



DNAZone Classroom Kit #7

Kit title	Diffraction of Light and Discovery of DNA Structure
Appropriate grade level	Physics, Grades 9-12
Abstract	<p>The diffraction and interference of light are easily observed phenomena that give direct, tangible evidence of the wave nature of light. Diffraction is at the root of many technologies, scientific techniques, and common visual phenomena. Students explore diffraction phenomena by shining a laser at a hair, a variable single slit made from pencils, and wire meshes of various size. After this introduction to the general principles of diffraction of light, students will use an ICE DNA transform slide to develop an understanding of the use of light diffraction in the discovery the structure of DNA.</p>
Time	Two 40-minute class periods
PA Department of Education standards met with this kit	<p>Pennsylvania Common Core Standards for Reading in Science and Technical Subjects</p> <ul style="list-style-type: none">• cc.3.5.9-12.B-F State Standards <p>Pennsylvania Academic Standards for Science & Technology addressed Unifying Themes</p> <ul style="list-style-type: none">• 3.1.10-12.C-D <p>Inquiry & Design</p> <ul style="list-style-type: none">• 3.2.10-12.B-C
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Diffraction of Light and Discovery of DNA Structure Overview

Behavioral Objectives:

Upon completion of this lab activity, students should be able to:

- Explain diffraction as it relates to Huygen's Principle and Babinet's Principle.
- Describe how x-ray diffraction was used to develop a model for the structure of DNA.

Class Time Required:

120 minutes for main lab activities

Teacher Preparation Time:

Minimal. Setting out materials and background reading.

Tips for the Teacher:

- Check all meters and batteries (hand microscopes) prior to lab
- Study background information prior to lab.

Assumed Prior Knowledge of Students:

- Wave nature of light.
- Law of wave superposition (interference of light)

PART I: Diffraction of Light

Objectives:

- To recognize that light is a wave with a small wavelength.
- To learn that diffraction is the bending of waves around an obstacle, and to differentiate this from projection.
- To gain familiarity with single-slit and multi-slit diffraction patterns.
- To learn that the dimensions of features in a diffraction pattern are inversely related to the dimensions of the object causing diffraction for small angles.
- To apply the diffraction equations to determine the size of features on some common objects, including CDs and DVDs, hairs, and lycopodium spores.

Background information:

The particle vs. wave debate (or "Saved by Diffraction")

1. Isaac Newton (1642-1727) felt light must be a particle
 - a. Light cast sharp shadows, and it was commonly felt that only particles would go in perfectly straight lines in order to cast sharp shadows.
 - b. Water and sound waves were observed to bend around obstacles, not go in straight



- lines to cast sharp “shadows.” They were the waves.
- c. Newton had incomparable status in the scientific community, so his view dominated throughout the 1700’s. (Young’s experiments in 1803 finally gave the concept of light waves common acceptance.)
 2. Christiaan Huygens (1629-1695) felt light must be a wave
 - a. (~1690) – attempted to correct Newton and show that light was a *longitudinal* wave, like sound waves
 - b. Light reflects and refracts like sound and water waves do
 - c. If wavelength (λ) was small enough, diffraction should be minimal and sharp shadows should occur.
 3. Francesco Maria Grimaldi (1618-1663) felt light must be a wave
 - a. Passed a beam of light through two consecutive narrow slits, and onto a surface
 - i. The band of light on the surface was a bit larger than the band of light entering the first slit. The beam had bent slightly outward at the edges of the slit. But, his findings were neglected!
 - ii. Called this bending of the light diffraction
 - iii. Observed some colored bands of light towards the outside (interference)
 4. Thomas Young (1773 - 1829)

“Young (proved that light is a wave - ed.) in 1803 by sending light through very narrow openings and showing that separate bands of light appeared where there should have been nothing but the sharply shadowed boundary of the edge of the opening. These bands of light arose from the kind of diffraction around corners that Grimaldi had noted, and it could not be explained by the particle theory.”

“Young had a more conclusive piece of evidence. From his study of sound he grew interested in the phenomenon of beats, in which two different pitches of sound produced periods of intensified sound separated by periods of silence. This was easily explained, since the two pitches had different wavelengths and therefore did not keep step. ...”

“Now, then, would two light waves add up to produce darkness? If they were particles, they couldn’t; if they were waves, they could. Young introduced light beams through two narrow orifices. They spread out and overlapped. The overlapping region was not a simple area of intensified light but formed a striped pattern of alternating light and darkness, a situation (interference) exactly analogous to beats in sound.”

