experimental apparatus.

Figure 1 shows the schematic diagram of the MUSE setup. The UV LED illumination is at an oblique angle and focused on the sample directly under the objective. This system was used to view plant and animal microanatomy with a quick, simple, and inexpensive process. This system was also used to image tissues without any preparation, as well as stained tissue samples. Imaging experiments of various objects, plants, and animal tissues were performed toward (a) exploring the spectrum of MUSE imaging suitable for an education setting and (b) developing laboratory experiments relevant to the high school science classroom. Laboratory procedures, background, and examples were written for each experiment.

These exercises utilize MUSE technology while complementing the current life science curriculum standards. The labs bring a personalized learning approach to obtaining high-quality images of tissue microstructure that reinforce material learned through classwork. Each laboratory exercise is discussed in further detail in the next sections.

3.1 Introduction to MUSE

The first laboratory exercise titled “*Introduction to MUSE*” serves the purpose of exposing students to MUSE technology while promoting individual interest in microscopy and microanatomy. In this exercise, students gather everyday objects from around the classroom and school and image each object’s autofluorescence with the microscope. Each student has unique samples; consequently, the set of images captured by each student is different. The examples of captured images in Fig. 2 show foam, paper towel, and sugar crystals, respectively. Students are able to complete every step of this lab exercise individually and obtain distinct resulting images, which allows for flexibility and creativity in the process. This approach has the potential to be more interesting and engaging for students while still being effectively informative. The exercise introduces students to the concept of microstructures, allowing them to view firsthand the microscopic structure of objects with which they are familiar. This reinforces knowledge of structural hierarchy and exposes students to the idea of fluorescence and the understanding of MUSE technology and how it can be used to capture fluorescence images.

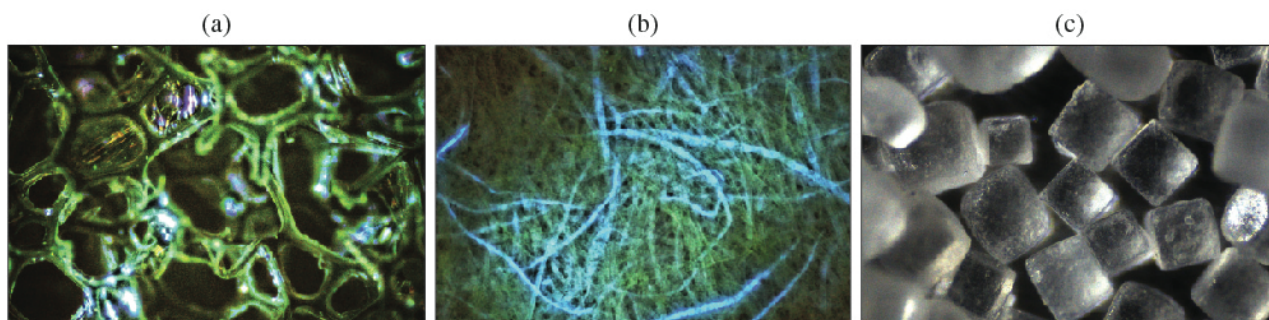


Figure 2: MUSE microscopy images based on native fluorescence (autofluorescence) from a foam sample (a), a piece of paper towel (b), and sugar crystals (c).

3.2 Living Organisms

The next exercise, “*Living Organisms*,” introduces students to the staining process and reinforces students’ knowledge of plant structures. The procedure for this experiment includes gathering living organisms such as leaves and flowers from the outdoors and imaging these samples with MUSE using their native fluorescence (autofluorescence) or staining to view in more detail. Figure 3 presents images obtained from a maple leaf: (a) the conventional color image of the leaf’s surface, (b) the autofluorescent image, and (c) the maple leaf after it was exposed to staining. Students can gather samples from various plants or other small organisms and perform each step on their own, so they are free to choose samples that are of personal interest. Students can also identify structures from captured images, such as nuclei, cell walls, pollen, and leaf veins. In this experiment, students should focus on the staining process and identifying notable parts of each living sample.

