Appendix 1 – Online Simulations

Free:

NC Community College virtual microscope simulations:
https://www.ncbiotnetwork.org/iet/microscope/
https://www.ncbiotnetwork.org/educational-resources/videos/use-and-care-microscope
https://www.ncbiotnetwork.org/educational-resources/videos/microscope-beginners-qa
https://www.ncbiotnetwork.org/iet/microscope/
System requirements – Hardware requirements: operating system: Windows XP or higher, Mac OS X 10.7 or higher; graphics card: requires DX9 (shader model 2.0) capabilities or higher (example: GeForce 6600 and above)
Note: GPUs made before 2003 (Nvidia), 2002 (AMD) and 2004 (Intel) are not supported; CPU: requires SSE2 instruction set support. SSE2 is supported by almost all processors sold since 2006

FSU:
https://micro.magnet.fsu.edu/primer/virtual/virtual.html
System requirements - students download latest version of java at https://www.java.com/en/ and need an up-to-date web browser

Virtual SEM:
https://myscope-explore.org.au/virtualSEM.html
System requirements - computer/mobile device that is able to open a webpage

Minnesota State University:
https://virtuallabs.nmsu.edu/micro.php
System requirements - computer/mobile device that is able to open a webpage

University of Washington:
https://depts.washington.edu/vurchin/
System requirements - modules are available in HTML, and thus fully mobile-compatible; larger screens are preferred if available

Paid for:

Labster:
https://www.labster.com/
“The Gram Stain: Identify and differentiate bacteria”

System requirements: Processor: Dual-core 2 GHz or higher, RAM: 4 GB or more, Graphics card: Intel HD 3000 / GeForce 6800 GT / Radeon X700 or higher, OS: Latest version of Windows (64-bit) or Mac OS or ChromeOS, Supported browsers: Latest version of Firefox and Chrome, A stable internet connection, same internet capacity as a low-quality YouTube video (500 kbps)

McGraw-Hill:
https://www.mhedu.co.uk/higher-education
“Microscopy: Operation of Bright Field Microscope”
“Microscopy: Oil Immersion Objective”
“Smear Prep”
“Gram stain”
“Acid fast stain”
“Endospore Stain”
“Capsule Stain”
System requirements: https://mhedu.force.com/CXG/s/article/McGraw-Hill-System-Requirements-HigherEd
Appendix 2:

Microscopy Materials Taken Home by Monmouth University Students and Assessment Highlights

Materials taken home:

Students picked up lab materials at the beginning of the semester. Exercises were carried out throughout the semester. Students returned all materials at the end of the semester.

1. Microscope: Amscope B120 binocular microscope
   https://amscope.com/products/b120?variant=40347597766831

2. Microscopy Materials: lens paper, immersion oil, cleaner for objective lenses, glass cleaner for cleaning slides if students did not have this already available, lab tissues, nitrile gloves, glass slides, methylene blue solution https://www.carolina.com/pdf/msds/methbluestaininghs.pdf, bibulous paper, plastic containers for staining, paper towels, lab bench paper, plastic garbage bag, weigh boat, plastic cups, baker’s yeast, probiotic capsule

3. Commercially prepared slides:
   - Acid fast stain - acid fast bacilli
   - *Amoeba proteus*
   - Bacterial capsules
   - Bacterial flagella; polar and amphitrichous
   - *Balantidium coli* trophozoites
   - *Entamoeba histolytica*, trophozoites
   - *Enterobius vermicularis*
   - Gram negative bacillus
   - *Ixodes damini*
   - Mold types – containing *Rhizopus, Aspergillus* and *Penicillium*
   - *Neisseria gonorrhoeae*
   - *Sarcina lutea*
   - *Spirillum volutans*
   - *Staphylococcus aureus*
   - *Streptococcus* spp.
   - *Trichinella spiralis* encysted larvae
   - *Trypanosoma gambiense*