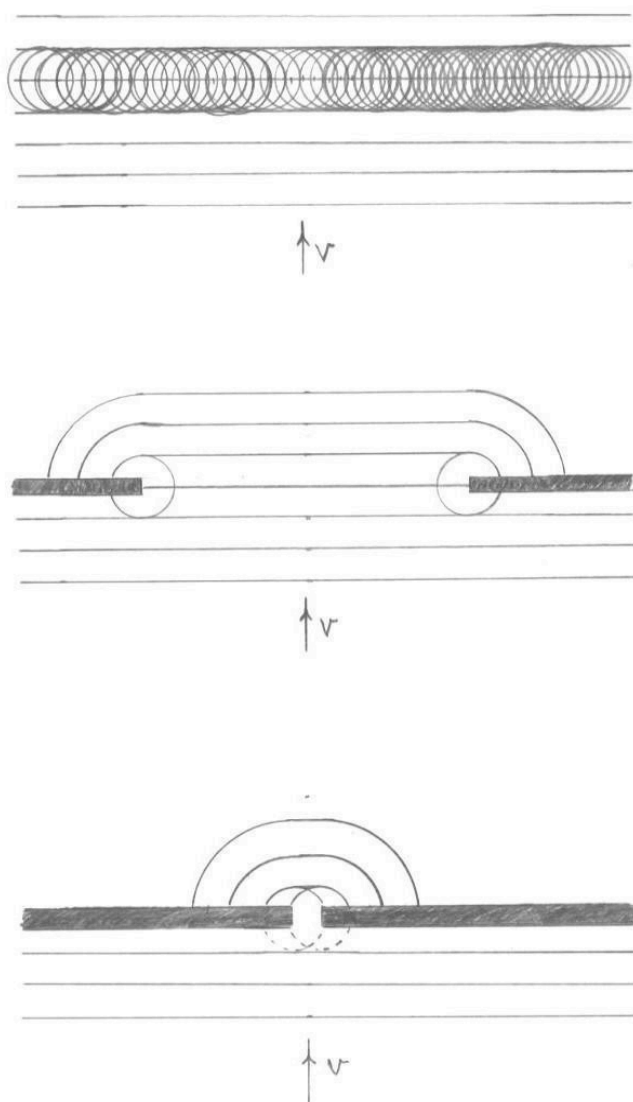
“From his diffraction experiment Young was able to calculate the wavelength of visible light, for it was only necessary to figure out what wavelength would allow the observed degree of small bending. ...” (Isaac Asimov’s Biographical Encyclopedia of Science & Technology, Isaac Asimov, Avon Books, ©1972

 - Interesting side-note #1: Young suggested that varying proportions of the three primary colors will allow a person to see the full range of color tones. This is the basis of color photography and color television.
 - Interesting side-note #2: Young used double refraction to show that light waves must be transverse



5. Babinet (1794 – 1872) - Diffraction around an obstacle vs. through a matching opening in a barrier.
 - a. Babinet's Principle: The diffraction pattern of *any* opaque obstacle is exactly the same as the diffraction pattern of an exactly matching opening in a barrier.
 - b. Interesting side-note: In 1827, Babinet suggested that fundamental SI units be based on stable natural properties rather than variable artifacts, like a portion of the circumference of the earth. He proposed using something like the wavelength of some particular color of light in standardizing the meter. In 1960, technology had ripened, and his idea became a reality.

Christiaan Huygens' Model of How Diffraction Occurs



When one drops a pebble into the water, circular periodic waves are seen traveling outwards. The wavelength is the distance between the wavefronts. Huygens model suggests that each of the infinite number of points on *any* wavefront acts as a source of circular waves like those from a pebble dropped into the water.

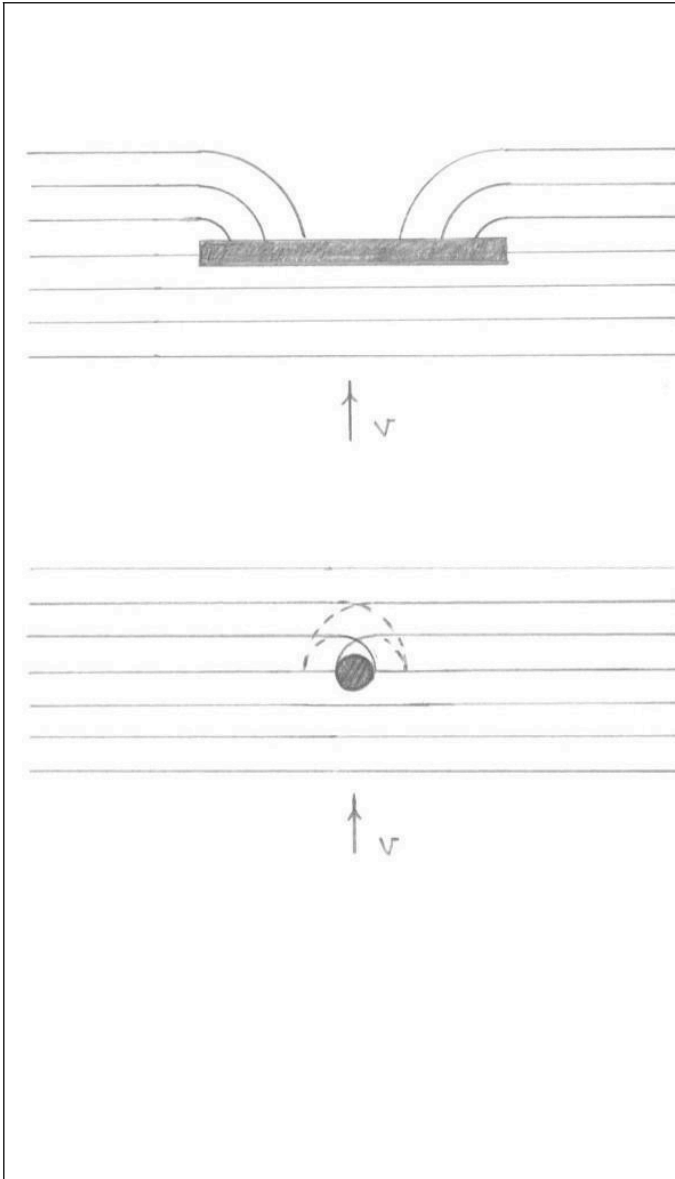
The first diagram here shows seven straight wavefronts. One of them shows a small sample of the infinite number of point sources creating the circular waves Huygens' model describes. Note that they have the same wavelength as the straight wavefronts had. Note also that they add together constructively to 'propagate' the next wavefront with just the right wavelength!

