Supplemental Materials
for
Increasing Student Understanding of Microscope Optics by Building and Testing the Limits of Simple, Hand-Made Model Microscopes

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Building The Microscope

Materials
1 glass Pasteur pipette or capillary tube, or whatever glass source is handy
5 x 10 cm piece of 1mm poster board (thick side)
5 x 10 cm piece of cardstock (thin side)
1 small dab (about 1 ml) of tacky putty or chewing gum
Wood block (e.g. small piece of 2 x 4 or particle board)
Razor blade or X-acto knife
Goggles

Tools
Drill with 1/16 bit
Stapler
Flame (portable plumbing torch or Bunsen burner)
Caliper

Instructions

1. Cut out microscope plates. Cut out two roughly equal sized pieces of poster board and cardstock. They can be any size and shape, but if you want to pay homage to van Leeuwenhoek’s microscopes they should be about 6 X 3 cm, with a slight taper at one end (approximately the dimensions of the adjacent outline).
2. **Drill light path.** Sandwich the poster board and cardstock together and place on the wood block. Drill a 1 mm diameter hole (1/16 inch) approximately 1.5 cm from each side and the top, and about 4.5 cm from the bottom. Carefully shave off any protruding paper around the holes with a sharp razor blade as lose paper fibers can be magnified along with your specimen.

3. **Creating the lens.** The lens will be a sphere of glass whose diameter dictates the magnification. You will be making two lenses, one "large" and one "small". Aim for a lens of about 2mm in diameter since this is big enough to work with and gives a decent magnification. There are two main steps to this (wear eye protection when melting glass):

   **Stretch some glass**
   1. Put on safety goggles and light the torch or Bunsen burner.
   2. Holding the glass rod or pipette at both ends, place the center in the flame and hold it there until the glass melts. It helps to roll it in the flame to equally expose all sides of the heated area (*Figure 1*).
   3. When the glass is soft, remove it from the flame and immediately pull the two ends apart to stretch the glass very thinly. You are aiming for a glass tube of >0.5mm. Too thick and your lens tends to be teardrop shaped rather than spherical, too thin and you have to feed a lot of glass into the flame during the actual formation of the lens (*Figure 2*).
Figure 1. To stretch the glass, first place it in the flame, rolling it to heat it evenly until it is quite soft and wobbly.

Figure 2. Once the glass is thoroughly soft, remove it from the flame and immediately pull apart the two ends so the softened part is stretched to a uniformly thin filament or tube.
Form the lens

1. Keep your safety goggles on.
2. Once the stretched glass has cooled sufficiently to handle, break it somewhere in the middle of the stretched portion.
3. Position the flame horizontally, and slowly feed the stretched glass into the flame from above (Figure 3).
4. A small, white hot glass sphere will grow at the tip of the tube as you feed it into the flame. It is critical to keep this sphere in the flame and not to let it cool before it is done. If you move the lens in and out of the flame, bubbles will form in the glass ruining the lens.
5. Keep feeding the tube into the flame until the sphere is about 2 mm in size. A nice trick to help keep this motion constant and the sphere in the flame is to feed the stretched glass through the loop of a pair of scissors so that about 5 cm of glass extends from the loop. Then hold the scissors by the blade and twist slightly so there is mild tension on the glass. This will hold the end of the glass steady as you feed it into the flame.
6. Once the sphere is the size you want, remove it from the flame, let it cool completely, and break the tube off about 0.5 cm from the sphere. This gives you a short handle, like a lollipop, so you can avoid touching the lens during later construction. This also ensures the light path will be perpendicular to the 'wound' inevitably caused by breaking the lens from the tube.
7. Make a couple of lenses and choose two of different sizes for constructing your microscopes.
Figure 3. Slowly feed the thin glass filament into the flame. The tube will melt and a small ball will form at the end of the filament (upper panel). Once you are happy with the size of the ball remove it (bottom left). These are easy to make, so make several and chose the best sphere with no visible imperfections (like bubbles). Snap off the sphere leaving a short length of tube attached to it.

Troubleshooting Forming the Lens
1. Your sphere should be a sphere, and not a teardrop shape. If you get a teardrop your glass was likely not stretched sufficiently, so go back to step one and stretch a new one to be thinner.

Measuring the Diameter of the Lens
1. Before assembling the microscope, you will need to measure the diameter of your lens.
2. Record your lens diameters and write the lens diameter on one of the microscope plates for future reference.

