How Rosalind Franklin Discovered the Helical Structure of DNA: Experiments in Diffraction

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Rosalind Franklin, a chemical physicist (1920-1958), used x-ray diffraction to determine the structure of DNA. What exactly could she read out from her x-ray pattern, shown in Fig. 1?[1] In lecture notes dated November 1951, R. Franklin wrote the following: "The results suggest a helical structure (which must be very closely packed) containing 2, 3 or 4 co-axial nucleic acid chains per helical unit, and having the phosphate groups near the outside."[2] This was 16 months before J. D. Watson and F. Crick published their description of DNA, which was based on R. Franklin's x-ray photos. How they gained access to her x-ray photos is a fascinating tale of clashing personalities and male chauvinism.[2,3]

In this paper we suggest four experiments that enable students to follow in the footsteps of Rosalind Franklin's discovery. We will increase the scale so that it is doable in a high school or undergraduate lab; instead of a tiny DNA molecule, we examine the diffraction pattern of a helical spring from a retractable ballpoint pen, and instead of x-rays we use light rays.[4,5] These experiments are relatively simple expansions of a regular single-slit or multiple-slits diffraction lab. They have the advantage of giving students a sense for the usefulness of diffraction techniques, something they often miss in "pure" diffraction labs.

The four experiments vary in equipment and difficulty. Experiment 1 uses only simple equipment to illustrate how diffraction reveals the structure of an object. Experiment 2 requires a slightly more elaborate setup, but also gives more information about the diffracting object. Experiment 3 is an analysis of *Photo 51* (Fig. 1), and Experiment 4 is a computational simulation of the diffraction of a helix. Experiments 1, 2, and 3 are also good classroom demonstrations.

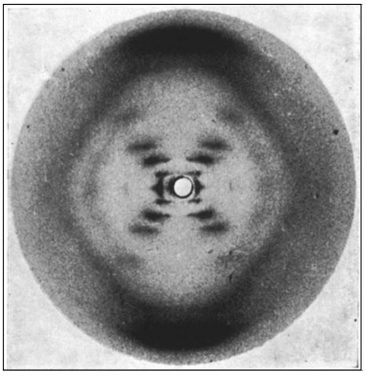

Fig. 1. X-ray diffraction pattern of B-DNA labeled *Photo 51* by Rosalind Franklin. The diameter of the largest circle has an original size of 94 mm. At the position of the zero order, a lead disc covered the film to avoid overexposure. The first order is almost blocked by this disc, too. Therefore, we used the second, third, and fifth order for calculation. The fourth order is missing, as explained in the text. *(Reprinted with permission from Macmillan Publishers Ltd, Ref. 1).*

Experiment 1: Why does the x-shaped pattern reveal the helical structure?

This is a variation on the single-slit experiment with readily available equipment, allowing the student to both see a diffraction pattern similar to Franklin's and to determine the pitch angle α of the helix, as in Fig. 2. If you take the spring from a typical retractable pen and place it on an overhead projector, you observe that the projection of a helix is a sine wave pattern (Fig. 2). We place a laser pointer[6] or a diode laser (from PASCO) on one end of the lab table and direct the beam (diameter ~2 mm) through the side of the spring. (The distance between the laser and spring does not matter.) Holding a white card a couple of centimeters past the spring shows the projection is indeed a sinusoidal pattern and also reveals the number of illuminated pitches. (The shadow will not look sinusoidal if the helix is not oriented perpendicular to the beam.) Since the diameter of the beam is small, it will only illuminate one pitch, i.e., one turn of the helix and only the relatively straight parts of the sine wave, not the curved maxima or minima. We project our diffraction pattern on a screen or wall at least 2 m away, with a larger distance (~4 m) better, since it gives a larger pattern. A mirror can be used to fold the path, allowing for a larger distance on a shorter table.

