Microscope and Microscopy

Subtitle

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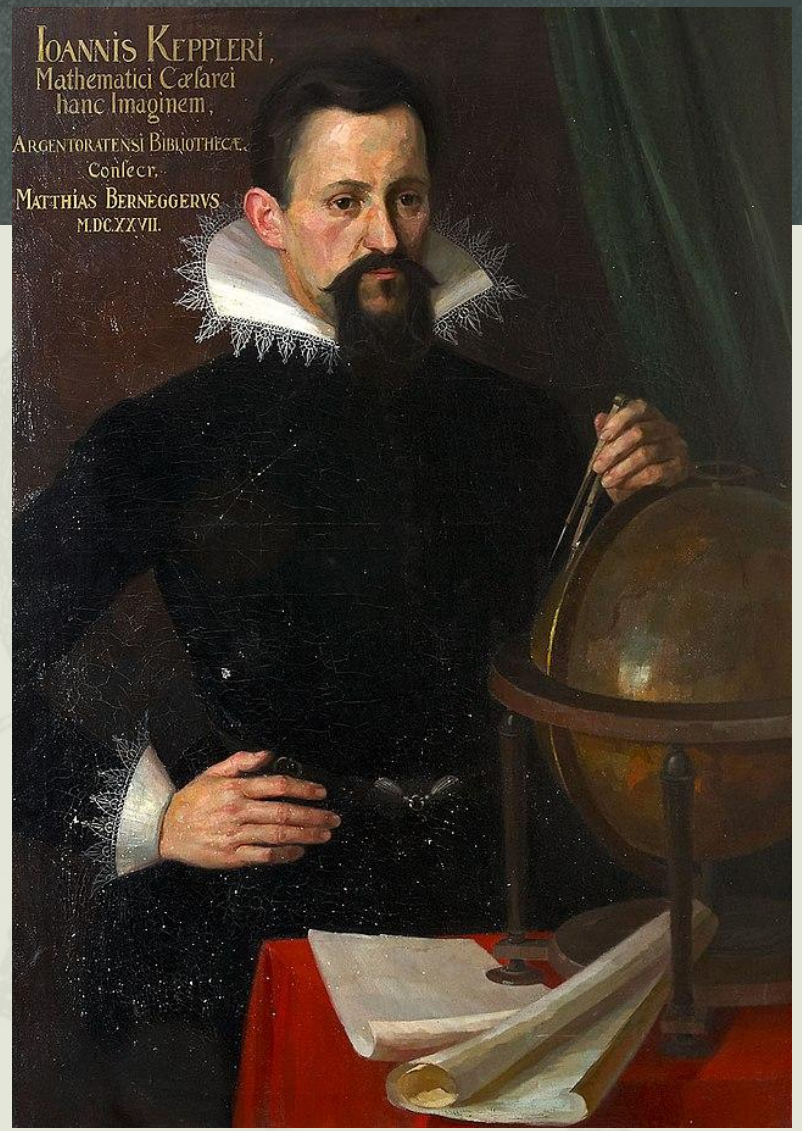
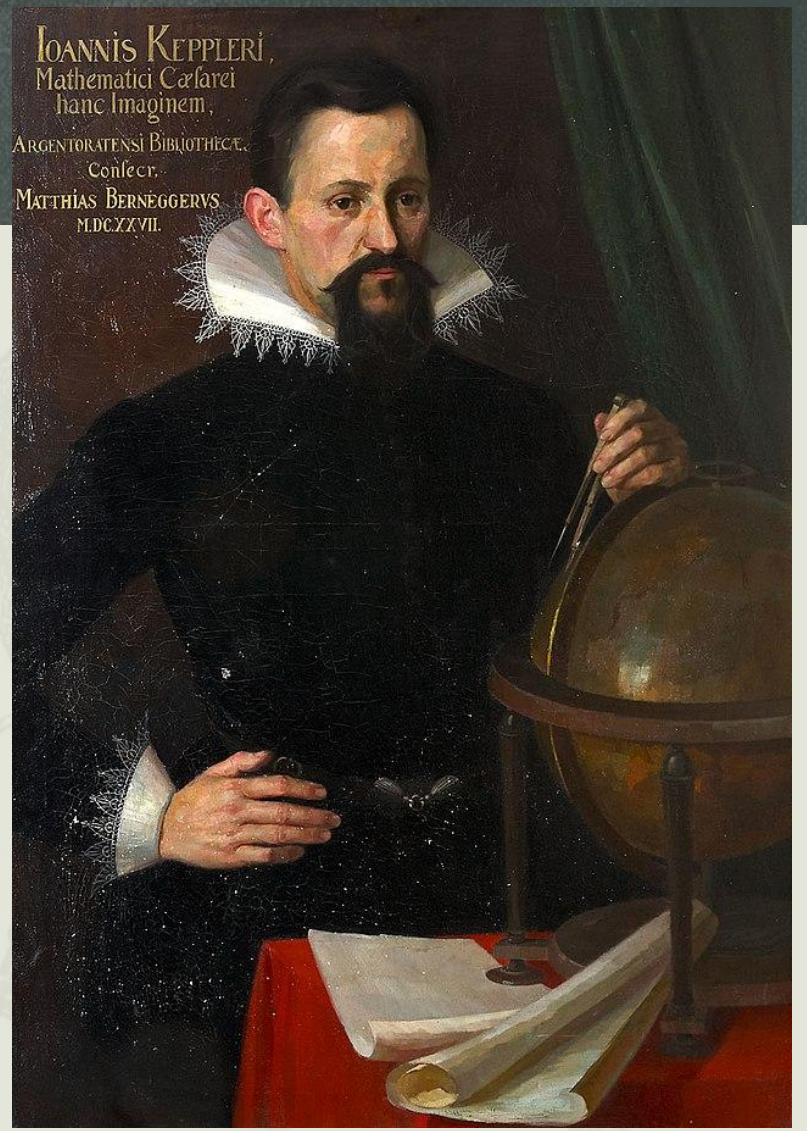


History of Microscopy

- Seventeenth century – Dutch people find out how to use lenses
- 1611 – Johannes Kepler – Magnifies using convex ocular and convex objective lens : KEPLER OCULAR
- 1625 – Giovanni Faber – coined “Microscope”



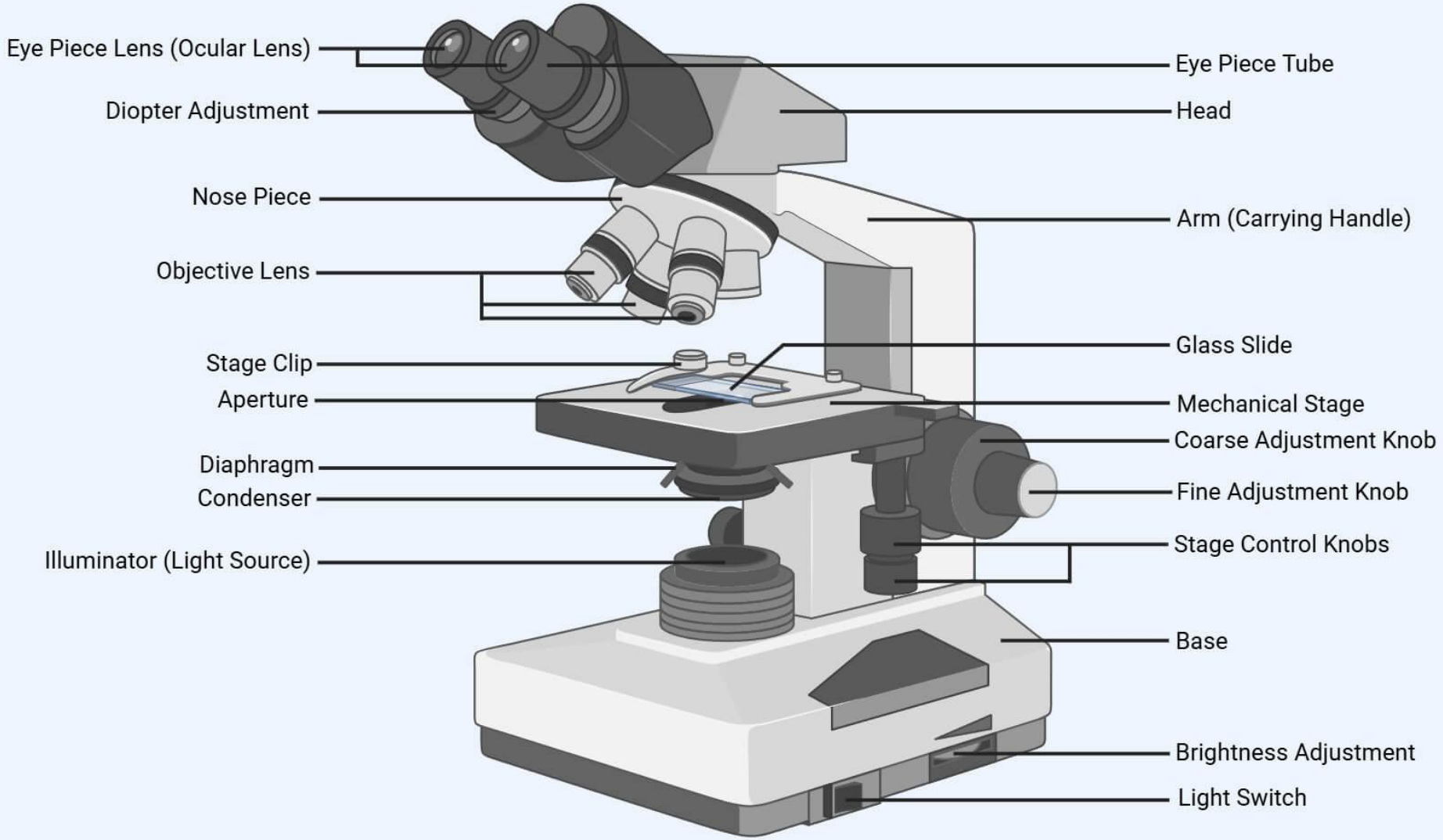
History of Microscopy

- First used for microorganisms by –
  - Dutch merchant – Anton von Leewenhoek – made 400 different microscopes with a combination of glass lenses
  - Used them to see ‘animalcules’
- 1600s – First light microscope
- 1920s – First electron microscope
- 1980s – Confocal laser scanning microscope, atomic force microscope

