



# ***IMMUNOHISTOCHEMISTRY***

## **INTRODUCTION TO IMMUNOHISTOCHEMISTRY**

Dr. Ahmad H. Ibrahim

- Semester 2

- Week 1

Date 7/2/2024

**University of TISHK**

***Immunohistochemistry***

**Introduction to Immunohistochemistry**

*Dr. Ahmad H. Ibrahim Lecture 1, 2 and 3*

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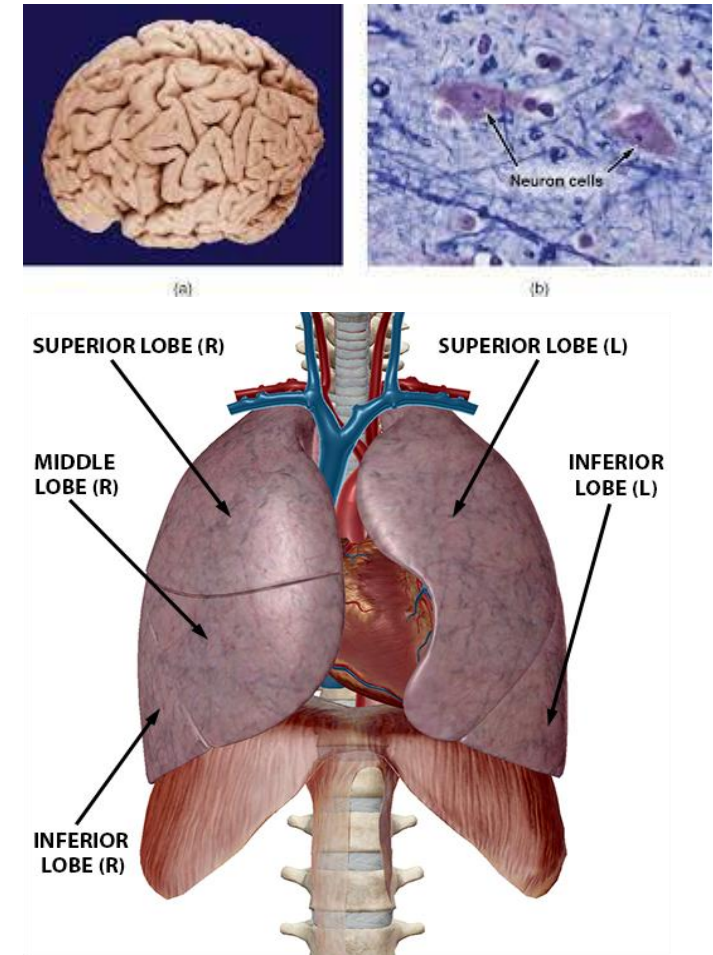
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# Objectives

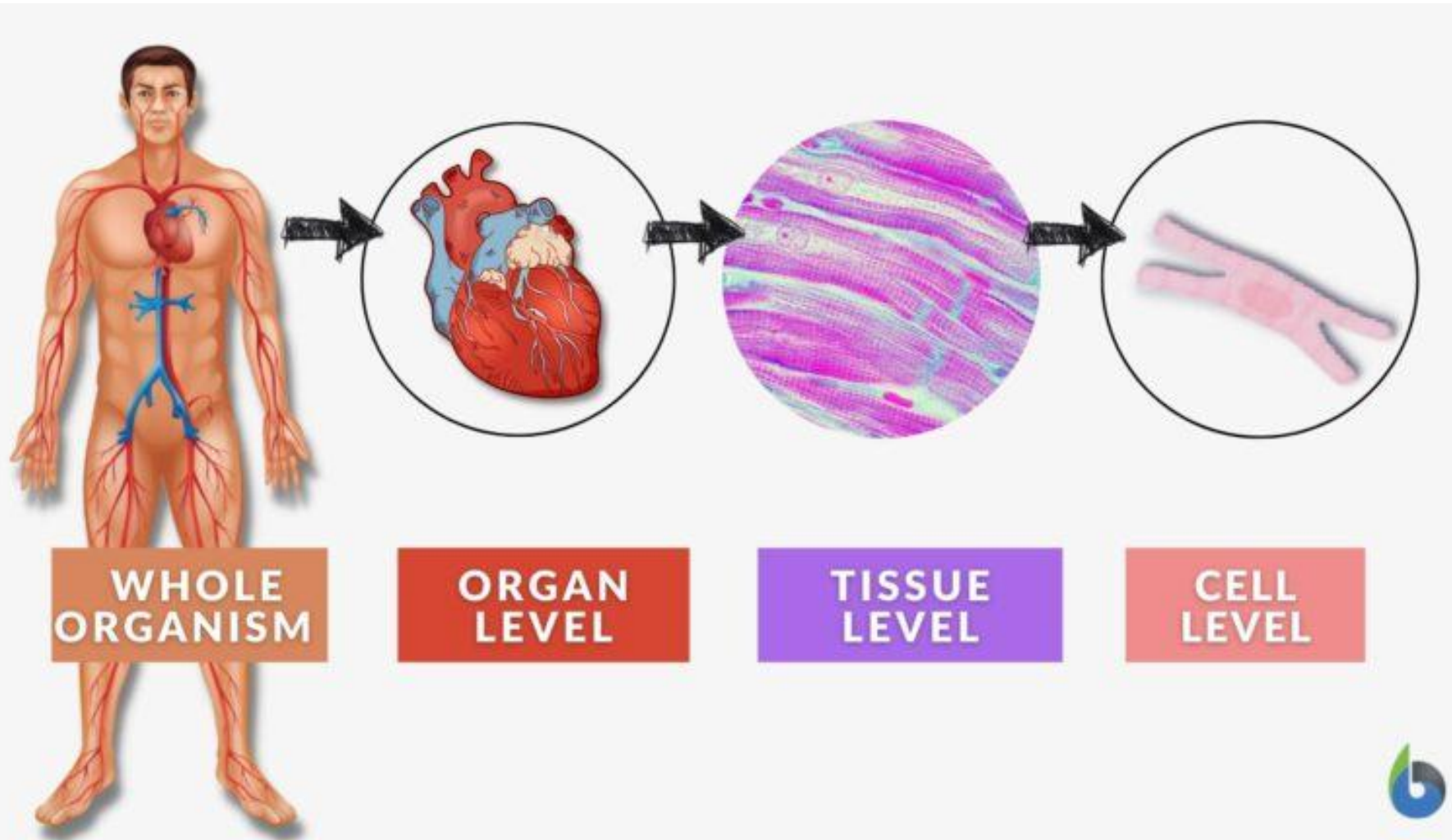
- 1. To Study the Historical review on Immunohistochemistry
- 2. To know about the Classification of Immunohistochemical methods
- 3. To know Types of
  - a. Chemical methods
  - b. Physical methods
  - c. Biological methods
- 4. To learn about Immunohistochemistry: general and special

# Histochemistry

- 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 **histochemistry 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:

