How to implement laboratory projects in microscopy

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Abstract
Laboratories are a great place for projects. Projects give students the opportunity to design experiments, model hypotheses, analyze data, construct new knowledge, and communicate results—all key activities recommended by the American Association of Physics Teachers (AAPT) in their "Recommendations for the Undergraduate Physics Laboratory Curriculum". Yet, projects are difficult to implement since time, resources, and instructor knowledge may be limited. Here, we describe how to implement laboratory projects in microscopy in introductory courses (electromagnetism or optics) or more advanced courses (optics, advanced laboratory, or biophysics) with standard 3-hour laboratory periods. Specifically, we describe the building of a complex microscope in stages using the engineering strategy of "minimum viable product", which we rename "minimum viable project" for this context. In this strategy, students start by building the minimum viable project—the simplest microscope with a single lens—and work their way up to more complex microscopes. To aid instructors, we describe how to build a single-lens microscope, a compound microscope, a high-magnification microscope, a darkfield microscope, a reflection microscope, a fluorescence microscope, and a total internal reflection fluorescence (TIRF) microscope. (Other possible microscopes are Leewenhoek's, Galileo's, Hooke's, differential interference contrast, phase contrast, or confocal.) The different microscopes give students the thrill of building something new, but the limitation of building a microscope constrains the required parts, time-to-build, and physics know-how.

Introduction
Recently, there have been calls to overhaul traditional undergraduate laboratories to make them more authentic (1-5). In the traditional laboratory or "cookbook" laboratory, students follow a recipe to complete a laboratory assignment and hopefully gain experience using physics concepts. However, there is evidence that these cookbook laboratories are not meeting a stated goal to improve physics understanding (1). At the same time, there are calls to teach experimental skills. In their 2014 recommendations, the American Association of Physics Teachers (AAPT) released new guidelines for laboratories to teach six focus areas: constructing knowledge, modeling, designing experiments, developing technical and practical laboratory skills, analyzing and visualizing data, and communicating physics (2). With the traditional labs not meeting their stated goal and new goals needing to be met, it is time for an overhaul of the undergraduate physics laboratory, but how?

One way to update laboratories to meet these new guidelines is to use course-based undergraduate research experiences (CUREs). In a CURE, students complete research-grade experiments in the classroom laboratory (6,7). This is not a new idea. Physics laboratory courses with research-grade experiments were implemented over 100 years ago in Germany by Fredrich Kohlrausch (5), but the experiments at that time (like today!) were too expensive, not scalable, and too complex for the students. This may be why CUREs are much more common in biology and chemistry. So, the question still is: how can we update the laboratory while maintaining its low cost, scalability, and simplicity?

Another method is to use projects (8,9). In a project, students choose their experiment (often from a list) and must model, design, construct, and communicate something new to the class, but don't

produce an entirely new outcome for science as a whole, as in research-based courses. Still, projects require more money, time, and effort than the traditional "cookbook" laboratory. Projects in optics and electronics, courses often taught in the sophomore year, are attractive because instructors can purchase a finite set of parts that students can then use to build an almost infinite set of projects. In addition, optics and electronics are taught in the introductory course as well, so instructors can offer projects in both the introductory and advanced courses with the same equipment. Here, we describe one such optics project to build a microscope.

An optical microscope is a device with a rich history that uses one or more lenses to magnify objects. Since lenses were invented before recorded history, it is hard to pinpoint the origins of the microscope. Some of the first lenses were found in the eyes of statues in Egypt in 2400 BC (10), and lenses were often used in ancient times in the Middle East and the Mediterranean (most notably Greece) to focus sunlight (11,12). Early lenses also appeared in China and Peru. Liu An (179-122 BC), a philosopher and king of Huai-Nan, used an ice lens to light a fire (13) and a spherical quartz medallion in a Pre-Columbian necklace (0-800 AD) in the Larco Museum in Peru has a magnification of 10X (12,14)! The first lens used as a magnifier may have been the Nimrud lens from the 8th century BC, which was found in the Assyrian palace of Nimrud (modern Iraq) (15). Single magnifying lenses used as eyeglasses were first recorded by Roger Bacon in 1268 (16). Microscopes (or telescopes) with multiple lenses were developed much later. The first telescope patent was in 1608 by Dutch spectacle maker, Hans Lippershey, but some accounts credit Lippershey's neighbors, father-son team of Hans Marten and Zacharias Janssen for the invention in 1590 (17). Robert Hooke used a compound microscope with a magnification of 20-50X in 1665 and recorded elaborate pictures of cork cells in his book *Micrographia* (18). At about the same time, Dutch biologist Antonie van Leeuwenhoek, the father of microbiology, used single-lens magnifiers that were able to magnify objects by 275X so that he was able to see bacteria, blood cells, and spermatozoa (19).

Today, recent advances in microscopy allow us to see even more. Super-resolution microscopes, which were awarded the Nobel Prize in Chemistry in 2014, allow us to observe nanoscale molecules within cells, including actin and microtubule fiber networks (20). Many of these types of microscopes rely on localizing fluorescent particles, rather than resolving structures with optical light, making fluorescence microscopy (21)—first realized in 1911 by Oskar Heimstaedt—incredibly important. One type of fluorescence microscope that removes a lot of background fluorescence is total internal reflection fluorescence (TIRF) microscopy. TIRF microscopes allow us to see the motion and binding of single molecules that are just at the surface of the cell (22). Today, we also have other microscopes, atomic force microscopes or electron microscopes, that don't use light at all. While students could complete undergraduate labs using these types of microscopes (23), here we will describe how to implement projects in optical microscopy.