**Figure 4** Measure the diameter of the lens using a caliper.

5. Assembling the microscope

1. Holding your sphere by its handle, place the sphere over the hole you drilled in the poster board with the “handle” laying flat on the inside surface of the poster board (**Figure 5**).
2. Set the cardstock on top so the lens and handle are sandwiched between the two layers. Hold the two layers firmly together with the holes and lens lined up, and staple it twice about 0.5 cm from either side of the hole (**Figure 6**) with the cardstock side up.

**Figure 5.** Place the lens in the hole on the inside of the poster board, laying the remaining tube flat on the surface.
Figure 6. Staple the two pieces of paper together with the lens between them, and your microscope is assembled.

Troubleshooting Assembling the Microscope

1. These lenses have very short working distances (the distance between the object you are viewing and the lens), so your sample needs to be very close to the lens. With the simple magnifying lenses you are making, when viewing a sample the object is placed between the lens and the focal point.
2. Due to the short working distance the microscope needs the thinner cardstock on one side. Your microscope only works in one direction: you look through the poster board side and place your sample on the cardstock side.

6. Making Your Focus Mechanism

The biggest challenge to making a microscope from paper is how to focus your specimen. The solution is to take advantage of the simultaneously elastic and sticky properties of tacky putty (e.g., Elmer’s Tack Adhesive Putty: the kind used to stick posters on a wall) to both mount the specimen and pivot it in relation to the lens. Recently used chewing gum works as well if you can’t find putty. To illustrate how this works, we will use mounting the barb of a feather as an example, but you can also mount a variety of other kinds of samples.

1. To start, put your feather barb on a flat surface.
2. Take a dab of putty about the size of a pea and press it over one edge of the feather barb (Figure 7). Pick the putty off the surface, and the barb will stick to the putty and project from it.
3. Stick the putty to the cardstock side of the microscope just below the hole so the barb is right over the hole (Figure 8). Be careful not to get putty on your lens. This will be your stage, focusing device, and your movement controls.
4. To view the specimen, hold the microscope as close to your eye as is comfortable, looking from the poster-board side directly into a light source a light bulb, or a bright sky. Never look directly at the sun with any optical device.
5. To focus, place your thumb on the putty. Pushing the putty up towards the top of the microscope will cause the sample to pivot closer to the lens, pulling down
will cause it to pivot away from the lens. Movement from side to side will position the sample over the lens as desired (Figure 9).

6. Use the same approach to mount and view other kinds of samples.
   a. To mount any dry, solid specimen place it on a surface and press the putty over it and attach it to the microscope as described (I like feather barbs, insect wings, or onion skin to see the microscope in action easily).
   b. To mount a wet specimen, place a glass coverslip on a surface and mount it as described. Then drop your sample onto the coverslip and hold the microscope horizontally with the coverslip side up (so your sample does not drip) and look from the bottom (Figure 10).
   c. Wet samples can also be mounted between two coverslips. For this I suggest diatoms since they are big and regularly shaped. Forams and radiolaria are also good, but not as easy to find in nature.

Figure 7 Take a pea size piece of tacky putty and press it on the subject (a feather barb is shown). The subject should stick to the putty when you remove it from the surface.
Figure 8 Press the putty with subject attached to the cardstock (thin) side of the microscope with the subject laying over the lens.

Figure 9 To focus, place your thumb on the putty. Pushing up will pivot the sample towards the lens, while pulling down will pull the sample away from the lens.
Figure 10 To view a liquid sample, follow the procedure of Figures 1-8 to 1-10 using a glass cover slip, and once attached to the microscope, drop the liquid sample on to the glass.

Some examples of images:
These were taken with a Canon G9 point and shoot camera and do not do the image quality justice because to get the field of view to a reasonable size you must zoom in fully and Canon cameras do not function in macro mode while zoomed in (so the depth of field is bad).

Figure 11 Clockwise from upper left: the hairs on the trailing edge of an insect wing, cells in an onion skin, and the spikes on the surface of a radiolarian skeleton and a vein of an insect wing.
Figure 12 A example of an image with a lower magnification lens of live cells in liquid on a cover glass. This is the hypermastigote parabasalian *Trichonympha* from the hindgut of the termite *Zootermopsis angusticollis*.

Figure 13 Comparison of the same Radiolarian skeleton with three different microscopes. At left is a commercial McArthur-Cooke field microscope set at 400X. In the center is the same skeleton magnified using a home-made lens photographed with the same camera and at the same scale (showing the power of this lens to be about 200X). On the right is the same sample again but photographed using the Canon G9 and a different microscope with a much higher power lens.
Microscope Optics:  
Building a Model van Leeuwenhoek Microscope

Introduction:
Antony van Leeuwenhoek, known today as the “father of microbiology”, is well known for his work on microscope design and the quality of microscope lens he was able to produce. His lenses enabled the viewer to magnify a sample up to three hundred times. Because of his technological advances, van Leeuwenhoek discovered many single-celled organisms such as bacteria and protozoa, which were unknown to science at the time. What he original called “animalcules” we now call microorganisms.

