

PROCEDURE

Stage 1 – Introduction to the Microscope. Initially, students are given time to physically familiarize themselves with the compound light microscope and its components after having gone through pre-lab instruction online and completing a worksheet (Appendix 1).

Stage 2 – Observation Skills. After this ‘get to know your scope’ session, students are given microscope slides containing already prepared and fixed (permanent) specimens, which were purchased from Carolina Biological Supply and Nebraska Scientific. This allows students a chance to interact with the microscope, learn how it works, and develop basic skills such as focusing, using an authentic sample of known high quality. Also at this stage is the introduction of immersion oil and its proper use and care of the microscope, including proper clean up protocols. Detailed protocols of all steps for the instructor are given in Appendix 3. An optional addition to help students familiarize with movement of the stage, focusing, and adjusting the field of view is using prepared slides with newsprint and/or the letter “e”. This is best for students where this is their first exposure to a microscope.

Stage 3 – Slide Preparation. Next, students are shown how to make a wet mount by the instructor and then asked to make one. For this, we use locally collected pond water (or liquid cultures) and ask the students to observe their wet mount using the microscope and sketch what they see. Manual sketching requires students to consider details in the image. Later in the semester students can take digital images for recording results, but sketching first requires thinking and adds kinesthetic value. This exercise illustrates the difficulties of viewing unstained microorganisms and finding specimens in a mixed sample. Larger and colored eukaryotic cells in pond water, such as algae, are easier for students to view than bacteria. This stage also adds the skills of placing samples on a standard slide and usage of glass cover slips. Students continue to improve focusing skills, and learn the importance of the condenser and iris diaphragm. Note that while the ASM Biosafety Guidelines (Emmert, 2013) state that all work with isolated unknown organisms should be performed at BSL-2, the microorganisms in the pond water are not isolated or grown up to high levels and thus the safety risk is very low.

Stage 4 – Basic Stain Techniques. A simple stain is performed using the same sample from stage 3 by making a smear and heat-fixing, followed by a one-minute stain using methylene blue dye. Students are asked to observe and sketch the results of the stain, emphasizing that staining reagents help alleviate the issue of low contrast, which is clearly visualized by comparison to the unstained wet mount. A methanol-fix can be used in place of the heat-fix,

which alleviates the need for flame or a heat block. After fixing, all organisms are inactivated so the slides are treated as BSL-1.

Stage 5 – Complex Staining. For the last step in the first lab period, students are asked to perform a Gram stain on *Bacillus subtilis*, incorporating all of the skills learned up to that point. *B. subtilis* is used since it is a large, Gram-positive rod which stains intensely.

At the end of the first lab, students have made their way up a pyramid of microscopy skills (Figure 1) where each previous skill learned is incorporated into learning the next, thus stepping them up to the ultimate goal.

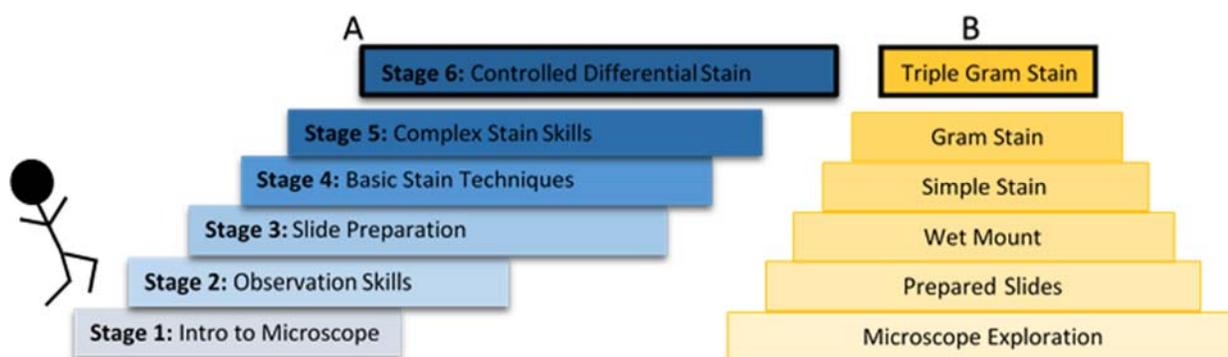


FIGURE 1. A) Stages in which students begin to learn microscopy and staining, beginning with the most basic skill, learning the microscope. B) The procedures utilized in the laboratory setting by which students acquire their microscopy skills in a stepwise fashion.

Stage 6 – Controlled Differential Stain. In the second microscopy lab, students perform a triple Gram stain, where two controls, Gram-negative rods (i.e. *E. coli*) and Gram-positive cocci (i.e. *S. epidermidis*) are smeared and stained on the same slide as an unknown, pure, culture (Figure 2). Before this complex stain is performed, there is a lab discussion of how the Gram stain works and how to put three separate smears on one slide. The triple Gram stain serves as a skills assessment for the students that helps them identify errors in their staining technique (Table 1) since there are dual controls for Gram reaction and morphology on the same slide. All strains utilized are BSL-1: *E. coli* K-12, *S. epidermidis* Evans (ATCC 14990), *B. subtilis* Cohn (ATCC 6051).

