Activity 5

MICROSCOPY

Applied Physics 167

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Objectives

1. Capture images of samples through one of the eyepieces of a stereomicroscope.

2. Discuss how (a) a polarizing microscope and (b) a phase contrast microscope work.

Methodology

A stereomicroscope from the Advanced Physics Laboratory was used for this activity. Various objects were selected as samples, including a 1 peso coin, a seashell, and a tablet screen. The magnification was meticulously adjusted to suit each of the specimens, and subsequently, images were captured using a phone camera.

1

from: https://docs.google.com/presentation/d/1m4aH97alejQNiqIYV-wqdxAIbBypNw9pgLhDd4H2x_k/edit#slide=id.p13

Methodology

In the exploration of two distinct microscope types, specifically polarizing and phase contrast microscopes, I researched to understand their configurations, functions, and applications. I provided the summary of the knowledge I acquired in this report.

2

Results & Analysis

(a) (b)

Figure 1. Images of the (a) front and (b) back of a one-peso coin observed on a stereomicrosope.

Figure 1 shows images of a one-peso coin as observed under a stereomicroscope. Evidently, discernable scratches on the coin's surface are noticeable, which are likely due to contact with other objects. By manipulating the magnification, the distinct features of Jose Rizal's visage in Figure 1a become apparent, and one can accurately enumerate the petals of the WalingWaling flowers in Figure 1b. These are features that are not readily observable to the unaided eye.

Results & Analysis

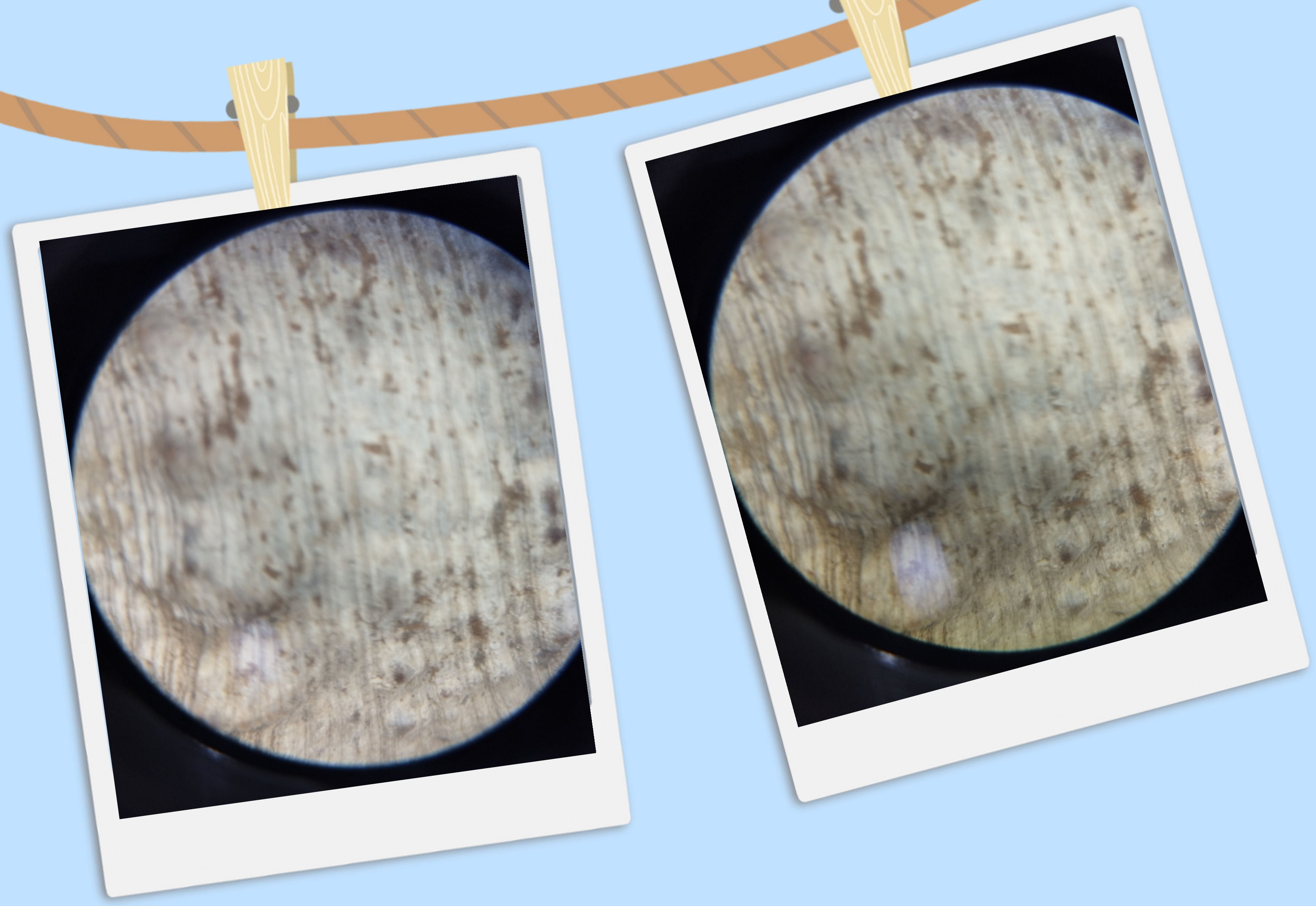

Figure 2. Images of a seashell observed on a stereomicroscope.

In Figure 2, we are presented with images of a seashell, as observed through the right eyepiece of a stereomicroscope. These images reveal distinct lines resembling colorful bands with varying hues. Additionally, the presence of diminutive bumps of diverse sizes is evident. These unique characteristics of the shell offer valuable insights into its biology, ecology, and evolutionary history.