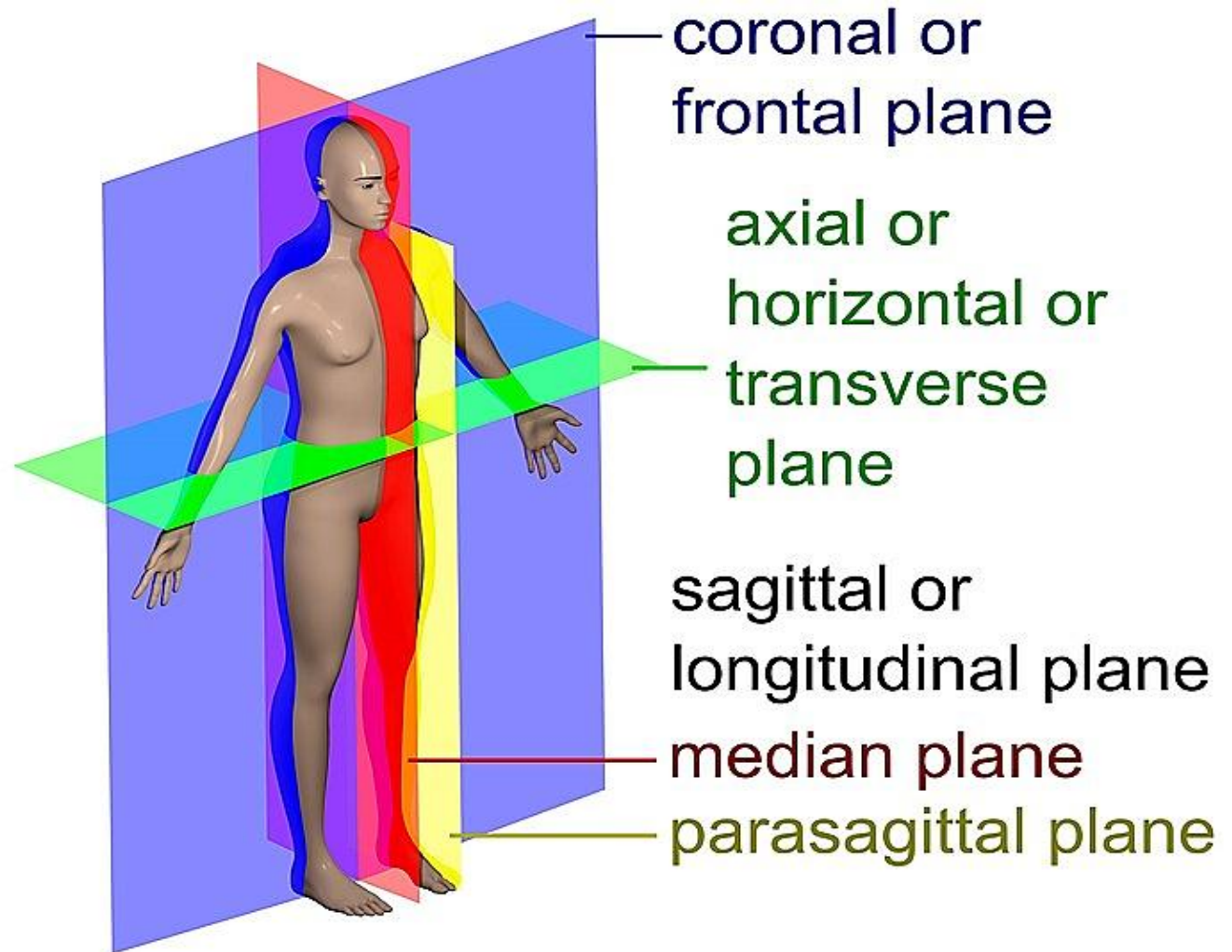
1. Cellular Morphology
2. Tissue Morphology
3. Organ Morphology
4. The Whole Organism

# 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.

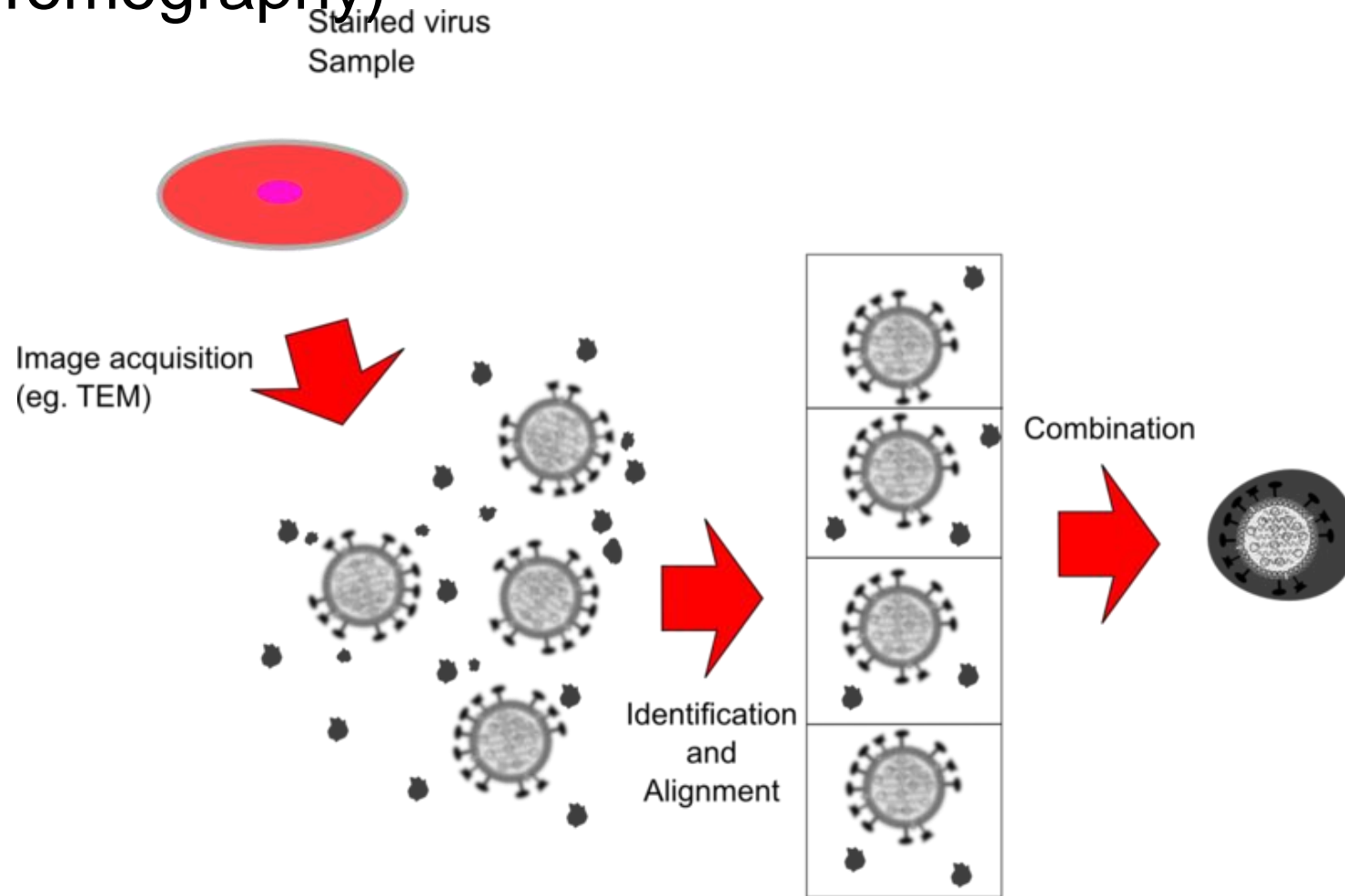


# Physical methods

- 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)

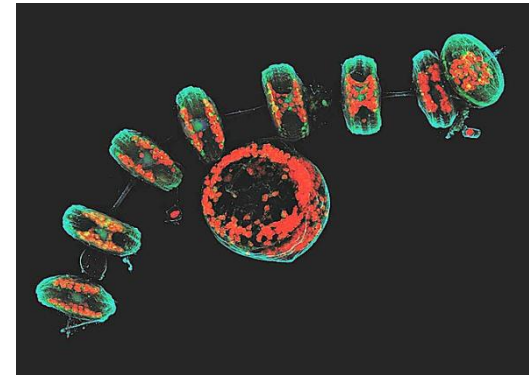
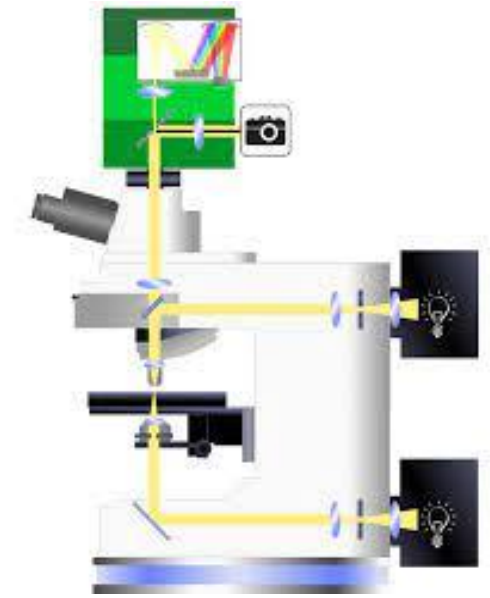


Single particle analysis segments and averages many particles from a sample, allowing for computer algorithms to process the individual images into a combined "representative" image.