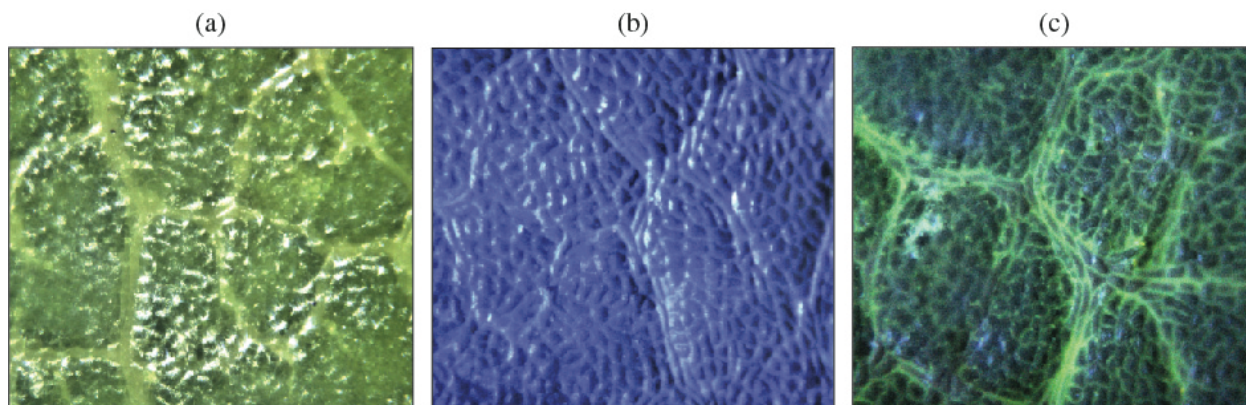


Figure 3: Images from the surface of a maple leaf: conventional white light illumination (a), autofluorescence (b), and following staining with Hoechst 33342 and Eosin Y (c).

3.3 Plant and Animal Cells

The third laboratory exercise titled “*Plant and Animal Cells*” focuses on emphasizing the structural and functional similarities and differences between plants and animals. In this exercise, students can use an onion, a flower, or a leaf as the plant sample and a cheek swab as the animal sample. Onion cells have been found to produce the best images, and due to the nature of MUSE, there is no need for thin sectioning. Autofluorescence or stained samples can be viewed and imaged, and the plant and animal

cell images can be compared in order to find structural similarities and differences. Figure 4(a) shows a stained onion sample, where the rectangular cell walls and dark red stained nuclei are clearly visible. Figure 4(b) shows an autofluorescent image from a cheek swab, allowing one to directly view the irregular-shaped cell membrane.

Through MUSE microscope captures, students can visualize the cellular organization and combine that with their knowledge of the functions of plant and animal cells. For example, students can compare rigid plant cell walls to the more abnormally shaped and flexible animal cells and study the structural and functional reasons for these differences. In this exercise, the presence or absence of fluorescing stains, as well as the different regions within a single sample, should be noted by students. Students can also focus on visualizing multiple types of specialized cells within a single sample.