Results & Analysis

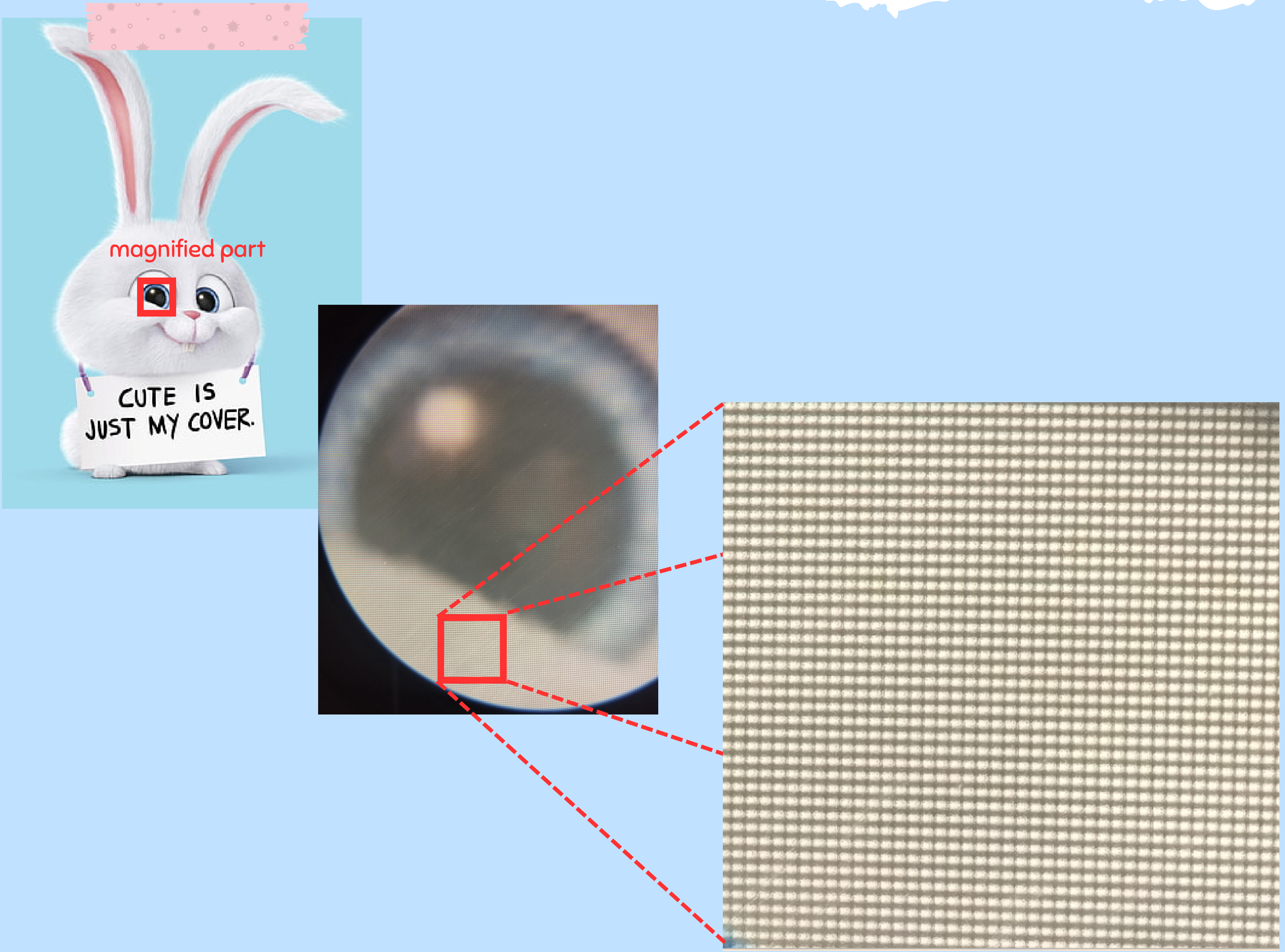

Figure 3. Image of a tablet screen as observed on a stereomicroscope.

Figure 3 shows an image of a tablet screen as observed under a stereomicroscope. I intentionally focused on the “eye” portion of the wallpaper revealing the intricate interplay of various blue shades that constitute the iris. Furthermore, upon closer examination, individual pixels constituting the screen’s display become discernible, manifesting as diminutive, well-defined squares. It is worth noting that, thus far, no pixel irregularities or defects have been observed *phew*.

Polarizing Microscope

Polarized light microscopy enhances image quality for birefringent materials compared to other techniques like darkfield, brightfield, and phase contrast. It's useful for qualitative and quantitative studies on various anisotropic specimens. Qualitative polarizing microscopy is common, while quantitative aspects are typically limited to specific fields like crystallography. Recent advancements have also made it accessible for biologists to study anisotropic sub-cellular assemblies [1].

https://www.microscopeworld.com/t-polarizing_microscopes.aspx

Figure 4. Eclipse LV100N POL

Polarizing Microscope

ISOTROPIC

diamond

ANISOTROPIC

sapphire

Figure 5. Examples of isotropic and anisotropic minerals.

This technique distinguishes between isotropic and anisotropic substances and provides information on absorption color and optical path boundaries based on refractive indices. Isotropic materials have consistent optical properties in all directions, while anisotropic materials, which make up the majority of solid substances, vary their optical properties with light orientation, refractive indices, and vibrational planes. Polarizing microscopy exploits the interference of split light rays, revealing details about anisotropic materials [1].

Polarizing Microscope

The microscope consists of a polarizer before the specimen and a second polarizer, called an analyzer, between the objective rear aperture and the observation tubes or camera port. Image contrast results from the interaction of plane-polarized light with the birefringent specimen, producing two wave components. These components, known as ordinary and extraordinary wavefronts, have different velocities and directions as they pass through the specimen. They exit the specimen out of phase but are recombined through constructive and destructive interference by the analyzer. Figure 6 outlines these concepts and the essential optical and mechanical components of a modern polarizing microscope [1].