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

# Physical methods

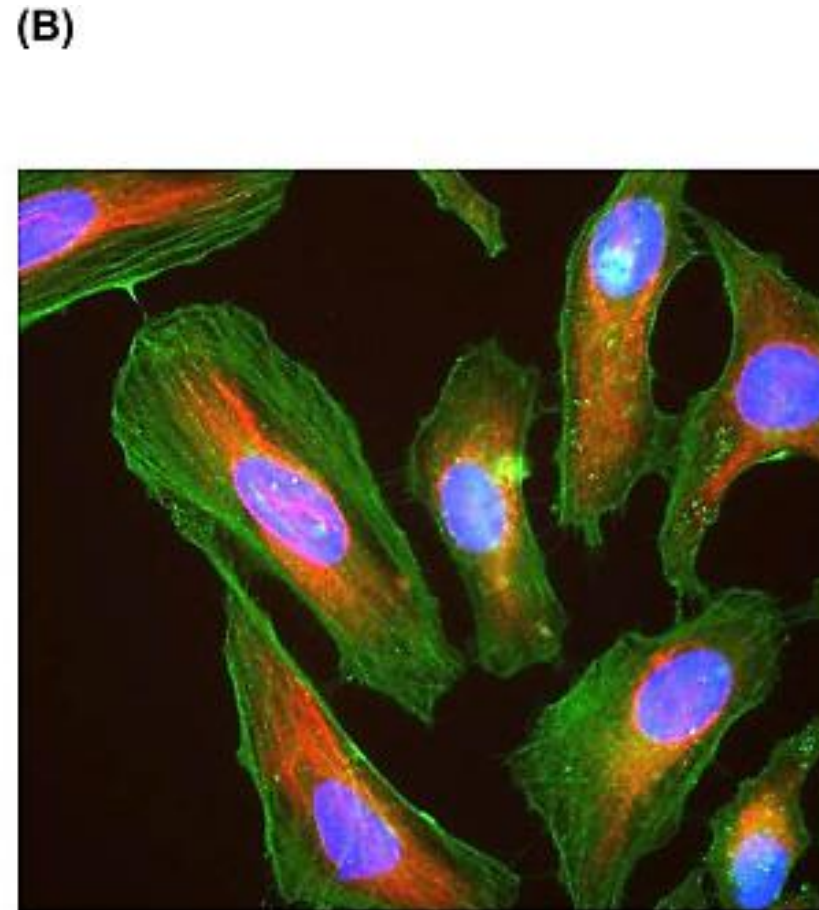
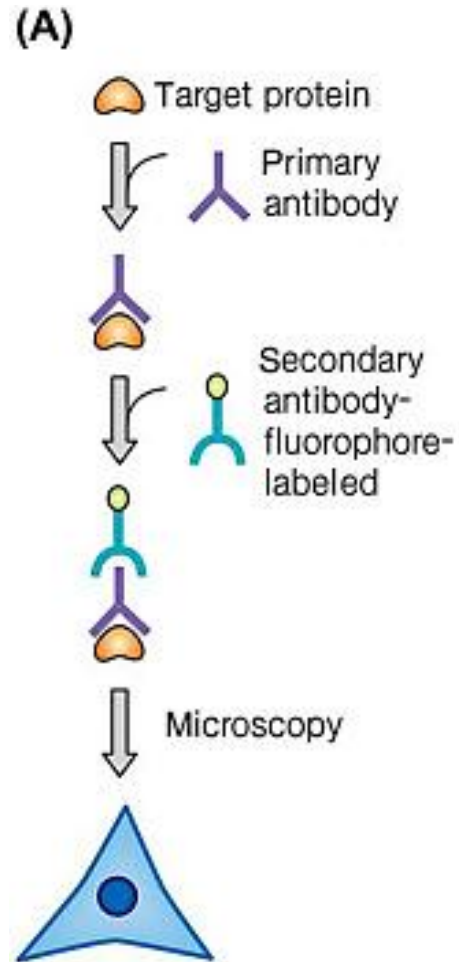
- Or effects of wavelength on absorption such as:
  - 1. Microspectrophotometry,
  - 2. Fluorescence microscopy,
  - 3. Confocal laser scanning microscopy.
- Or utilization of radiation such as
- radioautography and X-ray microanalysis.



# 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

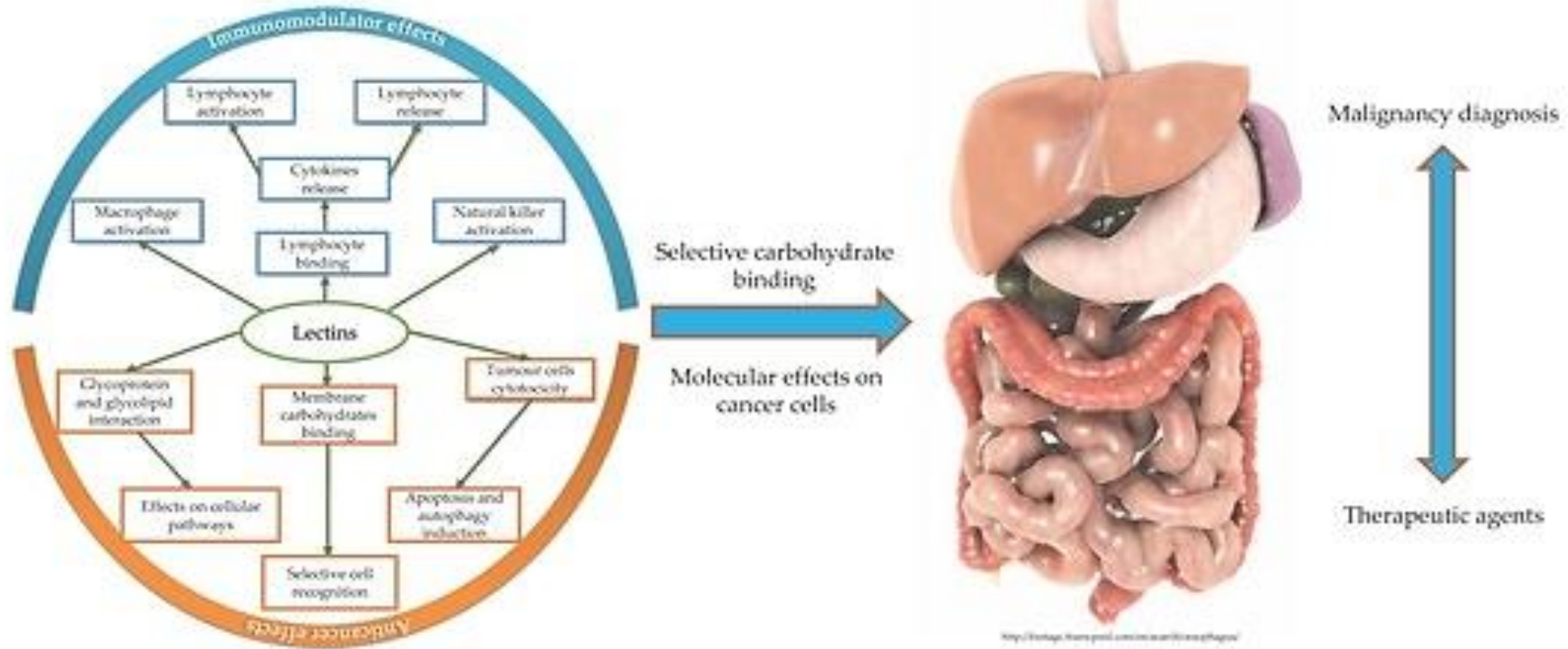
# proteins by the fluorescent antibody



# 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

# Appendix and extra readings

- Question What is Symmetry?
- Most animals and plants have spherical, radial, or bilateral symmetry. A spherically symmetrical body has the same shape throughout and may be sliced in half in any plane, for example, the body of a protozoan (e.g. Heliozoa).
- Starfish and mushrooms have radially symmetrical bodies with distinct top and bottom and body sections radiating from a central axis.
- Any plane with an axis passing through the core of the starfish may divide it in half. The anterior end has a mouth and the posterior end has an anus.
- Some species, including amoebas, slime molds, and some sponges, have no plane of symmetry.