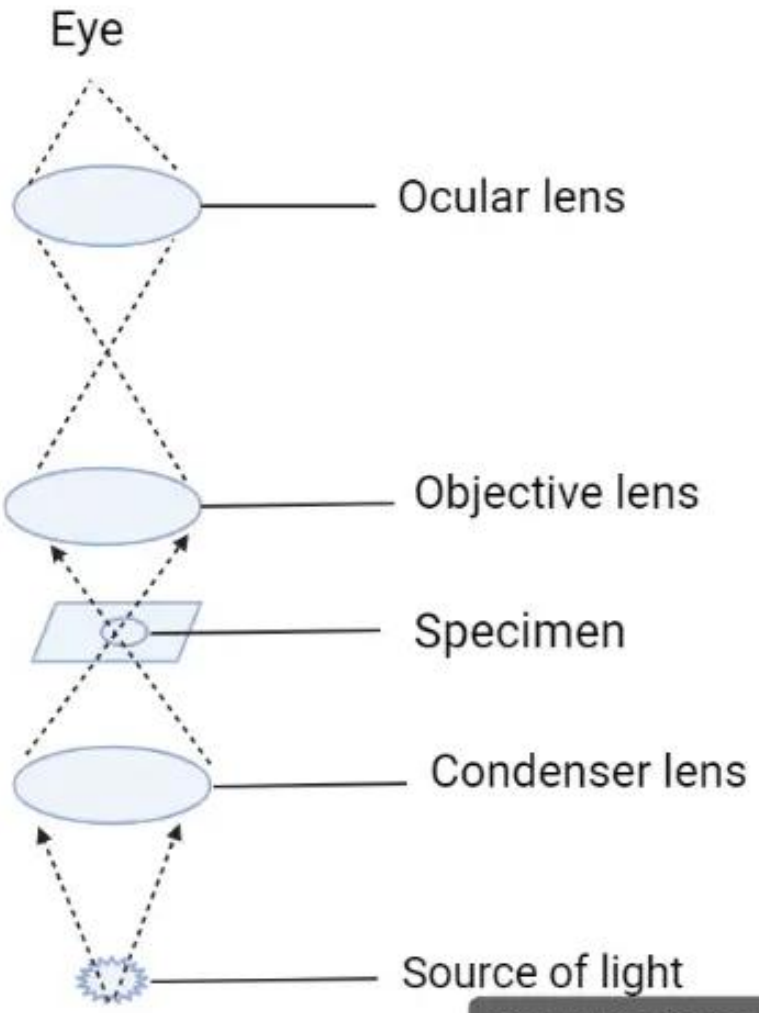
Human Eye can magnify upto 150 µm

Objective of Microscopy – Increase the resolution of the human eye

Parts of a Microscope



HOW DO THE LENSES WORK?



Microscopy and Micrometry

- Microscopy:

  Microscopy is the technical field of using microscopes to view samples & objects that cannot be seen with the unaided eye (objects that are not within the resolution range of the normal eye).

- Micrometry:

  The purpose of micrometry is to measure the dimensions (length, breadth, diameter and thickness) of microscopic objects (microorganisms, pollen grains, etc.) under microscope.

Micrometry – Principle

- Measurement of the dimensions of microorganisms is done under microscope with the help of two micro-scales called ‘micrometers’.
- Both the micrometers have microscopic graduations etched on their surfaces.
- One of them, the ‘ocular micrometer’ is a circular glass disc, which fits into the circular shelf inside the eyepiece. It has arbitrary graduations etched on its surface. However, the distance between the etched graduations is constant for a particular ocular micrometer.
- The other micrometer, called ‘stage micrometer’, is a special glass slide, which is clipped to the stage of the microscope. It has standard graduations etched on its surface, which are 10 µ apart.

Source:
http://courseware.cutm.ac.in/wp-content/uploads/2020/05/Micrometer.pdf

Micrometry – Calibration

- Graduations on the ocular micrometer are calibrated against the standard graduations on the stage micrometer.
- Calibration is required, because the distance between ocular graduations varies depending on the objective being used, which determines the size of the field. For calibration, both the micrometer etchings are superimposed by rotating the eyepiece.
- The number of ocular divisions (O.D.) coinciding with the number of stage divisions (S.D.) is found out.
- From this, the calibration factor for one ocular division (O.D.) is calculated as follows:
- If 10 O.D. coincide with 2 S.D., then 10 O.D. = 2 S.D. = 2 x 10 µ = 20 µ (Since 1 S.D. = 10 µ)
  01 O.D. = 20/10 µ = 2 µ
- Thus, the distance between two adjacent ocular etchings is 2 µ (i.e. calibration factor is 2 µ).

Source:
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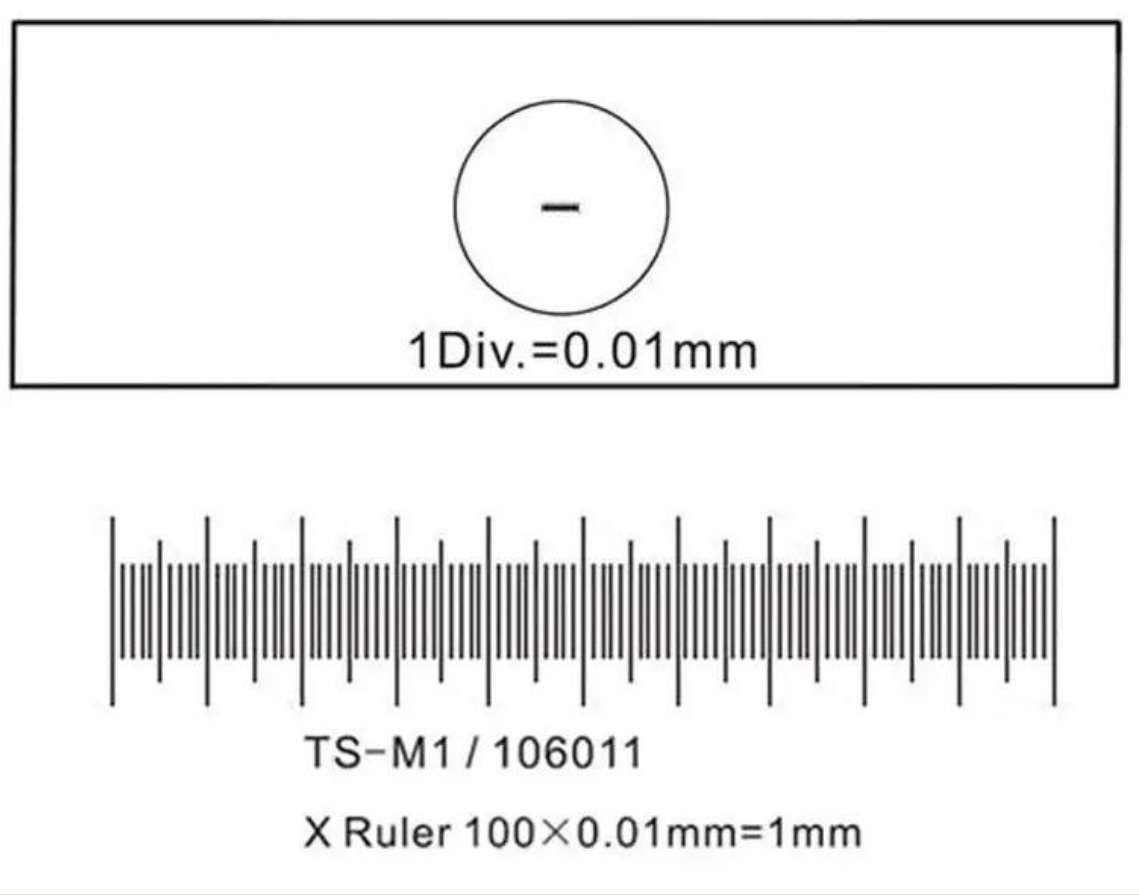
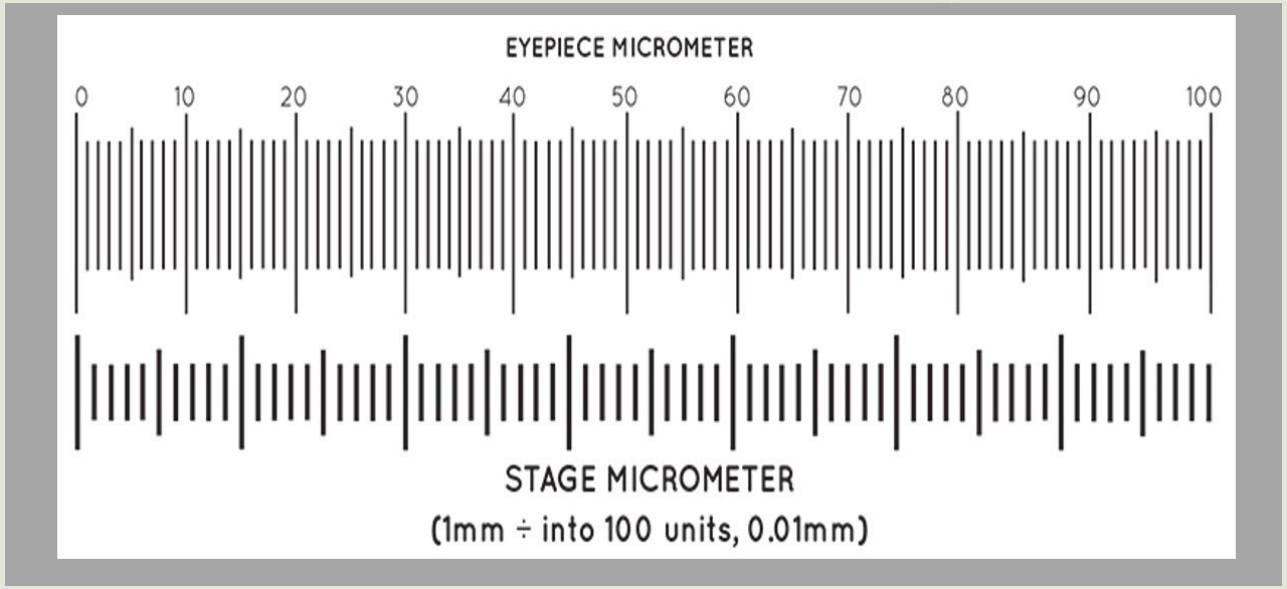
Micrometry – Calibration