4. Slides prepared at MU:
   - Gram-stained mix of *Staphylococcus epidermidis* and *Enterobacter aerogenes* – rendered inactive using methanol fixation (10)
   - Slides labeled as unknowns containing either *Staphylococcus epidermidis* or *Enterobacter aerogenes* – rendered inactive using methanol fixation (10)

Cell Phone/Microscope Attachment used by instructor: Gosky Microscope Lens Adapter, Microscope Smartphone Camera Adaptor - for Microscope Eyepiece Tube 23.2mm, Built-in WF 16mm Eyepiece
https://www.amazon.com/gp/product/B07412S738/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&psc=1

Biosafety:

Students wore lab coats, safety goggles and nitrile gloves for all laboratory procedures. Students were required to sign lab safety rules sign off sheet stating they would abide by all rules provided. All materials, including all slides made, were returned to the MU. Slides prepared at MU were fixed using methanol (1) approximately 4-6 weeks.
prior to distribution to students. Students did not culture live specimens and were provided with plastic cups and paper towels to absorb methylene blue (so that no dye would be washed into sinks and placed into water systems). Materials and procedures followed ASM biosafety guidelines (2) https://asm.org/Guideline/ASM-Guidelines-for-Biosafety-in-Teaching-Laborator, https://asm.org/ASM/media/Education/ASM-Biosafety-Considerations-with-At-Home-Microbiology-Kits-1.pdf and were cleared via MU’s Laboratory Supervisor and Compliance Officer.

If biosafety concerns are an issue, in-house made slides and/or staining can be omitted.

**Assessment highlights:**

1. All slides viewed required students to take and post pictures with accompanying drawings to allow for assessment of student interpretation. For some slides, annotation was required. Students received credit if their pictures and drawings clearly indicated that they viewed the correct organism or feature. Slide identifications were included among exam questions. A few slides pertaining to the lecture topic were assigned each week. Assessment excerpt:

   Microscopy -10 pts

   As you view each slide, take a picture and make a drawing at the specified objective lens.

   1. *Sarcinae* – 100X – picture and drawing – 5 pts
   2. *Streptococcus* – 100X – picture and drawing – 5 pts

2. An unknown identification of shape and Gram reaction was performed by each student as the Gram stain was covered in lecture. Assessment excerpt:

   The Gram Stain – 10 pts

   a. View Gram mix using 100X oil immersion objective (4x→10x→40x→100X). Do not wipe oil off of the slide once you’ve added the oil. Be sure you can distinguish between Gram-positive cocci and Gram-negative bacilli based on shape and Gram reaction (colors). Take a picture and submit along with a drawing. If you cannot color your drawing, use shading vs. no shading with purple being shaded and pink with no shading.

   b. View your gram unknown (this slide is numbered) using 100X oil immersion objective lens (4x→10x→40x→100X). Do not wipe oil off of your slide once you’ve added the oil. Report your unknown number. Take a picture and submit along with a drawing with color. State the Gram reaction and shape of your unknown. 10 pts

3. A formal virtual individual meeting with each student was conducted once during the semester in which students were required to annotate slides of stained and unstained baker’s yeast, a slide of stained contents of the probiotic pill and a slide of the stained specimen of their choice as the instructor asked questions. Informal meetings were scheduled as necessary. Assessment excerpt:

   Preparing a Wet Mount and Staining Microbes -25 pts

   Part A: Wet Mounts

   1. Perform the Microscopy – Pond Water Wet Mount McGraw-Hill virtual lab
   2. View the materials included on e-campus pertaining to this week’s lab. You’ll need familiarity with this material to identify a specimen that you will collect.
   3. Make a wet mount of pond water, puddle water, water from the Koi pond at MU or another fresh water source. You may also take soil (from a plant, or outside, etc.) and mix it with a very a drop of water.
a. Label a glass slide with your name and the name of the place you’ve taken your sample from. Use a transfer pipette to remove a small amount of liquid from your chosen sample location.