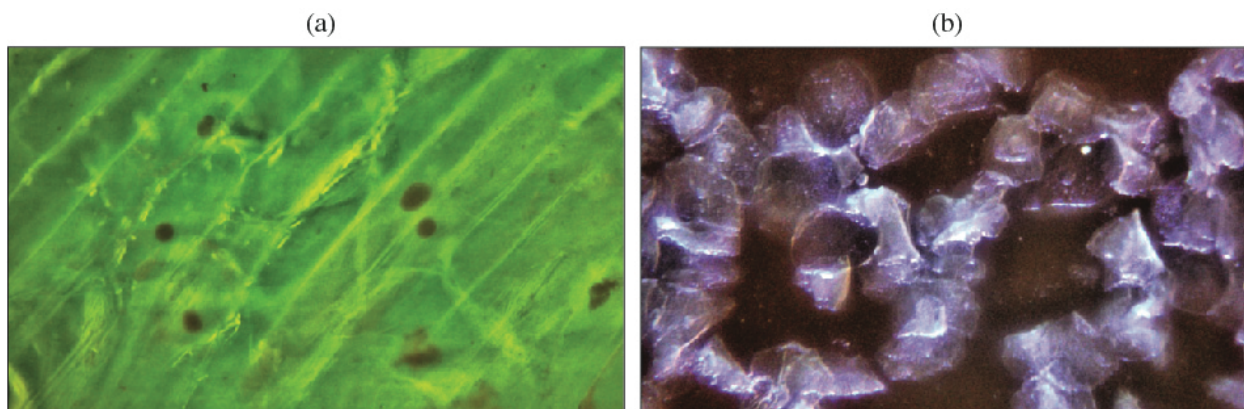


Figure 4: Onion cells in a thick sample visualized after staining with Hoechst 33342 and Eosin Y (a). Autofluorescence image of cheek swab cells (b).

3.4 Animal Dissection and Microanatomy

The next laboratory exercise is “*Animal Dissection and Microanatomy*,” in which students dissect a preserved animal to view gross anatomy and use tissue samples to view microanatomy. This experiment can be implemented into units on the digestive or circulatory system, as students first identify the organ systems present in the animal. Then, students can identify individual organs and other structures, taking a tissue sample for further use. A kidney cross section, heart cross section, skeletal muscle surface, lung

surface, and liver cross section were found to be relatively easy to identify, cut, and view under the microscope. The representative organs used in this report are from a frog, but other animals may be used as well. Students can use images of the organs to locate and view various microstructures.

In Fig. 5 the images of a ranine lung obtained following nuclear and cytoplasmic stain clearly show the bronchioles and the nuclei. Specifically, Fig. 5(a) is a digitally stitched image comprised of a number of individual image captures that offer an enlarged view of the lung cross section. Figures 5(b) and 5(c) are individual images (single captures) that allow one to appreciate the information detail and volume available to the student with the higher magnification.

Figure 6 shows a stitched image of a kidney cross section. The kidney tubules are visible in the cross section either through their exposed surface (mostly on the right side of the image), or as cuts through individual tubules that expose the rounded tubular walls (mostly on the center and left sides of the image), where the higher concentration of exposed nuclei gives them the appearance of ring structures.

Figure 7(a) shows striated cardiac muscle, while Fig. 7(b) shows skeletal muscle fibers. This exercise directly exposes students to the structural similarities and differences between various organs from the same organism. Cardiac muscle is striated while skeletal muscle has fibers, and the lungs have bronchioles while the kidneys have tubules,⁵ each displaying a unique structure that is related to its function within the body.

Through these experiments, students can focus on identifying how each organ's microstructure relates to its function. This questioning with an actual organism gives students a concrete example of hierarchal differentiation in multicellular organisms, which is an important concept in life science education.