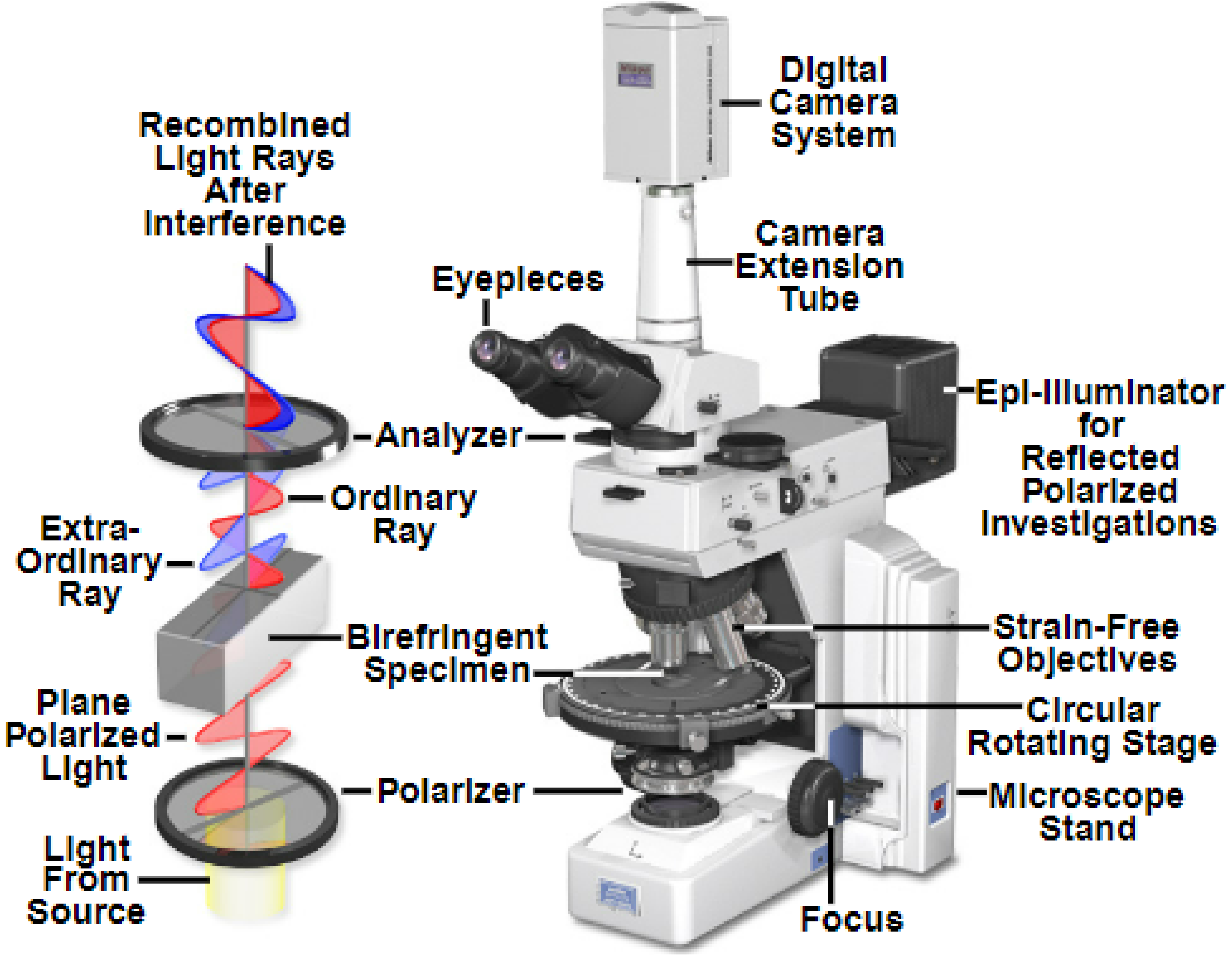

Figure 6. Configuration of the Polarizing Microscope.

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Polarizing Microscope

Modern polarized microscopes typically include a lamphouse with a 50 to 100-watt tungsten-halogen lamp, controlled by a built-in transformer and a potentiometer for voltage regulation. A filter cassette houses color correction, heat, and neutral density filters between the lamphouse and the base. The base contains a collector lens, field iris aperture diaphragm, and a first surface reflecting mirror to control light size, intensity, and distribution for Köhler illumination. This setup ensures vibration-free, controlled light, producing even illumination despite the lamp's uneven spectrum [2].