Babinet's principle states that the diffraction pattern of an obstacle is the same as the diffraction pattern of an aperture of the same shape.[7] According to this principle, the diffraction pattern formed by the two straight sections of the wire (one on each side) is equivalent to the diffraction pattern of two single slits oriented at a certain angle with respect to each other (see Fig. 2). When comparing the diffraction pattern of the helical spring (similar to Fig. 4) with the x-ray diffraction pattern of

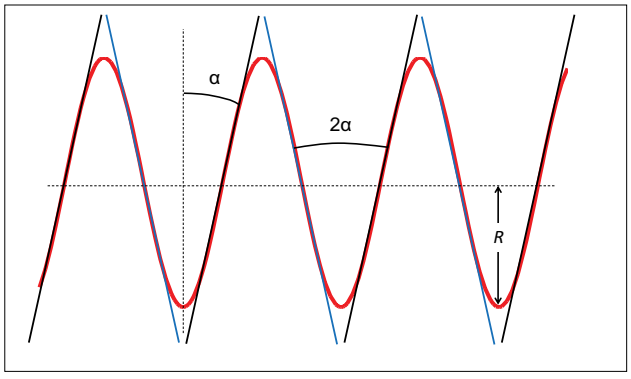

Fig. 2. Here we see the approximately parallel lines formed by the sine wave. We also see the pitch angle α and the radius *R*.

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Table I. An overview of the results acquired with the described experiments. The values can be obtained from the diffraction patterns in Fig. 1 and Fig. 4. The Franklin values are taken from R. Franklin's paper.[1,9]

	spring (Exp. 2)		DNA (Exp. 3)	
	diffraction	calipers/ protractor	diffraction	Franklin
2α	19°	20°	71°	72°
pitch, P (Eq. 2)	1.79 mm	1.87 mm	3.4 nm	3.4 nm
radius, R (Eq. 4)	1.70 mm	2.06 mm	0.74 nm	~1 nm

Fig. 1, students will immediately understand what convinced Rosalind Franklin that the DNA has a helical shape. They can deduce the pitch angle α of the helical spring by measuring the angle between the two main diffraction streaks and dividing by two. In order to get a sense of whether their result is correct, we suggest having the students draw an angle 2α and then try to align the pitch with the two lines. They can also project the helix with an overhead projector onto a sheet of paper, draw lines through the straight parts in the projected image, and then measure the angle. Doing the angular measurement in the vertical conical section of the famous *Photo 51* (Fig. 1) will render the correct pitch angle of DNA, which is listed in Table I.

In her paper that appeared in *Nature*, in the same issue as the paper by J. D. Watson and F. Crick, R. Franklin determined the radius of the DNA helix.[1] How did she do this? There is no way to figure out the radius with a single-slit (or in our case single-wire) diffraction. (We will see it *is* possible to find the radius in Experiment 2.) The only information that we can get from the distance between the diffraction minima is the thickness a of the wire in our spring. Minima in the diffraction pattern occur at angles $\theta_{\min}$ given by:[7]

$$a \sin \theta_{\min} = m\lambda . \quad (1)$$

The DNA molecule does not have a "thickness" because the x-ray diffraction actually measures the location of the heavy phosphorous nuclei. Nevertheless, it is a nice exercise for the students to determine the thickness of the wire of their helical spring. In our case, we measured the distance to the fifth minimum ($m = 5$) from the central spot (2.2 cm) divided by the distance between helix and screen (3.25 m), which yielded $\sin \theta_{\min} = 0.0068$ and subsequently the thickness $a = 467\ \mu$m. Students can then verify their results by directly measuring the thickness of the wire with calipers.

Experiment 2: How did Rosalind Franklin determine the diameter of DNA?

By expanding the beam with two lenses, students can illuminate multiple pitches of the wire, letting them find the radius R of the helix, by first finding the pitch P. The pitch is the spacing between turns in the helix and is analogous to the wavelength of our sine function. A look at Fig. 3 reveals that the pitch P is connected to the distance d between multiple parallel wires (or equivalently multiple parallel slits) by:

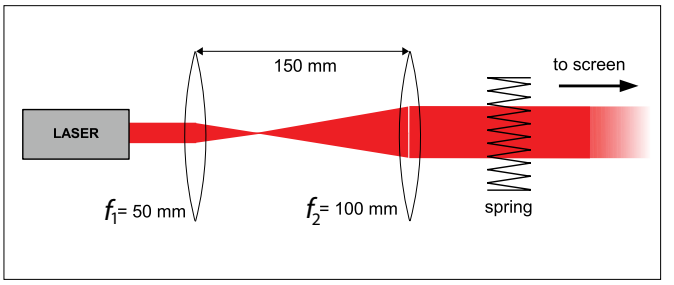

Fig. 3. We can expand the small laser beam by using two converging lenses, with the distance between them equal to the sum of their focal points. Increasing this distance results in converging light instead of a plane wave and shortens the distance needed to produce the far-field diffraction pattern.

