



IMMUNOHISTOCHEMISTRY

INTRODUCTION TO IMMUNOHISTOCHEMISTRY

Dr. Ahmad H. Ibrahim

- Semester 2

- Week 1

Date 1/2/2024

University of TISHK

Immunohistochemistry

Introduction to immunohistochemistry

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Objectives

At the end of the class, students should be able to:

- Define Immunohistochemistry
- Elucidate the goals of immunohistochemistry
- Appreciate the historical development of histochemistry as a scientific discipline
- Identify the related disciplines to Immunohistochemistry
- Classify the different methods used in Immunohistochemistry

Course Outline

LECTURE ONE:

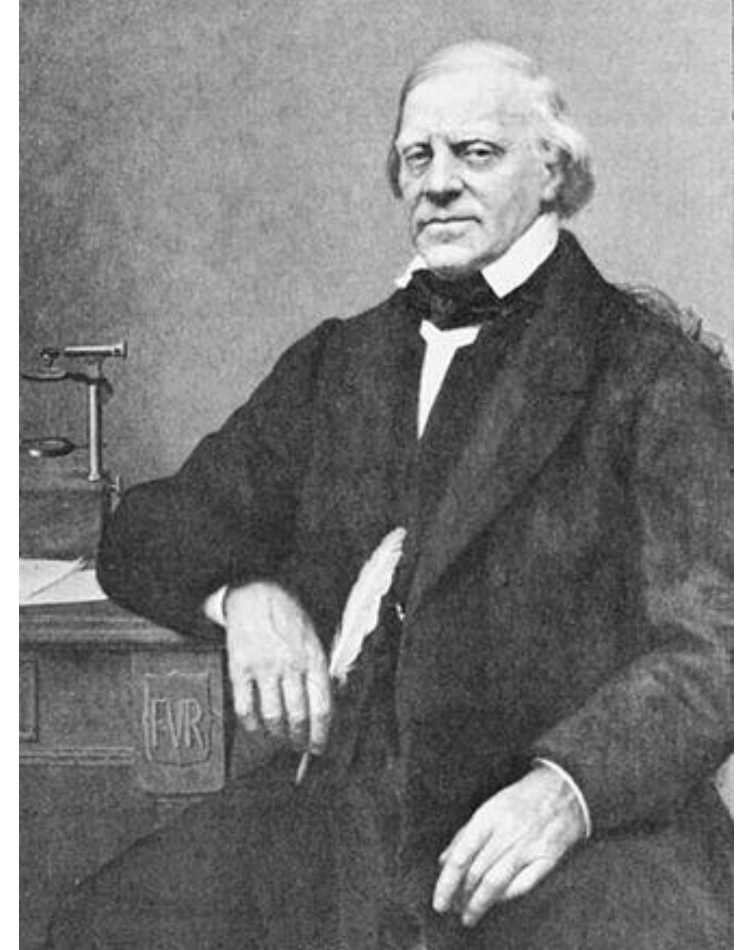
- 1.1 Historical review on immunohistochemistry and cytochemistry
- **1.2 Classification of histochemical methods**
 - 1.2.1 Chemical methods
 - 1.2.2 Physical methods
 - 1.2.3 Biological methods

What is Immunohistochemistry?

- *Histo* = histology: *chemistry* = chemical reactions
- The branch of science concerned with the qualitative and quantitative assessment of chemical compounds in a cell/tissue using stains/dyes and microscopy.
- It is a marriage between bio/chemistry and cyto/histology

Historical Review on Immunohistochemistry

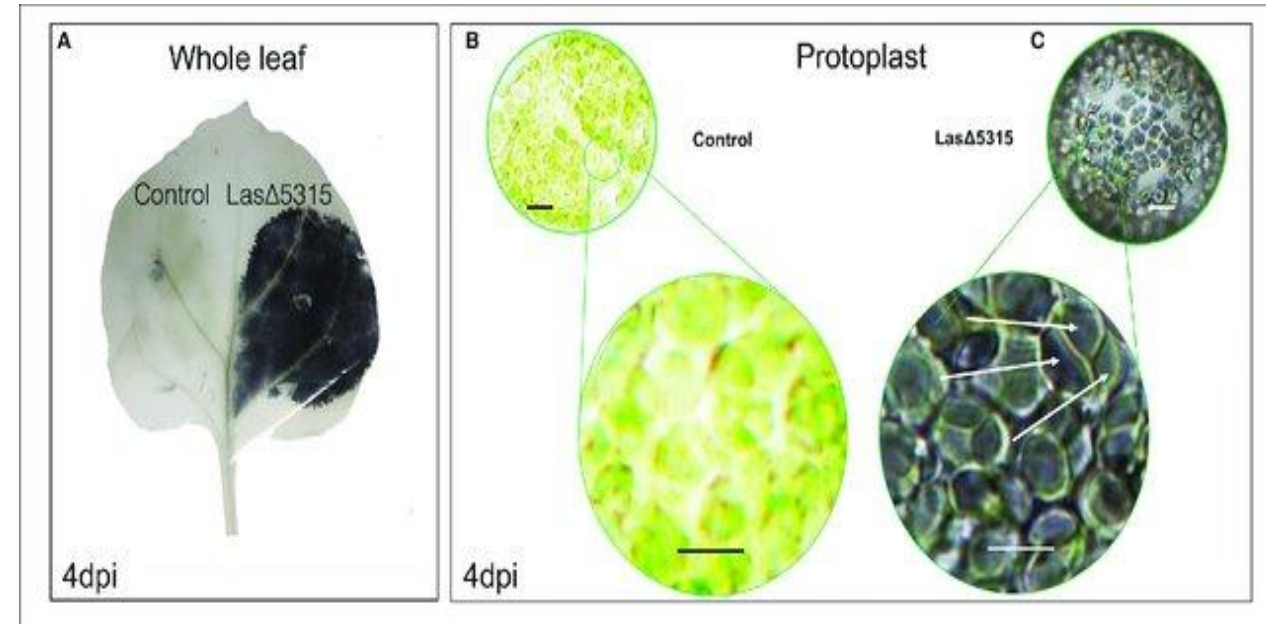
- Histochemistry/cytochemistry is as old as histology itself
- In early 19th century, Immunohistochemical researches to study chemical components of biological structure in combination with chemistry and biology started first in botany in France (Raspail, 1825).
- Raspail was one of the founders of the cell theory in biology. He coined the phrase *omnis cellula e cellula* ("every cell is derived from a [preexisting] cell")