In laboratory today, you have the opportunity to construct your own model van Leeuwenhoek microscope. You’ll use this microscope to make observations, just like van Leeuwenhoek, about microscopic cells and organisms. However, unlike van Leeuwenhoek, we have the technology and understanding to calculate the magnification AND resolving power of our microscope lenses.

Objectives:
After successful completion of this exercise, students will be able to:
1. Describe how a simple lens allows for sample magnification by bending light.
2. Explain the effect of lens diameter and focal length on the magnification power of a simple lens.
3. Demonstrate and explain the effect of working distance on the resolution of a simple lens.
4. Define and calculate the numerical aperture of a homemade lens.
5. Predict the effect of using different wavelengths of light on the resolution of a lens.

Building the microscope:
1. Cut out microscope plates. Cut out two roughly equal sized pieces of posterboard and cardstock. To pay homage to Leeuwenhoek’s microscopes they should be about 6 X 3 cm, with a slight taper at one end.
2. Drill light path. Drill an approximately 1 mm (1/16 inch) hole in the posterboard and cardstock. Drill both at once and to drill into a wood or block additional posterboard to give a clean hole. The hole should be 1.5 cm from each side and the top, and about 4.5 cm from the bottom. Carefully shave off any protruding paper around the holes with a sharp razor blade as lose paper fibers can be magnified along with your specimen. See figure 1.
3. Create the lens. The lens will be a sphere of glass whose diameter dictates the magnification. Aim for a lens of about 2mm in diameter since this is big enough to work with and gives a decent magnification. There are two main steps to this (wear eye protection when melting glass):
   - Step 1. Holding the pipette at both ends, place the centre in the flame of a candle and hold it there until the glass melts and wobbles freely between your hands. It helps to roll it in the flame to equally expose all sides of the heated area. When the glass is soft, remove it from the flame and immediately pull the two ends apart to stretch the glass very thinly. How far you pull depends on the thickness of your glass source. You are aiming for a glass tube of >0.5mm. Too thick and your lens tends to be teardrop shaped rather than spherical, too thin and you have to feed a tedious length of glass into the flame or the lens can break off during step 2.
   - Step 2. Once the stretched glass has cooled sufficiently to handle, break it somewhere in the middle of the stretched portion. Position the flame horizontally, and slowly feed the stretched glass into the flame from above. Watch this step carefully. A small, white-hot glass sphere will grow at the tip of the tube as you feed it into the flame. It is critical to keep this sphere in the flame and not to let it cool before it is done (or when you reintroduce it bubbles will form in it). Keep feeding the tube into the flame until the sphere is about 2 mm in size. A nice trick to help keep this motion constant and the sphere in the flame is to feed the stretched glass though the loop of a pair of scissors so that about 5
cm of glass extends from the loop. Then hold the scissors by the blade and twist slightly so there is mild tension on the glass. This will hold the end of the glass steady as you feed it into the flame. Once the sphere is the size you want, remove it from the flame, let it cool completely, and break the tube off about 0.5 cm from the sphere. This gives you a short handle, like a lollipop, so you can avoid touching the lens during later construction. This also ensures the light path will be perpendicular to the ‘wound’ inevitably caused by breaking the lens from the tube.

4. Measure the diameter of your lens with a micrometer and record your results for your laboratory report.

5. **Assemble the microscope.** Holding your sphere by its handle, place the sphere over the hole you drilled in the posterboard with the handle laying flat on the inside surface of the posterboard. Set the cardstock on top so the lens and handle are sandwiched between the two layers. Hold the two layers firmly together with the holes and lens lined up, and staple it twice about 0.5 cm from either side of the hole (figure 2). These lenses have very short working distances, so your sample has to be very close to the lens. This is why you use the thinner cardstock on one side, and it means your microscope works in one direction: you look through the posterboard side and place your sample on the cardstock side. When you staple, do so with the cardstock side up so the staple is less likely to get in the way of the sample.
Observing Samples:

1. **Make your focus mechanism.** The biggest challenge to making a microscope from paper is how to focus your specimen. The solution is to take advantage of the simultaneously elastic and sticky properties of adhesive putty to both mount the specimen and pivot it in relation to the lens. See figure 2.

