LABROTORY INSTUMENTATION AND TECHNIQUES

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LECTURE THREE
POLARIZATION MICROSCOPE& ELECTRON MICROSCOPE

Polarization microscopy

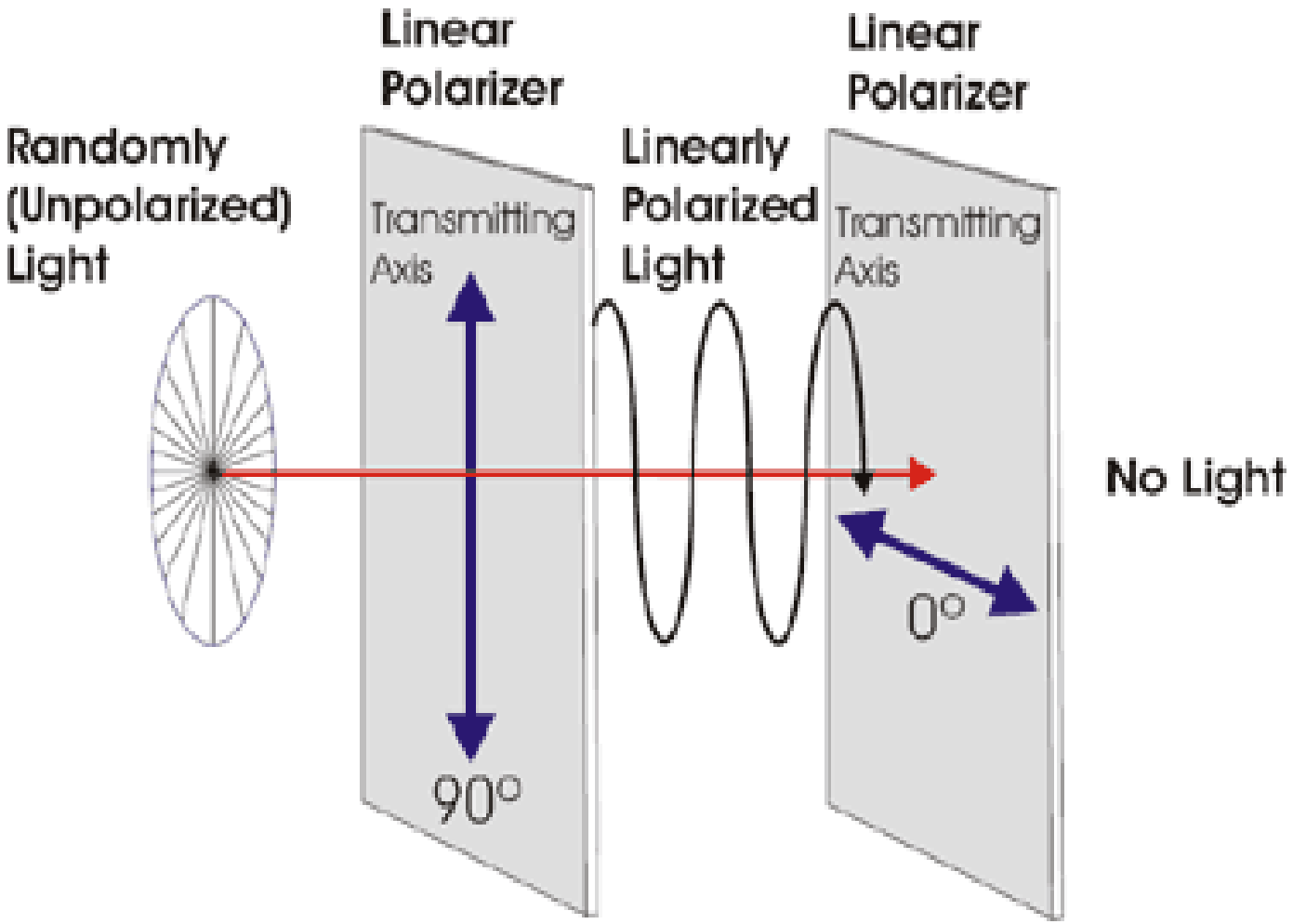
is an optical technique used to make birefringent structures like starch grains or cellulose visible without staining.

Component of polarized microscope:

Besides optical component like the condenser aperture diaphragm and objective, some additional components have to be inserted into the light path to achieve polarized microscopy:

1. Polarizer: is an optical filter located under the condenser that passes light oscillating within single plane" linearly polarized light".

