Polarization Microscopy: A Useful Tool for Biological Imaging*

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Polarized light microscopy allows for detailed imaging of birefringent structures, such as cell membranes, that are not visible with bright-field imaging[1]. The goal of this project was to view the structure of an onion skin using a polarization microscope. We built the microscope using a light source, condenser lens, 20× objective, and two polarizers. The setup produced about 33× magnification. Compared to bright-field imaging, the polarized image showed a darker background and clearer cell outlines.

I. INTRODUCTION

A brightfield microscope is one of the most common tools used to image biological samples. It works by lighting up the entire specimen, making it simple and efficient for imaging [2]. However, this also means that the resulting images can appear washed out. One limitation of using a brightfield microscope is that it does not capture fine details in transparent samples, showing little difference in brightness between the specimen and the background [2]. As a result, the image often appears faded or nearly invisible unless staining is used to add contrast.

Unlike a bright-field microscope, a polarization microscope can image transparent samples without the need for stains [2]. It works by using two filters called polarizers, which control how light passes through.

Light from the source starts out unpolarized, meaning its electric field oscillates in all directions. It first passes through the first polarizer, which filters the light so that only the electric field oscillating in one direction continues [1]. The filtered light then passes through the specimen. As it travels through the specimen, the electric field of the light is slightly rotated because of the organized structure of the material. This change in orientation allows some light to pass through the second polarizer, since only light that is parallel to its transmission axis can get through. The microscope then creates an image where we can see the difference between the specimen and the background [1].

Here, our goal is to view the structure of an onion skin using a polarization microscope. To test the microscope's capacity to resolve fine structural details, we first needed to confirm that the optical setup could produce a clear, magnified image. This required calculating the microscope's magnification, which is the ratio between the image height (h) and the object height (h), given by the equation

$$M = h'/h, \tag{1}$$

Measuring magnification allowed us to verify that our microscope could enlarge the specimen enough to observe the onion's cell walls.

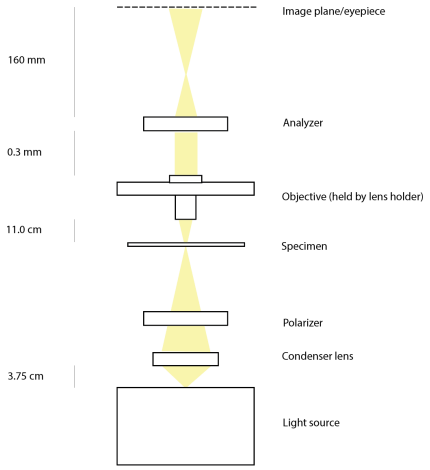

FIG. 1. This is an image of the polarization microscope setup and its light path. *Figure created by Cynthia Feng.*

In this paper, we built a polarization microscope to measure its magnification and use it to view the structure of onion skin. We compared images taken with and without polarizers to examine how polarization affected image contrast and how well the microscope could distinguish between adjacent cells.

II. METHODS

Microscope Assembly: Figure 1 shows the optical path of the polarization microscope used in this experiment. The setup began with a light box (Pasco OS-8470) that served as the light source. A plano-convex condenser lens with a 75 mm focal length was placed 3.75 cm from the light box to focus the light into a parallel beam.

To collect the transmitted light and form a magnified image, we used a 20× objective lens with a 160 mm tube length, numerical aperture (NA) = 0.4, and an estimated working distance of 0.3 mm. The onion skin specimen

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was placed on a standard glass slide and secured in a slide holder 11 cm from the condenser. The slide holder was also placed 0.3 mm from the objective.

Verification of Image Formation To confirm that the optical setup produced a magnified image, we assembled a microscope using the same optical path described in Figure 1, excluding the polarizers. For this test, two strips of clear tape placed 1 mm apart on a glass microscope slide served as the specimen. The slide holder was positioned 11 cm from the condenser, and the resulting image was projected onto a screen placed 160 mm from the objective, which corresponds to the tube length of the 20× objective lens.

The magnification was measured in the control setup by comparing the heights of the objects and images. The height of the object (h) was defined as the 1 mm spacing between the two pieces of tape on the slide. The corresponding image height (h') was measured directly on the projected screen using a ruler. The ratio of h'/h provided the experimental magnification value.

Specimen Preparation Two samples were prepared for this experiment. For the control sample used to measure magnification, two strips of clear tape were placed 1 mm apart on a clean glass microscope slide. For the biological sample used in polarized imaging, a thin layer of red onion skin was peeled from the inner surface of the onion, flattened, and placed on a standard glass slide. The slide was secured in a holder aligned along the optical axis of the microscope.