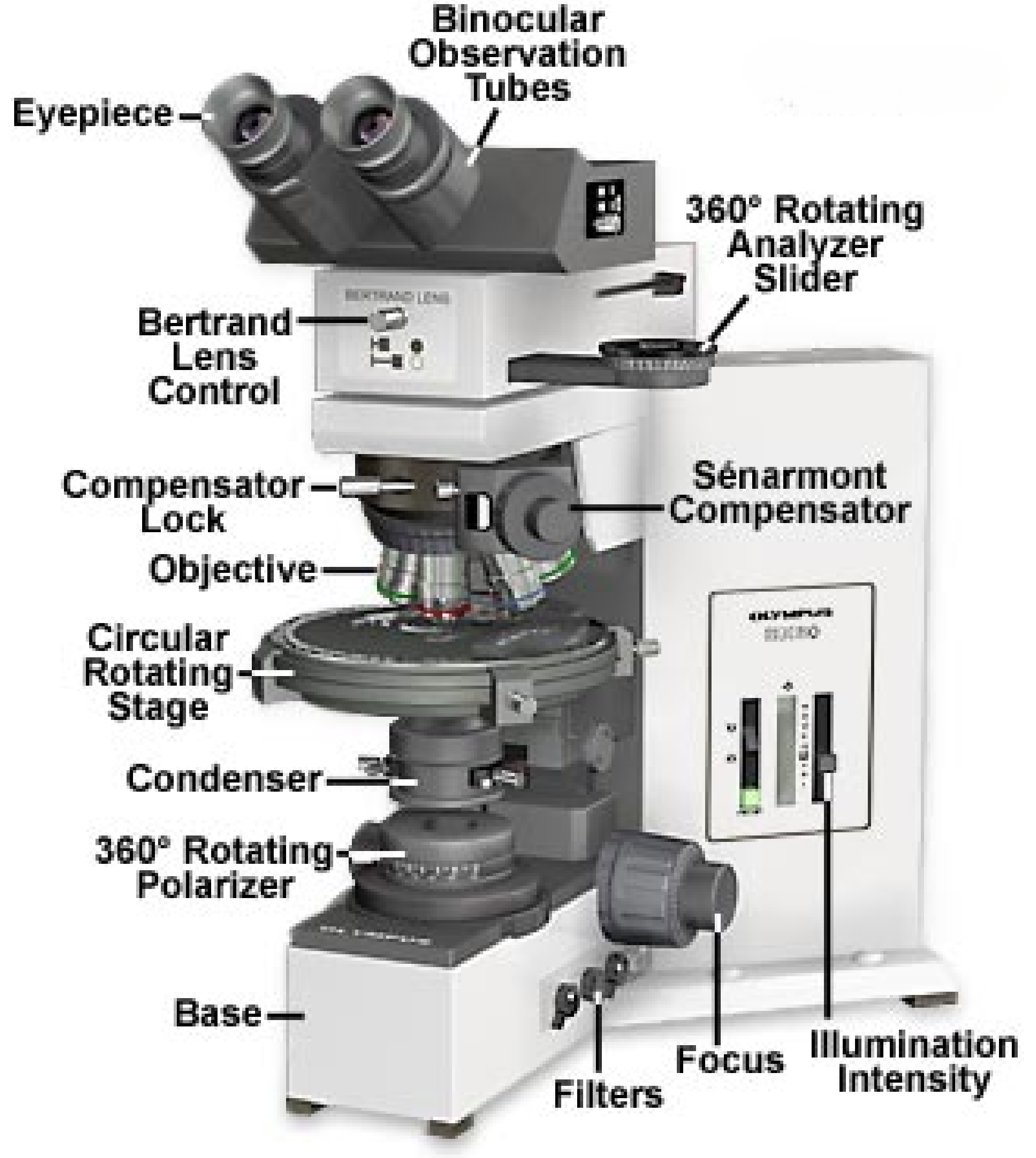

Figure 7. Modern polarizing microscope configuration.

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Polarizing Microscope

In certain polarized light microscopes, an external light source can be directed at a plano-concave substage mirror, angled toward the polarizer under the condenser aperture, which is useful for monochromatic light sources. This adaptation allows for observing weakly birefringent specimens with high-intensity light sources, unconstrained by a permanent tungsten-halogen lamp [2].