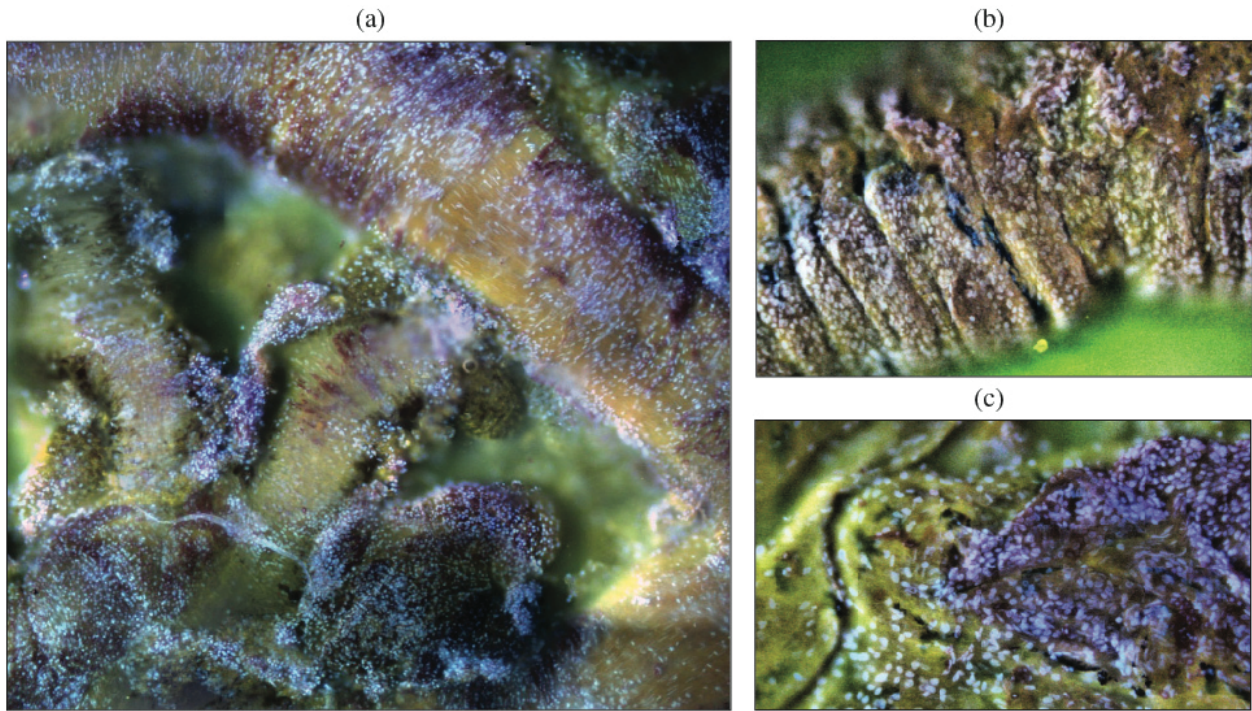


Figure 5: Ranine lung with nuclear and cytoplasmic stain. (a) This image is digitally stitched to show a larger field of view. The blue illuminated dots are nuclei of lung cells. Single image captures (b and c) as recorded by the microscope system reveal the microstructure of the cross sectioned tissue.