# Home work

## Application to the class Alopecia Case



# Scenario case

- A woman, 30 years of age, presented with patchy hair loss on her head. It started 1 year ago when she developed an itchy, slightly painful, red scaling rash on her scalp 1 month after she dyed her hair. She had been treated with antifungal and other treatments unknown to her by several primary care doctors. However, the lesion worsened, and new lesions appeared. She was referred to a dermatologist.



# Scenario case

- Aside from the devastating psychological effects from the disfigurement and the effect on her occupation as a cosmetics sales promoter, she had no other symptoms. There was no significant past or family history. She did not smoke, but her husband smoked heavily in the house.



# Scenario case

- An examination of the scalp revealed focal coin-shaped, scaly, erythematous lesions, some of which had coalesced . Closer examination revealed shiny, central hypopigmentation and absence of hair follicles, and some thickened areas of hyperpigmentation peripherally. Where there were no lesions, her hair was black, thick, straight and long. At the periphery of the lesions, her hair became sparse and thin. A full examination of her face, neck, body and nails revealed no other skin lesions.



# Question

What differential diagnoses should be considered?

# Answer

On the basis of the clinical features, including nummular-shaped lesions with central permanent scarring alopecia due to total loss of hair follicles, and peripheral hyperpigmented adherent thick scales, the most likely diagnosis is **discoid lupus erythematosus** (DLE).<sup>1–4</sup> DLE is the most common form of chronic cutaneous lupus erythematosus and is usually localised on the face (eg cheeks, nose and ears). It is termed 'generalised' if found on the neck, upper back and dorsum of hands.

On the scalp, it causes dilatation and plugging of hair follicles with adherent scales. This leads to destruction of hair follicles and permanent balding. Initial manifestation of DLE can be difficult to recognize. Early referral to a dermatologist is recommended if in doubt, or if there is disease progression and treatment failure.

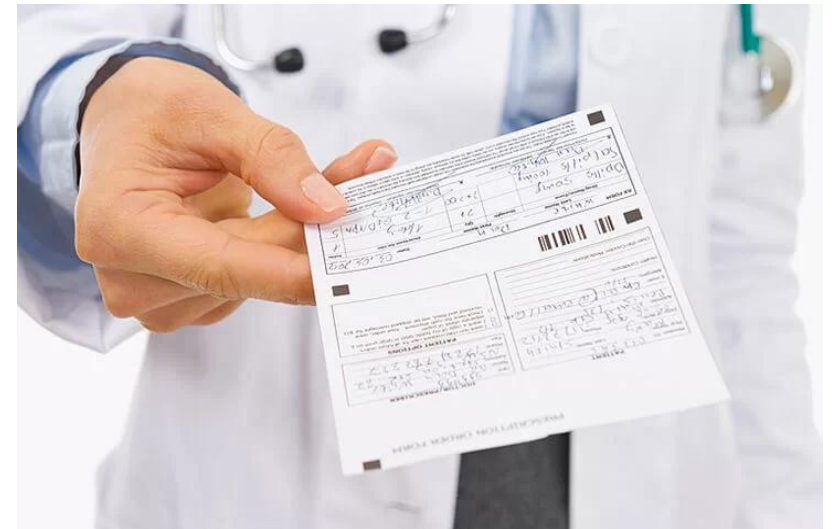




# Answer

On the scalp, it causes dilatation and plugging of hair follicles with adherent scales. This leads to destruction of hair follicles and permanent balding. Initial manifestation of DLE can be difficult to recognise.

Early referral to a dermatologist is recommended if in doubt, or if there is disease progression and treatment failure.



**University of TISHK**

***Immunohistochemistry***

**Fine Needle Aspiration Cytology - an overview**

***Dr. Ahmad H. Ibrahim***

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**Lecture 2**

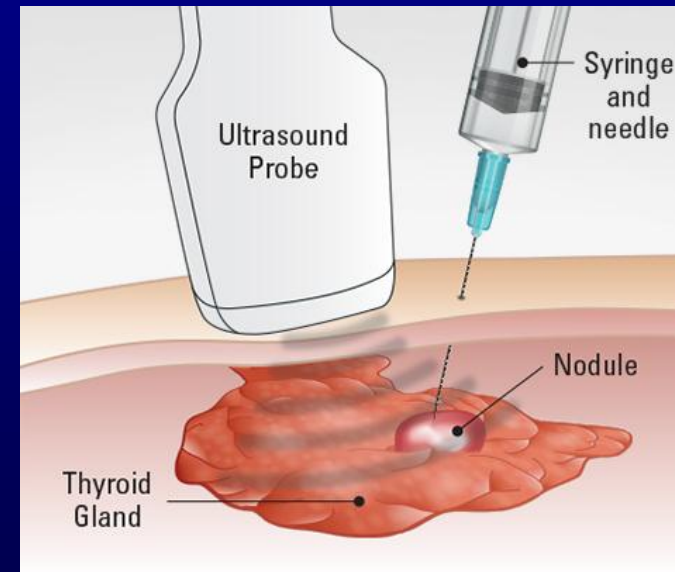
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# **Fine Needle Aspiration Cytology - an overview**

**Dr. Ahmad H. Ibrahim**  
**Pharmacy Dept. TIU**

# Fine Needle Aspiration Cytology

- Performed by ‘professional hybrids’ - clinicians who used it for rapid diagnosis



# **FNAC - definition**

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- **Aspiration of cells/ tissue fragments using fine needles ( 22 , 23, 25 G) ; external diameter 0.6 to 1.0 mm**
- **1.5 inches long needle ( radiologists use longer needles)**
- **Diagnostic materials in the needle and not in the syringe even in cystic lesions**

# Clinical skill required

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- Familiarity with general anatomy eg thyroid vs other neck swelling
- Ability to take a focused clinical history
- Sharp skill in performing physical examination eg solid vs cystic, benign vs malignant lesions

# Clinical skill required -2

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- **Good knowledge in normal cellular elements from various organs and tissue and how they appear on smears eg fats cells vs breast tumour cells**
- **Comprehensive knowledge of surgical pathology**