2. Put an onion skin on a flat surface. Take a dab of putty about the size of a pea and press it over one edge of the skin. Pick the putty off the surface, and stick it to the cardstock side of the microscope just below the hole (don’t get putty on your lens) so the skin is right over the hole. This will be your stage, focusing device, and your movement controls. To view the specimen, hold the microscope as close to your eye as is comfortable, looking from the posterboard side directly into the light source provided (figure 3). To focus, place your thumb on the putty. Pushing the putty up towards the top of the microscope will cause the sample to pivot closer to the lens, pulling down will cause it to pivot away from the lens. Movement from side to side will position the sample over the lens as desired. Record your results.

3. The same principle is used to mount other kinds of samples. To mount an eyelash or hair, place it on a surface and press the putty over it and attach it to the microscope as described above and record your results.

4. To mount a wet specimen, place a glass coverslip on a flat surface and drop your sample onto the coverslip. Place a second coverslip on top, gently taping to seal the two together and mount on to your scope as above. Record your results.

**Magnification Calculations**

To determine magnification power, mount your microscope onto the laser platform. This apparatus uses a green laser pointer (532 nm) to magnify a tiny copper grid mounted on the opposite side as your microscope. The extent of magnification, as projected on the wall, is a function of how your specific lens was made.

There are two equations to help you determine the power of your lens. The power of the lens is dependent on the distance you are from it. However, there is a convention in optics to record the power as being from 250 mm. Therefore, the first equation converts projected size to what it would be if the focal length were 250 mm. The second equation compares the size of the projected grid to the size of the actual grid. This number is the magnification you achieved with your microscope.

Using the following equations, determine the magnification of your lens.

Equation 1. \((X / d) \times 250 \text{ mm} = D\)
Equation 2. \((D / A) = P\)

Where:
- \(d\) = distance between lens and projected image
- \(X\) = measured size of grid hole
- \(D\) = size of projected grid hole at 250 mm
- \(A\) = actual size of grid hole (0.204 mm)
- \(P\) = power of lens

**Resolution Calculations**

To determine resolving power, you need to perform some basic trigonometry. (Yikes!) Recall that resolving power is determined by the Abbe equation.

Abbe equation: \(d = 0.5\lambda / \text{numerical aperture}\)

Numerical aperture: \(\text{N.A.} = n(\sin \theta)\)

Where:
- \(d\) = resolving power
- \(\lambda\) = wavelength of light
- \(n\) = refractive index
- \(\theta\) = the half-angle of the maximum cone of light that can enter the lens

Where:
\[ \theta = \tan A = a/b \text{ (assume } a = b \text{ because the lens is physically touching the grid) } \]
1. Draw the specimens in the following circles.

Onion Skin  Eyelash or Hair  Protist

Crucial Variables for Calculations

Magnification:
Diameter of lens: ________  Distance from lens to projected image: ________
Measured size of grid hole: ________

Resolution:
Diameter of lens: ________  Wavelength of light: ________
Refractive index: ________  θ: ________

2. Calculate the magnification power of your lens using the equations given to you in the instructions. Convert all measurements to metric. (1 in. = 2.54 cm) Write your results on the board and record the results of the other groups.
3. Compare the group data and your calculations above. Generally speaking, what effect does diameter have on the magnification power of a lens? Explain your answer in terms of light refraction.

4. Calculate the magnification power of a lens that is twice as large as yours and a lens that is half as large.

5. Determine the numerical aperture of you lens and then use the Abbe equation to determine the resolving power of your microscope.
6. Calculate the resolving power of your microscope, if the working distance was doubled and if the working distance was halved.

7. Compare your resolving power calculations. What effect does working distance have on the resolving power? Explain why this is the case.

8. Calculate the resolving power of your microscope using a red laser pointer (650 nm) and the resolving power using a blue laser pointer (450 nm).
9. Compare your resolving power calculations. What effect does the wavelength of light use have on resolving power?

10. In your own words, define what the resolving power of your microscope means.
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   - Step 2. Once the stretched glass has cooled sufficiently to handle, break it somewhere in the middle of the stretched portion. Position the flame horizontally, and slowly feed the stretched glass into the flame from above. Watch this step carefully. A small, white-hot glass sphere will grow at the tip of the tube as you feed it into the flame. It is critical to keep this sphere in the flame and not to let it cool before it is done (or when you reintroduce it bubbles will form in it). Keep feeding the tube into the flame until the sphere is about 2 mm in size. A nice trick to help keep this motion constant and the sphere in the flame is to feed the stretched glass though the loop of a pair of scissors so that about 5
cm of glass extends from the loop. Then hold the scissors by the blade and twist slightly so there is mild tension on the glass. This will hold the end of the glass steady as you feed it into the flame. Once the sphere is the size you want, remove it from the flame, let it cool completely, and break the tube off about 0.5 cm from the sphere. This gives you a short handle, like a lollipop, so you can avoid touching the lens during later construction. This also ensures the light path will be perpendicular to the ‘wound’ inevitably caused by breaking the lens from the tube.