Historical Review on Immunohistochemistry

He stained starch in plant tissues blue with potassium iodide solution under the light microscope and demonstrated its localization microscopically

Then, he published an essay on microscopic chemistry for the first time (Raspail, 1825)



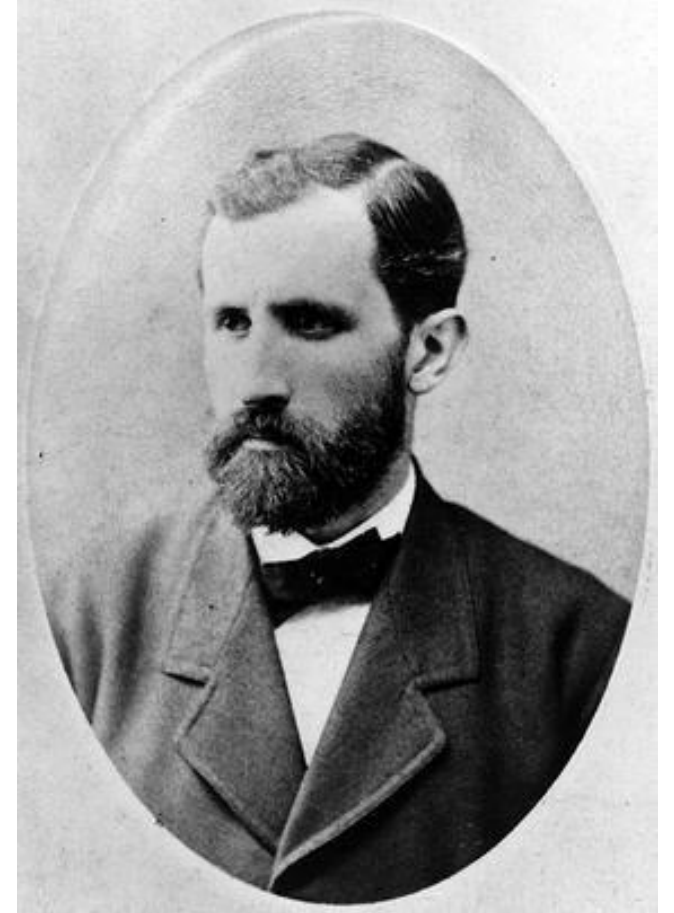
Historical Review on Immunohistochemistry

Later, from the 1840's to 1870's, histochemistry in zoology and medicine was developed mainly as biological chemistry, together with histology (Lehmann, 1842).



Historical Review on Immunohistochemistry

Among these zoologists, anatomists and pathologists who were at that time interested in analyzing chemical constituents in animal tissues including human, Miescher (1874) was the first to introduce cell fractionation to analyze nucleic acids in nuclei of leukocytes.



Fredric Miescher, He discovered DNA

Historical Review on Immunohistochemistry

During these times in the 19th century, this new field was called as “microchemie” in French or “Mikrochemie” in German, which meant microchemistry in English.