The second diagram shows the same straight wavefronts approaching a barrier that has a large opening compared to the wavelength of the wave. The wave energy that hits the barrier simply reflects back (not shown here). The wavefront right *in* the opening in the barrier would generate plenty of circles to add up to a straight wavefront *except at the edges of the opening*. At the edges of the opening, the circular wavefronts created from the outer points do not have other circles to the outside to interact with. The overall result is a mostly straight wavefront passing through the opening with just a bit of curving at the edges, as only a bit of the wave energy spreads out behind the barrier.

The final diagram on this page shows the outcome if the size of the opening in the barrier is small compared to the wavelength of the wave. In this case, there is not enough straight wavefront actually in the opening in the barrier to propagate a straight wavefront on the backside of the barrier. The outside edge points are so close to each other that the wave behind the barrier is, for all intents and purposes, a semi-circle.

Summary: When the size of the opening in a barrier is large compared to the wavelength of the wave, little diffraction occurs. When the reverse is true, much diffraction occurs, and the waves appear circular.

Christiaan Huygens' Model of How Diffraction Occurs (Diffraction around an obstacle)



The first diagram on this page shows straight wavefronts approaching an obstacle that is large compared to the wavelength of the wave. The wave energy that hits the obstacle simply reflects back (not shown here). The wavefronts on either side of the obstacle generate plenty of circles to add up to a straight wavefront *except in the region directly behind the obstacle*. At the edges of the obstacle, the circular wavefronts created from the outer points do not have other circles behind the obstacle to interact with. The overall result is a straight wavefront on each side of the obstacle with just a bit of curving at the edges, as only a bit of the wave energy spreads into the region behind the obstacle.

The final diagram on this page shows the outcome if the size of the obstacle is small compared to the wavelength of the wave. In this case, the straight wavefronts continue on either side of the obstacle; and the outside edge points of the obstacle are so close to each other that the associated circles on the two sides almost totally overlap. The wave fills in almost completely behind the obstacle, there is no „shadow“ cast, and it is as though the obstacle isn't there at all!

Summary: When the size of an obstacle is large compared to the wavelength of the wave, little diffraction occurs and the obstacle effectively blocks the wave energy from the region behind it. When the size of an obstacle is small compared to the wavelength of the wave, there is a great deal of diffraction, and the wave fills in behind the obstacle.



PART II: Discovering the Structure of DNA

Credits:

Part II of this worksheet is based on an activity originally developed by the University of Wisconsin Materials Research Science and Engineering Center. See mrsec.wisc.edu/Edetc for more information.

Purpose:

To explain how x-ray diffraction was used to determine the structure of DNA. Students will use an *optical transform slide* for modeling the structural elements of DNA and generate diffraction patterns from each structure using a laser. This experience will introduce students to x-ray diffraction as a tool for investigating the structure of biological molecules and in general to the field of biophysics.

Background: (<http://www.sdsc.edu/ScienceWomen/franklin.html>)



There is probably no other woman scientist with as much controversy surrounding her life and work as Rosalind Franklin. Franklin was responsible for much of the research and discovery work that led to the understanding of the structure of deoxyribonucleic acid, DNA. The story of DNA is a tale of competition and intrigue, told one way in James Watson's book *The Double Helix*, and quite another in Anne Sayre's study, *Rosalind Franklin and DNA*. James Watson, Francis Crick, and Maurice Wilkins received a Nobel Prize for the double-helix

model of DNA in 1962, four years after Franklin's death at age 37 from ovarian cancer.

Franklin excelled at science and attended one of the few girls' schools in London that taught physics and chemistry. When she was 15, she decided to become a scientist. Her father was decidedly against higher education for women and wanted Rosalind to be a social worker. Ultimately he relented, and in 1938 she enrolled at Newnham College, Cambridge, graduating in 1941. She held a graduate fellowship for a year, but quit in 1942 to work at the British Coal Utilization Research Association, where she made fundamental studies of carbon and graphite microstructures. This work was the basis of her doctorate in physical chemistry, which she earned from Cambridge University in 1945.

After Cambridge, she spent three productive years (1947-1950) in Paris at the Laboratoire Central des Services Chimiques de L'Etat, where she learned X-ray diffraction techniques. In 1951, she returned to England as a research associate in John Randall's laboratory at King's College, London.



Rosalind Franklin's x-ray photograph of DNA



It was in Randall's lab that she crossed paths with Maurice Wilkins. Their groups and had separate projects, although both were concerned with DNA. When Randall gave Franklin responsibility for her DNA project, no one had worked on it for months. Wilkins was away at the time, and when he returned he misunderstood her role, behaving as though she were a technical assistant. Both scientists were actually peers. His mistake, acknowledged but never overcome, was not surprising given the climate for women at the university then. Only males were allowed in the university dining rooms, and after hours Franklin's colleagues went to men-only pubs.