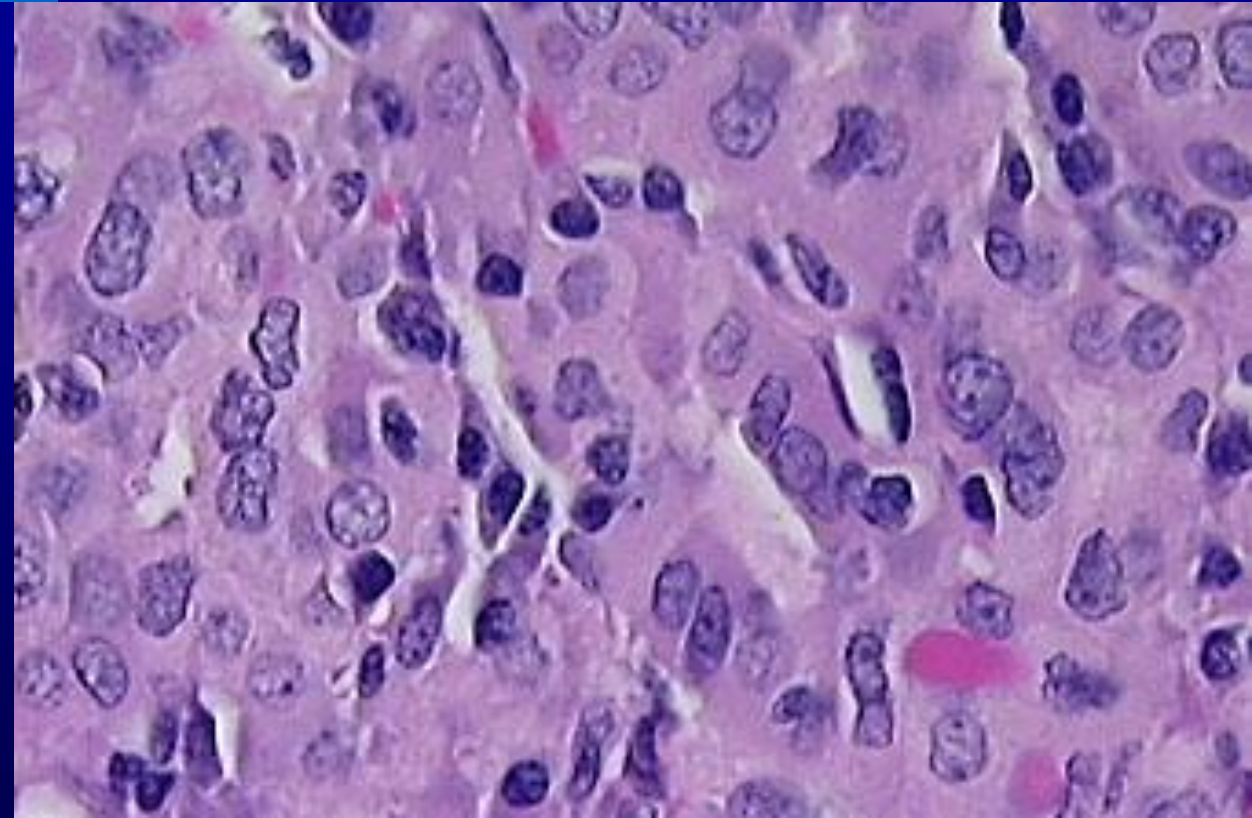
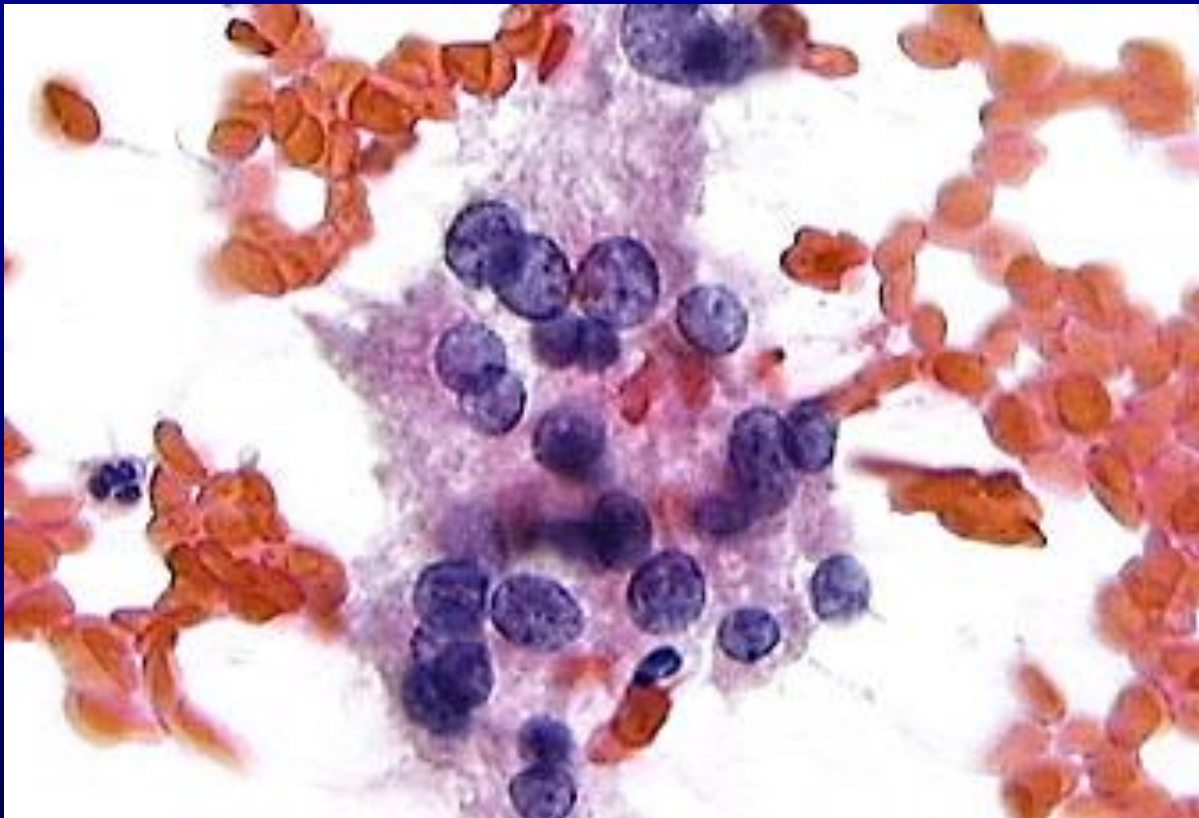
# Clinical skill required -3

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- Ability to translate traditional tissue patterns of lesions to their appearance in smears