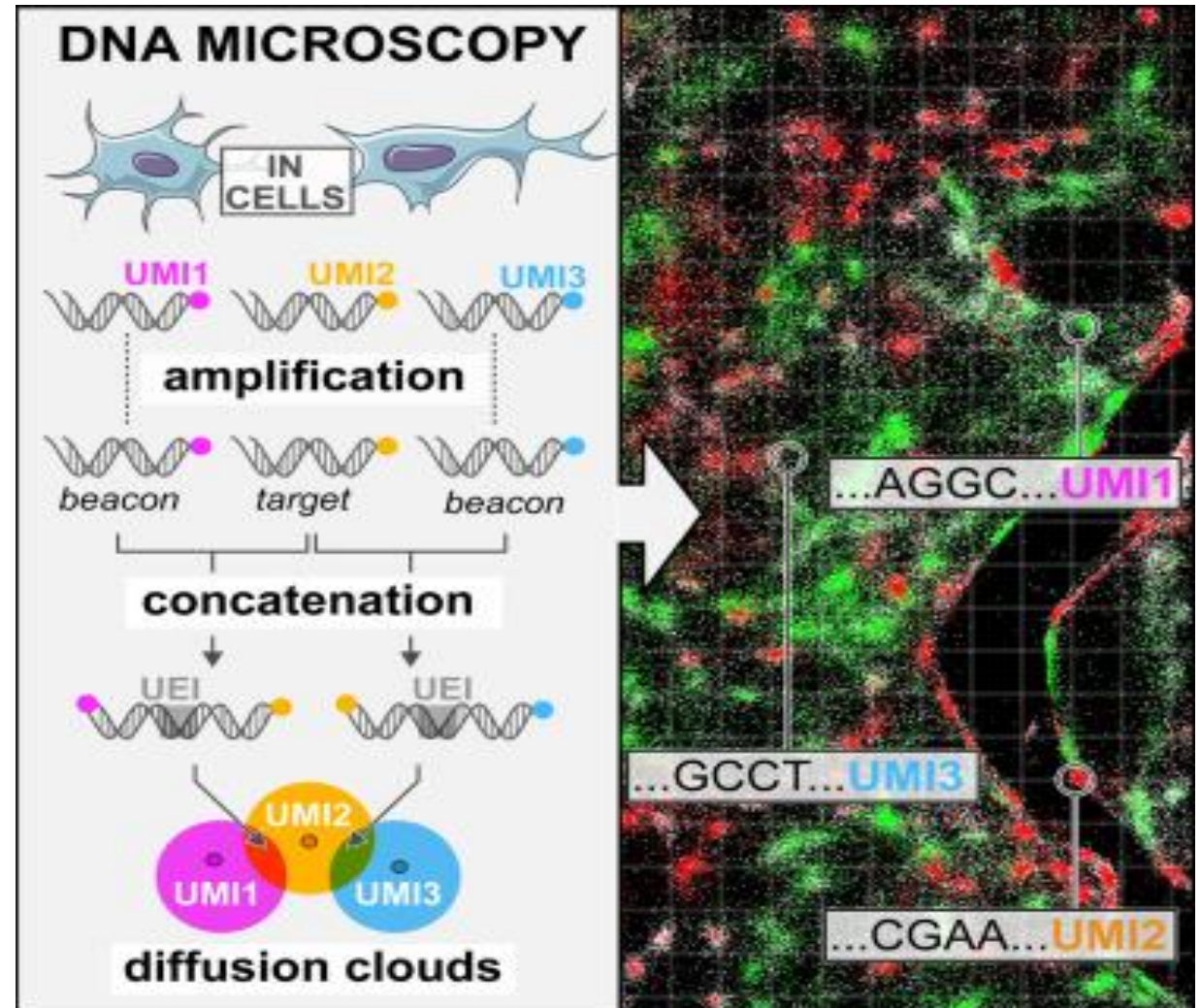


Mikrochemie

Der Beginn des Nano-Sturms in der Welt!

Historical Review on Immunohistochemistry

- Microchemistry was, in other words, microscopic chemistry or chemical microscopy and meant to observe chemical reactions *in situ* under microscopy.



Historical Review on Immunohistochemistry

In early 20th century, aniline dyes were frequently used to stain tissues in anatomy and pathology.

- Histologists and pathologists were much interested in new dyes and less interested in histochemistry at that time.



**HAVE A NICE
DAY!**



University of TISHK

Immunohistochemistry

Introduction to Immunohistochemistry

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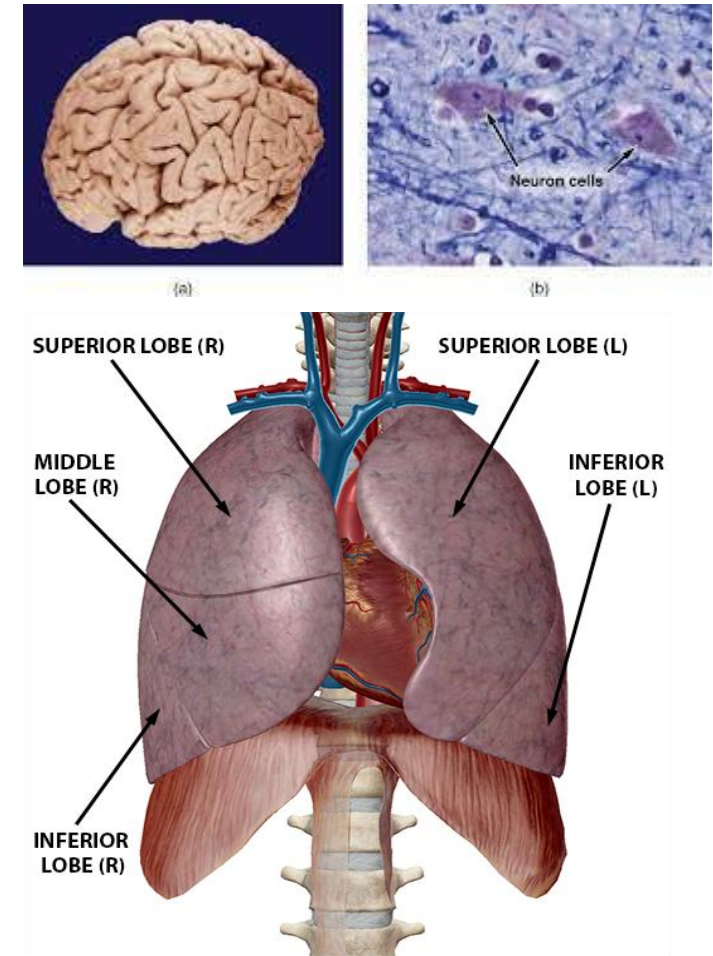
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Objectives