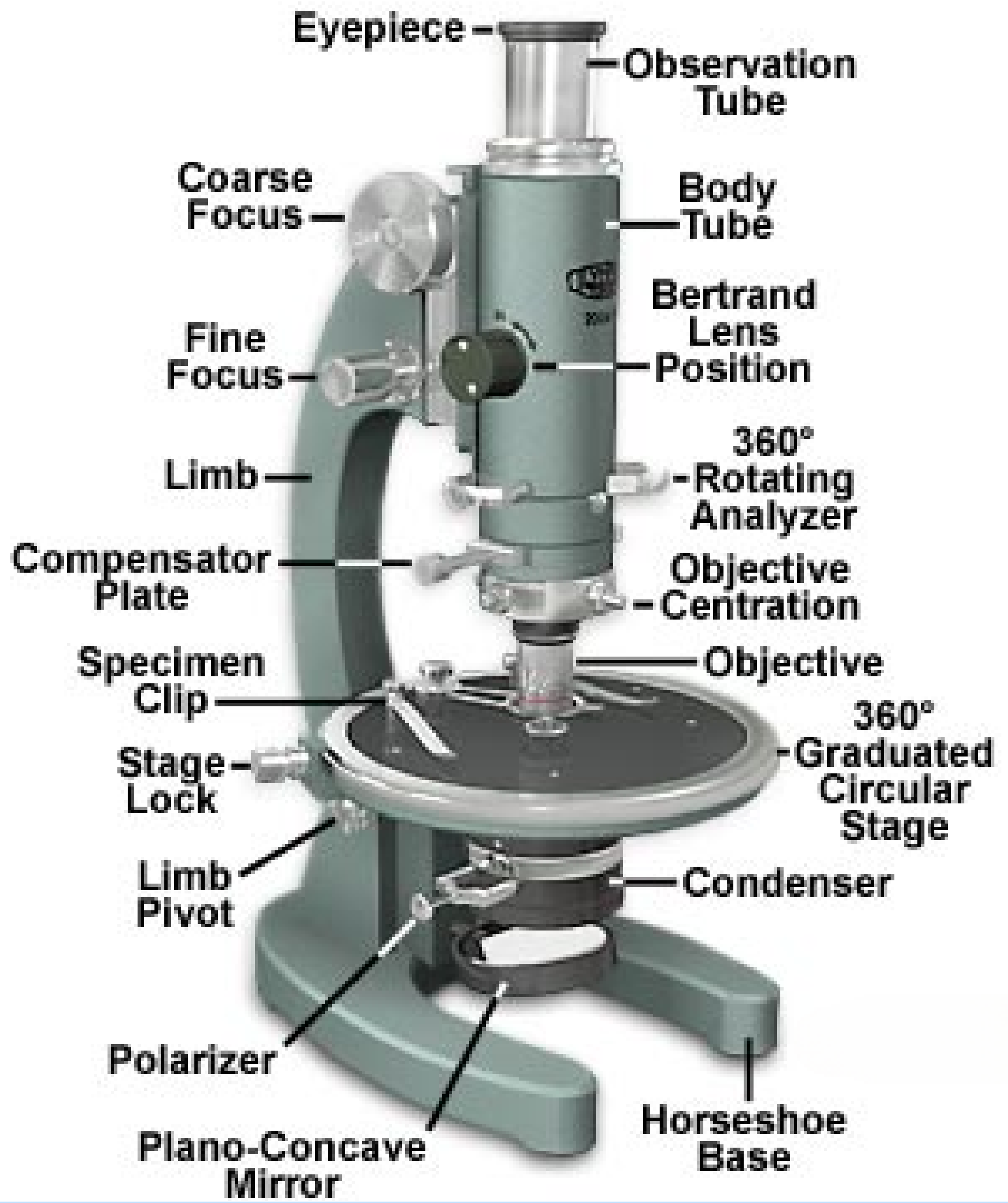

Figure 8. Monocular polarizing microscope configuration

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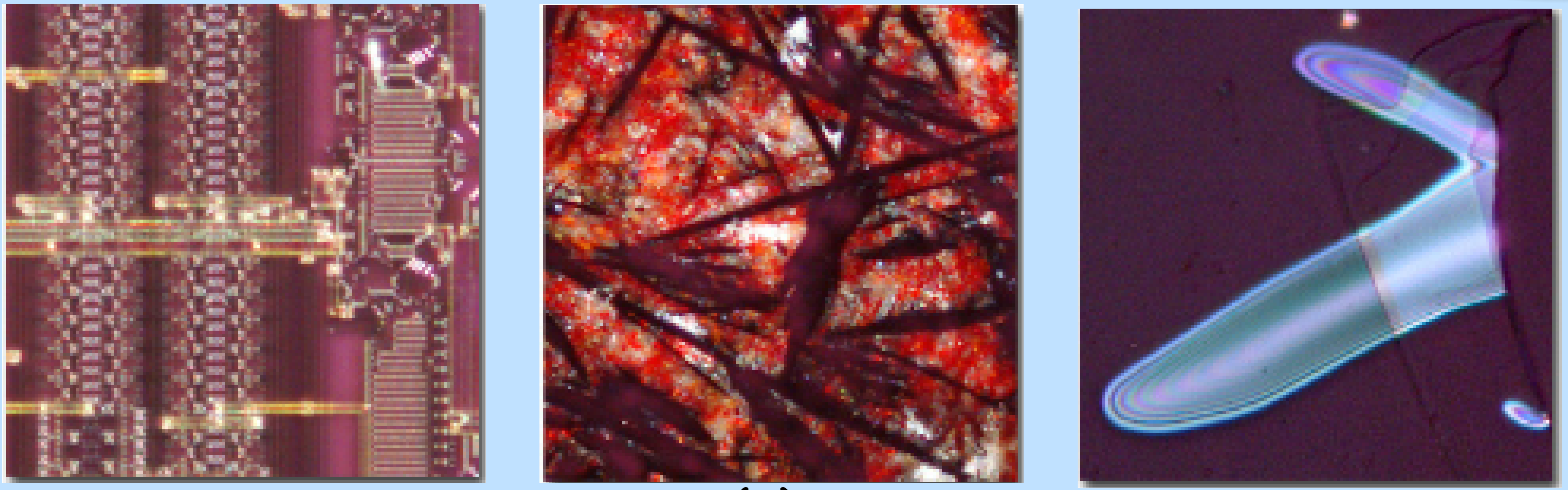
Polarizing Microscope



(a) (b) (c)

Figure 9. Reflected polarized light photomicrographs of (a) microprocessor, (b) bismuth base, and (c) blisters in thin film of copper.

While polarized light microscopy is often associated with geological mineral analysis in thin rock sections, it offers versatile applications across various fields. It can effectively examine natural and industrial minerals, cement composites, ceramics, mineral fibers, polymers, starch, wood, urea, and biological macromolecules. This technique is valuable for both qualitative and quantitative analyses, making it a powerful tool in materials science, geology, chemistry, biology, metallurgy, and even medicine. Figure 9 shows photomicrographs of some specimens viewed under a polarizing microscope.

Phase Contrast Microscope

Phase contrast microscopy, developed by Dutch physicist Frits Zernike in 1934, is an optical technique enhancing contrast to produce high-contrast images of transparent specimens like living cells, microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles. One of its most notable advantages is that it enables the observation of ongoing biological processes in living cells without the need for fixation, staining, or killing the organisms. This provides sharp clarity of specimen details, making phase contrast microscopy invaluable for studying dynamic biological processes [3].

Figure 10. Phase contrast microscope OBL series OBL 145 binocular

https://www.microscopeworld.com/t-polarizing_microscopes.aspx

Phase Contrast Microscope

The core concept in phase contrast microscope design involves separating surround and diffracted wavefronts from the specimen and introducing a quarter-wavelength phase shift to enhance specimen-background contrast. This is achieved by employing specialized accessories, including an annular diaphragm in the condenser front focal plane and a phase plate in the objective rear focal plane [3].