But Franklin persisted on the DNA project. J. D. Bernal called her X-ray photographs of DNA, "the most beautiful X-ray photographs of any substance ever taken." Between 1951 and 1953 Rosalind Franklin came very close to solving the DNA structure. She was beaten to publication by Crick and Watson in part because of the friction between Wilkins and herself. At one point, Wilkins showed Watson one of Franklin's crystallographic portraits of DNA. When he saw the picture, the solution became apparent to him, and the results went into an article in *Nature* almost immediately. Franklin's work did appear as a supporting article in the same issue of the journal.

A debate about the amount of credit due to Franklin continues. What is clear is that she did have a meaningful role in learning the structure of DNA and that she was a scientist of the first rank. Franklin moved to J. D. Bernal's lab at Birkbeck College, where she did very fruitful work on the tobacco mosaic virus. She also began work on the polio virus. In the summer of 1956, Rosalind Franklin became ill with cancer. She died less than two years later.

Materials:

- Hand held laser pen
- Optical transform slide (ice.chem.wisc.edu)
- Copy of magnified image of transform slide
- 30X hand microscope
- Slide-holder block
- Laser support block
- Display screen
- Small transparent metric ruler
- Protractor
- Red and blue colored pencils
- Wire mesh (coarse, medium, fine)
- Diffraction grating



"The instant I saw the picture my mouth fell open and my pulse began to race.... the black cross of reflections which dominated the picture could arise only from a helical structure... mere inspection of the X-ray picture gave several of the vital helical parameters."