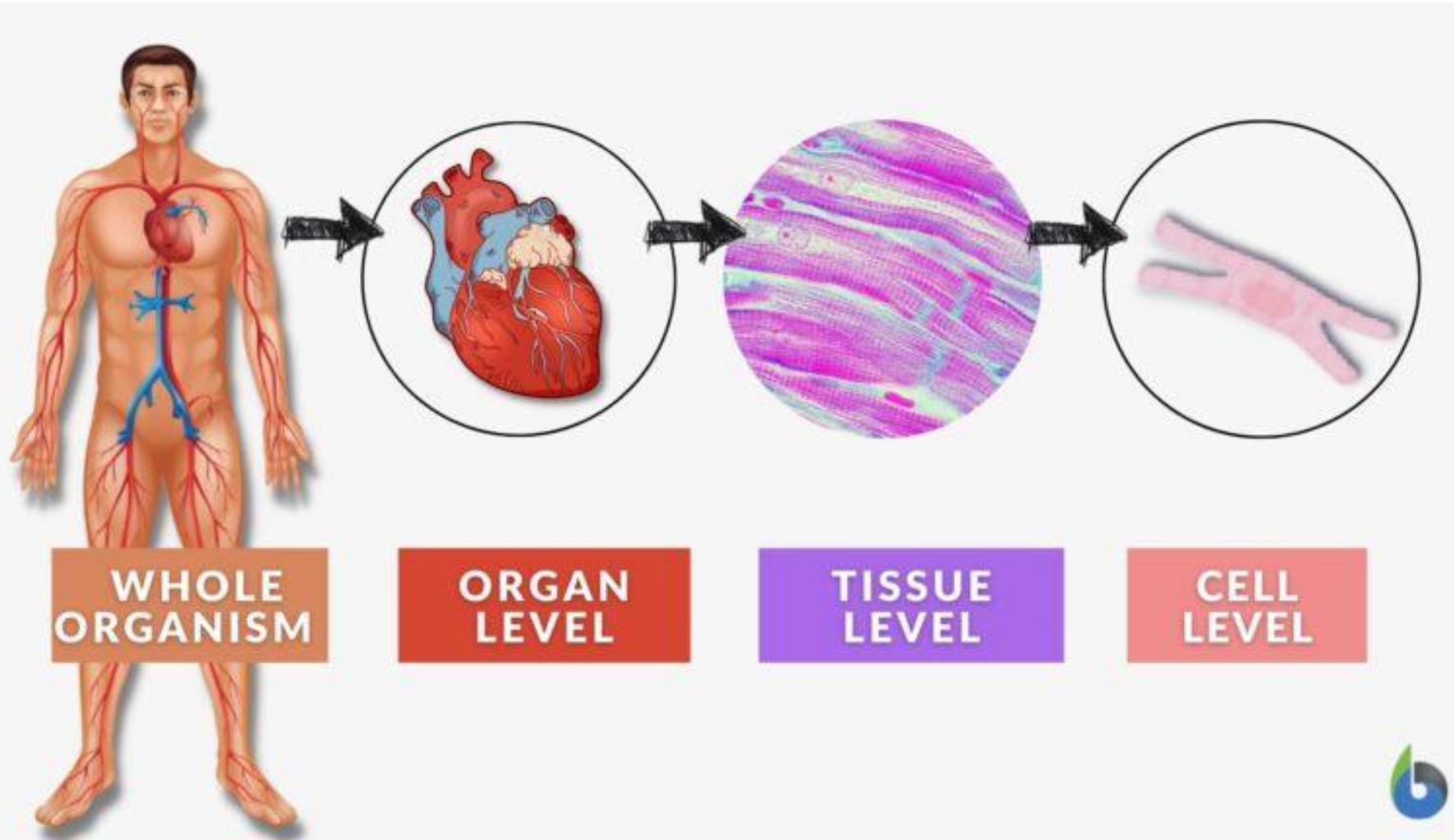
- 1. To Study the Historical review on immunohistochemistry
- 2. To know about the Classification of immunohistochemical methods
- 3. To know Types of
 - a. Chemical methods
 - b. Physical methods
 - c. Biological methods
- 4. To learn about Immunohistochemistry: general and special

Immunohistochemistry

- It is a recent advances in research methodology in medicine, Pharmacy and biological sciences have been made at the borders between the **morphology and functionology**, between anatomy-pathology and biochemistry-physiology.
- This new frontier is designated as **immuohistochemistry and cytochemistry**, which is a field to localize chemical components of cells and tissues on histological sections by using various techniques and analyze the functions based on morphology.