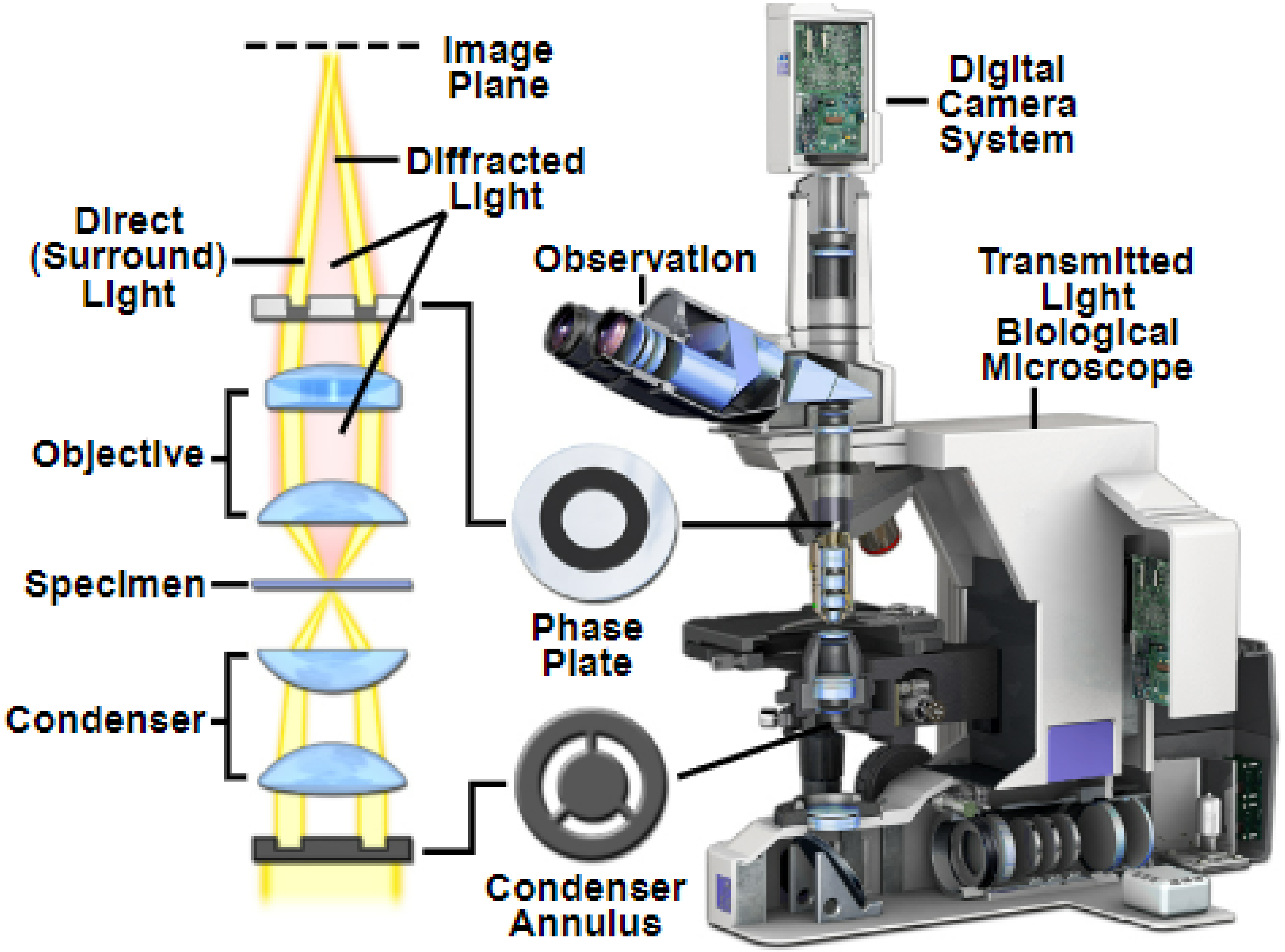


Figure 11. Phase contrast microscope configuration

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Phase Contrast Microscope

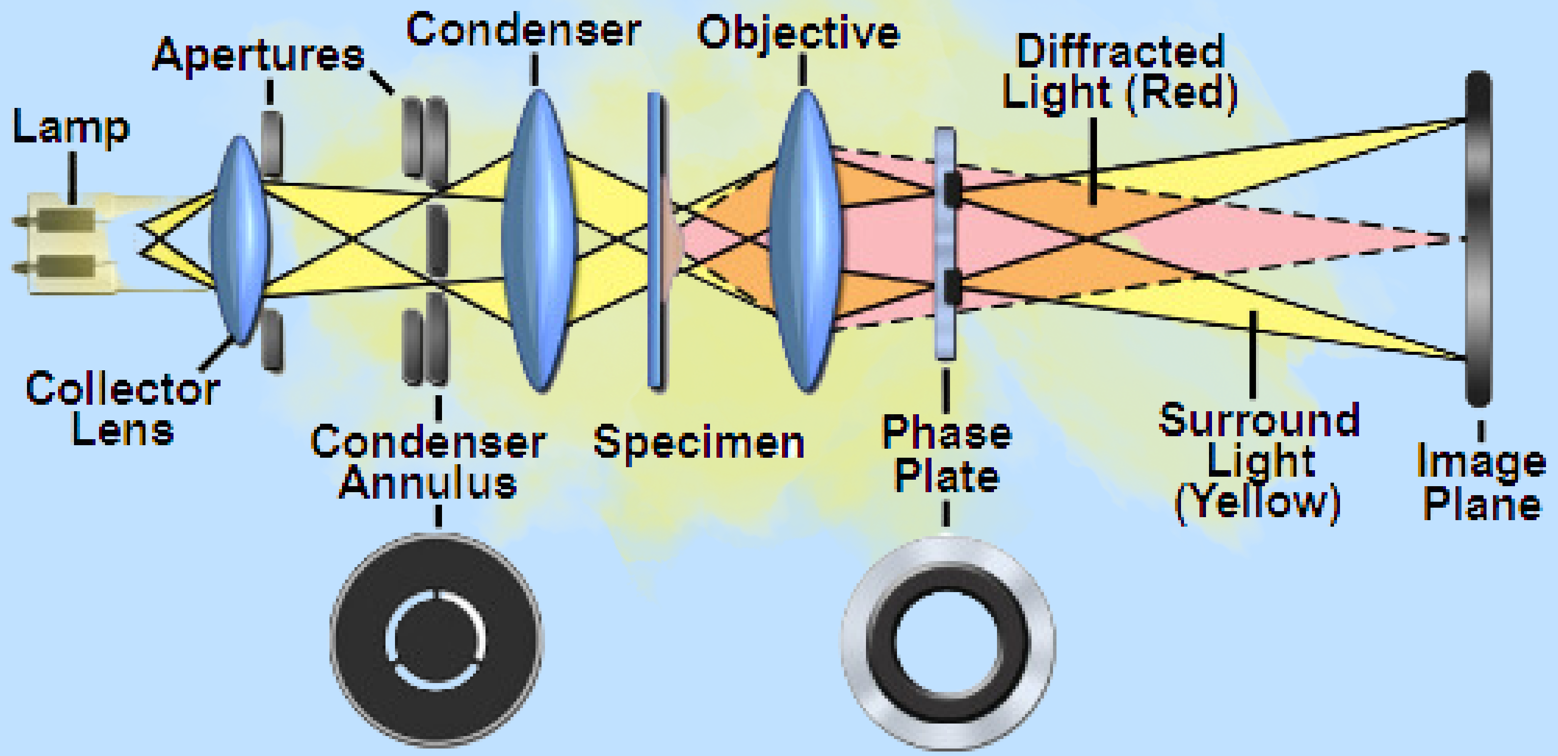

Figure 12. Phase contrast microscope optical train

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The phase contrast microscope uses a specialized condenser annulus, consisting of an opaque flat-black plate with a transparent ring, positioned in the front focal plane of the condenser to illuminate the specimen with defocused, parallel light waves. In Köhler illumination, non-diffracted light forms a bright image of the annulus at the rear aperture of the