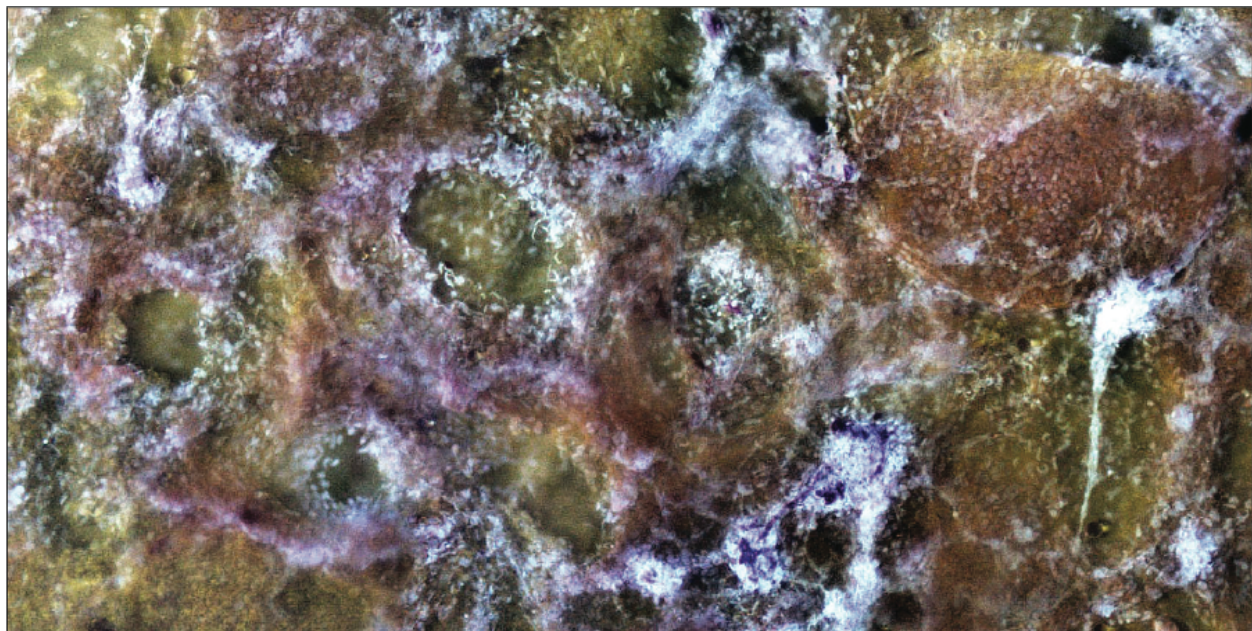
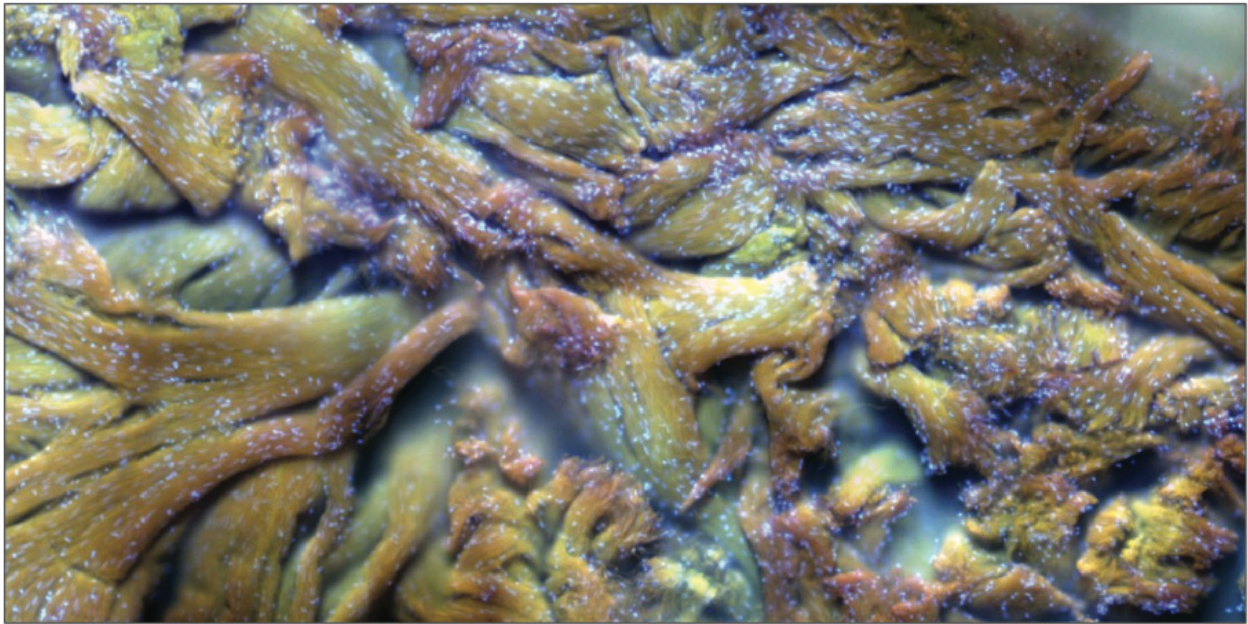


Figure 6: Ranine kidney cross section with nuclear and cytoplasmic stain. This image is digitally stitched to show a larger field of view. The round shapes in the image are the cross sections of kidney tubules.