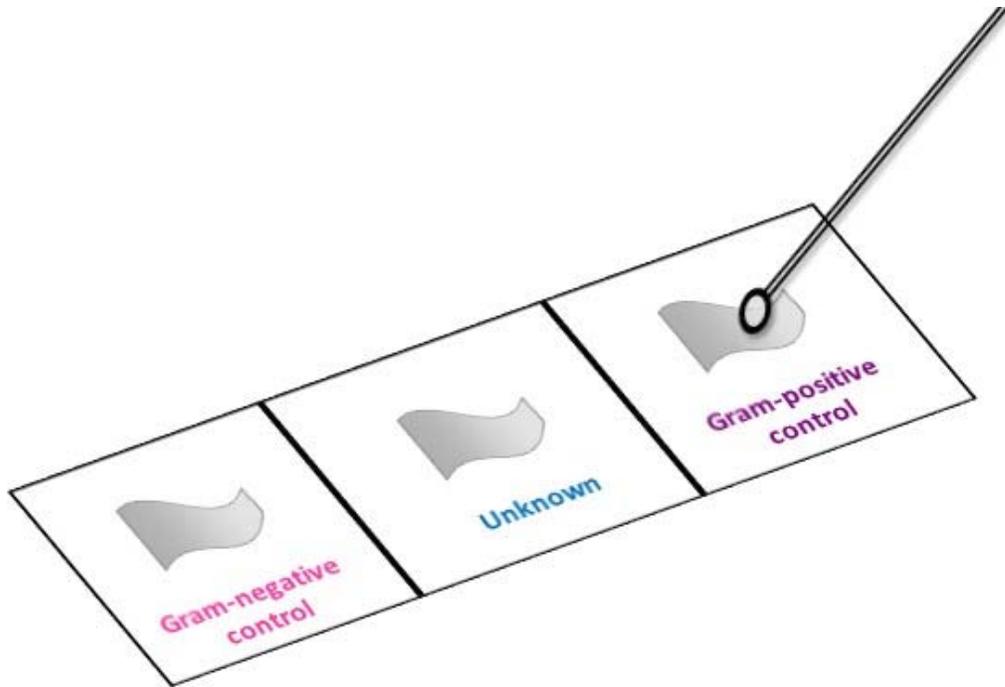


FIGURE 2. Example of a triple Gram Stain on one microscope slide, accomplished by performing three separate smears, simultaneously dried and fixed. This allows for internal Gram positive and Gram negative controls as well as rod and cocci morphology controls.

TABLE 1: Triple Gram Stain Diagnostic

Ability of the triple Gram stain method to aid students in assessing their microscopy skills via easily detectable errors.

Common Error	Detection
Destain too long	G(+) cocci is pink
Insufficient destain	G(-) rod is purple
Slide not fully dried before fixation	Streaks of 'no cells' amongst lots of cells
Insufficient heat fixation	Not many cells visible on slide
Smear too heavy	Cells too clustered to see individual cells

Timeline Summary

1. Physical introduction to the microscope and materials used in staining; ~10 minutes (optional; first-time users)
2. Viewing of prepared slides, TA ensuring proper use of microscope; 10 – 15 minutes
3. Wet mount (pond water or broth culture) and viewing, with sketches; 10 – 15 minutes & discussion
4. Simple stain (same liquid used in step 3) including smear & heat-fixing, using methylene blue, with sketches; 15 minutes & discussion
5. Gram stain with Gram positive (such as *B. subtilis*) and sketches; 15 – 20 minutes & discussion
6. Triple Gram Stain (a positive, a negative & an experimental on the same slide); 20 minutes

*Total time for this stepped learning approach is approximately 1.5 hours, depending on student feedback at the end of each step; accounting for discussions, clean up at each step, viewing and sketching, preparation of materials (described in Appendix 2) at each step and clean up at the end of the lab section. Our practice is to complete stages one to five in one laboratory period, and then assess with stage six in the next period.

CONCLUSION

Proficient microscopy skills are essential for success in various disciplines in the biological sciences and even in select career fields. Thus, it is important that college students be able to learn the basic skills of staining and microscopy with confidence and be able to adequately perform complex stains. By separating the various components of staining into stages, they can be presented in a stepped process that allows students to incorporate the previously learned microscopy skill into the next one being learned. This process eases the introduction of so many techniques at one time and can help raise the success rate of student-performed complex stains, such as the Triple Gram stain, acid-fast and endospore stains. No official assessment of this procedure has been performed, yet in our experience the quality of the final Gram stains is much improved and students report that they feel less overwhelmed by the process.

REFERENCES

1. Emmert, E.A.B. (2013). Biosafety Guidelines for Handling Microorganisms in the Teaching Laboratory: Development and Rationale†. *J. Microbiol. Biol. Educ. JMBE* *14*, 78–83.

SUPPLEMENTAL MATERIALS

Appendix 1: Student handout “Use of a microscope”

Appendix 2: Lab supplies

Appendix 3: Detailed protocols