objective, while diffracted light passes through the objective rear aperture, depending on specimen characteristics. The spatial separation of these wavefronts in the objective rear focal plane allows for selective manipulation of the phase of either component (surround or diffracted), without interference, contributing to phase contrast's effectiveness in specimen observation without orientation-dependent artifacts. Moreover, phase contrast is insensitive to polarization and birefringence, making it advantageous for examining living cells in plastic tissue culture vessels [3].

Phase Contrast Microscope

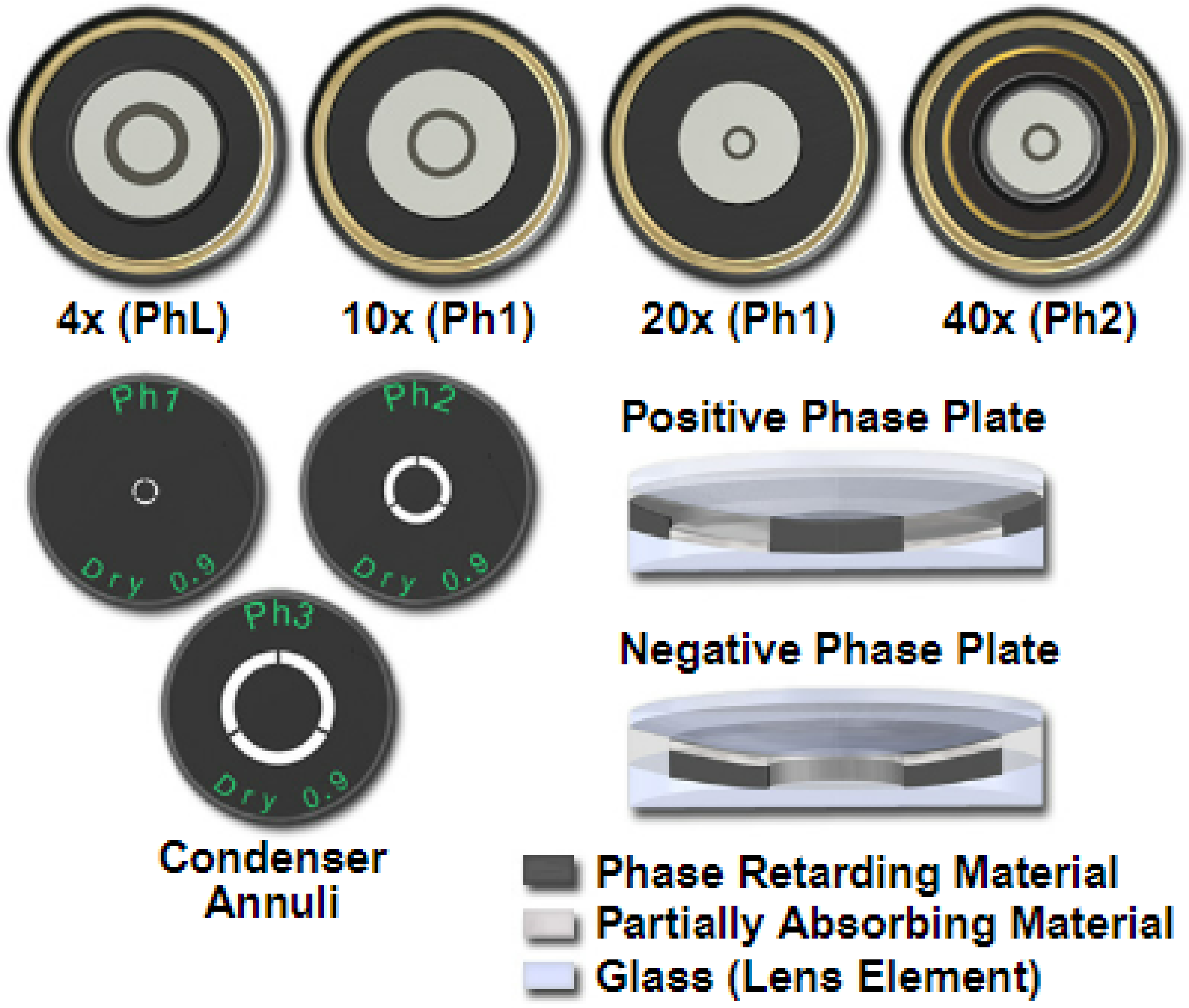
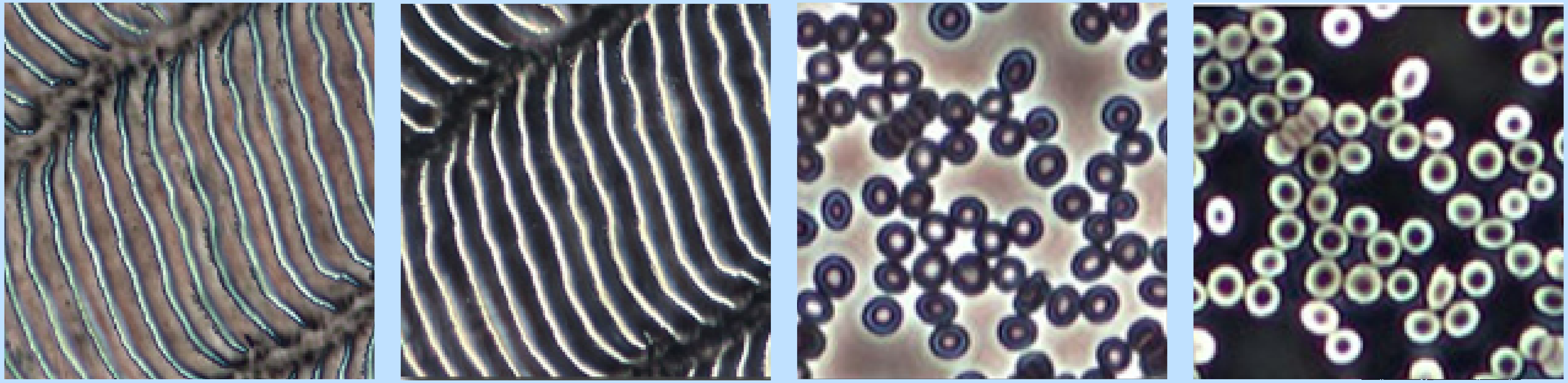