$$P = \frac{d}{\cos\alpha}. \quad (2)$$

We see why we need to illuminate more than one pitch to deduce the distance d between wires from a multiple-wire diffraction pattern. The maxima in the diffraction pattern occur at angles $\theta_{\min}$ according to the equation[7]

$$d \sin \theta_{\max} = m\lambda . \quad (3)$$

We place the laser in front of a beam expander, constructed from a lens with short focal length ($f_1 = 50$ mm) and a lens with a longer focal length ($f_2 = 100$ mm), as in Fig 3. Other lens choices are possible and result in more or fewer illuminated pitches. We used an optics track and lens holders by PASCO and lenses from Sargent-Welch. The distance between the two lenses should be equal to $f_1 + f_2$.[8] We thus expand the diameter of the beam to $w = 7$ mm and illuminated five pitches. Care should be taken that the beam is almost parallel and not diverging. This can be tested by holding a white card at various distances from the laser into the beam and marking the diameter on the card. Adjusting the position of the lenses back or forth along the beam axis helps to make the beam parallel. At a distance D given by $D \geq w^2/\lambda$, where w is the beam diameter and λ is the wavelength, the diffraction pattern is a far-field diffraction pattern, as seen in Fig. 4. In our case the distance D would be approximately 77 m, which of course is not very practical in the lab. In order to get a diffraction pattern at the much closer screen (4.20 m), we slightly increase the distance between lenses until a slightly converging beam produces a focus on the screen. The helical spring is then placed into the beam. Its far-field diffraction pattern on the screen is recognizable by the bright but small spot in the center. In the broad minima some bright spots are faint or missing from the pattern. These minima occur because of interference caused by the *thickness* of *each single wire*. Be sure to count these missing spots. We picked the 18th maximum from the central spot,

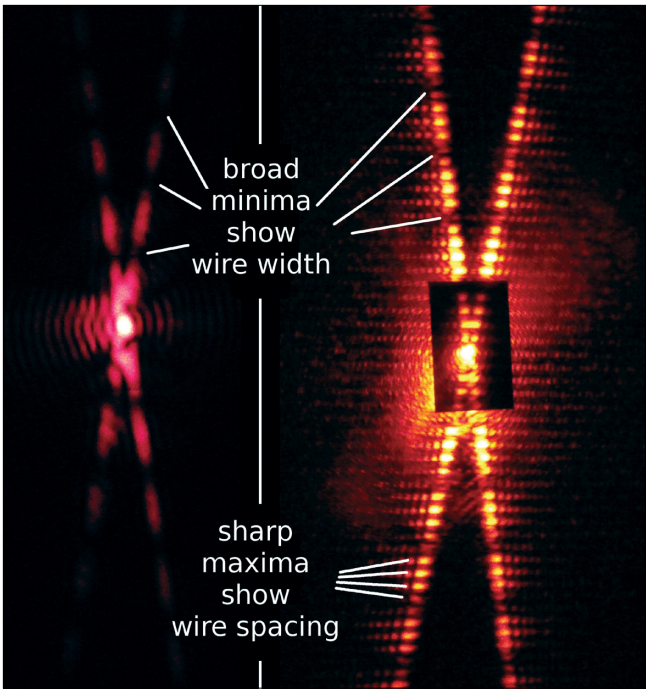

Fig. 4. The left photo is a pattern due to a setup like Experiment 1, and the right like Experiment 2. These were taken with a larger spring-to-screen distance (~12 m) so as to be better photographed. The broad minima can be used to determine the thickness of the wire. The structure in the areas with maxima is caused by the multiple pitch diffraction, and so is visible only in Experiment 2. Note that the center of the right pattern was covered with a polarizer set almost to extinction to avoid overexposure at the very bright center maximum. Franklin used a lead disc for the same purpose in the x-ray photo.

measured its distance to the central bright spot (27 mm), and used Eq. (3) to calculate the grating constant d, i.e., the distance between parallel wires (1.77 mm). Now that the grating constant d and the pitch angle α are known, we can find the pitch P by using the Eq. (2). Measuring the distance of one of the broad minima to the central spot yields the thickness of the wire as in Experiment 1.