4. Measure the diameter of your lens with a micrometer and record your results for your laboratory report.

5. **Assemble the microscope.** Holding your sphere by its handle, place the sphere over the hole you drilled in the posterboard with the handle laying flat on the inside surface of the posterboard. Set the cardstock on top so the lens and handle are sandwiched between the two layers. Hold the two layers firmly together with the holes and lens lined up, and staple it twice about 0.5 cm from either side of the hole (figure 2). These lenses have very short working distances, so your sample has to be very close to the lens. This is why you use the thinner cardstock on one side, and it means your microscope works in one direction: you look through the posterboard side and place your sample on the cardstock side. When you staple, do so with the cardstock side up so the staple is less likely to get in the way of the sample.

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**Figure 1**

**Figure 2**

**Figure 3**
Observing Samples:

1. **Make your focus mechanism.** The biggest challenge to making a microscope from paper is how to focus your specimen. The solution is to take advantage of the simultaneously elastic and sticky properties of adhesive putty to both mount the specimen and pivot it in relation to the lens. See figure 2.

2. Put an onion skin on a flat surface. Take a dab of putty about the size of a pea and press it over one edge of the skin. Pick the putty off the surface, and stick it to the cardstock side of the microscope just below the hole (don’t get putty on your lens) so the skin is right over the hole. This will be your stage, focusing device, and your movement controls. To view the specimen, hold the microscope as close to your eye as is comfortable, looking from the posterboard side directly into the light source provided (figure 3). To focus, place your thumb on the putty. Pushing the putty up towards the top of the microscope will cause the sample to pivot closer to the lens, pulling down will cause it to pivot away from the lens. Movement from side to side will position the sample over the lens as desired. Record your results.

3. The same principle is used to mount other kinds of samples. To mount an eyelash or hair, place it on a surface and press the putty over it and attach it to the microscope as described above and record your results.

4. To mount a wet specimen, place a glass coverslip on a flat surface and drop your sample onto the coverslip. Place a second coverslip on top, gently taping to seal the two together and mount on to your scope as above. Record your results.

Magnification Calculations

To determine magnification power, mount your microscope onto the laser platform. This apparatus uses a green laser pointer (532 nm) to magnify a tiny copper grid mounted on the opposite side as your microscope. The extent of magnification, as projected on the wall, is a function of how your specific lens was made. There are two equations to help you determine the power of your lens. The power of the lens is dependent on the distance you are from it. However, there is a convention in optics to record the power as being from 250 mm. Therefore, the first equation converts projected size to what it would be if the focal length were 250 mm. The second equation compares the size of the projected grid to the size of the actual grid. This number is the magnification you achieved with your microscope.

Using the following equations, determine the magnification of your lens.

- **Equation 1.** \( (X / d) \times 250 \text{ mm} = D \)
- **Equation 2.** \( (D / A) = P \)

Where:

- **d** = distance between lens and projected image
- **X** = measured size of grid hole
- **A** = actual size of grid hole (0.204 mm)
- **D** = size of projected grid hole at 250 mm
- **P** = power of lens

Resolution Calculations

To determine resolving power, you need to perform some basic trigonometry. (Yikes!) Recall that resolving power is determined by the Abbe equation.

- **Abbe equation:** \( d = 0.5\lambda / \text{numerical aperture} \)
- **Numerical aperture:** \( \text{N.A.} = n(\sin \theta) \)

Where:

- **d** = resolving power
- **\( \lambda \)** = wavelength of light
- **n** = refractive index
- **\( \theta \)** = the half-angle of the maximum cone of light that can enter the lens

Where:

\[
\theta = \tan A = a/b \text{ (assume } a = b \text{ because the lens is physically touching the grid)}
\]
Microscope Optics

Names: ___________________ ___________________
Lab Section ______________________

1. Draw the specimens in the following circles.

Onion Skin
Eyelash or Hair
Protist

Crucial Variables for Calculations

Magnification:
Diameter of lens: 1.5 mm
Distance from lens to projected image: 980 mm
Measured size of grid hole: 160 mm

Resolution:
Diameter of lens: 1.5 mm
Wavelength of light: 532 nm
Refractive index: 1.0 (for air)
θ: 45 degrees

2. Calculate the magnification power of your lens using the equations given to you in the instructions. Convert all measurements to metric. (1 in. = 2.54 cm) Write your results on the board and record the results of the other groups.