Micrometry – Measurement

- After calibration, the stage micrometer is removed and the microbe/pollen grain, whose dimensions are to be measured, is placed on the stage on a slide and focused.
- Now, the number of ocular divisions occupied by the microorganism is counted.
- Then, by multiplying this number of divisions with the calibration factor, the size of the microbe is determined as follows.
- If the microbe/pollen grain occupies 6 O.D. in length, then length of microbe = 6 × calibration factor = i.e. 6 x 2 = 12 µ.

Parallax Error in Micrometry

- **Parallax Error**

Parallax error is a common type of observational error that can occur in a wide range of scientific and practical applications. It arises when the position or direction of an object appears to differ when viewed from different positions. This is due to the angle of observation changing as the observer's position changes, causing the object to appear to move or shift.

TABLE 9.1 Comparison of Types of Microscopy

Microscope	Maximum Practical Magnification	Resolution	Important Features
Visible Light as Source of Illumination			
Bright-field	2000 ×	0.2 μm (200 nm)	Common multipurpose microscope for live and preserved stained specimens; specimen is dark, field is white; provides fair cellular detail
Dark-field	2000 ×	0.2 μm	Best for observing live, unstained specimens; specimen is bright, field is black; provides outline of specimen with reduced internal cellular detail
Phase-contrast	2000 ×	0.2 μm	Used for live specimens; specimen is contrasted against gray background; excellent for internal cellular detail
Differential interference	2000 ×	0.2 μm	Provides brightly colored, highly contrasting, three-dimensional images of live specimens
Ultraviolent Rays as Source of Illumination			
Fluorescence	2000 ×	0.2 μm	Specimens stained with fluorescent dyes or combined with fluorescent antibodies emit visible light; specificity makes this microscope an excellent diagnostic tool
Electron Beam Forms Image of Specimen			
Transmission electron microscope (TEM)	1,000,000 ×	0.5 nm	Sections of specimen are viewed under very high magnification; finest detailed internal structure of cells and viruses is shown; used only on preserved material
Scanning electron microscope (SEM)	100,000 ×	10 nm	Whole specimens are viewed under high magnification; external structures and cellular arrangement are shown; generally used on preserved material
Surface Forces Forms Image of Specimen			
Atomic force microscope (AFM)	1,000,000 ×	0.5 nm	Can examine live or preserved specimens. Provides surface detail at very high resolution

Fluorescence Microscopy

- Use of UV rays as the source of illumination
- Uses fluorescent dyes like acridine orange, fluorescein
- Direct staining and counting
- Useful in detection of fluorescently labelled probes in hybridization techniques

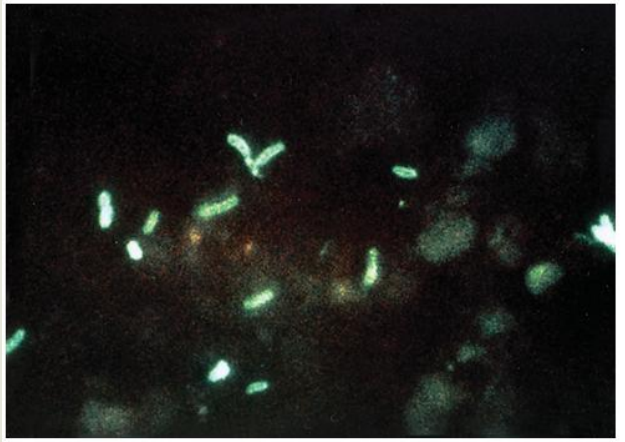


FIGURE 9.13 Use of fluorescent antibodies coupled to fluorescein isothiocyanate to detect antigens. Here rhizobia fluorescence in response to UV irradiation is shown. Photo courtesy I.L. Pepper.