Morphology and Functionology



Divisions of Morphology
There are different types of morphology:

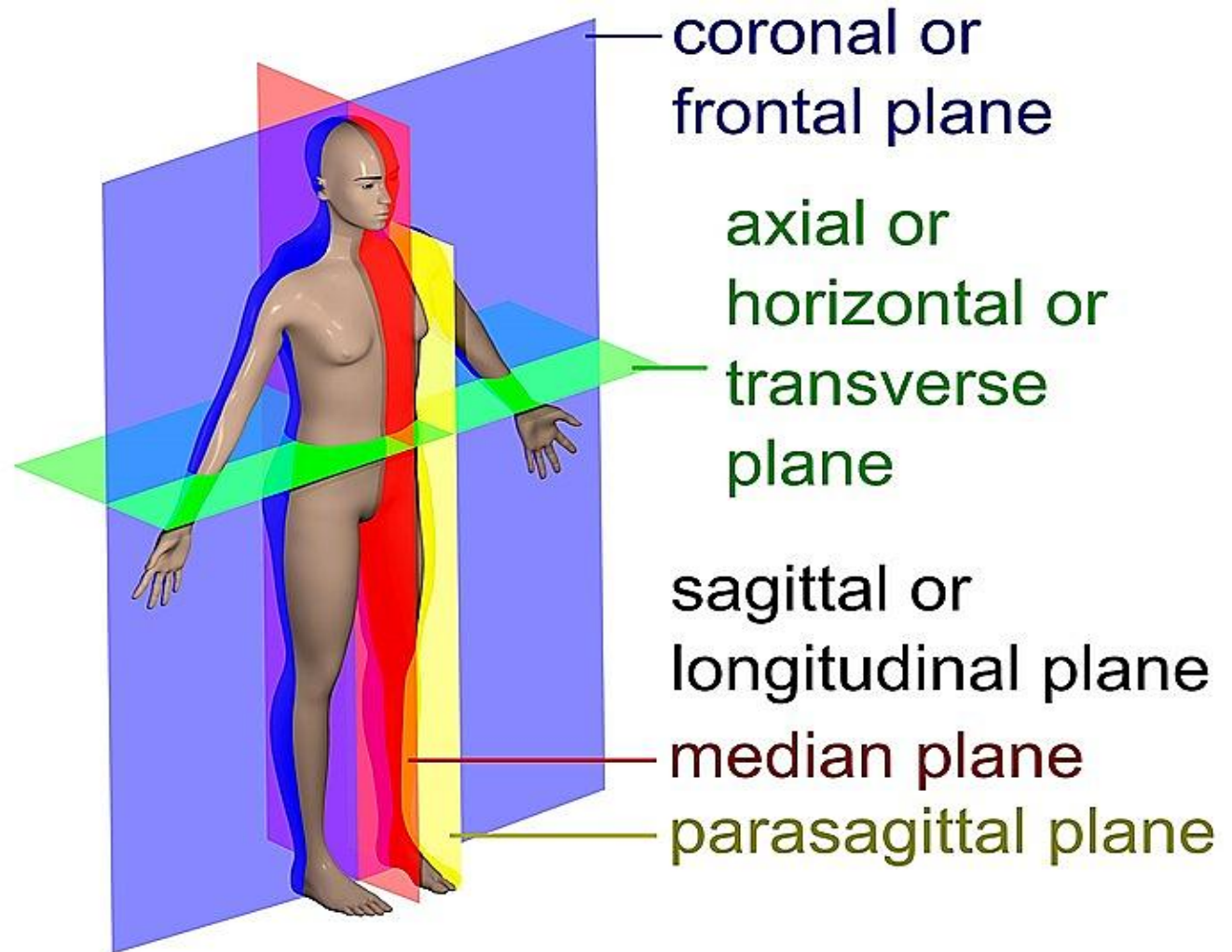
1. Cellular Morphology
2. Tissue Morphology
3. Organ Morphology
4. The Whole Organism

Human Body plan and symmetry

In higher animals like humans, only a cut from head to foot in the middle separates the body into equal halves.

Anterior and posterior ends may be differentiated, as **dorsal** and **ventral** sides.

Certain human internal organs (such as the **heart**) are not symmetrical, even the right and left sides of the body are not perfectly equal.