Projects involving optical microscopes are advantageous because: 1) microscopes are ubiquitous in research labs giving students an authentic experience, 2) there are many different types of microscopes that students can build, 3) only an introductory physics knowledge is required to design, model, and build these microscopes, and 4) students can view interesting objects once the microscope is built. The disadvantage is that building a microscope is tricky! However, this disadvantage can be overcome with the correct implementation.

To implement projects in microscopy, we emphasize that building something complex means building in stages (**Figure 1**). But, the stages are not what students expect! Let's say a group of students decides to build a TIRF microscope for their project. They might look up an optical diagram for a TIRF

system and use stages 1-4 to put all of the parts in the diagram onto their optical breadboard. Then, once they have all of the parts in place, they move to stage 5 where they view a sample with their microscope. The problem with this plan is that the students don't have the skills to understand the optical diagram or how to build the system in the optical diagram. Even if they do build everything correctly, they will still have no idea if what they are seeing in stage 5 is what they are supposed to be seeing! These stages will never work! Instead, we have students plan the stages of their project so that they create a minimum viable project (MVP), which is a play on the engineering term, "minimum viable product" (24). A minimum viable product is a product that has just enough capability—it is minimally viable—to be considered a product. The MVP has just enough capability to be considered the next stage in the project. In the case of a TIRF microscope, the most minimal MVP is a single-lens microscope, so this is stage 1. Students can model this system, take measurements, and view the sample without much help from the instructor. Stage 2 is to build a compound microscope. The second lens adds some complications to the model, design, and measurements, but after completing the single-lens microscope, the students should be able to overcome many of the challenges present in building a compound microscope. Then, each stage gets more complicated: stage 3 is to build a high-magnification microscope, stage 4 is to build a fluorescence microscope, and then finally in stage 5 the students build the TIRF microscope. As students build the next MVP, then they learn as they go, are happy with their accomplishment at each stage, and can redirect the project if they encounter issues.

These MVP stages are also much easier to implement in the classroom laboratory than the stages the students might have picked. In the first laboratory session, all of the students receive the project prompt to design and build a microscope, measure its magnification, and image something interesting. They choose a microscope and all begin their project in stage 1 by creating a single-lens microscope using Pasco kits from the introductory physics laboratory. Then, students add a lens to their system to build a brightfield, compound microscope in stage 2. This gives students the opportunity to get comfortable with the laboratory parts, make simple models and designs, and take some initial magnification measurements. After building this system with the Pasco kits, they complete the task again with the research-grade components. In the second laboratory session, the students tackle stages 3-5 where they work toward the more complex microscope they selected for their project. Here, we will discuss building a "high-magnification" (100X) microscope, a darkfield microscope, a reflection microscope, a fluorescence microscope, and a TIRF microscope. Other microscope choices could include: Leewenhoek's, Galileo's, Hooke's, differential interference contrast, phase contrast, or confocal; though we will not discuss these choices here. One of the best parts of the microscopy project is viewing the beautifully magnified images of milk, tissue paper, insects, bacteria, and minerals that the students take!

Materials

Compound Microscope: Pasco Kit. For stages 1 and 2, students will build a single-lens microscope and a compound microscope. In our introductory physics laboratory, we have students complete this task using the optical kits from Pasco (OS-8515C, $589 per kit). These kits come with an optical bench, lenses (focal lengths of -150 mm, +100 mm, +200 mm, and +250 mm), lens holders, a light source, and a viewing screen. This is good for introductory courses since the optical bench makes alignment easy, but it is not good for advanced courses because the optical bench is too limiting for projects. Still, we use these Pasco kits in the advanced course in stages 1 and 2. When we offer microscopy projects using these Pasco kits in the introductory course, we have students build either a projector (with a single lens and the largest real image they can make), Leewehoek's microscope (with a single lens and the largest

magnification possible), Hooke's microscope (with two convex lenses) (25), or Galileo's microscope (with one convex lens and one concave lens).

Compound Microscope: Research-grade components. To complete stage 2, the students will want to build a compound microscope again, but, this time using research-grade optomechanical parts (Thorlabs or Newport). These optomechanical parts are adaptable to the different microscope configurations that we want to build, are often available in bulk on second-hand sites (eBay), and are standard in research laboratories. One "kit" consists of a small, 12" by 12" optical breadboard (MB12, Thorlabs, $170), light source (OS-8470, Pasco, $120; another choice is the gooseneck lamp from Amscope LED 6W, $120), two plano-convex lenses (focal length of 100 mm and 75 mm, LA1509-ML and LA1608-ML, Thorlabs, $40 each), lens holders (CP35, Thorlabs, $100 for five), posts (TR2-P5, Thorlabs, $30 for five), post holders (PH3-P5, Thorlabs, $50 for five), bases (BA1-P5, Thorlabs, $30 for five), base clamps (CL2-P5, Thorlabs, $40 for five), and ¼-20 screws and washers (SH25S075 and W25S050V for 25 pack, Thorlabs, $10 each). This kit is $640, which is comparable to the Pasco system. Instead of buying separate mounted lenses, another choice is to purchase a lens kit (LSB01, $630) that has various focal lengths and gives students more diversity in their microscope design.