b. Cover with a cover slip (found in a Petri dish). When placing your cover slip over your sample, hold the cover slip at a 45° angle, let it touch the sample and allow the sample to spread along the bottom of the slide, then gently allow the cover slip to fall on top of the sample to cover it.

c. View under the 4X, 10X and possibly the 40X objective lens of your microscope. You will likely need to adjust the lighting of the microscope to view your specimen by closing the iris diaphragm and by moving your condenser to a lower position. Take a picture and make a drawing using the magnification that provides optimal viewing of your specimen (see item d). Report the place you took your specimen from and the magnification.

d. Using the materials on e-campus, identify one organism that you see in your specimen. Point out this organism in your picture and drawing. If you have trouble with this, let me know.

e. Make a wet mount of yeast by placing a small drop of water on a glass slide. Add a few grains of the yeast (contained in a tube – use the tiny transfer device to dispense the yeast -save the transfer device for exercise #4 below) and cover with a cover slip.

f. View under the 4X, 10X and 40X objective lenses. Take a picture and make a drawing using the magnification that provides optimal viewing of your yeast. Report this magnification.

Part B: Staining

4. Label a glass slide with your name and “yeast”. Make wet mount of yeast using as little water as possible. Allow this to air dry overnight in a safe place with no cover slip.

5. Label a glass slide with your name and “probiotic pill”. Make a wet mount of a few grains of the probiotic pill, again using very little water and again dispensing and mixing with the transfer device. Allow to air dry overnight in a safe place with no cover slip.

6. Make a wet mount of the specimen of your choice from the following choices: fermented dairy products such as yogurt and kefir, fermented food such as pickles, sauerkraut, kombucha, kimchee, foods to which probiotics have been added or soil. Check with me if you have other ideas. No human or animal material is permitted and will violate the lab safety agreement in your signed waiver and the lab safety rules. Label your glass slide appropriately. Allow to air dry overnight in a safe place with no cover slip.

7. Spread bench paper on a flat surface. Place your three slides in a weigh boat on top of the bench paper. Cover the area where you have placed your specimen with methylene blue (in dropper bottle). Let it stain for 2 minutes. Take out the plastic cup provided and place the cup inside of a garbage pail lined with the garbage bag provided. Carefully rinse into the plastic cup using a small amount of water. Use paper towels to absorb the stain in the plastic cup. Do not rinse the stain down a sink. You can also use the plastic container provided to keep things neat.

8. Gently blot the slides dry using bibulous paper. You will place each slide, one by one, in between pages of the bibulous paper taking care not to crack the slides or scratch the surface of the slides. Move a few times to dry areas until the slides are completely dry. Observe the yeast in 4X, 10X and then 40X. Take a picture at 40X and make a drawing. Observe the probiotic pill slide and your other slide using the 4X, 10X, 40X and finally 100X objective lens. Take a picture of each using the 100X objective lens.

Grading:

Pond Water Wet Mount MH virtual lab -3 pts.

Wet Mount of Yeast – good quality picture and drawing -5 pts.

Wet Mount of Your Specimen and ID – good quality picture, drawing and proper ID- 5 pts.

Stained yeast – good quality picture and drawing – 5 pts.

Stained bacteria from probiotic pill – good quality picture and drawing – 5 pts.

Stained bacterial specimen – good quality picture and drawing – 7 pts.
1. Minnerath, JM, Roland, JM. Rossi, LC. Weishalla, SR, Wolf, MM. 2009. A Comparison of Heat versus Methanol Fixation for Gram Staining Bacteria, Journal of College Biology Teaching, 35:36-41.

2. Byrd J, Emmert E, Maxwell R, Townsend H. 2019. ASM Guidelines for Biosafety in Teaching Laboratories. Am Soc Microbiol. https://asm.org/Guideline/ASM-Guidelines-for-Biosafety-in-Teaching-Laborator