Classification of Immunohistochemical methods

- To classify the immunohistochemical techniques into 3 categories;
 1. Chemical methods such as chemical reactions by staining .
 2. Physical methods such as radiations
 3. Biological methods such as immunity

Chemical methods

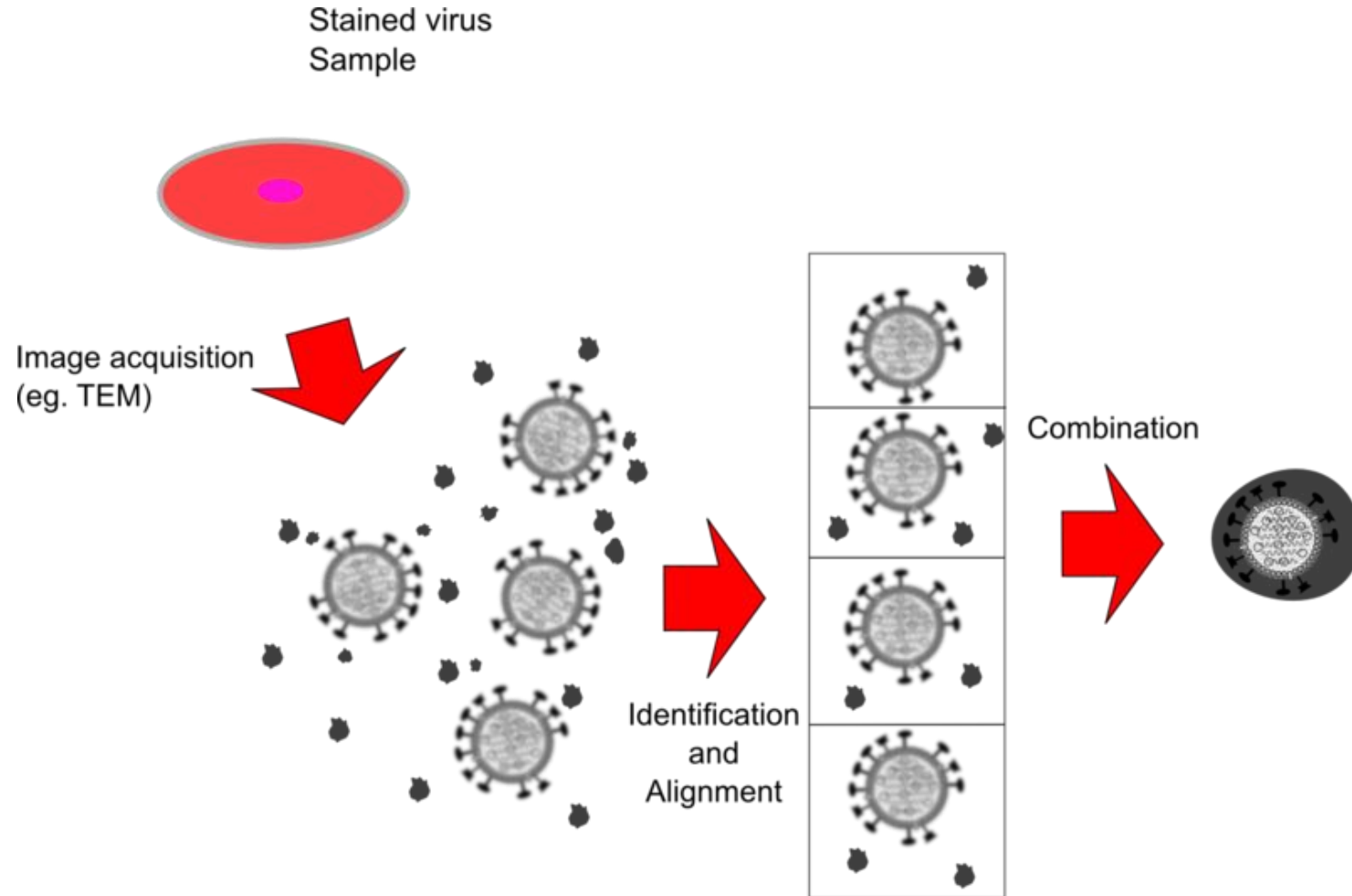
- The chemical methods in Immunohistochemistry consist of various chemical reactions, e.g. the color staining of DNA with Feulgen reaction.
- The principles and methods categories the color reactions for light microscopy and dense deposits for electron microscopy.
- The color reactions were first developed in Immunohistochemistry such as:
 - 1. DNA Feulgen reaction, staining of proteins with Millon reactions,.
 - 2. Lipids with Sudan
 - 3. Some enzymes such as alkaline and acid phosphatases.

Physical methods

- The principles and methods using physical reactions consist of various physical reactions such **changes of temperature** of specimens as microincineration or cryo-techniques, and effects of wavelength on absorption such as:
- Electron cryotomography (redirect from Cryo Electron Tomography) in other electron cryomicroscopy techniques.