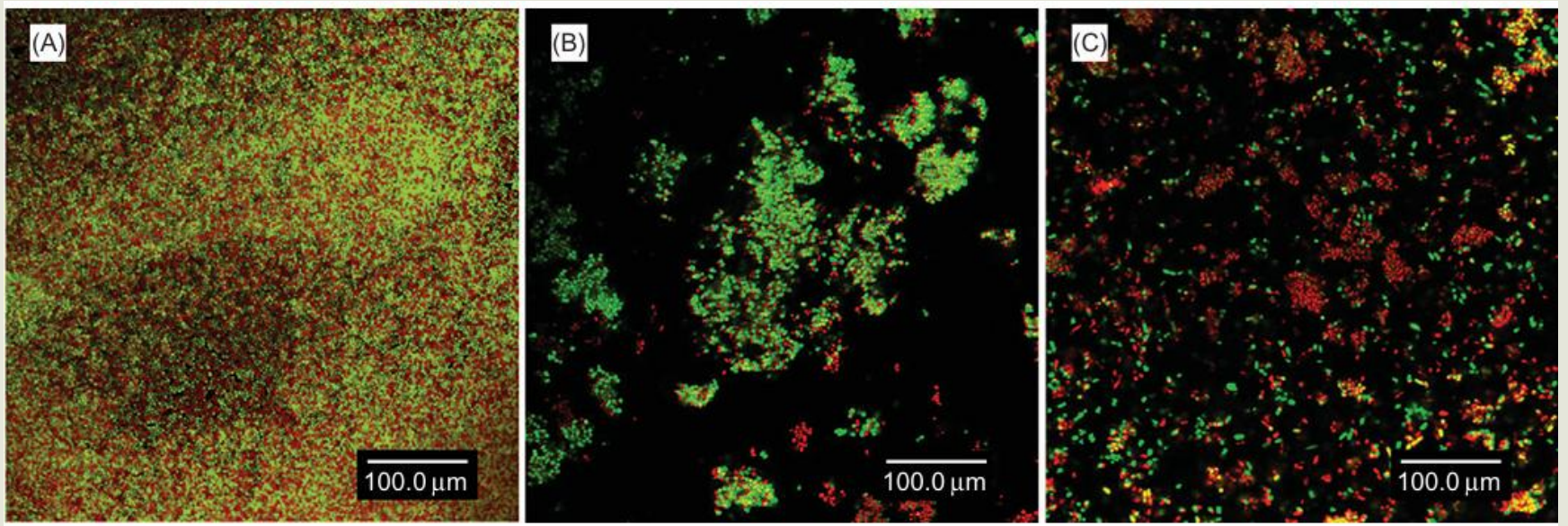
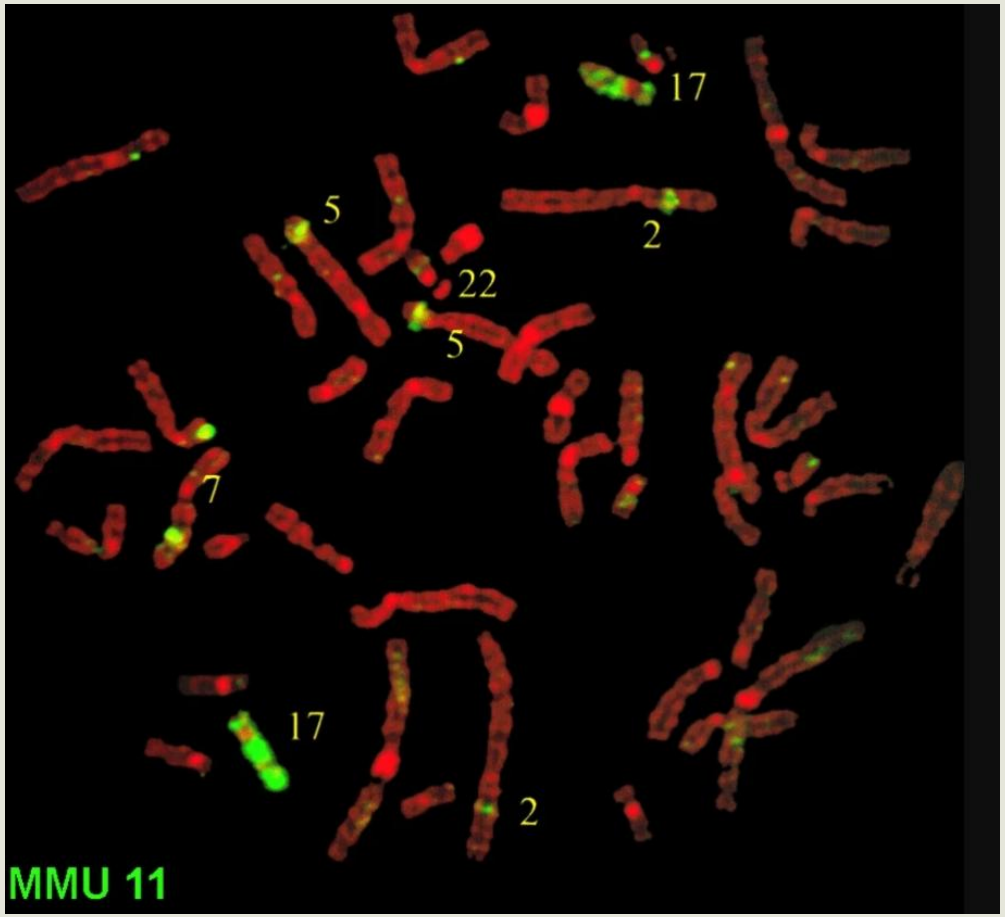
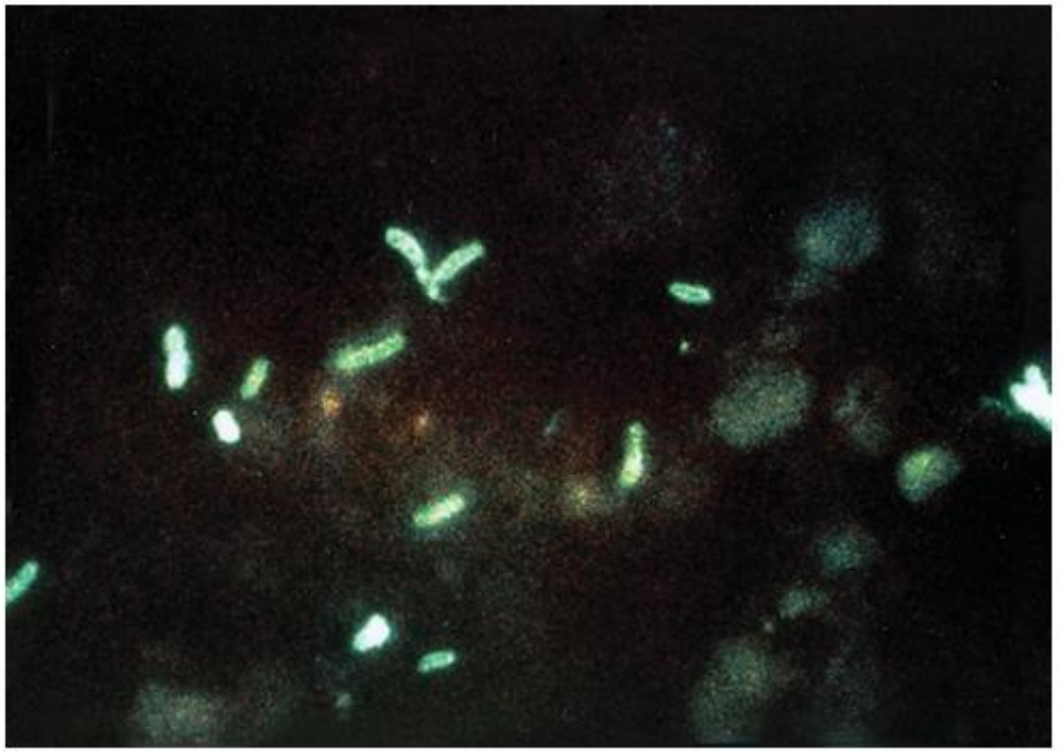


FIGURE 9.12 Bacterial cells stained with LIVE/DEAD BacLight bacterial viability stain to directly visualize the effects of an added antibiotic, vancomycin. These are confocal scanning laser microscope images of *Staphylococcus epidermidis* (SE6) intact biofilms (A), disrupted biofilm (B) and planktonic cells (C) on plastic coverslips after incubation for 24 h with 500 µg/ml of the vancomycin. These images suggest that the antibiotic is more effective on planktonic cells and disrupted biofilms than it is on an intact biofilm. From El-Azizi *et al.* (2005).





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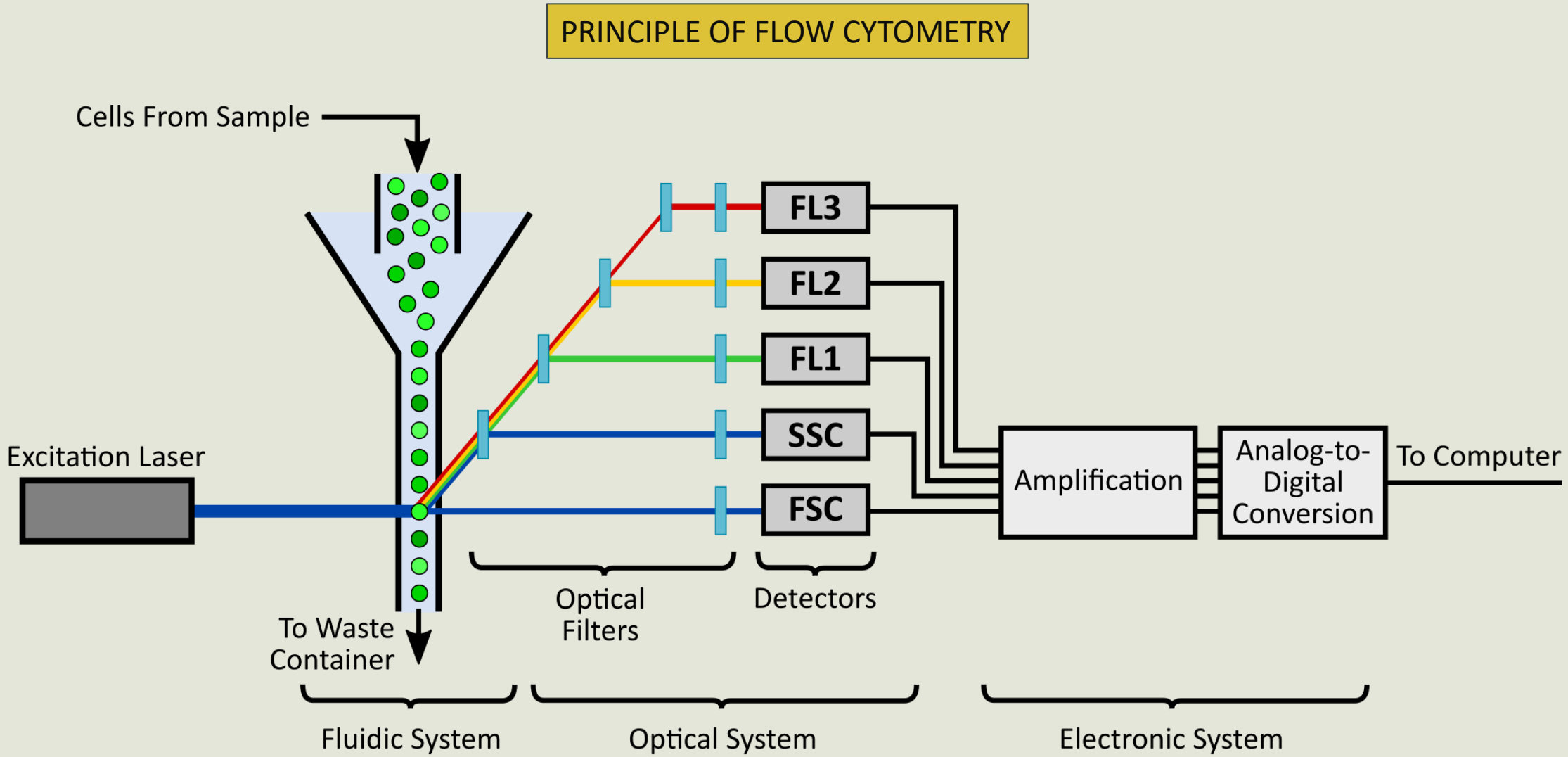
Human chromosomes painted with DNA from mouse chromosome 11 showing hybridization signals on human chromosomes 17, 5, 2, 7, and 22 and some other chromosomes. That is, an ancestral chromosome broke up into multiple fragments that can still be found in many human chromosomes (Source: Wikipedia)

Fluorescence Microscopy



Vaccines

Vaccines

- Edward Jenner – 1796 – small pox vaccine
- 1881 – Loius Pasteur – Anthrax vaccine in sheep, rabies vaccine
- Vaccine – A biological preparation that stimulates the body's immune system to recognize and fight off a specific disease, using weakened or killed forms of a pathogen, its toxins, or parts of it.