High-magnification Microscope. For the high-magnification microscope, we have students use an objective lens. We use older objectives that are standard sizes of 20X (similar to M-20X, Newport, 0.4 numerical aperture, 160 mm tube length, 9 mm effective focal length, 1.7 mm working distance, $213) or 10X (similar to M-10X, Newport, 0.25 numerical aperture, 160 mm tube length, 16.5 mm effective focal length, 5.5 mm working distance, $159). Adjustable lens holders (LH1, Thorlabs, $50) are useful for holding the objectives. Samples that are interesting to view are bugs, minerals, colloidal liquids, and paper fibers. Sample chambers for liquids can be made out of glass microscope slides and coverslips ($10 for both) that are held together with double-sided tape (26). Bugs and minerals can be epoxied on to a microscope slide. We mount samples vertically in an adjustable mount (VG100, Thorlabs, $100) or in a slide holder (MAX3SLH, Thorlabs, $145).

Darkfield or Reflection Microscope. Darkfield or reflection microscopes do not require any additional parts. To switch to either, we just change the orientation of the light source.

Fluorescence Microscope. For the fluorescence microscope, we need a fluorescent sample, a laser to excite the sample, and a color filter so that we collect only the emitted light. One choice for a sample is to draw on an index card with a yellow highlighter (Hi-liter, 24063 Avery, $2) and excite with a 405 nm laser pointer or a 395 nm blacklight flashlight ($10 on Amazon). The flashlight or laser pointer can be mounted in an adjustable lens holder. Often, laser pointers will need to be clamped into the "on" position, so buying one with an on/off button is helpful. Another choice is to use fluorescent, micron-sized beads (FluoSpheres F8819, Invitrogen, diameter = 1.1 μm, Nile Red fluorophore) and excite with a laser pointer or laser diode (532 nm, OS-8458B, Pasco, $270). Color filter sets (24 different filters, Amazon, $40) for cameras can be used to isolate the fluorescent emission. Other fluorescent samples that might be fun to image are honey, milk, olive oil, tonic water, and egg shells (27).

TIRF Microscope. A TIRF microscope can be assembled from the parts for a high-magnification microscope and a fluorescence microscope. In addition, the TIRF microscope requires a prism to create the total internal reflection. The typical prism used in TIRF is a pellin-broca prism (ADB-10, Thorlabs, $80) (28,29), but other possibilities are a triangular prism (PS10, Thorlabs, $80) or a dove prism (PS992, Thorlabs, $110). We used a triangular prism that we mounted to a base plate (on a post) with double-sided tape. For longer term use, the prism could be held by a kinematic platform (KM200B, Thorlabs, $110) and clamp (PM4, Thorlabs, $25).

Single-Lens Microscope

To start the project, students design a single-lens microscope (**Figure 2**). A single-lens microscope is often called a magnifier and has been around since ancient times (15). To design the single-lens microscope, students can choose the focal length of the lens, *f*, and the distance of the object to the lens, *o*. Once they decide on these distances, they will need to calculate the image distance, *i*. The image distance can be found by rearranging the imaging equation,

$$\frac{1}{o}+\frac{1}{i}=\frac{1}{f}\,. \tag{1}$$

If the object distance is greater than the focal length, then the image distance will be positive, producing a real image that must be viewed with a screen. If the object distance is less than the focal length, then the image distance will be negative, producing a virtual image that can be seen by looking through the lens. The magnification, *M*, for the single-lens microscope is given by the magnification equation,

$$M=\frac{h_i}{h_o}=\frac{-i}{o}\ , \tag{2}$$

where h_i is the height of the image and h_o is the height of the object. (The relationship between the heights of the image and object and the distances to the image and object can be related using geometric relations.) The absolute value of *M* gives the amount of magnification. If the absolute value of M is greater than one, then the image is enlarged; if it is less than one, then it is reduced. The sign of *M* gives information about the orientation of the image. A negative value means that the image is inverted with respect to the object, while a positive value means that the image and object have the same orientation. To see how *M* depends on the two design parameters—*o* and *f*—we can plug **Eq. 1** into **Eq. 2** to get,

$$M=\frac{-1}{\left(\frac{o}{f}-1\right)}\,. \tag{3}$$

Students can calculate this magnification to see if they have a microscope that produces an enlarged image. We see that the image will be enlarged if the object distance is within two focal lengths of the lens. The greatest magnification will be when the object distance is close to the focal length. When the object is at the focal length, no image will be produced. In **Figure 2**, we show two designs for a single-lens microscope, one design with a virtual image, similar to magnifiers used in ancient times (12), and one design with a real image.

After designing the system, students should build the single-lens microscope and measure its magnification. To make sure measurements go smoothly: students will need to use a different procedure for measuring the magnification of a real image vs. a virtual image (30). For a real image, students can measure the image height with a ruler and can compare to the object height, also measured with a ruler. Or, students can set a camera at a particular distance from the object and take a picture, and then set the same camera with the same orientation and same particular distance from the real image and take a picture. Students can open both pictures on a computer and use an application (Paint, Photoshop, or ImageJ) to measure the height of the object and real image using the pixels in the picture. In **Figure 2**, we measure the distance between millimeter lines in the picture of the object (17.8 ± 0.8 px) and in the picture of the real image (-37 ± 1 px), finding a magnification of -2.1 ± 0.1X, which agrees with our nominal value of 2X. For the virtual image, students must use a camera to take a picture of the virtual image (30). Students should place the camera right at the lens when they take the picture, as this will produce a picture of the virtual image at a distance equal to *i*. Then, students can take a picture of the object with the camera a distance of *i* from the object. This will make sure that the camera

is the same distance away from both the virtual image and the object. In **Figure 2**, we compare the height of the object (34 ± 2 px for the distance between the millimeter lines) and the height of the image (64 ± 2 px) to get a magnification measurement (1.9 ± 0.2X) that agrees with the nominal value.