2. The analyzer: another polarizer filter located after the objective lens, and rotated 90° relative to the polarizer.


Linear
Polarizer
Linear
Polarizer
Randomly
(Unpolarized)
Light
Transmitting
Axis
Linearly
Polarized
Light
Transmitting
Axis
No Light
90°
0°

Principle:

1. The first polarizer produces polarized light which is focused on the specimen by the condenser
2. When the polarized light passes through **birefringent material in the specimen, the polarization plane of a portion of the light is turned 90° ,light that did not pass through birefringent material can have any polarization plane.**
3. The image is then magnified by the objective lens
4. The light then will pass through the analyzer which is rotated 90° compared to the first polarizer, light that did not pass through birefringent material will be filtered out since it does not have a polarization plane of 90° .while light passing by birefringent material will be able to pass through the polarizer since it is rotated by 90° and continuous to produce the image.

Fluorescent Microscopy

Fluorescence Microscopy is a special form of light microscopy. It uses fluorescence to highlight structures in fixed and living biological specimens instead of using absorption.

Applications:

These microscopes are often used for -

Imaging structural components of small specimens, such as cells

Conducting viability studies on cell populations (are they alive or dead?)

Imaging the genetic material within a cell (DNA and RNA)

Viewing specific cells within a larger population with techniques such as FISH

Electron microscope (EM) is a type of microscope that uses an electron beam to illuminate a specimen and produce a magnified image.

It has a greater resolving power than a light microscope and can detect the structure of smaller objects. They can achieve better than **50 picometer resolution and magnifications of up to about 10,000,000x whereas ordinary light microscopes are limited to about 200 nm resolution and magnifications below 2000x.**

Electron microscopes are used to investigate the structure of a wide range of biological and inorganic specimens including microorganisms, large molecules, biopsy samples, metals, and crystals.

The main component of electron microscope :-

1. Electron gun (cathode): a heated filament or crystal, made from the metal tungsten that releases electrons when a high voltage is pass through.

2. Electromagnetic lens: is a coil of wire through which current flows, creating magnetic fields that manipulate the electron beam, much the same way that optical lenses focus and direct light.

3. Anode: to accelerate the electron beam. 2

4. Vacuum system to ensure that the microscope is operated under a high vacuum to maintain the integrity of the electron beam.

5. Camera or detector

6. Computer

Principle

1. The beam is produced by the electron gun at the top of the instrument.

2. The electron beam then passes down through the vacuum system.

3. Because electrons change their path when pass in electromagnetic field (electromagnetic lens) the beam can be focused.

4. First lens is a condenser which focuses the beam of electron on the specimen.

5. Some electrons interact with the specimen and are modified while other crosses the specimen without interacting.

6. Electron passing through the specimen reaches the objective lens, which form a focused magnified images that is then magnified further through other lenses and captured on screen

Types

1.Transmission electron microscope is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen that permits high resolution , this high resolution allow magnification of up to 400,000 times with isolated molecules or particles

2. Scanning electron microscope is a microscopy technique permits a three dimensional image of the surfaces of cell, tissue, and organs. Like the transmission electron microscope this microscope produces and focuses a very narrow beam of electrons, but in this type the beam does not pass the specimen. Instead the surface of the specimen is first dried and coated with a very thin layer of metal which electron interact with and produces reflected electrons these are captured by the detector and results a black and white image on monitor.