Electron cryotomography (redirect from Cryo Electron Tomography)

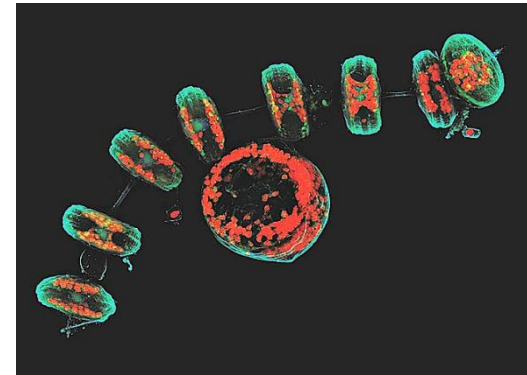
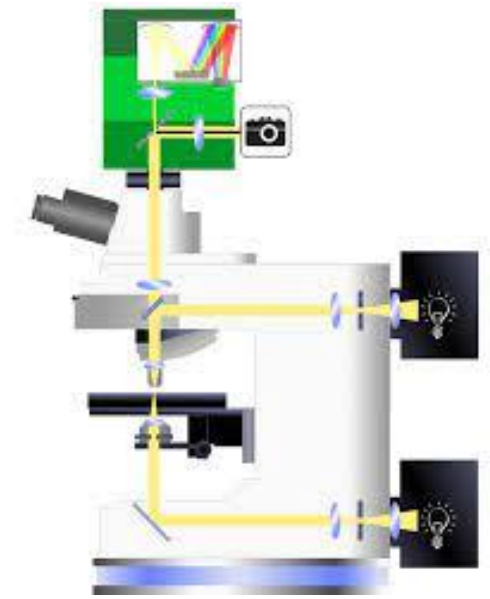


Single particle analysis segments and averages many particles from a sample, allowing for computer algorithms to process the individual images into a combined "representative" image.

samples for CryoET (typically small cells such as Bacteria, Archaea, or viruses) are prepared in standard.

Physical methods

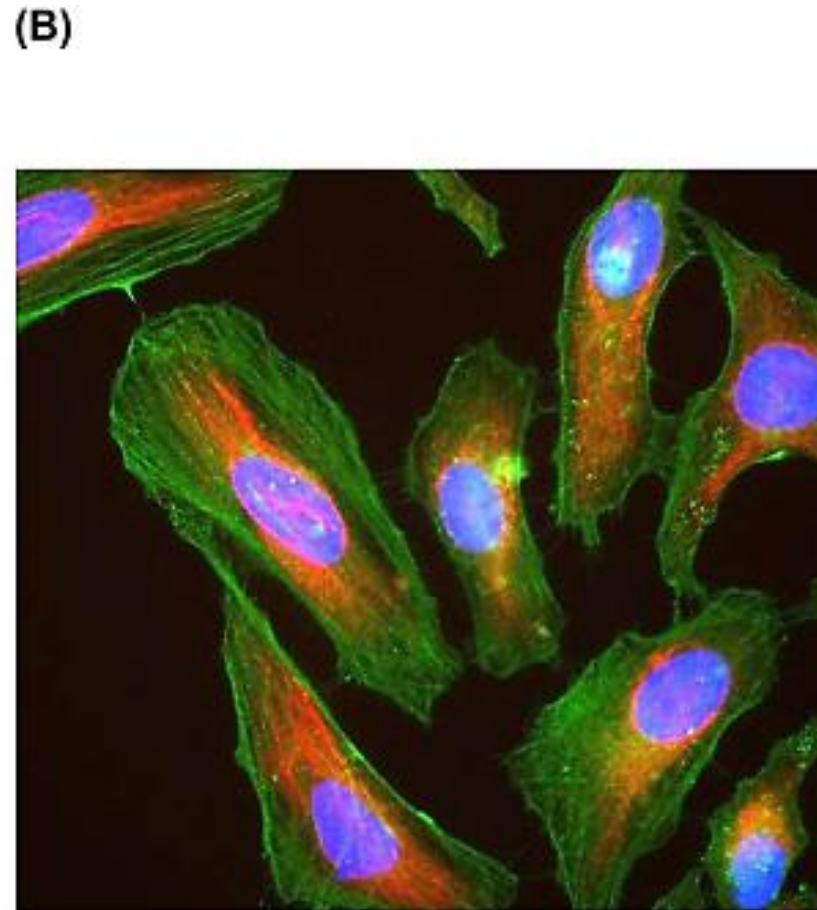
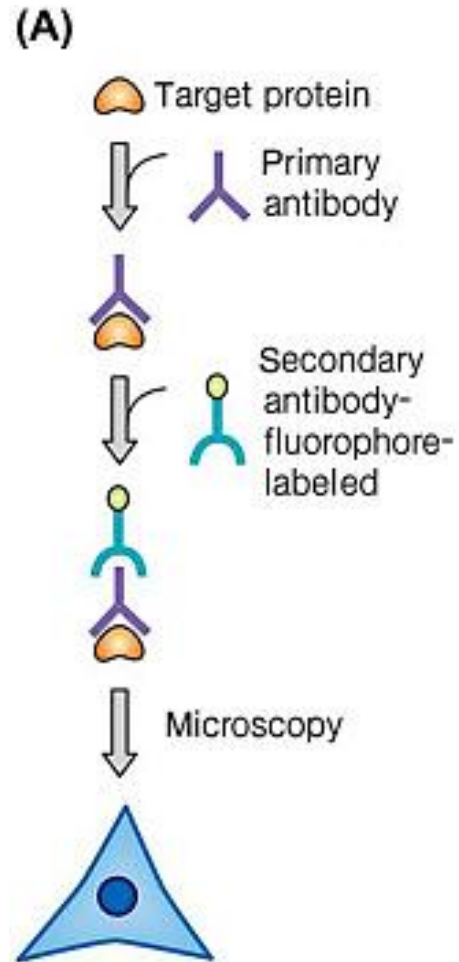
- Or effects of wavelength on absorption such as:
 - 1. Microspectrophotometry,
 - 2. Fluorescence microscopy,
 - 3. Confocal laser scanning microscopy.
- Or utilization of radiation such as
- radioautography and X-ray microanalysis.