Figure 13. Objective appertures and phase contrast optics of a phase contrast microscope

In phase contrast microscopy, a phase plate near the objective rear focal plane selectively modifies surround light's phase and amplitude. It usually has a ring with reduced thickness, coated in a metallic film, unique to phase contrast objectives. Different configurations control the surround and diffracted wavefronts. The phase plate's conjugate area alters surround light's phase, and it's wider than the annulus image to limit spread. With higher numerical aperture and magnification, phase plate dimensions decrease, while the annulus size increases. There are positive and negative phase plate types, with the positive creating dark contrast and reducing surround wavefront amplitude, and the negative attenuating and retarding the undiffracted surround wavefront by 90 degrees [3].

https://www.microscopeworld.com/t-polarizing_microscopes.aspx

Phase Contrast Microscope



(a) (b) (c) (d)

Figure 14. Specimens viewed under the phase contrast microscope in positive and negative contrast.

In phase contrast microscopy, when thin, evenly distributed specimens are examined using positive phase contrast, they appear darker than the surrounding medium if their refractive index is higher. This enhances contrast near specimen edges, creating high-contrast images resembling density maps. In contrast, negative phase contrast attenuates and retards the surround light, causing the specimen to appear brighter than the medium. Both techniques rely on variations in refractive index and optical path length for image contrast. Organelles with increasing density appear progressively darker in positive phase contrast, while negative phase contrast brightens the specimen. Artifacts and contrast fluctuations can be present, particularly in large specimens, and symmetry influences how specimens appear [3]. Figure 14a and 14b shows the ctenoid fish scale in positive and negative contrast, respectively, while Figure 14c and 14d shows the human erythrocytes in positive and negative contrast, respectively.

https://www.microscopeworld.com/t-polarizing_microscopes.aspx

Phase Contrast Microscope

Phase contrast microscopy has widespread applications in biological and medical research, particularly in cytology, histology, tumor cell diagnosis, and cell culture studies. It's also valuable in areas like hematology, virology, bacteriology, parasitology, paleontology, and marine biology. In industrial and chemical contexts, it aids in mineralogy, crystallography, polymer morphology, and the examination of microcrystals, powders, particulate solids, and various commercial products, including clays, oils, paints, foods, and textiles. This technique is utilized for refractive index measurements and identification purposes [3].

Figure 10. Phase contrast microscope OBL series OBL 145 binocular

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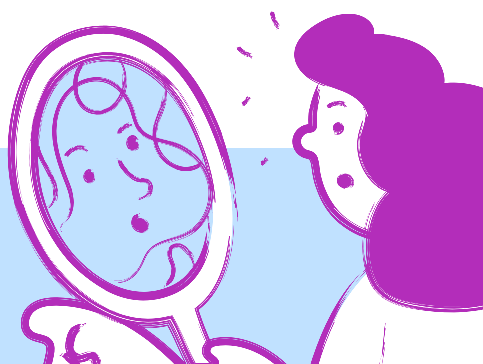
Conclusions

This activity involved presenting images captured through a stereomicroscope's eyepiece, along with detailed discussions on the functions and applications of two specific microscope types: polarizing and phase contrast microscopes. Modern microscopes have significantly impacted various fields by rendering tiny objects visible through the manipulation of light and adherence to optical principles. It's crucial to note that selecting the appropriate microscope for the intended specimens is essential due to their varying applications. In conclusion, the world of microscopy offers a fascinating journey into the microscopic realm, where each instrument, like the polarizing and phase contrast microscopes discussed here, brings to light the intricate beauty and hidden wonders of the unseen world, opening up endless possibilities for exploration and discovery.

Reflection

This is so far the most chill activity that I did. Also, the one of the most fun in my opinion because I get to experience observing an object through a stereomicroscope. It was amazing to see such fine details that I never would've seen clearly, thanks to my defective eyes, without the aid of the microscope. Moreover, I was able to acquire new knowledge about the functions and applications of the two microscopes that I chose. I randomly chose these two and I'm happy with my decision because it's cool what they can do. I'm particularly amazed with the phase contrast microscope because we get to see live microorganisms and you can even choose if you want to view it in a negative or positive contrast. Overall, I enjoyed doing this activity and it would be amazing actually to get to try these microscopes sometime in the future.



Self-Grade

CRITERIA	perfect score	my score
Technical correctness	30	30
Quality of presentation	30	30
Reflection	30	30
Ownership	10	10
TOTAL	100	100

I give myself a perfect score because I was able to achieve all the objectives for this activity. I gave insights to each of the specimens that I used for the first objective. Moreover, I thoroughly discussed the concepts, functions, and applications of the two microscopes that I chose.

References:

1. Polarized light microscopy. Nikon's MicroscopyU. (n.d.). https://www.microscopyu.com/techniques/polarized-light/polarized-light-microscopy
2. Microscope configuration. Polarized Light Microscopy - Microscope Configuration | Olympus LS. (n.d.). https://www.olympus-lifescience.com/en/microscope-resource/primer/techniques/polarized/configuration/
3. Polarizing microscope. Polarizing Microscopes Information |Microscope World.(n.d.). https://www.microscopeworld.com/t-polarizing_microscopes.aspx