(a)



(b)

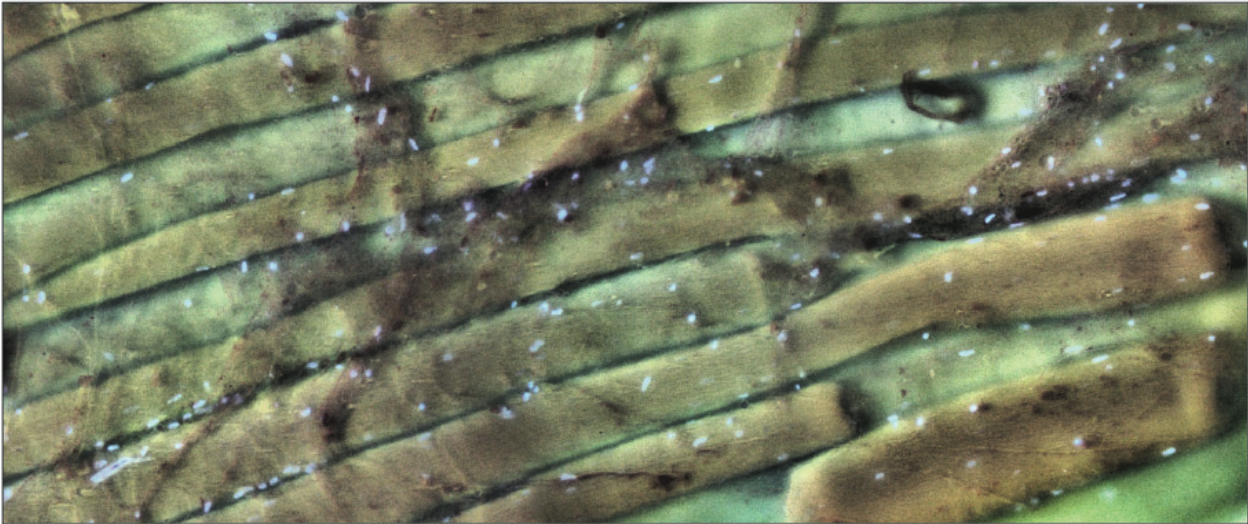


Figure 7: MUSE images of ranine cardiac muscle (a) and skeletal muscle (b) with nuclear and cytoplasmic stain. Muscle fibers and cell nuclei (illuminated dots) are visible. Each image is digitally stitched to show a larger field of view.

3.5 Leaf Cross Section and Microanatomy

The last laboratory exercise developed in this work, titled “*Leaf Cross Section and Microanatomy*,” allows students to use a physical model of a leaf to identify various microstructures,

including the cuticle, xylem, phloem, stomata, and mesophyll layer. Students collect leaves of their interest, cut a thin strip, and place the sample on the stage so that the cross section is facing the microscope objective. The autofluorescence generated by the UV light creates clear structural differences in color and intensity, resulting in an image that students can use to easily identify the different parts of the leaf. Images of maple leaf cross sections are shown in Fig. 8.

With this exercise, students gain a physical model of a leaf cross section, which is generally taught from cartoon diagrams. With the images of actual leaf cross sections, students can identify structures and compare them to the schematic representations taught in class. Students can view the structure of different leaf parts and can question its relation to each part's function. For example, the cuticle is a layer on the top of the leaf with thin cells that act as a protective barrier. The xylem and phloem are tubes in leaf veins that transport water and nutrients throughout the plant. The mesophyll layers in the center of the leaf have cells that face the top of the leaf since they have chloroplasts that use sunlight for photosynthesis.⁶ Students can explore these types of relationships with this exercise, which reinforces their knowledge of specialization and cellular differentiation.