Types of Vaccines

TYPE OF VACCINE	EXAMPLE
1. First generation (conventional) vaccines	Live attenuated vaccine Inactivated (killed) vaccine Toxoid vaccine Bacterins
2. Second generation vaccines	Subunit vaccine Peptide vaccine Conjugate vaccine Anti-idiotypic vaccine
3. Third generation vaccines (recombinant DNA vaccines)	Recombinant subunit vaccine Recombinant synthetic peptide vaccine Marker vaccine Deletion mutant vaccine

Live Attenuated Vaccine

Mechanism

- Pathogen virulence reduced; immunogenicity retained via adaptation to unfavorable conditions.
- Replicates in host to stimulate immune response without causing disease.

Attenuation Methods

- **Passaging**: Repeated growth in non-host cells (70–80 cycles).
- **Cold adaptation**: Grows at lower temps (e.g., nasal vaccine strains).
- **Temperature-sensitive mutants**: Restricted growth at elevated temps.

Live Attenuated Vaccines

Advantages

- Replication mimics natural infection → robust, long-lasting immunity (often 1 dose).
- Contains T-cell & B-cell epitopes → broad immune response.
- Effective against intracellular pathogens (e.g., viruses).
- Cost-effective; no adjuvant/boosters typically needed.

Disadvantages

- **Safety risks**: Reversion to virulence, viral shedding, transient immunosuppression.
- **Logistical challenges**: Strict cold chain required; contamination risks.
- **Side effects**: Possible reactions to residual pathogen components.

Inactivated/Killed Vaccines

Mechanism

- Pathogen inactivated via physical/chemical methods → retains immunogenicity but loses replication/virulence.
- Often uses **virulent strains** for higher immunogenicity.
- Used when live vaccines are unavailable or during outbreaks with uncharacterized pathogens.

Inactivation Methods

- **Chemical**: Formaldehyde, beta-propiolactone (cross-link proteins/nucleic acids).
- **Physical**: UV/gamma irradiation (disrupt genetic material).

Inactivated/Killed Vaccines

Advantages

- Safety: No risk of reversion or environmental shedding.
- Stability: Less dependent on cold chain.
- Contains T/B-cell epitopes → broad immune response.

Disadvantages

- **Limited efficacy**: Requires multiple doses, adjuvants, and boosters.
- **Production risks**: Improper inactivation → potential disease outbreak.
- **Cost**: Expensive to produce.
- **Side effects**: Higher antigen load → allergic reactions.
- Less effective against intracellular pathogens (no replication).

Adjuvants

- Adjuvants provide depots effect to an antigen at the site of administration which allows persistence and slow release of antigen over an extended period of time resulting in higher and prolonged immune response.
- Adjuvants increase immunogenicity of weak antigens.
- It helps in stimulation of cell-mediated immune response.
- Addition of adjuvant reduces the cost and dose of an antigen.
- Examples, mineral oils (Aluminium hydroxide, Liquid paraffin etc.) Vegetable oils (Ground Nut oil, Montanide etc.), Mycobacterial products (Freund's adjuvant) etc.
- Some other delivery systems are ISCOM (Immunostimulating complex), Nanoparticles etc. – particulate systems

Toxoid Vaccines

Mechanism

- **Exotoxins** (from Gram+/− bacteria) inactivated via formaldehyde/heat → non-toxic **toxoids** with retained immunogenicity.
- Used for toxin-mediated diseases (e.g., tetanus, anthrax).

Key Features

- **Anaculture**: Combines toxoid + formalin-killed bacteria (e.g., clostridial vaccines).
- **Enhanced immunogenicity**: Trypsinization improves efficacy.

Bacterins (Killed Bacterial Vaccines)

Mechanism

- Bacteria killed (e.g., formaldehyde) + adjuvant (e.g., alum) → triggers immune response.
- **Autogenous vaccines**: Farm-specific strains (e.g., fowl cholera).

Advantages

- Simple production; no reversion risk.

Disadvantages

- Short-term immunity (<6 months); frequent boosters needed.

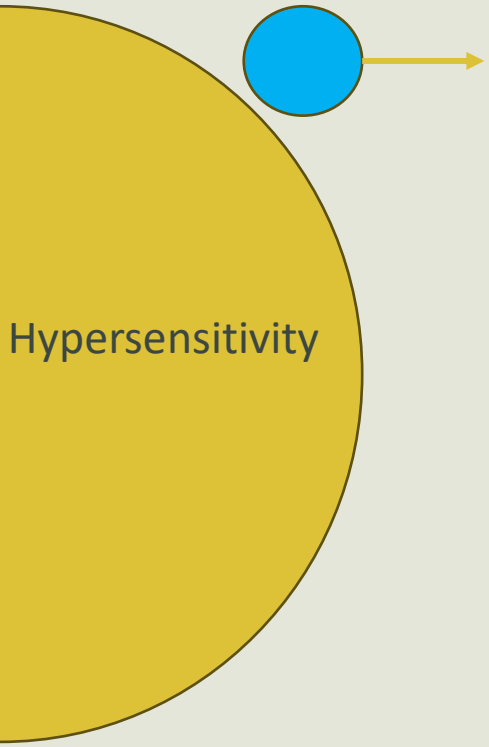
Vaccine Type	Description	Examples	Advantages	Disadvantages
Subunit/Conjugate	Purified antigenic subunits (viral/bacterial); polysaccharides conjugated to protein carriers.	Influenza HA vaccine, Pneumococcal conjugate	Avoids whole organism; fewer side effects; multiple epitopes.	Loss of immunogenicity; costly purification; cold chain needed; large size.
Peptide	Synthetic/natural immunogenic peptides; may require carriers for T-cell response.	Foot and mouth disease (VP1 peptide 141-160)	Defined antigens; stable; fits delivery systems.	Acts as haptens without carriers; rapid dissipation; costly identification.
Anti-Idiotypic	Antibodies mimicking pathogen antigens (idiotype-anti-idiotype principle).	Hepatitis B	No pathogen exposure.	Complex production; low efficacy; expensive.
Recombinant DNA	Cloned pathogen genes expressed in vectors (bacteria, yeast, viruses).	Hepatitis B, Rabies (vaccinia vector)	Avoids pathogens; strong immunity; cost-effective; stable.	Anti-vector antibodies; vector replication risks; glycosylation issues in E. coli.
DIVA/Marker	Uses deleted antigens or proteins to distinguish infection vs. vaccination.	Avian influenza control	Enables disease surveillance; aids eradication programs.	Requires companion diagnostic tests; limited to specific pathogens.

- Subunit/Conjugate: Loss of immunogenicity → requires carrier/adjuvant.
- Peptide: Short synthetic sequences → need carriers (T-cell help).
- Anti-Idiotypic: Mimics antigen structure → no live pathogen used.
- Recombinant DNA: Vector-based (e.g., vaccinia) → potential anti-vector immunity.
- DIVA: Deletion mutants → paired with serological tests to detect natural infection.

Hypersensitivity

- Antigen when enters into a body it leads to development of immunity or may cause an allergic reaction and results in tissue damage. This undesirable effect of tissue damage is known as hypersensitivity.
- Classification of Hypersensitivity (Gell and Coombs, 1963)
  - Type I hypersensitivity (Immediate hypersensitivity; Anaphylaxis)
  - Type II hypersensitivity (Cytotoxic reaction)
  - Type III hypersensitivity (Immune complex disease;Arthus Phenomenon)
  - Type IV hypersensitivity (Cell-mediated;Delayed hypersensitivity).