If students are enjoying single-lens microscopy, we often encourage them to choose the project where they build Leewenhoek's microscope (a single-lens microscope) and try to get the highest magnification they can. Can they get a higher magnification (275X) than Leewenhoek? Can they view microorganisms?

Compound Microscope

A compound microscope, first used by Lippershey, Janssen, Galileo, and Hooke (17), is a microscope composed of multiple lenses. Students can design a brightfield, compound microscope by following the optical diagram in introductory physics textbooks (31). In this optical diagram, the object is placed more than one focal length away from the first lens, creating an enlarged, real image. This real image is then placed within the focal length of the second lens to create an enlarged, virtual image that can be viewed through the lens. In this design, there are four parameters that students choose: the focal length of the first lens f_1, the object distance for the first lens, o_1, the focal length of the second lens f_2, and the object distance for the second lens, o_2. The total magnification of the system, M_T, is the magnification of the first lens, M_1, times the magnification of the second lens, M_2, or,

$$M_T = M_1 M_2 \, . \tag{3}$$

The magnification for each individual lens can be calculated by solving for the image distance for the lens using **Eq. 1** and plugging that value into **Eq. 2**.

While there are many microscope designs that students can create, we give two examples in **Figure 3**: Design 1 uses the Pasco optical kit (focal length of the first lens f_1 = 100 mm and focal length of the second lens f_2 = 200 mm) and Design 2 uses research-grade parts (f_1 = 75 mm and f_2 = 100 mm).

After designing the system, students build the microscope and measure the magnification. Here instructors should emphasize that students should place the first lens and measure its magnification before placing the second lens and measuring its magnification. Then multiplying the two magnifications together will produce the total magnification. Specifically, let's walk through the building and measurements for one particular design, Design 1. In Design 1, we place the object on the track at 50 mm and the first lens (f_1 = 100 mm) at 200 mm, creating an object distance o_1 of 150 mm. This creates a real image at i_1 = 300 mm (which is at 500 mm on the track). Next, we measure the magnification by using a ruler to measure the height of the object (the center of the arrow to the point), obtaining 20 ± 1 mm, and using the ruler to measure the same height on the real image, obtaining -41 ± 1 mm. The magnification of the first lens, M_1, is measured to be -2.1 ± 0.1X, matching the nominal value of -2X. Then, we place the second lens (f_2 = 200 mm) on the track at 600 mm, creating an object distance o_2 of 100 mm and a virtual image at i_2 of -200 mm. We take a picture of the second image (the virtual image) with the camera right at the exit of the lens, and we take a picture of the first image (the real image) with the camera at the i_2 distance of 200 mm. We open the pictures on a computer and use an application (Paint, Photoshop, or ImageJ) to measure heights in pixels of the millimeter spacing lines on the object (first image = 37 +/- 1 pixels and second image = 70 ± 5 pixels). Thus, the magnification of the second lens, M_2, is measured as 1.9 ± 0.1X (nominal magnification of 2X), and the total magnification of the microscope, M_T, is -4.0 ± 0.3X, as expected.

If students want to compare the final virtual image to the original object, then they need to take another picture of the object with the camera placed a distance of i_2 from the object. In Design 1, this

would mean taking a picture of the object with the camera 200 mm from the object. If we choose this experimental path, then we measure the size of the millimeter spacing lines on the object to be 17.8 ± 0.8 pixels, giving a total magnification of -3.9 ± 0.9X, which also matches our nominal magnification.

Finally, after students build a compound microscope with the Pasco parts, they design and build a compound microscope with the research-grade parts. This gives students two chances to design and take measurements, increasing their confidence. This also facilitates the introduction of the research-grade optomechanical parts. If students are interested in the history of microscopy, they could choose a project where they build a third compound microscope: Galileo's microscope (with one concave lens and one convex lens) or Hooke's microscope (25).

Higher-Magnification Microscope

One of the choices for a project is to build a higher-magnification microscope using an objective lens (**Figure 4**). Specifically, we want students to build a microscope with a 10X or 20X objective. To design a microscope with this objective, the students need to read the specification sheet for their objective to see if it has a "finite tube length" (the standard pre-1980's) or if it is "infinity-corrected" (the standard post-1980's). If the objective is infinity-corrected, it will create an image at infinity, requiring the microscope design to have a third lens—the tube lens (32). To simplify the design of the system, we will use an objective with a finite tube length.

But, how do we design a compound microscope with a finite-tube-length objective? We won't be able to find the image distances for the objective using the imaging equation for a single lens (**Eq. 1**) since objectives are composed of multiple lenses. Instead, we will think of the objective as a single lens with an effective focal length. If we place the object at the "working distance" of the objective, it is like we have placed the object at the effective focal length. Since this is a finite-tube-length objective, objects that are at the effective focal length will have a real image at the tube length. Thus, we design our high magnification microscope (**Figure 4A**) by replacing the first lens in the compound microscope with the objective. Then, we place the object at the working distance of the objective, which forms a real image at the tube length. The second lens is placed as before, a distance of o_2 from the real image.