Using equation 1 (pg. 3):
\[ D = \frac{X}{d} \times 250 \text{ mm} = \frac{160 \text{ mm}}{980 \text{ mm}} \times 250 \text{ mm} \]
\[ D = 40.8 \text{ mm} \]

Using equation 2 (pg. 3):
\[ P = \frac{D}{A} = \frac{40.8 \text{ mm}}{0.204 \text{ mm}} = 200 \]
\[ P = 200 \]

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</table>
3. Compare the group data and your calculations above. Generally speaking, what effect does diameter have on the magnification power of a lens? Explain your answer in terms of light refraction.

As the diameter of the lens gets larger the power of magnification decreases. This is due to the curvature of the lens because as the diameter increases, the light is bent (or refracted) to a lesser degree.

4. Calculate the magnification power of a lens that is twice as large as yours and a lens that is half as large.

To obtain this information, students are asked to graph the class data (see figure 3 of the article). If using a computer program, students can determine the best fit line of the data and solve for 2x or 0.5x, where x = their lens diameter. Additional properties of the lens can affect the power such as lens shape or aberrations in the glass itself. Therefore, each data point may not follow the expected line. This can be a good way to introduce the complexity of using experimental models to determine scientific principles.

5. Determine the numerical aperture of you lens and then use the Abbe equation to determine the resolving power of your microscope.

\[
\text{Numerical aperture: } N.A. = n \sin(\theta)
\]
\[
\text{Abbe equation: } d = 0.5\lambda / \text{numerical aperture}
\]

\[
\text{N.A.} = 1.0 \ (\sin(\tan^{-1} a/b)) = 1.0(\sin 45) = 0.71
\]
\[
d = 0.5 \ (532 \text{ } \text{nm})/ 0.71 = 375 \text{ } \text{nm}
\]
6. Calculate the resolving power of your microscope, if the working distance was doubled and if the working distance was halved.

Same equations as question 5, but adjusting for working distance (b).

For 2b
\[
\text{N.A.} = 1.0 \left( \sin \left( \tan^{-1} \frac{a}{2b} \right) \right) = 1.0(\sin 27) = 0.45 \\
\text{d} = 0.5 \left( \frac{532 \text{ nm}}{0.45} \right) = 591 \text{ nm}
\]

For 0.5b
\[
\text{N.A.} = 1.0 \left( \sin \left( \tan^{-1} \frac{a}{0.5b} \right) \right) = 1.0(\sin 63) = 0.89 \\
\text{d} = 0.5 \left( \frac{532 \text{ nm}}{0.89} \right) = 299 \text{ nm}
\]

7. Compare your resolving power calculations. What effect does working distance have on the resolving power? Explain why this is the case.

As the working distance increases, the resolution decreases. At increasing distances, less light from the sample enters the lens, thereby decreasing the numerical aperture of the lens and the overall resolution.

8. Calculate the resolving power of your microscope using a red laser pointer (650 nm) and the resolving power using a blue laser pointer (450 nm).

Same equations as question 5, but adjusting for wavelength of light (\(\lambda\)).

For 650 nm
\[
\text{N.A.} = 1.0 \left( \sin \left( \tan^{-1} \frac{a}{b} \right) \right) = 1.0(\sin 45) = 0.71 \\
\text{d} = 0.5 \left( \frac{650 \text{ nm}}{0.71} \right) = 458 \text{ nm}
\]

For 450 nm
\[
\text{N.A.} = 1.0 \left( \sin \left( \tan^{-1} \frac{a}{b} \right) \right) = 1.0(\sin 45) = 0.71 \\
\text{d} = 0.5 \left( \frac{450 \text{ nm}}{0.71} \right) = 317 \text{ nm}
\]
9. Compare your resolving power calculations. What effect does the wavelength of light use have on resolving power?

As the wavelength of light increases, the resolution decreases.

10. In your own words, define what the resolving power of your microscope means.

Using a green laser pointer (532 nm), the resolving power of our microscope is 375 nm. This means that using this lens two objects can be distinguished as separate objects as long as they are not closer than 375 nm to each other.
Pre-Assessment Questions:
The following questions will allow your instructor to measure the effectiveness of today’s laboratory exercise. Please do not write your name on the quiz; you will not be graded on the outcome.

1. In your own words, explain how a lens is used to magnify an image. Draw a diagram demonstrating how light from the sample travels through the lens and reaches the viewer.

