# Developing a Curriculum for the Translation of Microscopy with Ultraviolet Surface Excitation (MUSE) into a High School Science Classroom

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The translation of microscopy with ultraviolet surface excitation (MUSE) into a high school science classroom is investigated with the goal of providing a suitable new modality to enhance life science education. A key part of this effort is the development of laboratory exercises that can integrate the advanced capabilities of MUSE into a classroom setting. We consider that MUSE in education can eliminate the need for premade microscope slides and provide a far more engaging and rewarding experience for students.

MUSE is a new microscope technology originally developed to locate defects in optical materials that were responsible for introducing laser-induced damage in optical materials for large-aperture laser systems such as OMEGA. This method is based on the salient property of UV light at wavelengths between 250 and 285 nm to propagate into only the top 10 $\mu$m 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. This 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]

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] 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. 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. The goal of this work was to develop a curriculum involving MUSE that can be adapted to life science education. To do this, laboratory exercises for a high school science class were created to enhance student interest with a personalized learning approach.

A MUSE imaging setup was configured similar to that described in Ref. 4. In short, the UV LED illumination is at an oblique angle and focused on the sample directly under the 10× 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. 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.[5]

The first practice that this curriculum emphasizes is the identification of major structures in plant samples. Students can view organisms such as leaves (see Fig. 1), 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 Next Generation standards HS-LS1-1 and HS-LS1-2.

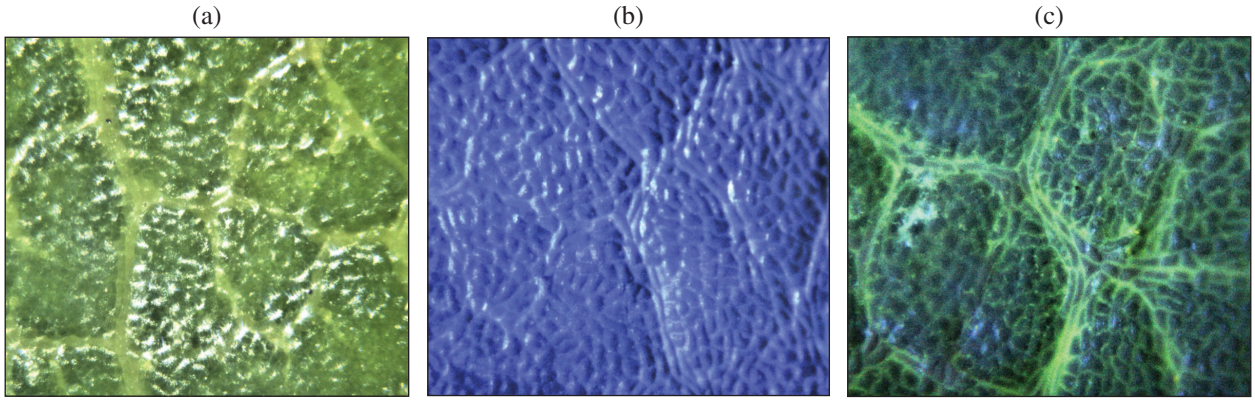


Figure 1
Images from the surface of a maple leaf: (a) conventional white-light illumination, (b) autofluorescent image, and (c) staining with Hoechst 33342 and Eosin Y.

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 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 distinctive experiences to students such as experiments that enable one to monitor the dynamic response of cells and tissues to an external stimulus.

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