To calculate the magnification of our high-magnification microscope, we need to multiply the magnification for the objective by the magnification of the second lens. The magnification for the objective, M_{obj}, is given by the tube length L and the effective focal length f_{obj}, using the equation,

$$M_{obj} = \frac{-L}{f_{obj}}. \quad (4)$$

Notice that **Eq. 4** is just the regular magnification equation (**Eq. 2**) at the specific object distance of one focal length and image distance of one tube length. For our 20X and 10X objectives, the magnification using **Eq. 4** is 17.7X (160 mm/9 mm) and 9.7X (160 mm/16.5 mm), respectively. If we replace the first lens in Design 2 with the objective, the total magnification for the new system is 88.5X.

Using this high-magnification microscope, we can image some interesting objects (**Figure 4B**), including the fibers in paper (20-30 µm in diameter), particles in milk (5 µm in diameter), ant hairs (10 µm in diameter), and hairs on a bee wing (10 µm in diameter)!

Other Microscopes

By changing the illumination system, students can turn the high-magnification microscope (**Figure 4**) into several different microscopes (**Figure 5**), including darkfield, reflection, fluorescence, and TIRF. To see the differences between the different microscopes, we image a sample of 1-µm-diameter

fluorescent beads (green excitation, red emission) at a concentration of either 1% or 0.01% on all of the microscopes.

Darkfield. To create the darkfield microscope, we move the light source off-center so that the illumination light does not pass straight into the lens. With the light source off-center, the background or field looks dark (instead of bright) and any features that scatter light look bright (instead of dark). The advantage of a darkfield microscope is the higher level of contrast. Human eyes have a logarithmic response, so it is much easier to see dim objects as bright features against a dark field.

Reflection. Students could also choose a project where they create a reflection microscope (29). In a reflection microscope, the light source is moved so that it is on the same side as the objective.

Fluorescence. In the fluorescence microscope, we excite a fluorescent sample with one wavelength of light and collect the lower energy wavelength that is emitted. This is useful when we would like to image molecules tagged with fluorophores. To create the fluorescent microscope, we replace the light source in the darkfield microscope with a green laser. Using the darkfield setup is advantageous for two reasons: 1) students won't look directly into the laser when they look into the microscope, and 2) students won't have to filter out as much green excitation light. We add a red color filter after the sample to block the green excitation light and pass the red emission light. With the fluorescence microscope, we see only the portions of the sample that are fluorescent, which in this case is the fluorescent beads (and the autofluorescence from the scotch tape). To improve this microscope, students could build a microscope with: 1) a higher magnification (400X instead of 100X) so that is easier to localize the beads, and 2) a better excitation/emission collection so that the beads are not as dim. With this improved microscope, students could measure Brownian motion of the beads (33).

TIRF. A TIRF microscope is a microscope that uses total internal reflection to illuminate the sample. In total internal reflection there is no refracted beam, but there is an evanescent wave that penetrates the sample a few hundred nanometers. This means that fluorophores within this depth will be illuminated and will emit light, but fluorophores outside of this depth will not emit light, getting rid of a lot of background emission. To create the TIRF microscope, we use the fluorescence microscope and mount a prism so that it touches the glass slide of the sample. The purpose of the prism is to angle the incoming laser beam so that the incident angle at the glass-water interface of the sample is greater than the critical angle. (Bouncing the laser off the glass sample without the prism won't work due to the refraction angle at the air-glass interface.) When total internal reflection occurs at the glass-water interface, an evanescent wave occurs within the water, illuminating the portion of the sample that is close to the glass surface. To see the TIRF microscope in action, we place a sample with a large amount of fluorescent beads (concentration 1%) on the microscope and view the sample with and without the prism. The large number of beads is useful to get enough fluorescence so that we can see emission from the TIRF illumination. With the prism in place, we see a definite change in the emission of the sample. Specifically, we see much less background emission, and the emission we see is coming from one focal plane located at the surface!

Conclusion

One method to overhaul undergraduate physics laboratories to make them meet the AAPT laboratory guidelines (2) is to use multi-week projects. Projects give students the chance to model a system, create designs, analyze data, and communicate findings to the larger class (8), without requiring research-grade experiments (6,7). But, projects can be problematic to administer since resources, time, and instructor knowledge may be limited. Here, we discuss how to implement projects in microscopy using three

laboratory sessions of 3 hours each and a strategy of building a series of MVPs. Building the simplest system in the first stage and then increasing in difficulty in each subsequent stage results in increased skill level, confidence, and knowledge. By the end of the third laboratory session, students will have built their microscope and have viewed an interesting sample. Perfect!