Clinical features

Anemia, fatigue, depression, low grade fevers

Dehydrated/cracked and pale lips and **dark circles around the eyes**

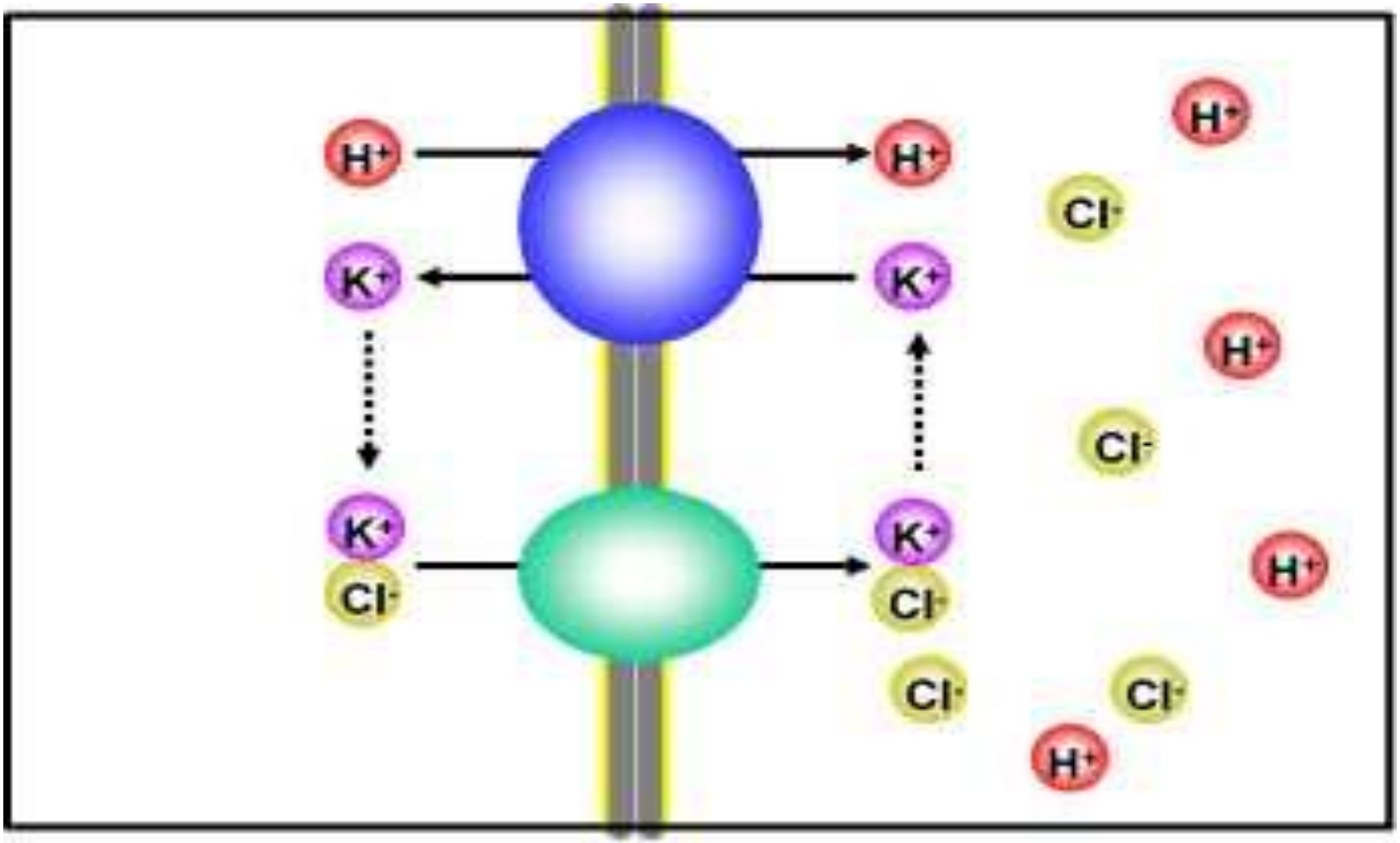
difficulty in proprioception, memory changes and sluggish responses, referred to as **brain fog**

Etiology

Genetic factor

Autoimmune factors:- An autoimmune condition in which the body's immune system attacks intrinsic factor (IF) protein or the cells that make it, by producing an auotoantibodies against intrinsic factor, this substance attaches itself to vitamin B12, and both are absorbed in combination into the lowest portion of the small bowel (*ileum*), just before the small bowel enters the colon.

Although the exact role of *Helicobacter pylori* infection in PA, evidence indicates *H.pylori* is involved in the pathogenesis of the disease. A long-standing *H. pylori* infection may cause gastric autoimmunity by a mechanism known as **molecular mimicry**. Antibodies produced by the immune system can be **cross-reactive** and may bind to both *H. pylori* antigens and those found in the gastric mucosa. The antibodies are produced by activated B cells that recognize both **pathogen** and **self-derived peptides**


H+
K+
K+
Cl-
H+
K+
K+
Cl-
H+
Cl-
H+
Cl-
H+
Cl-
Cl-
H+
Parietal cells of gastric mucosa
Lumen of stomach