--J. D. Watson

Answers to Questions: *send request for answers to cipt_contact@cornell.edu*

Equipment

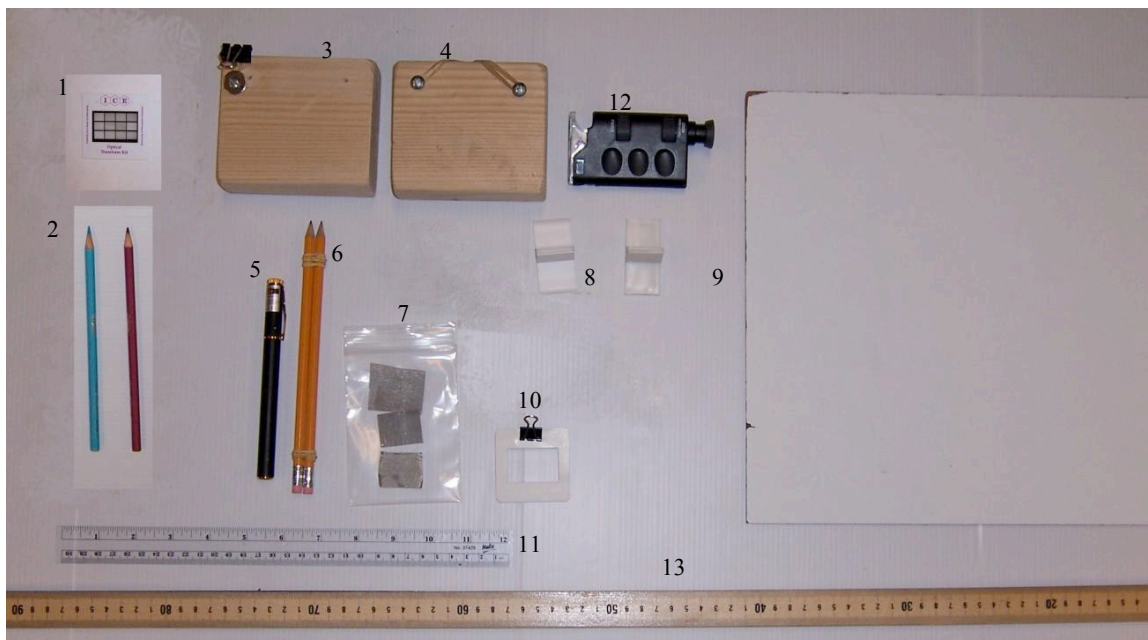


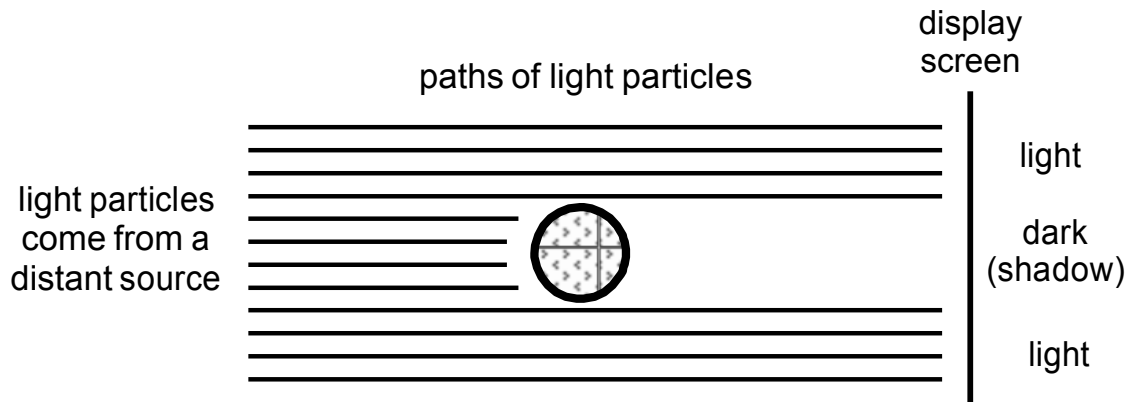
Photo ID	No.	Item
1	1	Optical transform slide (ice.chem.wisc.edu)
2	1 each	Red and blue colored pencils
3	1	Wooden Sample Holder
4	1	Wooden Laser Holder
5	1	Laser Pointer
6	1	2 pencils connected with rubber bands
7	4	Wire mesh (2, 4, 6, 8 wires/mm)
8	2	Projection screen stand
9	1	Projection screen
10	1	Empty slide holder with binder clip
11	1	12- inch clear plastic ruler
12	1	Hand held microscope
13	1	Meter stick



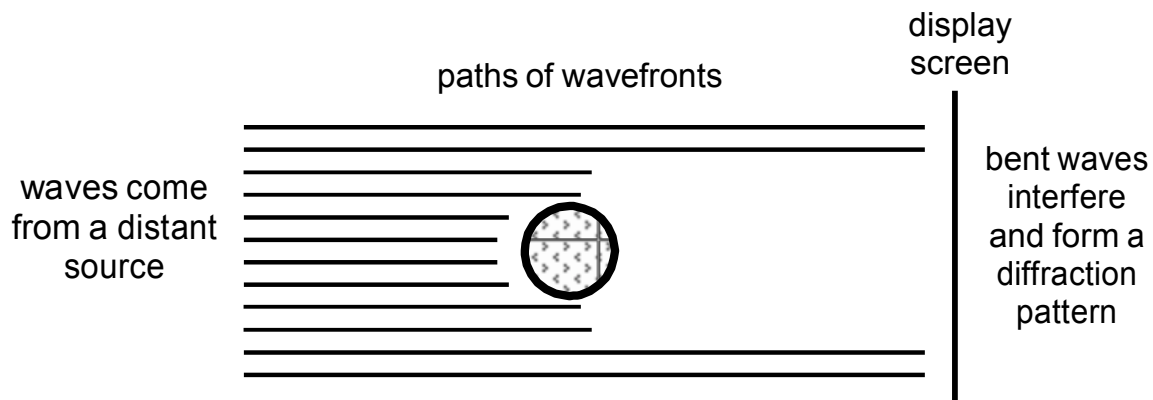
DIFFRACTION OF LIGHT AND THE DISCOVERY OF THE DNA STRUCTURE

PART I: Diffraction of Light

What is the nature of light? Is it a wave? Is it a particle? Isaac Newton (1642-1727) thought that light must be a particle. He noticed that light makes sharp-looking shadows of objects. This can be explained by particles of light that travel in straight lines until they are stopped by some object that lies in their path.



Waves, such as water wave and sound waves, can bend around obstacles in their path. For example, you can hear someone talking around a corner. This bending is called diffraction. The diffracted (bent) waves collide with other waves and interfere to form interesting patterns called diffraction patterns.



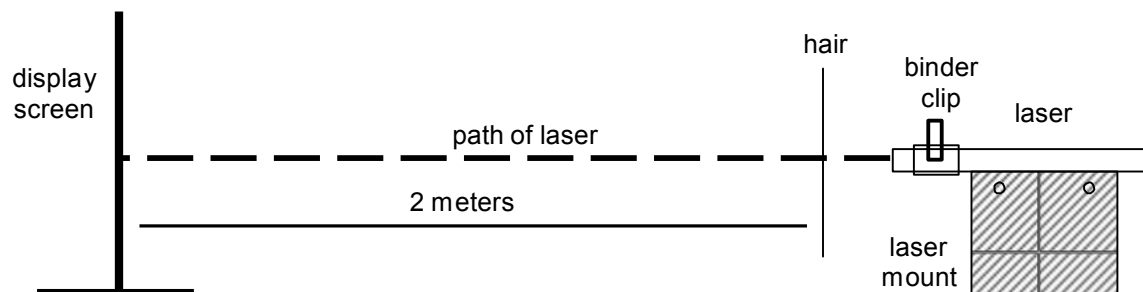
Christiaan Huygens (1629-1695) thought light must be a wave because it reflects and refracts like sound and water waves do. He attempted to correct Newton and pointed out that if wavelength of light were small enough, the diffraction or bending would be a very small effect and for most objects and sharp-looking shadows would occur.

Due to Newton's greater status within the scientific community, his particle theory of light dominated through the 1700's. But was Newton right? You get to decide with the aid of a modern invention, the laser, which produces a bright, parallel beam of light.