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Figures

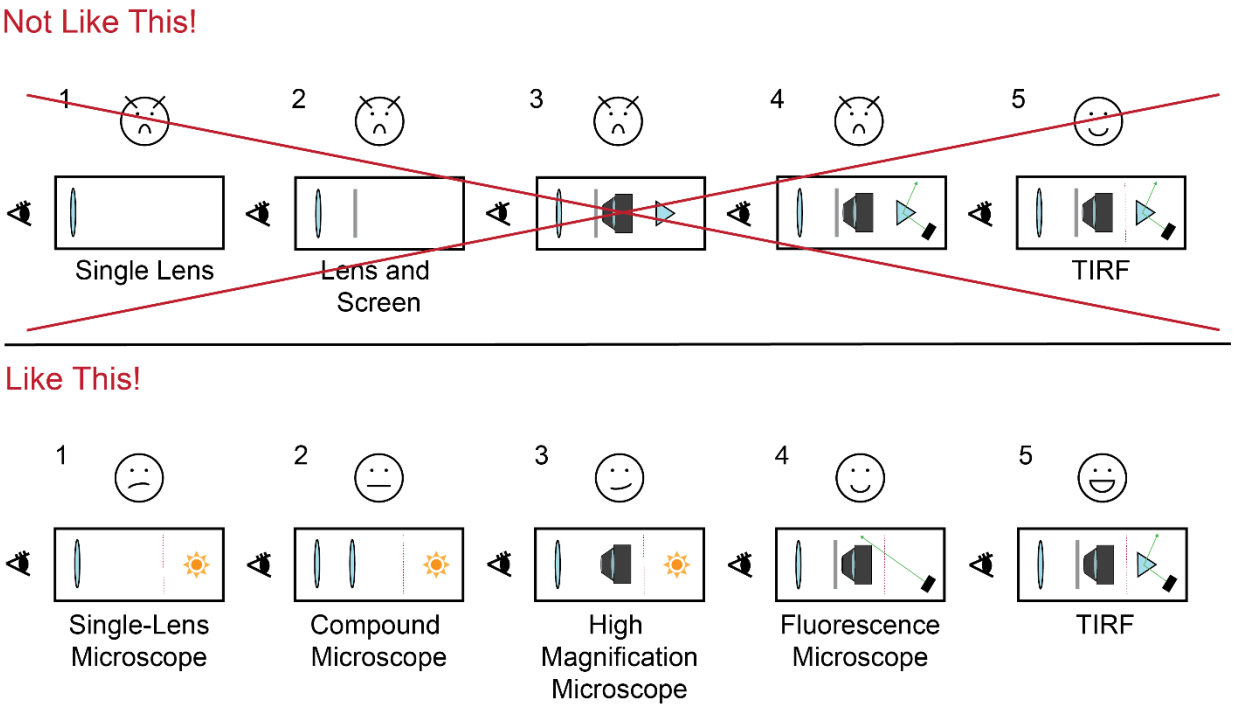


Figure 1. Strategy for building a TIRF microscope. Students should not build a TIRF microscope by looking up the optical diagram for the microscope and laying out all of the parts on an optical breadboard. Instead, students should build a TIRF microscope by building a series of very simple systems, minimum viable projects, and work up to the TIRF microscope.

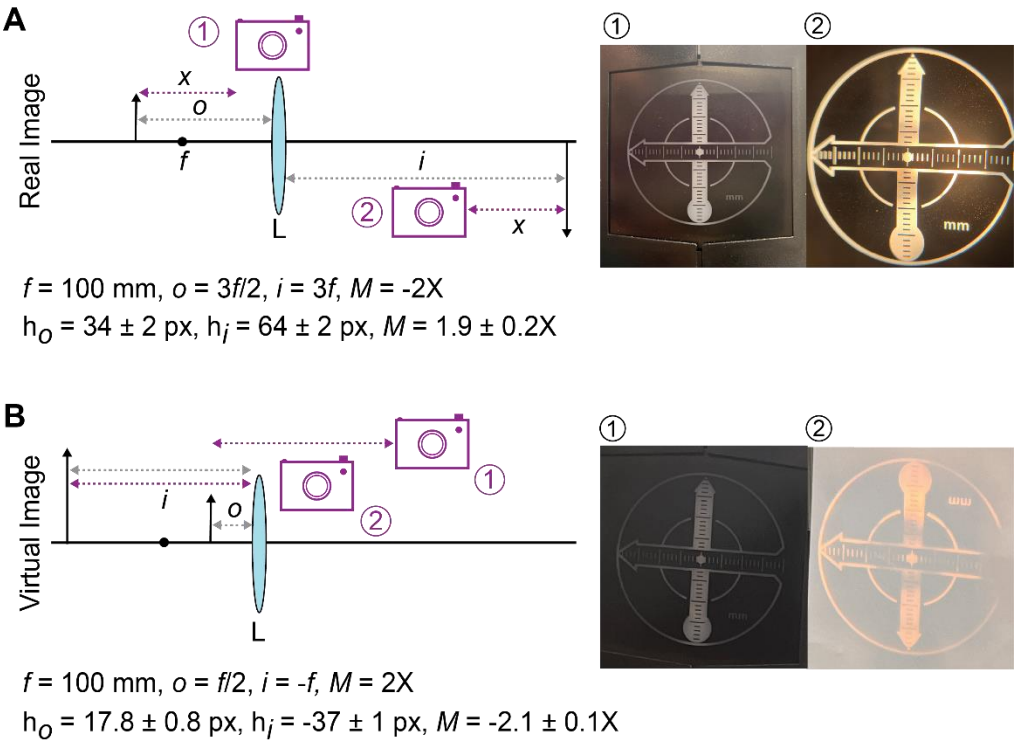
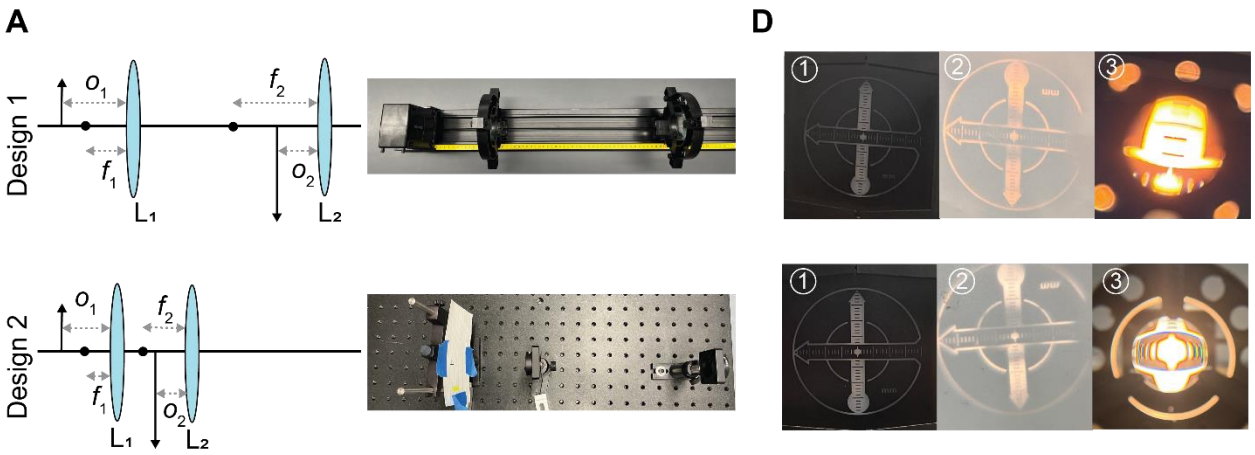