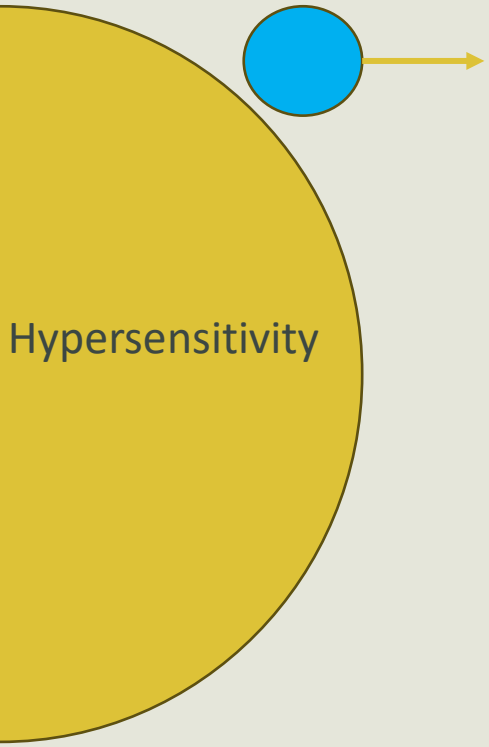
Hypersensitivity



- Type I Hypersensitivity
- Type II Hypersensitivity
- Type III Hypersensitivity
- Type IV Hypersensitivity

- Immediate Type
- IgE bound to mast cells and basophils
- Induced by allergens
- **Atopy:** When individuals make IgE continuously – against common environmental antigens or hereditary

Hypersensitivity



- Type I Hypersensitivity
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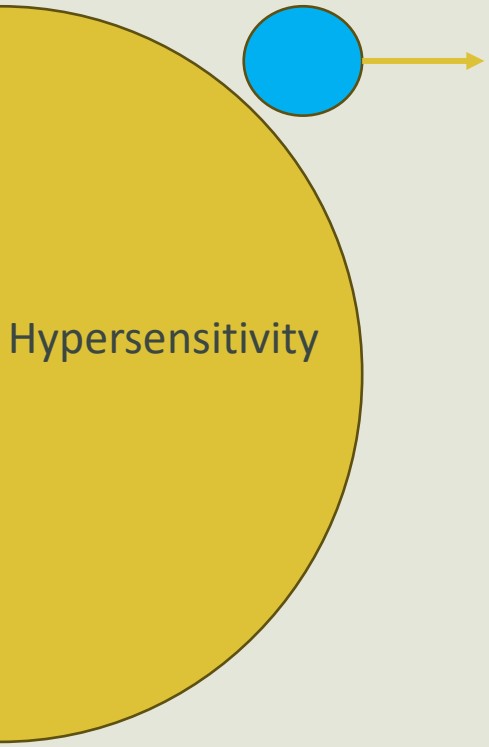
- Type IV Hypersensitivity

Mechanism:

- Plasma cells secrete IgE – binds to mast cells and basophils – causes degranulation
- Release of histamine – smooth muscle contraction in bronchi, GIT, Uterus and bladder
- Increase in vascular permeability leading to *wheal* formation
- Stimulates mucous production, lacrimation & salivation
- Serotonin – vasoconstriction – high BP
- In rats: wheal & flare reaction

Hypersensitivity



- Type I Hypersensitivity
- Type II Hypersensitivity
- Type III Hypersensitivity
- Type IV Hypersensitivity

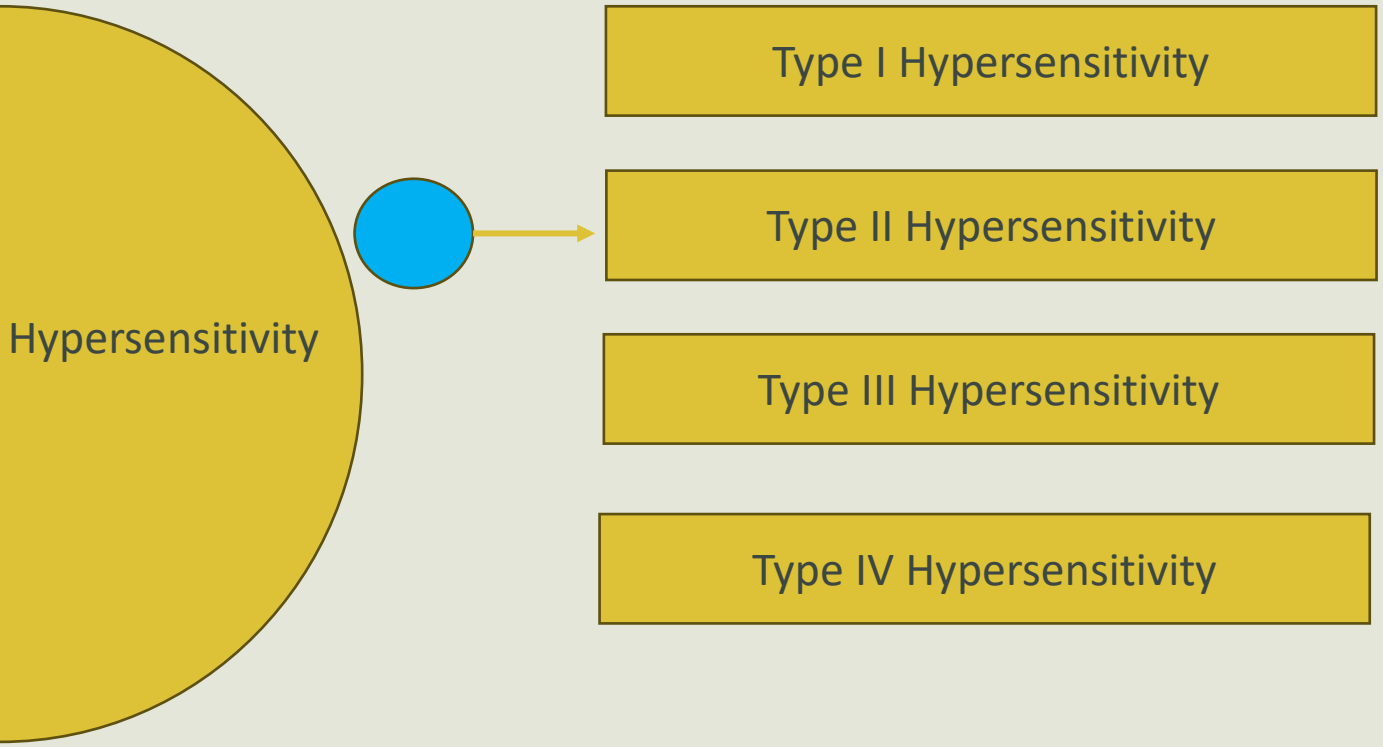
Mechanism:

- Mast cell granules: release eosinophil chemotactic factor –
- Characteristic eosinophilia of Type I reaction

- **Clinical Hypersensitivity I reactions:**

Milk Allergy, Food allergy, Atopic dermatitis, Drug/vaccine hypersensitivity, Allergy to parasite etc.

Hypersensitivity



IgG/IgM Mediated

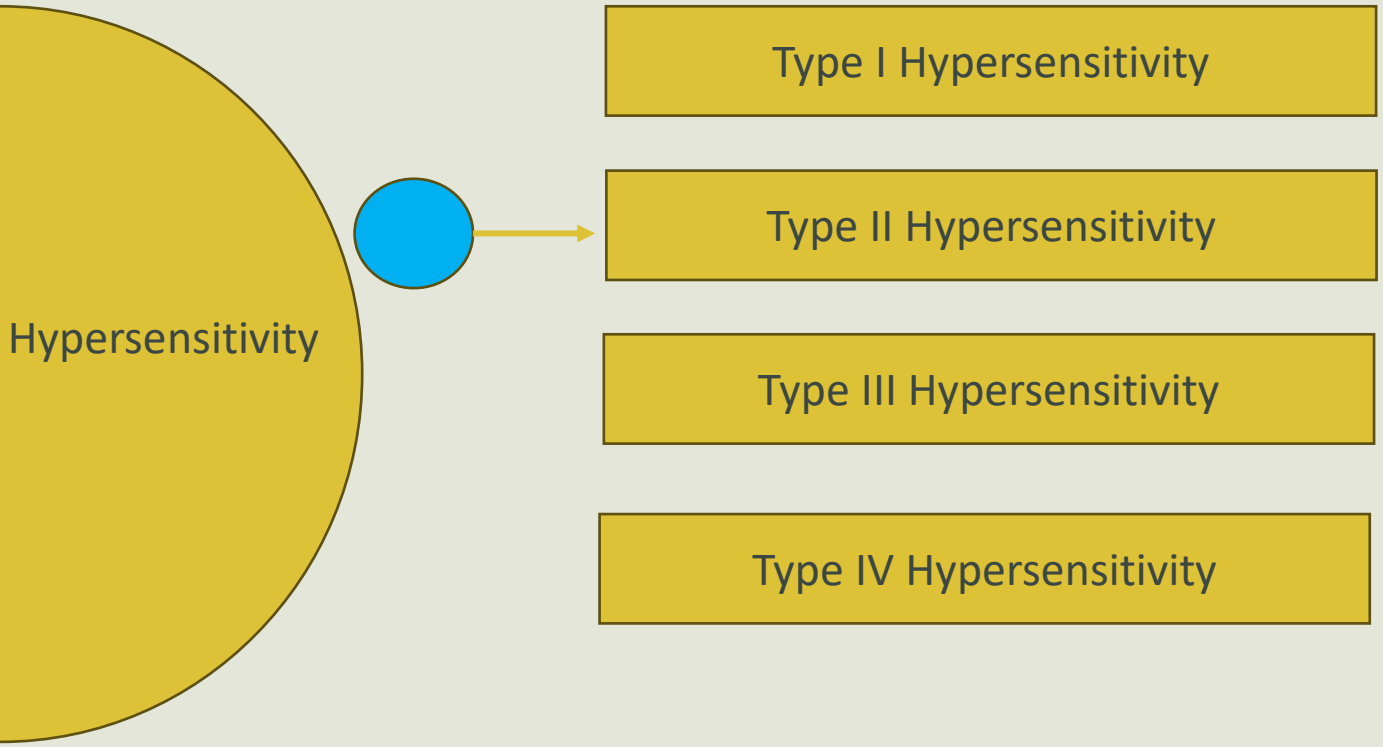
Cytotoxic/cytolytic reaction

Antibodies recruit inflammatory cells and complement system – bridge between antigen and effector cells leading to lysis

Complement activation causes production of enzymes which cause osmotic changes and lysis