The radius can be found as follows: The slope of the projected sine wave pattern is equivalent to the derivative of the sine wave at $R \cdot \sin(2\pi/P \cdot x)$ at $x = 0$, where R is the yet-unknown radius (Fig. 2):

$$R \cdot \frac{d}{dx}\sin(2\pi / P \cdot x)\Big|_{x=0} = R \cdot 2\pi / P \cdot \cos(2\pi / P \cdot 0) = 2\pi R / P. \quad (4)$$

This slope is also equal to the tangent of the complement of α (see Fig 5.):

$$2\pi R/P = \tan(90^{\circ} - \alpha).$$

Since the period P is identical to the pitch, the radius of the helix can be readily determined. Students can measure the diameter of the helical spring with calipers and compare this value with the result that they achieved by diffraction. This is a good time to point out to them that the diameter of DNA or other small chain molecules cannot be measured with calipers and that our sole information stems from diffraction.

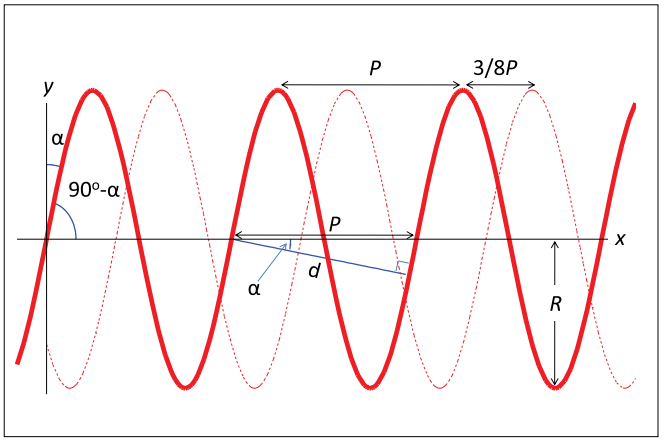

Fig. 5. The relationship among *d*, *P*, and α can be seen here, as well as that between α and the slope of the sine function.

Experiment 3: Simple diffraction calculations with Rosalind Franklin's photo

Students can apply the same considerations to Franklin's *Photo 51*, which should be made available to them in original size (diameter 94 mm). We assumed that Rosalind Franklin used the wavelength of the copper K_α line ($\lambda = 0.15$ nm). This let us work backward and determine a distance between sample and film of about 9 cm.[9] Given these dimensions of the experimental setup, along with the photo, the students can determine the angle, pitch, and radius of the DNA molecule. The zero-order and the first-order maxima had been blocked by a lead disk because they would have overexposed the film. So the order closest to the hole in the center of *Photo 51* is the second order. We used the second, third, and fifth order to calculate the pitch according to Eqs. (2) and (3). Rosalind Franklin correctly attributed the missing fourth order in the diffraction pattern to a second helix, the double helix (see Fig. 5), which is offset by 3/8 of the helical pitch:[1] "The structural unit probably consists of two co-axial molecules which are not equally spaced along the fibre axis,… If one molecule is displaced from the other by about three-eighths of the fibre-axis period, this would account for the absence of the fourth layer line maxima and the weakness of the sixth." The fourth maximum of the pattern due to one helix occurs precisely at the second-order minimum of the pattern due to the spacing between the helices, similarly to how bright spots from the double-slit pattern do not appear when they occur at a minimum of the single-slit pattern. The patterns from each helix are out of phase with each other at this point, and so no light appears. This can be verified by the student with the equation $n\lambda = d\sin\theta$, by showing that the $n = 4$ maximum of the full pitch pattern is at the same angle as the $n = 1.5$ minimum of the 3/8 pitch pattern.

Table I gives an overview over our results for the helical spring of the retractable pen that we used and the DNA.