Figure 2. Stage 1: build a single-lens microscope. There are two choices for a single-lens microscope: A) one that produces a real image and B) one that produces a virtual image. If the object distance, *o*, is beyond the focal length, *f*, of lens L, then the image distance, *i*, is positive and the image is real. If the object distance is less than the focal length, the image is virtual. To measure the magnification of a real image, students should take a photograph of the object and real image at the same distance, *x* (*purple*). For the virtual image, the camera should be placed at the lens to take a picture of the virtual image and a distance *i* (*purple*) away from the object. Then, the height of the object h_o and height of the image, h_i, in the photographs can be compared to calculate the magnification, *M*.



B

Variable	Calculation	Design 1	Design 2
f_1	selected	100 mm	75 mm
f_2	selected	200 mm	100 mm
o_1	selected	150 mm	120 mm
o_2	selected	100 mm	80 mm
i_1	$(1/f_1 - 1/o_1)^{-1}$	300 mm	200 mm
i_2	$(1/f_2 - 1/o_1)^{-1}$	-200 mm	-400 mm
M_1	$-(i_1/o_1)$	-2X	-1.67X
M_2	$-(i_2/o_2)$	2X	5X
M_T	M_1M_2	-4X	-8.35X

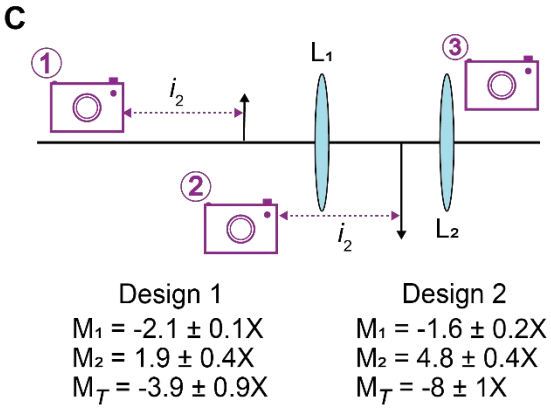
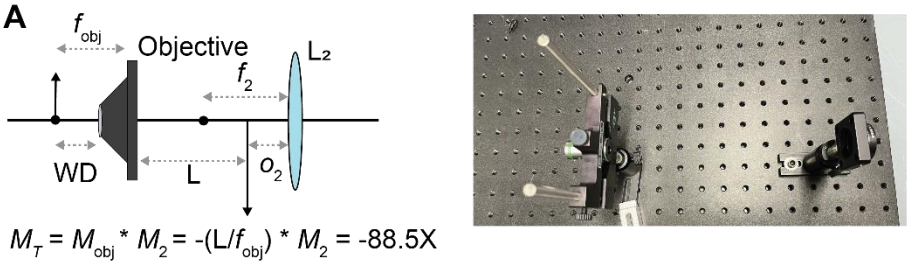


Figure 3. Stage 2: build a compound microscope. A) We present two designs for a compound microscope. Design 1 uses the parts from the Pasco kit. Design 2 uses research-grade components. In both designs, the object distance, o_1, is beyond the focal length, f_1, of the first lens L_1 so that a real image forms at i_1. Then, a second lens L_2 is placed o_2 from the real image, within the focal length of the lens, f_2. This creates a virtual image at i_2. B) Table shows nominal values for all of the variables for each design. M_1, M_2, and M_T are the magnification of the first lens, second lens, and total magnification, respectively. C) To measure the magnification of the system, the camera should be placed at the lens for the virtual image, and i_2 away from the real image and object. D) Images of the object (*1*), real image (*2*), and virtual image (*3*) for both designs allow for measurements of the total magnification of the microscope.