2. What effect, if any, does the diameter of the lens have on magnification power?

3. Define focal length and explain how it affects magnification power.
4. How does the working distance of a lens affect the numerical aperture?

5. What effect does the numerical aperture have on resolution?

6. Would shortening the wavelength of light used in a microscope increase or decrease resolution?
Microscope Optics:
Building a Model van Leeuwenhoek Microscope

The following questions will allow your instructor to measure the effectiveness of the previous laboratory exercise. Please do not write your name on the quiz; you will not be graded on the outcome.

1. In your own words, explain how a lens is used to magnify an image. Draw a diagram demonstrating how light from the sample travels through the lens and reaches the viewer.

2. What effect, if any, does the diameter of the lens have on magnification power?

3. Define focal length and explain how it affects magnification power.
4. How does the working distance of a lens affect the numerical aperture?

5. What effect does the numerical aperture have on resolution?

6. Would shortening the wavelength of light used in a microscope increase or decrease resolution?
**Laboratory Exercise Evaluation**

In reference to the laboratory exercise, "Microscope Optics: Building a Model van Leeuwenhoek Microscope", please answer the following questions.

<table>
<thead>
<tr>
<th>Question</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The exercise enhanced my knowledge of microscopes:</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2. The instructor provided sufficient resources:</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3. The instructions were easy to follow:</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4. The exercise was too technical:</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>5. I enjoyed the exercise:</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

**Comments:**

6. My favorite part of this exercise was:

7. My least favorite part of this exercise was:

8. Additional comments:
Microscope Optics:  
Building a Model van Leeuwenhoek Microscope

Pre-Assessment Questions:
The following questions will allow your instructor to measure the effectiveness of today’s laboratory exercise. Please **do not write your name** on the quiz; you will not be graded on the outcome.

1. In your own words, explain how a lens is used to magnify an image. Draw a diagram demonstrating how light from the sample travels through the lens and reaches the viewer.

   A lens magnifies an image by collecting light from a sample, which bends as it enters and exits the lens. This light is focused at the focal point and then expanded to the observer.

   ![Diagram of light through a lens](Image)

   **Light** | **Lens** | **Focal Point** | **Observer**

2. What effect, if any, does the diameter of the lens have on magnification power?

   A smaller lens diameter produces a higher the magnification power.

3. Define focal length and explain how it affects magnification power.

   **Focal length** is the distance from the center of the lens to the focal point. A smaller focal length produces a larger magnification.
4. How does the working distance of a lens affect the numerical aperture?

A lens with a shorter working distance will have a higher numerical aperture.

5. What effect does the numerical aperture have on resolution?

A higher numerical aperture will increase resolution.

6. Would shortening the wavelength of light used in a microscope increase or decrease resolution?

Shortening the wavelength of light would increase resolution.
Basic Microscopy Concepts

Aberrations
Aberrations are various types of image distortion due to the interaction of light with a lens or lens system. Aberrations reduce the resolution of a lens system and the quality of the image produced. In modern microscopes, lens and light systems are engineered to reduce the effect of aberrations on image quality. Some types of aberrations that you may encounter are: chromatic, spherical, and field curvature.

**Chromatic aberrations** result in colored halos around objects when samples are viewed using a light source composed of a number of wavelengths (e.g. white light). This is due to images created with different wavelengths being focused at different points.

**Spherical aberrations** reduce image sharpness, clarity and ultimately reduces the resolving power or resolution of a lens. Spherical aberrations are caused by differences in the focal point of light passing through the center of the lens and the edge of the lens.

**Field Curvature** results in an image that is either in focus in the center but not the edges or in focus at the edges but not the center. This type of aberration is caused by light passing through a curved lens.

Contrast
The difference in color or intensity (light or dark) between the specimen and the background or between structures within the sample. Low contrast is when there is little difference in color or intensity between an object and the background or adjacent objects.

Field Of View
The area of a sample you can observe with a particular microscope objective (lens). As the magnification increases, the field of view decreases. The useful field of view can be further reduced by image distortion or aberrations that occur towards the edges of lenses.

Magnification or Lens Power:
The ratio of the size of the image produced by a lens and the actual size of an object (Equation 1). The power of the lens is inversely related to the focal length (Equation 3).
Figure 1.5 The relationship between image height \((H)\) and object height for a converging lens. The focal length of a lens is the point at which light rays converge on the optical plane.