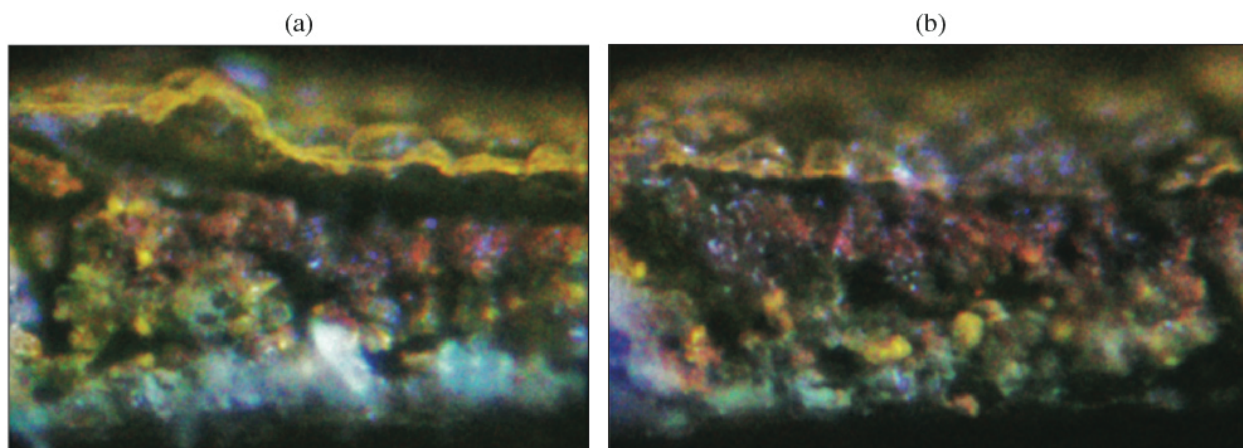


Figure 8: MUSE images of maple leaf cross-section using autofluorescence only.

4. Curriculum

The developed MUSE curriculum focuses on exposing high school students to scientific practices that reinforce their knowledge about life science. Each practice learned through the laboratory exercises is relevant to the public high school life science standards, and the coordinating Next Generation science standard is referenced.⁷

The first practice that this curriculum emphasizes is the identification of major structures in plant samples. Students can view organisms such as leaves, grass, and flowers under the microscope and use the resulting images to find certain microstructures, such as leaf veins. By looking at various cells and structures within one organism, students are learning about cellular specialization and organization. This practice reinforces the fact that while different cells within an organism have the same genetic information, they may not have the same structure or function, depending on which genes are expressed. This practice reinforces Next Generation standards HS-LS1-1 and HS-LS1-2.

The next scientific practice with which students are involved is the dissection of an organism to view gross anatomy as well as tissue microstructure. When students dissect an organism, they work to understand the hierarchical levels of organization within a multicellular organism. This corresponds to Next Generation standard HS-LS1-2. Students can identify organs and organ systems in an organism and can then take a tissue sample to view the same organism's microanatomy at the cellular level.

The last emphasized practice is the comprehension of the structure and function of plant and animal cells. This practice works on a microscopic scale and focuses on life processes at the cellular level. Plant and animal cells have different organelles to perform different functions, and each organelle and cell has specific structures that relate to its function. The various cells work together to perform life processes to maintain homeostasis within an organism. This is shown in Next Generation standard HS-LS1-2.

Additional laboratory exercises can be developed to offer unique experiences to a student. For example, experiments that enable dynamic response of cells and tissues to an external stimulus are

possible using the MUSE technology. In addition, MUSE can be used to visualize microanatomy of non-normal tissue such as tissue that has undergone some type of injury.

5. Conclusion

This work details the developed curriculum for the high school life science implementation of MUSE. Further optimization of the system, including the development of a design specifically for use of MUSE in education, must be done to enable the actual use of this technology in the classroom. Such an instrument can be based on the schematic diagram showed in Fig. 1 and should include a focused UV LED with wavelengths between 250 and 285 nm, a removable and adjustable sample holder, and an objective and tube lens for imaging. With the proper instrumentation and curriculum, MUSE can be effectively integrated into a high school life science classroom.

6. Acknowledgements

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7. References

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