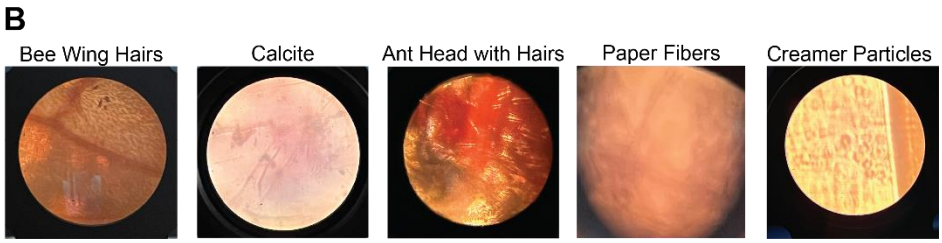


Figure 4. Stage 3: build a high magnification microscope. A) Optical diagram shows the placement of the object as one working distance, *WD*, from the front aperture of the objective. This places the object at the effective focal length, f_{obj}, of the objective. A real image forms at the tube length, *L*. Placement of the second lens, L_2, is as before with the distance to the real image, o_2, being less than the focal length. Total magnification of the microscope, M_T, is the magnification of the objective, M_{obj} times the magnification of the second lens, M_2. B) Images of various samples with the high magnification microscope.

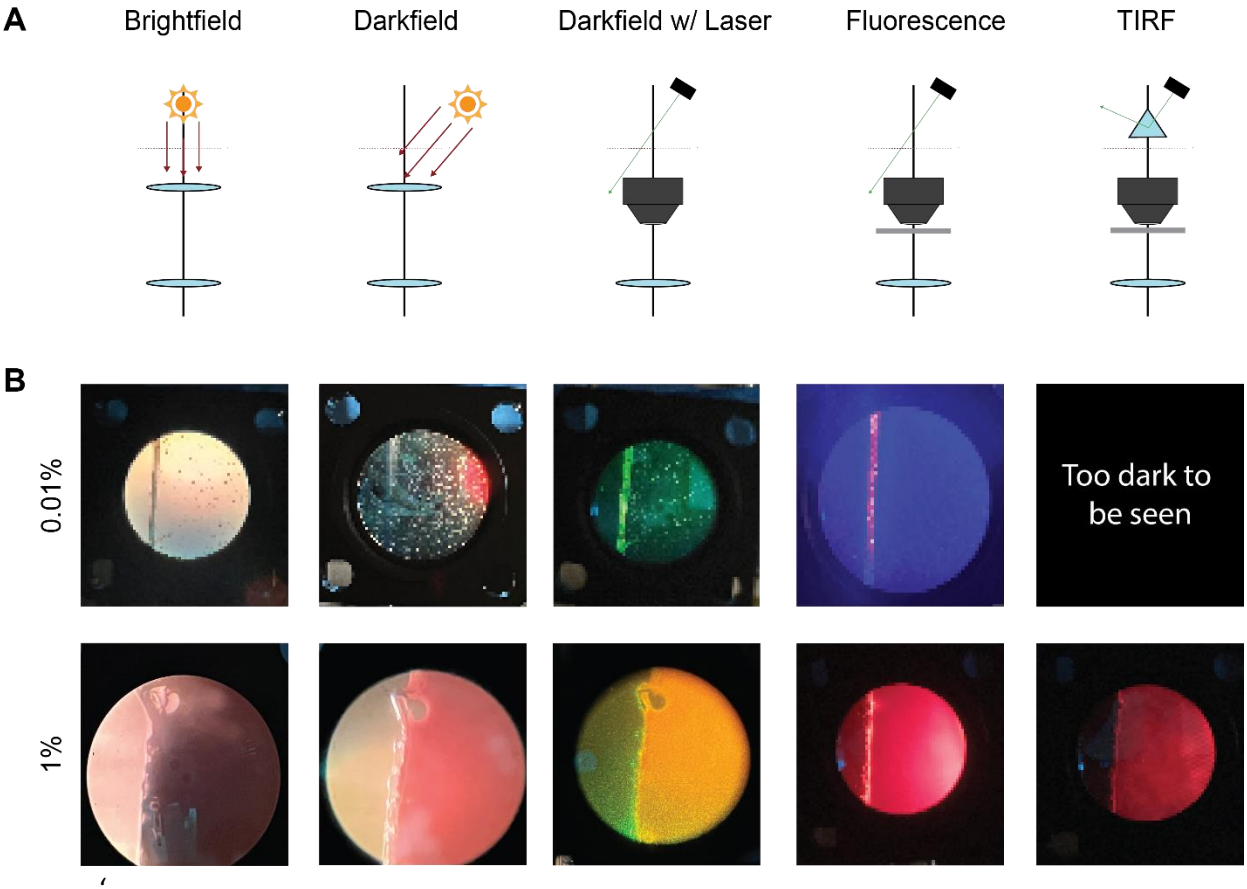


Figure 5. Stages 4-5: build more complex microscopes. A) Optical diagrams for several different microscopes. B) Images of fluorescent beads at a concentration of 1% and 0.01% for each of the different microscopes in A. The vertical line across the field of view is the tape at the edge of the sample chamber. Fluorescent beads are to the right of the tape.

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