Name _____

Exploration 1: The "shadow" from a strand of hair



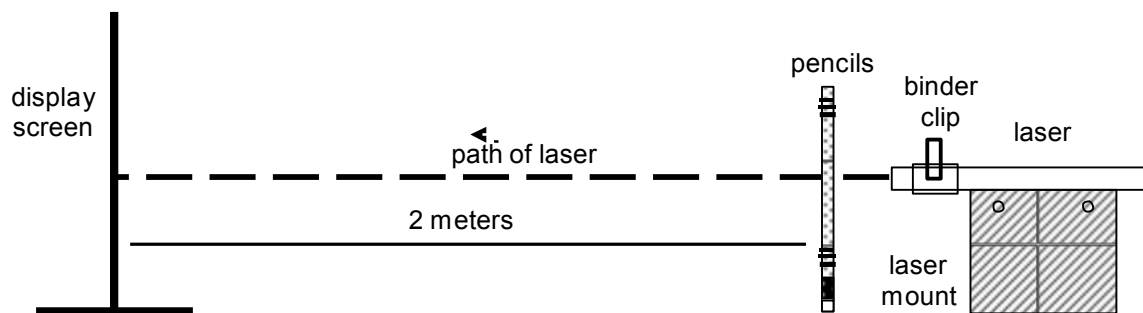
Instructions:

- Take a piece of your hair and tape it so that it hangs from the test tube clamp.
- Place the display screen 2 meters from the hair.
- Put the laser underneath the rubber bands on the mounting block, and aim the laser at the hair and the screen.
- Use the binder clip to clamp the laser on, and adjust the laser as necessary to strike the hair directly.
- Observe the laser light on the screen and answer the following questions.

Questions:

1. Sketch the pattern of the light on the screen with the laser aimed at the hair.
2. Does the pattern on the screen look like a shadow of the hair? Explain.
3. Why do you think the laser beam spreads out when you put the hair in the path of the laser?
4. What direction does the laser beam spread relative to the orientation of the hair?

Exploration 2: Light between two pencils



Instructions:

- Construct a narrow slit using two pencils and two rubber bands, as shown below:

loop each rubber band once around
one pencil, then wrap tightly around both

narrow slit

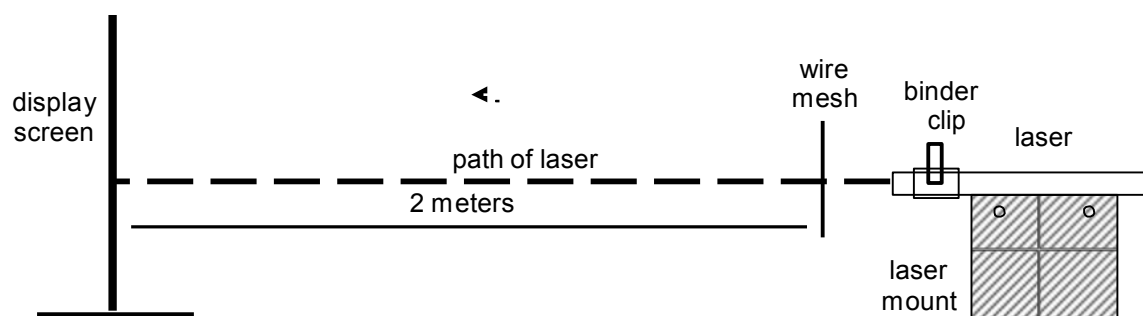


- Rest the erasers of the pencils on the table so that the laser shines through the narrow slit between them. Position the screen 2 meters behind the pencils.
- Observe the pattern of the light that falls on the screen. Try squeezing the pencils gently together to decrease the slit size. Observe any changes to the pattern of light on the screen.

Questions:

- Sketch the pattern of the light on the screen with the laser aimed through the slit.
- Does the pattern on the screen look like a shadow of the slit? Explain.
- What happens to the pattern when you squeeze the pencils to make the slit narrower?

Exploration 3: Wire meshes



Instructions:

- Find the coarse mesh and clamp it with a vertical orientation. Place the display screen 2 meters from the mesh.
- Aim the laser perpendicular to the mesh so that it shines through the mesh and onto the screen. Observe the pattern on the display screen.
- Record the distance between nearest bright dots on the display screen.
- Record the number of wires per millimeter of the mesh. Place the mesh between the clear plastic ruler and the sight saver, and hold this all up to the light.
- Repeat for the other two wire meshes.

Questions:

- Organize your data in the first two columns of the table. Calculate the distance between adjacent wires in the mesh and put your answers in the third column.

Mesh	Distance between nearest bright dots in diffraction pattern	Number of wires per millimeter	Distance between wires
Coarse			
Medium			
Fine			

- How does the diffraction pattern change as the wires get closer together?
- What is the mathematical relationship between the "distance between wires" and the "distance between nearest bright dots in the diffraction pattern"?