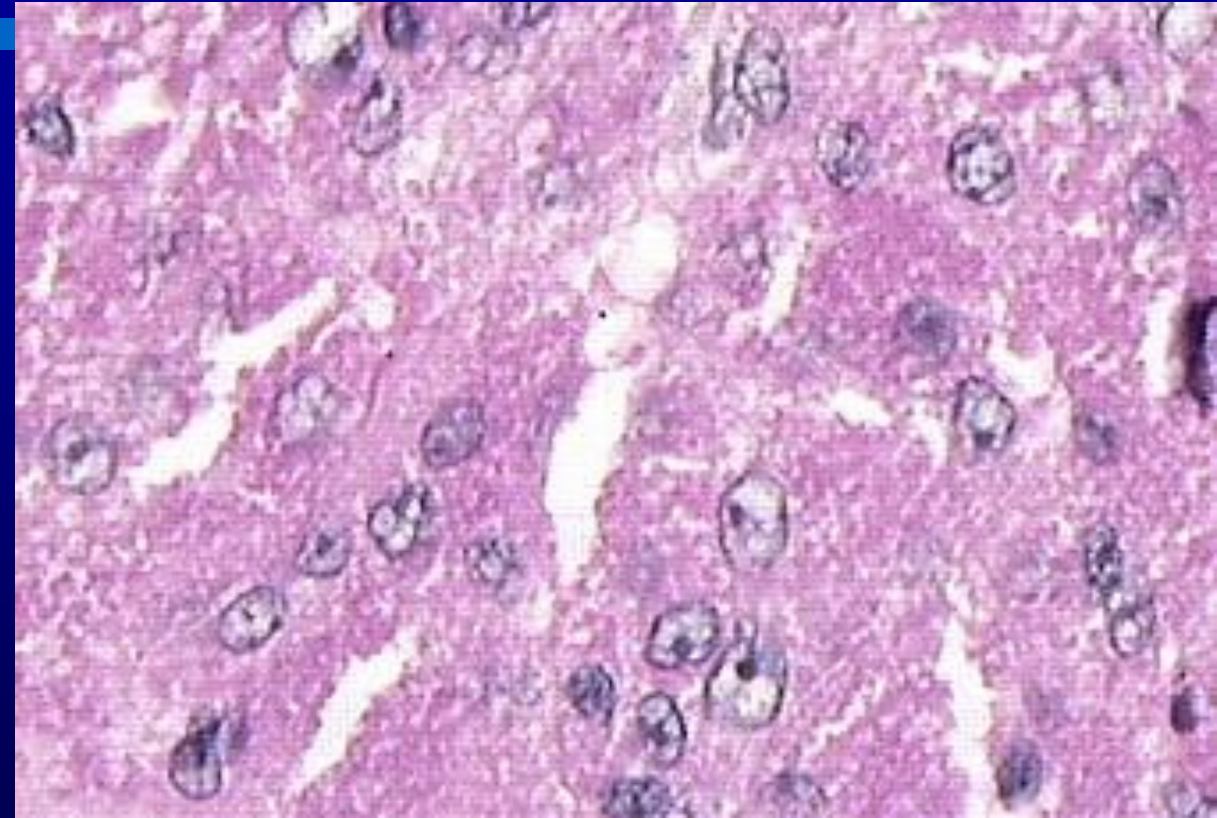
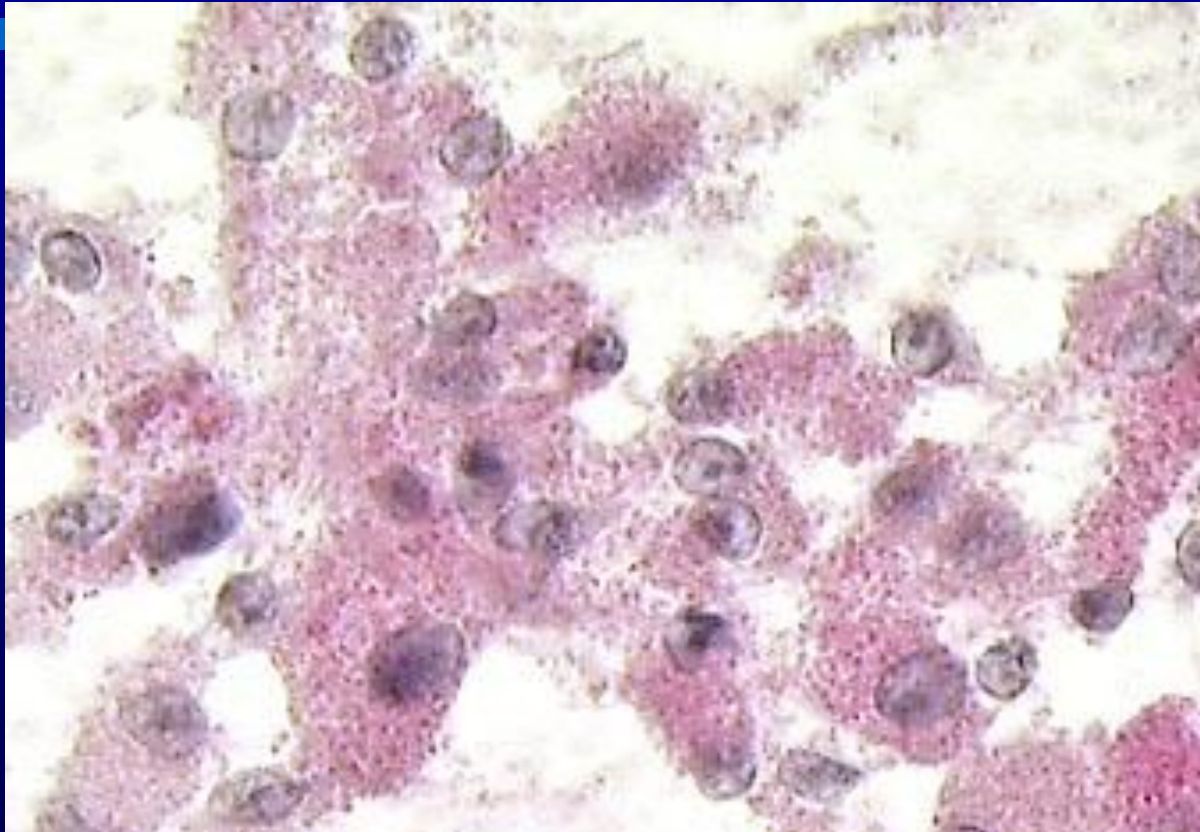


# Cytology vs Histology



**Papillary carcinoma of thyroid - follicular variant**

# Cytology vs Histology - 2



**Granular Cell Myoblastoma**

# Who should do FNA?

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- Clinicians
- Cytotechnologists
- Radiologists
- Pathologists

The one who examines the patients, does the aspiration, makes the smears, interprets the cytology is the best one to do FNA -

**PATHOLOGIST**

# Current status

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- **Palpable lesions**
- **Outpatients , in- patients**
- **Thyroid , breast, lymph nodes, salivary glands , soft tissue lumps...**
- **Lung, intra-abdominal and retroperitoneal by radiologic imaging : CT, ultrasound, flouroscopy**

# LIMITATIONS

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- **Soft vs hard ( bone) lesions**
- **Solid vs cystic lesions**
- **Poor cellular yield vs poor technique**
- **Reactive vs specific diseases eg reactive lymphadenitis vs Hodgkins disease**
- **Diffuse vs nodular lymphoma**

# Complications

- **Needle trauma**
  - granulation tissue formation
  - granuloma formation
  - Sarcoma like changes
  - Needle linear tract  
haemorrhage
  - tissue necrosis
- **Needle track seeding -  
testicular tm, chondrosar**
- **Hematoma**
- **Pain**
- **Pneumothorax???**

**Interfere with surgical  
pathology**

# ADVANTAGES

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- **Fast - early diagnosis**
- **Less pain, less trauma**
- **No anaesthesia**
- **Acceptable by patients and doctors**
- **Accurate**

# How to interpret?

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- **Aspiration materials eg colloid, blood, mucus?**
- **Cellular yield vs acellular yield**
- **Smear pattern - 3 dimensional balls vs flat monolayered sheet os cells**
- **Cohesiveness vs discreet cells**
- **Cell morphometry**