Imaging we used an iPhone 16 camera set to 10.3× zoom to capture images of the onion skin with and without polarizers. The camera was placed 16 cm from the objective lens, aligned with the optical axis. All microscope components remained fixed during image collection to ensure direct comparison between images taken with and without the polarizers.

III. RESULTS

Microscope Construction:

The polarization microscope was successfully assembled according to the setup described in the Methods section. The light box provided illumination, and the alignment of the condenser, polarizers, and objective produced an image at the expected tube length of 160 mm. Rotating the analyzer by 90° relative to the first polarizer caused a change in image brightness, confirming that the system effectively polarized the transmitted light.

Magnification Measurements:

Magnification was determined using the microscope setup without the polarizers, as the tape slide provided a simple and measurable reference. The two strips of clear tape, spaced 1 mm apart, allowed for direct comparison between object and image dimensions. The measured object height was 0.10±0.02 cm, while the image height was 3.18±0.02 cm. From these measurements, the experimental magnification was calculated to be approximately 33±7×.

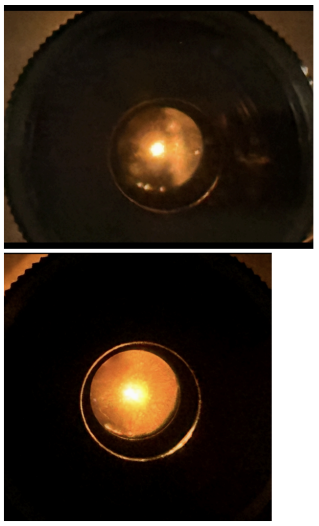

FIG. 2. Images of onion skin captured with an iPhone 16 camera using a 20× objective. The top image shows the specimen with polarizers, and the bottom image shows the specimen without polarizers.

Bright-Field Microscope (Without Polarizers) The image of the onion peel taken without polarizers appears very bright, with significant background light filling the entire field of view. This excess illumination makes it difficult to distinguish the onion tissue from its surroundings. The edge of the onion skin is difficult to distinguish from the background. The interior of the onion peel appears blurry, with fine structural details washed out by glare and stray light. In general, the lack of polarization causes the background and the specimen to blend together, reducing the clarity of the image.

Polarization Microscope (With Polarizers) When polarizers were added to the microscope, the overall background brightness was reduced. This shows that polarization was successfully achieved. The visual contrast between the onion peel and its background was improved, making the edge of the onion skin easier to identify. Although the specimen itself still appeared somewhat blurry, the darker background provided a clearer separation between the onion skin and the background compared to that of the microscope without the polarization filters.

IV. DISCUSSION

Microscope Performance We assembled a polarization microscope to examine how introducing polarizers affects image clarity and contrast. When the analyzer was rotated approximately 90° relative to the first polarizer, the change in brightness confirmed that polarization was achieved. The polarized setup reduced background glare by filtering out much of the unpolarized light, which made the sample's edges easier to distinguish. Some light

was still visible in the background, likely because the two polarizers were not precisely crossed at 90°

Magnification The measured magnification of $33\pm7\times$ was consistent with expectations based on the components used. Although the objective lens was labeled as $20\times$, additional magnification was likely introduced by the iPhone camera, which typically adds around $10\times$ optical zoom. This brings the expected total magnification to roughly $30\times$, closely matching our experimental result.

Biological Specimen To evaluate the imaging performance, we examined a red onion epidermis sample with cell sizes ranging from approximately 130 to 300 μm. According to the Rayleigh criterion, the minimum resolvable distance is given by

$$d = 0.61\lambda/NA \tag{2}$$

, where d is the minimum resolvable distance, λ is the wavelength of the illumination light, and NA is the numerical aperture of the objective lens [3]. Using a wavelength of 500 nm and the stated numerical aperture of the $20\times$ objective (NA=0.4), the theoretical resolution limit is d=$(0.61\times500\text{nm})/0.4= 0.76$ μm. This resolution is sufficient to clearly resolve structural features of onion cells, whose characteristic dimensions are significantly larger than the diffraction limit. This indicates that our microscope had sufficient resolving power to clearly visualize individual onion cells. However, the captured images appeared blurry because the iPhone camera was unable to maintain a consistent focus on the projected image. While the microscope itself produced a visible image, the phone's autofocus kept adjusting and shifting the focal point, making it difficult to capture a sharp photo.

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