Information on Diffraction:

For small angles only!:

$$\lambda = \frac{xnd}{nl}$$

For all angles:

$$n\lambda = d \sin \Theta$$

For two or more slits:

λ = wavelength

x = the distance between the center of the zeroth order antinode (bright fringe) and the n^{th} order antinode, measured along the screen surface

n = the order of the antinode being viewed

d = the distance between the slits

l = the perpendicular distance from the slits to the screen

Θ = the angular deviation from the 0th order antinode to the n^{th} order antinode on the screen

For one slit:

x = distances as above, but measured to *nodes*

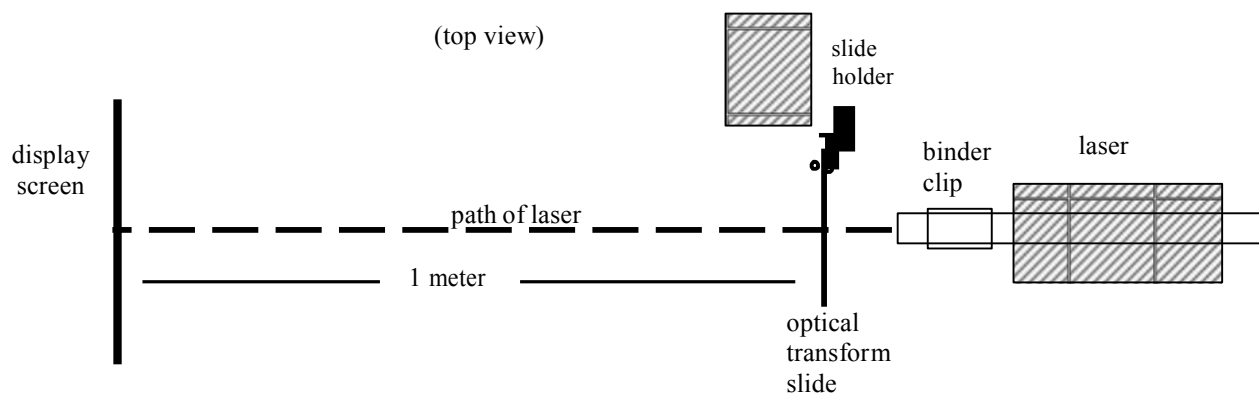
n = the order of the node being viewed

d = the width of the slit

Θ = the angular deviation as above, but measured to *nodes*

PART II: Discovering the structure of DNA

Exploration 4: The Black Cross



- Using the set-up illustrated above, shine the red laser through each of the masks (A, B, C) and look at the diffraction image on the screen. Carefully sketch the diffraction image from each mask in the table below.



Mask A	—	Mask B		Mask C	

11. Describe how the diffraction pattern relates to the lines on the mask for A, B, and C.

12. Shine the laser through mask D and look at the diffraction image on the screen. Carefully sketch the diffraction image in table below.

Mask D

13. How does the diffraction pattern for mask D relate to the diffraction patterns you sketched in table above?

14. Explain why the lines shaped like waves in mask D create a “cross.”

15. The lines on mask D looks like sine waves. How does this relate to the shape of a helix (spiral)?

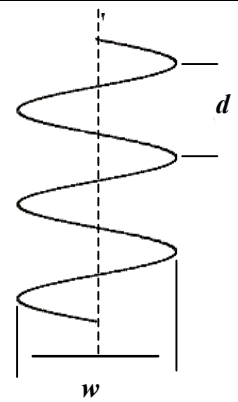


16. Notice that Rosalind Franklin's x-ray image of DNA has a cross. What does this tell you about the structure of DNA?

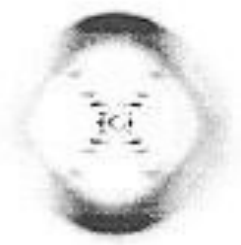
Exploration 5: Vital Helical Parameters

- Shine the laser through each of the masks D, E, G, H and look at the patterns on the screen.
17. Describe how the diffraction image changed as you moved the laser among masks D, E, G, and H.
18. What differences among the helices on masks D, E, G, H caused the changes in the diffraction images?
19. Using the *paper copy* of the optical transform slide, measure the width w and length d of a single spiral for the helices in masks D, E, G, H. Record your answers in the chart below. Compute the ratio w/d in the last column of the chart.

Mask	Width w	Length of one spiral d	w/d



20. Examine Rosalind Franklin's x-ray diffraction image of DNA. Using the laser, view the diffraction image of masks D, E, G, and H. Which mask appears most similar to Rosalind Franklin's data?

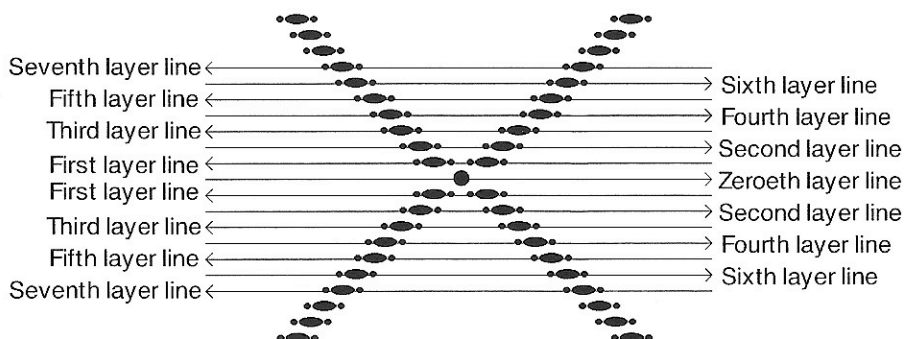


21. Based on your comparison, what is the ratio of the width w to the length d of one spiral for DNA (Franklin's image)?

Rosalind Franklin's x-ray photograph of DNA

Exploration 6: Layer Lines

- Using the set-up illustrated above, shine the laser through the mask H. Notice that the cross of the diffraction image is composed of individual layer lines as illustrated below.