Experiment 4: Computer simulations of the diffraction pattern

According to Huygens' principle, a diffraction pattern is generated by all elementary waves emerging from an aperture. We can therefore calculate the diffraction pattern by integrating over all sine waves (or cosine waves) emerging in the x- and y-direction from the aperture.[7,8] The helical spring can be approximated by a sinusoidal aperture that is formed by the two functions

$$R\sin\frac{2\pi x}{P}+\frac{a}{2} \quad \text{and} \quad R\sin\frac{2\pi x}{P}-\frac{a}{2}\ ,$$

where $x_1 < x < x_2$. The diffraction pattern $F^2(k_x, k_y)$ is then given by the square of the area integral:

$$F(k_x,k_y)=\int_{x=x_1}^{x=x_2}\int_{y=R\sin\frac{2\pi x}{P}-\frac{a}{2}}^{R\sin\frac{2\pi x}{P}+\frac{a}{2}}\sin k_x x\,\sin k_y y\,dx\,dy\ . \qquad (5)$$

The result is a function of the so-called spatial frequencies k_x and k_y, and can be plotted with k_x and k_y as variables. Students with experience in mathematical software such as Maple or Mathematica may have fun generating this pattern on the computer screen and comparing it to the experimental one. We chose as parameters $R = 2$, $a = 0.5$, $P = 1.5$, $x_1 = -3.5$, and $x_2 = +3.5$, since these reflected our helical spring (in mm, see Table I) and the number of illuminated pitches. We used the exponential form for the waves [$\exp(ikx) = \cos kx + i \sin kx$] and then plotted the absolute value of Eq. (5):

$$abs\left(\int_{x=-3.5}^{x=+3.5}\int_{y=2\sin 4.19x-0.25}^{2\sin 4.19x+0.25}\exp(ik_x x)\exp(ik_y y)\,dx\,dy\right) \qquad (6)$$

We want to point out that Eq. (5) describes the electric field distribution, whereas we see or photograph the intensity of the light. Therefore, Eq. (5) would need to be squared. However, this overemphasizes the central orders and suppresses the outer diffracted structures in the plot. Also, using the difference in two sine waves results in an aperture varying in thickness, which is not the case when we look at the projection of our helical wire. Note that it took a few hours for this to run on our laptop. The correct formula for the diffraction pattern of a helix was calculated by A. R. Stokes[10,11] and is explained in detail in Ref. 12.

These experiments allow the students to understand a vital discovery at the junction of physics, biology, and chemistry, and show an application of diffraction interesting to students of all disciplines. Such interdisciplinary discoveries are invaluable in sparking interest among both physics and non-physics students.

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6. We used a red laser pointer and also a 632.8-nm red laser, but green laser pointers also can be used (available for $8-12 at www.amazon.com or www.ebay.com)
7. E. Hecht, *Optics*, 2nd ed. (Addison-Wesley, 1974). In order to convince students that this is the case, it is useful to show first the diffraction pattern of a slit and then the pattern of a wire that has a similar thickness as the slit.
8. A similar setup for use in spatial filtering is described in A. Eisenkraft, "A closer look at diffraction: Experiments in spatial filtering," *Phys. Teach.* **15**, 199–211 (April 1977).
9. We do not know the details of Franklin's setup. The x-ray wavelength and the distance to the film are based on typical data for x-ray. We think this is close enough to the actual apparatus to illustrate the point.
10. M. H. F. Wilkins, A. R. Stokes, and H. R. Wilson, "Molecular structure of deoxypentose nucleic acids," *Nature* **171**, 738–740 (April 25, 1953).
11. F. Wang, www.maplesoft.com/applications/view.aspx?SID=4902&view=html.
12. C. Kittel, "X-ray diffraction from helices: Structure of DNA," *Am. J. Phys.* **36**, 610–616 (July 1968).
13. When you unscrew the retractable pens from various brands and take out the helical springs, you will discover that the springs vary in pitch, thickness of wire, and diameter. Different sized springs can be used to prevent all groups having the same values. In addition, some pen springs are right handed, while others are left handed. Although the handedness of the helix does not play a role in our simple analysis of the diffraction pattern, it is good to point out the difference, and to note that the DNA helix is right handed, although left handed can also exist in vivo. See for example Jaworski et al., "Left-handed DNA in vivo," *Sci.* **6**, 773–777 (Nov. 1987).

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