Destroys cells by **ADCC (Antibody Dependent Cell Mediated Cytotoxicity)**

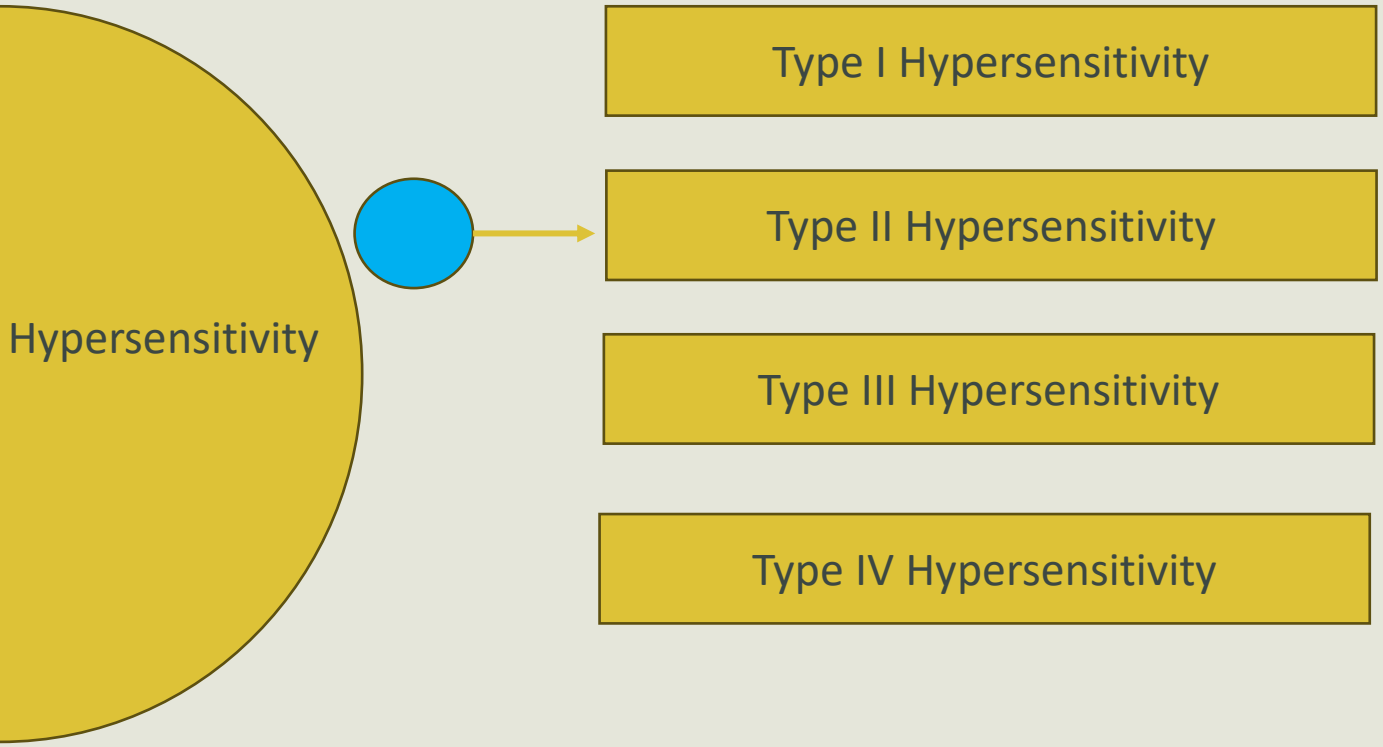
Hypersensitivity



Blood Transfusion Reactions:

- Receipient possessing natural antibodies to foreign RBCs (IgM type) – without antigen exposure
- Receipient possessing antibodies after immune response (IgG type) – after first transfusion
- Transfusion of other blood types causes Type II hypersensitivity reactions
- Agglutination of RBCs by hemagglutinin (antibodies) from another individual of same species is called isohemagglutination

Hypersensitivity

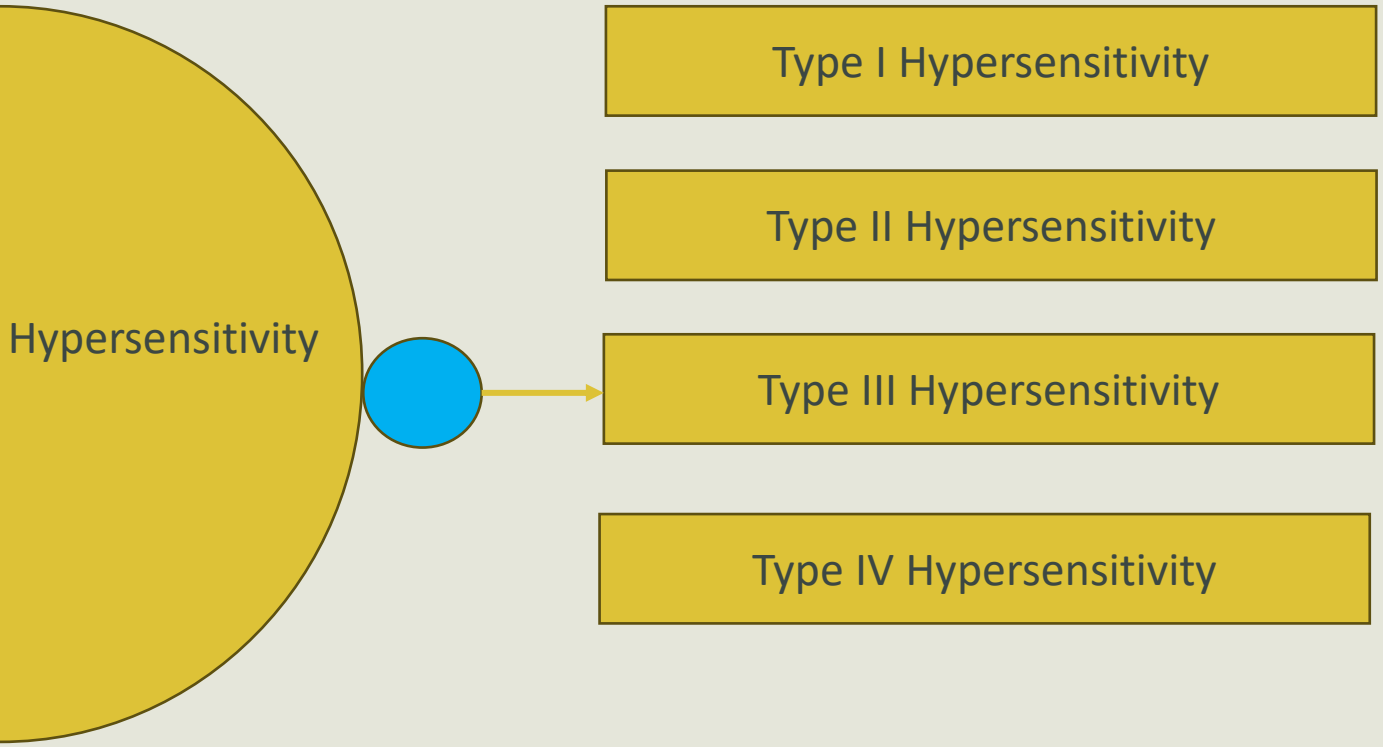


Hemolytic Disease of the Newborn

- Female animals sensitised to newborn RBCs through placenta
- Antibodies against fetal RBCs secreted in colostrum and absorbed by newborn through intestinal wall causing HDN or neonatal isoerythrolysis

Equine Infectious Anaemia, Trypanosomes, Anaplasma, Babesia – adsorbed onto red cells which get altered and are treated as antigens leading to Type II response

Hypersensitivity



Deposition of soluble antigen and antibody complex in tissues (on vascular endothelium)