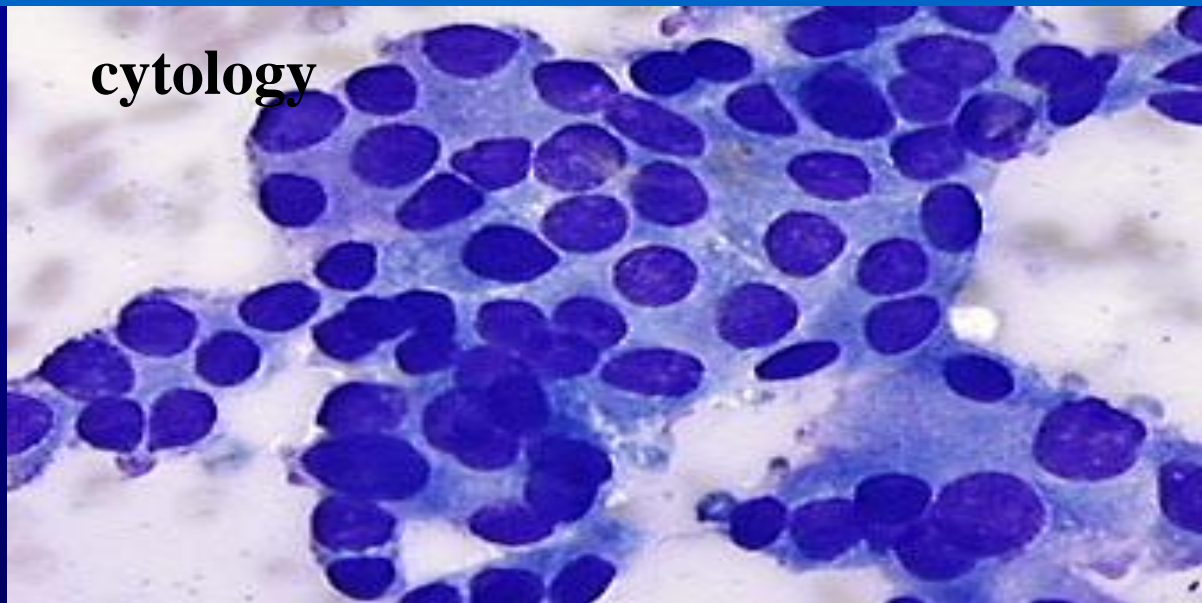
# Adjunct tools

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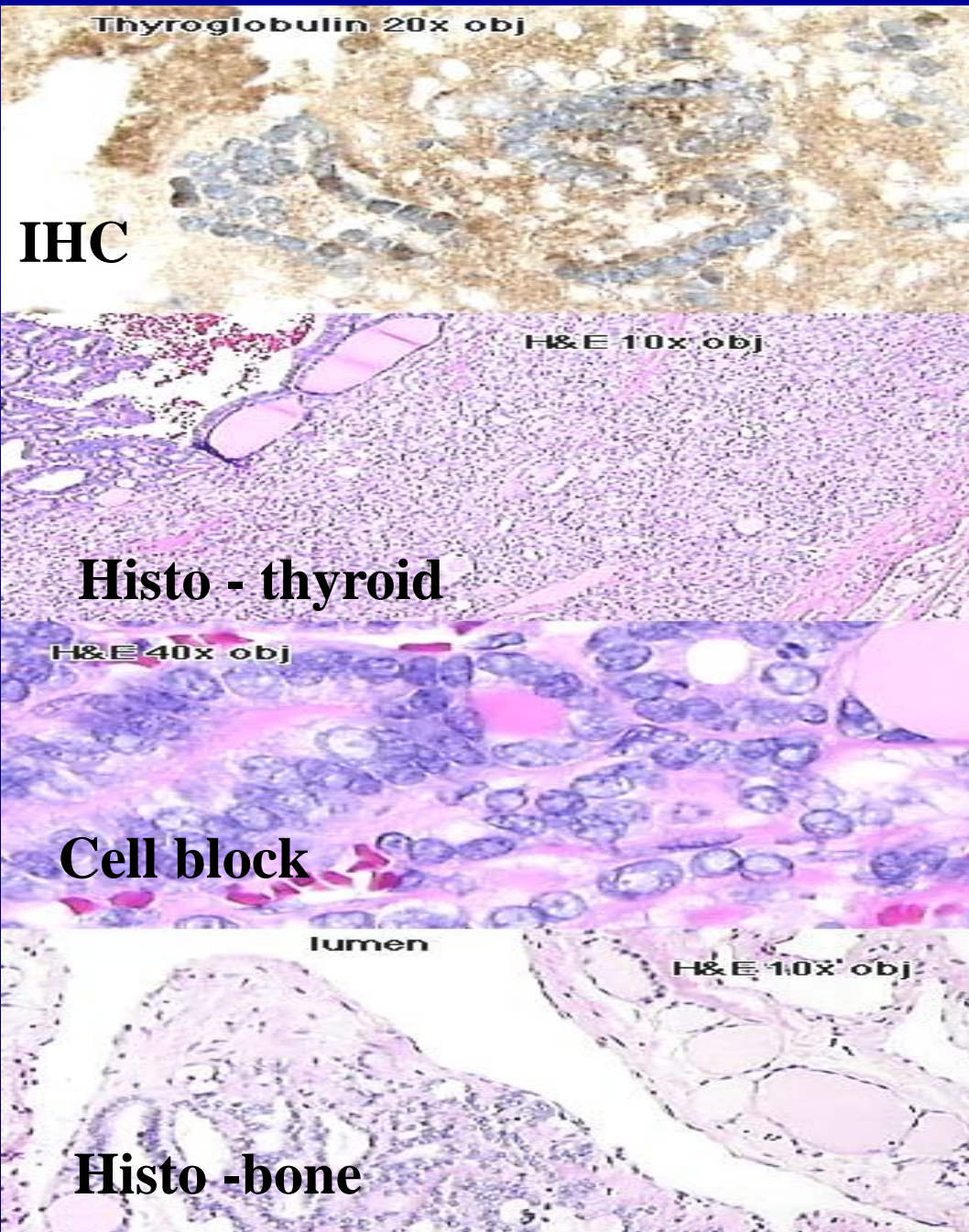
- Cell blocks
- Histochemistry
- Immunohistochemistry
- Electron microscopy
- Flow cytometry
- Immuno electron microscopy
- Molecular pathology -In situ hybridization, PCR etc

# Adjunct tools

cytology



45 yr old woman with  
lytic bone lesion



# Future directions

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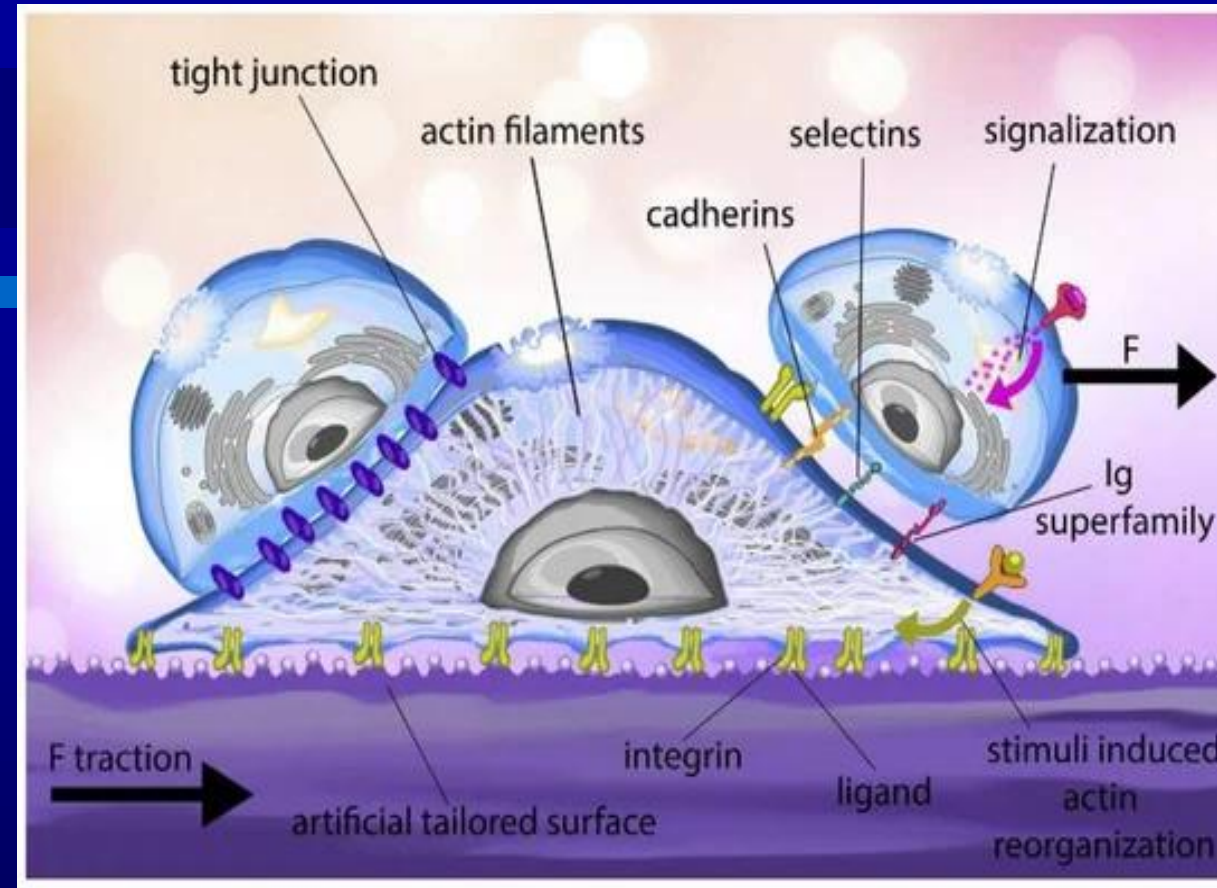
- Aspirating non palpable lesions using MRI
- Molecular pathology eg In Situ Hybridization
- Replacing diagnostic surgical pathology?
- Combined with MRI - replacing autopsy?