Biological methods

- The biological methods in cytochemistry consist of mainly two biological reactions, which can be observed in living organism. The biological reaction was first introduced into cytochemistry.
- It demonstrated by:
 1. The localization of **proteins by the fluorescent antibody** method using the immunity .
 2. Lectins obtained from plants were also introduced into cytochemistry as another biological reaction to demonstrate sugar residues of glycoproteins

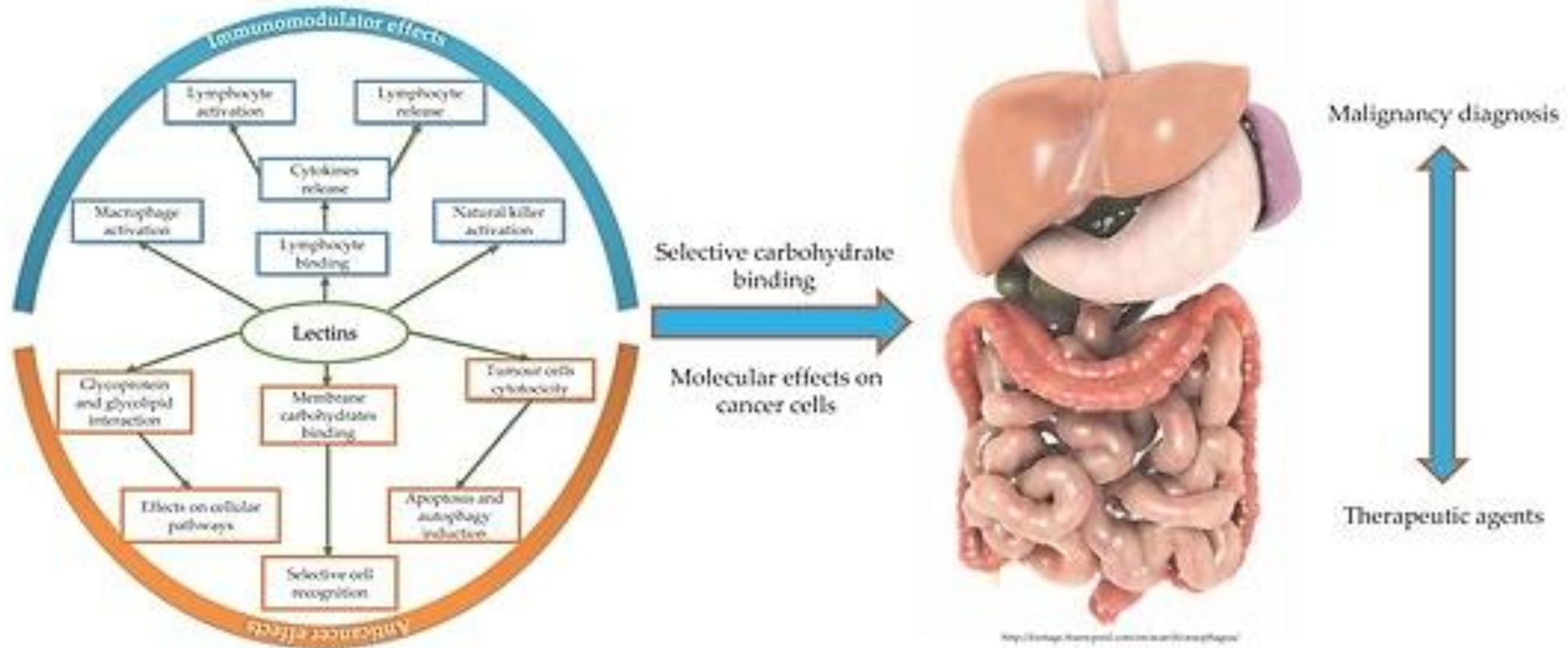
proteins by the fluorescent antibody



Plant Lectin

- Lectin-free diet
- foods that contain high amounts of lectins will prevent and cure disease. There is no clinical evidence that a lectin-free diet is effective to treat any disease.
- Digestive system cancers those of the esophagus, stomach, small intestine, colon-rectum, liver, and pancreas are highly related to genetics and lifestyle.
- Most are considered highly mortal due to the frequency of late diagnosis, usually in advanced stages, caused by the absence of symptoms or masked by other pathologies.

Lectins obtained from plants were also introduced into cytochemistry



Plant Lectin

- Different tools are being investigated in the search of a more precise diagnosis and treatment.
- Plant lectins have been studied because of their ability to recognize and bind to carbohydrates, exerting a variety of biological activities on animal cells, including anticancer activities.

Thank you

End