**Equation 1.5** The magnification of a lens is given by the formula:

\[
I = \frac{q}{p} = \frac{H}{h}
\]

- \(I\) is the magnification of the lens
- \(q\) is the distance between the lens and the image
- \(p\) is the distance between the lens and the object
- \(H\) is the image height
- \(h\) is the object height

**Equation 2** The focal length is given by the formula:

\[
\frac{1}{f} = \frac{1}{p} + \frac{1}{q}
\]

\[
F = \frac{p \times q}{p + q}
\]
**Equation 3** The magnifying power of a lens is given by the formula:

\[ I = \frac{250 \text{ mm}}{F} = \frac{250 \text{ mm} \ (p + q)}{pq} \]

By convention, lens power is calculated at a distance of 250 mm from the lens since this is the closest distance the average human eye can focus on an object. When you view an object under the microscope you are actually seeing a virtual image (an image that can't be captured by a camera) that appears as if it is ~250mm in front of the lens.

**Resolution**

The ability to perceive adjacent objects or structures as distinct. The resolution of a lens system is related to the ability of an optical system to correct for aberrations and the light gathering ability of an objective lens. The numerical aperture (NA) of a lens is a quantitative measure of light gathering ability of a lens which is in turn related to the working distance of the objective; the smaller the working distance the higher the numerical aperture, the greater the resolving power of the objective.

![Figure 1](image_url)

**Figure 1** A diagrammatic illustration of the concept of resolution. A) A highly resolved image, the two points appear as distinct. B) A less resolved image, the two points appear somewhat distinct but the boundaries between the points are blurred making them appear to be part of a single object. C) A poorly resolved image, the two points appear to be a single object not two distinct objects.

**Useful Magnification**

The upper limit of magnification that provides useful information or resolution of image detail. Beyond this point, additional magnification does not produce more resolution of a sample. For example, if increasing magnification of image C from Figure 1 does not result in improved resolution as in image A, then the limit of useful magnification has been reached. Increasing magnification beyond the resolving power of a lens system actually leads to image degradation.
**Working Distance**  
The distance between the object you are viewing and the lens.

**Activity 1 - Contrast**

1. Observe chicken feathers stained different colors (unstained white feathers, black, red etc.).
2. Which color or colors of feathers were easiest to view?

   
   ___________________________________________________________

3. What features made viewing this sample easier than the others?

   
   ___________________________________________________________

4. Sketch barbs of the feather in the space below.

5. Describe some of the difficulties you had when trying to view and draw the specimens.
Activity 2 (Field of View)
1. Affix a slide containing a basic micrometer to your stage.
2. Measure the field of view by determining the number of hash marks there are from one edge of the field of view to the opposite edge.
3. Record your results in the Table 1 (pg. 6).

Figure 2 An example of measuring the field diameter of a 20 x objective for a compound microscope using a stage micrometer. The circle represents the field of view. The distance between each of the small hatch marks is 0.01 mm (10 μm). The field diameter is 0.94 mm (940 μm; 940 micrometers)

Determining the Power of the Lens

In addition to using your microscope to look at tiny things, it is a good exercise in optics to determine the power of the lens. In this activity you will determine the power of your lens and compare it with other students in the class. Because everyone will make slightly different sized lenses, you will be able to see the relationship between the size of the lens and the power.

Materials
Micrometer
Laser pointer
Transmission electron microscope grid, 100-mesh (e.g. Pelco product 1GC100)

Instructions
1. **Project laser beam though TEM grid and lens**

1. To measure the power of the lens, set up the components in this order: the laser source, then the EM grid, then the lens, then a surface, with the EM grid and the lens situated very close to one another.

2. The EM grid is a small, usually copper disk with a fine metal mesh, the size of which is known exactly. For the 100-mesh grid suggested above, the width of each hole is 204µm and width of each bar is 50µm.

3. When the laser beam is passed through the grid and lens, it projects a greatly enlarged image of the grid pattern onto the surface. If you then measure the distance between two grid bars on projected image and compare it to the known size of the grid (i.e. 204µm), taking into account the distance it has been projected, you can calculate the power of the lens using the following equations.

For the lenses you have made, it is difficult to accurately measure the distance between the lens and the object \((p\) in Equation 1). Rearranging Equation 1 to solve for \(p\) gives:

**Equation 4**

\[
p = \frac{qh}{H}
\]

Which can be substituted into Equation 3 to give:

**Equation 5**

\[
I = \frac{250(h + H)}{Hq}
\]

\(I\) is the magnification of the lens  
\(q\) is the distance between the lens and the image  
\(H\) is the image height  
\(h\) is the object height

| **Table 1** Measurement of lens parameters. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Lens Number | Lens Diameter | Field Diameter | Power | Focal Length |