Q: What is Cell adhesion Process ?

Cell adhesion process in ECM extracellular Matrix. It is the ligand on the artificially tailored surface binds to the integrin receptors found on the cell membrane.

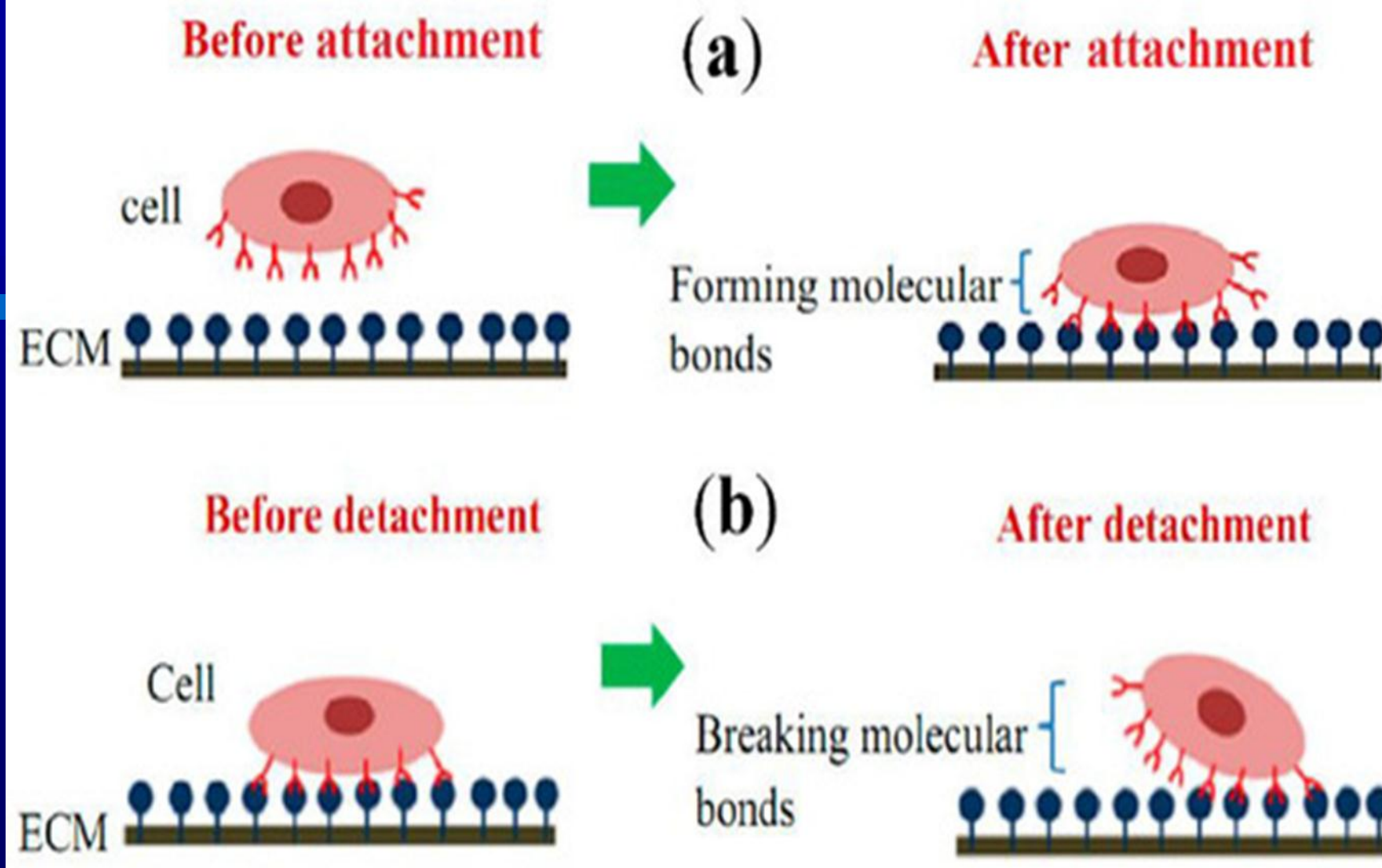
Throughout the adhesion process, the actin filament structure of the cell is reorganized, and a traction force is generated in the substrate.

External stimuli also regulate the reorganization of cytoskeletal.



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After surface adhesion, the cell can interact with other cells through membrane proteins such as cadherins, selectins and the Immunoglobulin (Ig) superfamily. In tissues, cell junctions, a variety of multiprotein complexes (e.g., tight junctions), can form between cells to promote intercellular communication and mechanical stability.



Schematic diagram of single-cell adhesion (a) attachment event via the formation of molecular bonds, (b) detachment event via breakage of molecular bonds. (ECM—Extracellular matrix)

Thank you

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**End**

# Homework

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- **Case Scenario: Fine Needle Aspiration Cytology - An Overview**
- **Patient Information:** Mrs. Smith, a 52-year-old female, presents to her primary care physician with a palpable lump in her left breast. She reports that she first noticed the lump about two weeks ago and it has been gradually increasing in size. She denies any history of trauma to the breast or any recent changes in her breast size or shape. Mrs. Smith is otherwise healthy and has no significant medical history.
- **Clinical Examination:** Upon clinical examination, the physician notes a firm, non-tender mass measuring approximately 3 centimeters in diameter in the upper outer quadrant of the left breast. There are no visible signs of skin changes, nipple retraction, or discharge. The axillary lymph nodes appear non-palpable.



# Homework Solution

- **Discussion:**
- **Fine Needle Aspiration Cytology (FNAC):** FNAC is a minimally invasive procedure used to obtain tissue samples for cytological examination. It is often performed as an initial diagnostic test for evaluating suspicious breast lumps, thyroid nodules, and other palpable masses.
- **Advantages of FNAC:** FNAC is a quick and cost-effective procedure that can provide rapid diagnostic information, allowing for timely management decisions. It is associated with minimal discomfort and has a low risk of complications.
- **Limitations of FNAC:** While FNAC is useful for providing a preliminary diagnosis, it may not always yield definitive results, particularly in cases where the sample is insufficient or non-representative. In such cases, additional diagnostic tests, such as core needle biopsy or surgical excision, may be required for further evaluation.

# Homework

- **Management Plan:** Based on the cytology results confirming invasive ductal carcinoma, Mrs. Smith is referred to an oncologist for further management. She undergoes additional imaging studies to assess for the extent of disease and is scheduled for surgery to remove the breast tumor. Subsequent treatment options, such as chemotherapy, radiation therapy, and hormone therapy, are discussed with Mrs. Smith as part of her comprehensive cancer care plan.
- **At the end:** Fine Needle Aspiration Cytology (FNAC) is a valuable diagnostic tool for evaluating suspicious lesions in various anatomical sites, including the breast. In the case of Mrs. Smith, FNAC played a crucial role in the timely diagnosis of invasive ductal carcinoma, facilitating prompt initiation of appropriate treatment measures.