- Shine the laser through each of the masks D, E, G, H and pay close attention to the layer lines of each diffraction image. Determine what property of the helix affects the vertical spacing of the layer lines in the diffraction image.
- Shine the laser through mask H. Measure the distance from mask H to the display screen and the vertical distance between layer lines. Fill in the table below.

22. Compare D,E,G and H and consider the following. Given red light, what property of the helix determines the vertical spacing of the layer lines in the diffraction image?
(d or w)

23. Fill in the table below related to the helix in mask H and its diffraction pattern. Use the diffraction formula in the last column to compute the length of one spiral d .

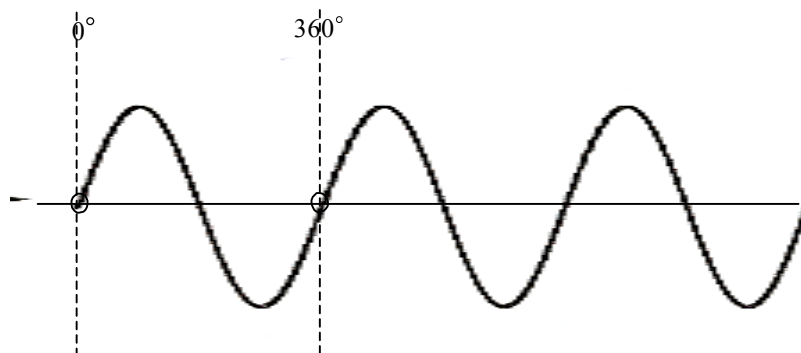
Mask	Distance from mask H to screen [L (mm)]	Vertical distance between layer lines [x (mm)]	$\theta = \tan^{-1}(x/L)$ [$\theta(^{\circ})$]	$d \sin \theta = \lambda$ ($\lambda = 6.7 \times 10^{-4}$ mm) [d (mm)]
H	2 m (2000 mm)			



24. Using a hand microscope and a clear plastic ruler, look at the optical slide mask H and determine the actual length d of one spiral of the helix.
Length of spiral d from direct measure: _____mm
25. How does the value in #26 compare to the value you computed from the diffraction pattern in the table above?
26. Using the value you found for the actual length of one spiral (d) in question #2 table, determine the width (w) of the helix on mask H without directly measuring it. Use the information from the diffraction pattern and the ratio (w/d) from the table in the previous section.

Exploration 7: The Double Helix

27. One full turn of the helix can be mapped to 360° . On the following illustration, label the position corresponding to the following angles: 0° , 45° , 90° , 135° , 180° and 360° .



- Study the diagram of the “layer lines” in the previous section. Notice that the layer lines can be numbered as in the diagram, with zero at the central bright spot of the laser beam.



28. Study the masks H, F, I, and L *on the transform sheet* and estimate the phase difference in degrees between the two helices on each mask (H has only one helix). Record your data in the table below.

<u>Mask</u>	Phase difference between helices (°)	Layer lines missing
H	-----	
F		
I		
L		

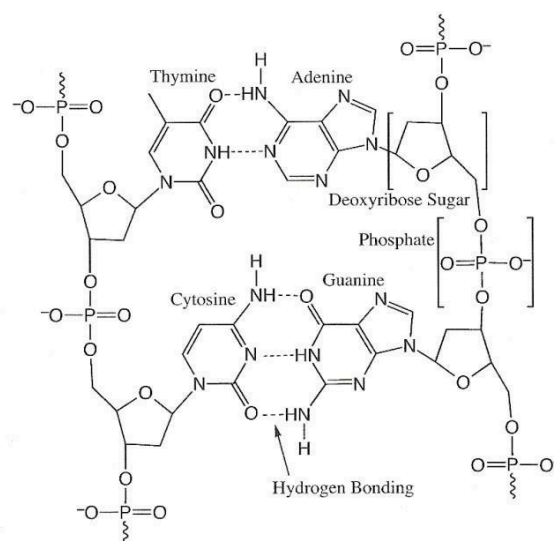
29. Now using the LASER, note which “layer lines” are missing from the diffraction image of each mask H, F, I, and L, and record your observations in the last column of the table above.
30. In general, how is the diffraction image of a single helix different from a double helix?
31. Rosalind Franklin’s x-ray diffraction image for DNA have a missing layer lines What is the number of the layer line that is missing?
32. Which mask has the same layer line(s) missing as Franklin’s image?
33. Referring your data table above and your answer to question #34, how many degrees offset are the two helices of a DNA molecule?

Exploration 8: Advanced Study

- Compare the double helixes represented in masks J, K and L on the copy of the optical transform slide. Answer questions below.
 - View the diffraction images of J, K and L. Answer questions below.
34. If masks J, K, and L all have the same double helix structure, describe the other observed differences between these three masks in the space below.

35. On the structural formula of DNA to the right, circle the **phosphate groups** in red, **base pairs** in blue and the **sugars** in black.

36. Given that atoms of highest atomic number will be the principal contributors to the diffraction pattern (their electrons cause the greatest scattering of the x-rays), which of the three components of DNA – phosphate, sugar, or the bases – do the dots in mask K represent?



37. Which of the three components of DNA do the horizontal lines in mask J represent?

12 Mask Patterns on the DNA Optical Transform Slide