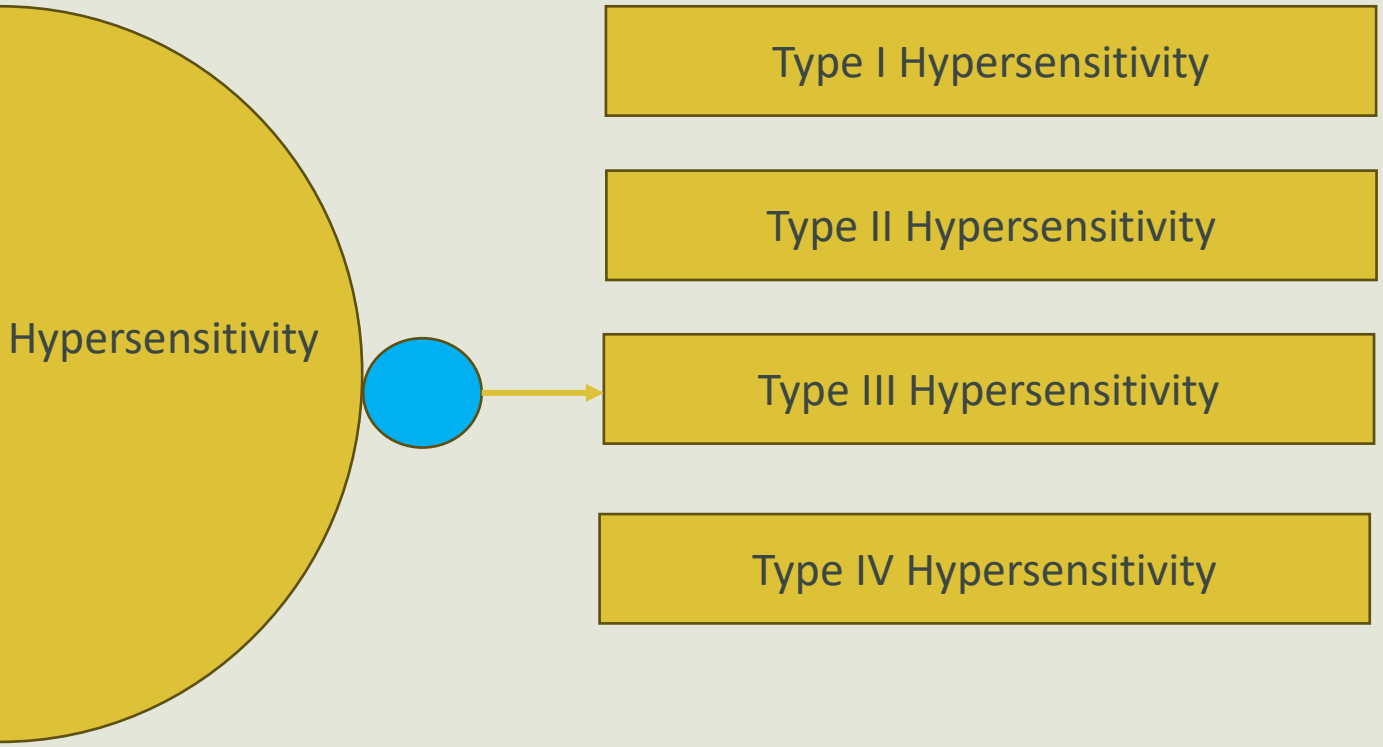
Leads to complement activation

Massive infiltration of PMNLs, release of vasoactive molecules, inflammation and tissue destruction

Two types of reaction:

1. Local – Arthus reaction
2. Systemic – Serum sickness

Hypersensitivity



Arthus Reaction

Subcutaneous injection of antigen to an already sensitised animal – leads to inflammation at site of injection

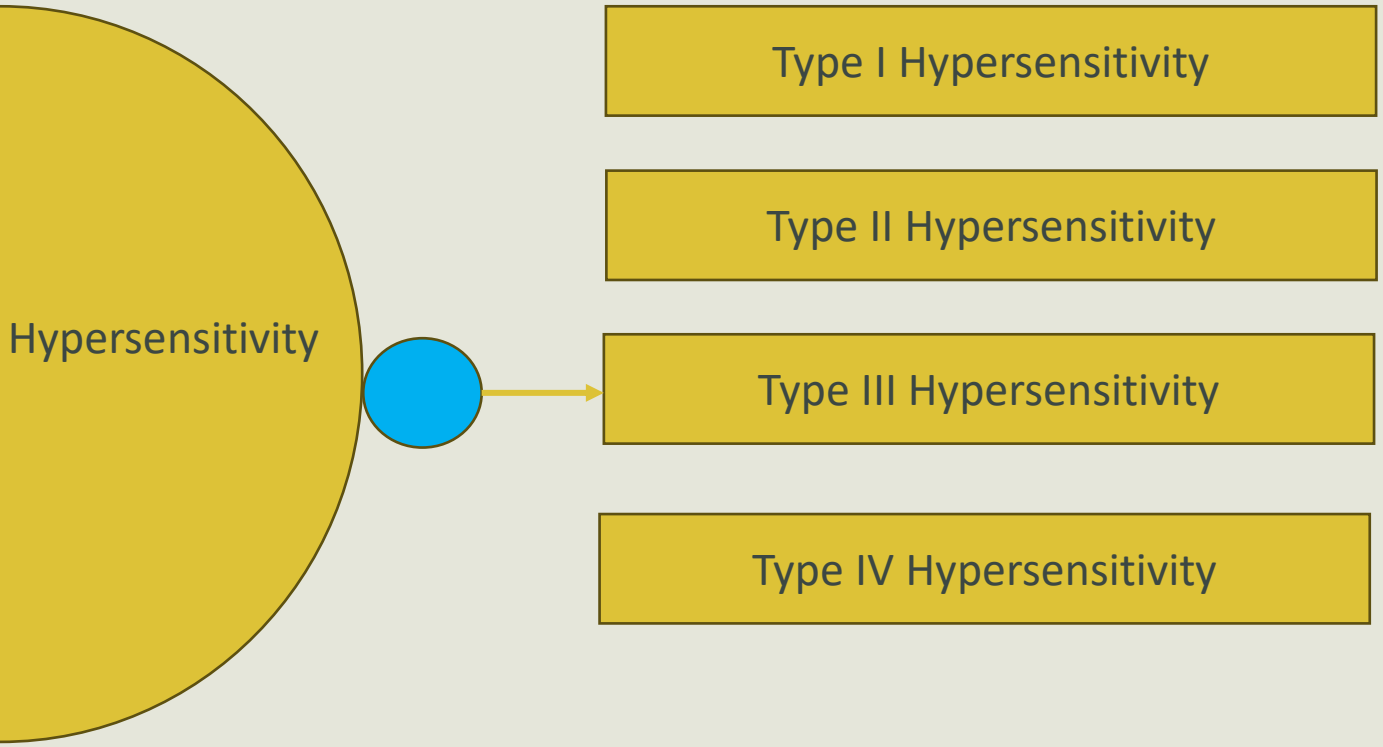
Erythematous, edematous swelling ->

Local hemorrhage and thrombosis ->

Local tissue destruction

Usually IgG class of precipitating antibodies

Hypersensitivity



Arthus Reaction

Reversed Arthus reaction:

Animals already having circulating antigen is injected with antibodies intradermally

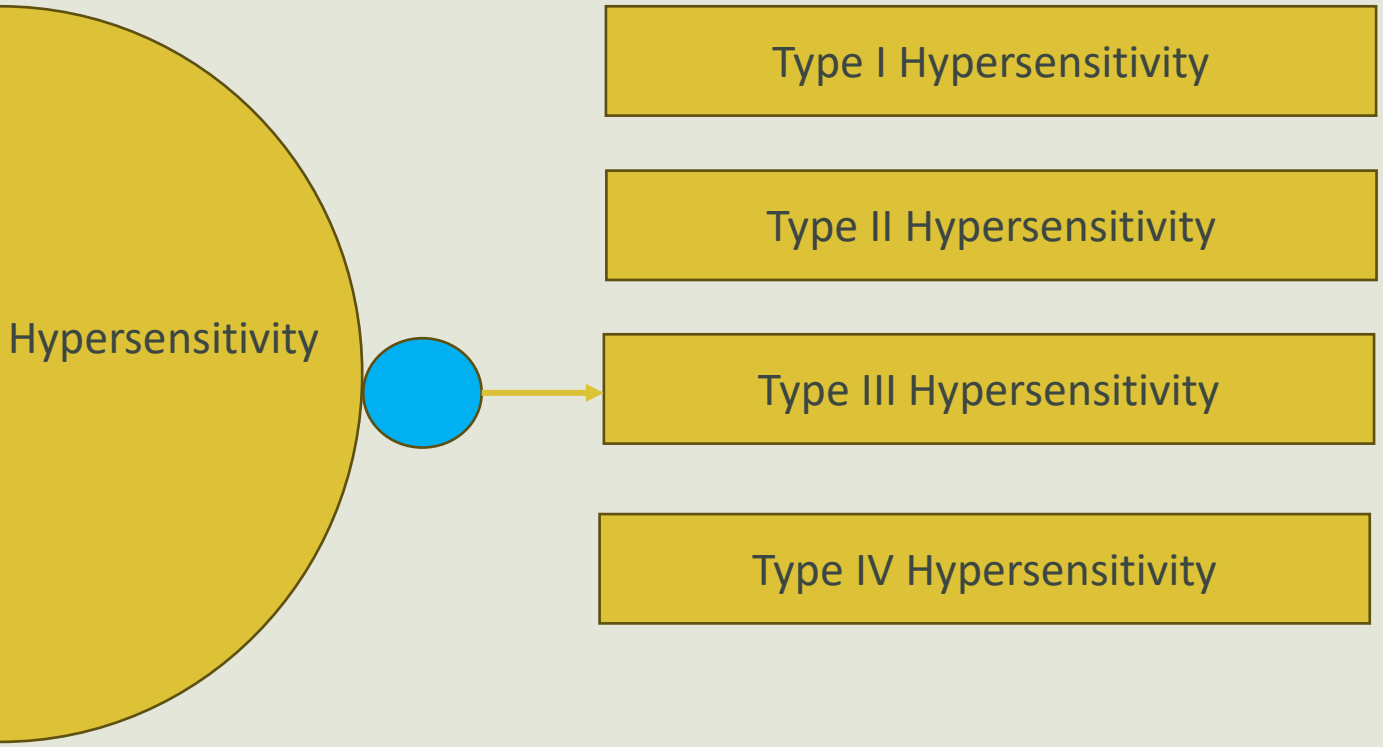
Eosinophil infiltration is not a common feature of Arthus reaction

Examples:

Blue eye (CAV-I) – anterior uveitis & virus-antibody complex deposition in cornea

Hypersensitivity Pneumonitis

Hypersensitivity



Examples:

Hypersensitivity Pneumonitis:

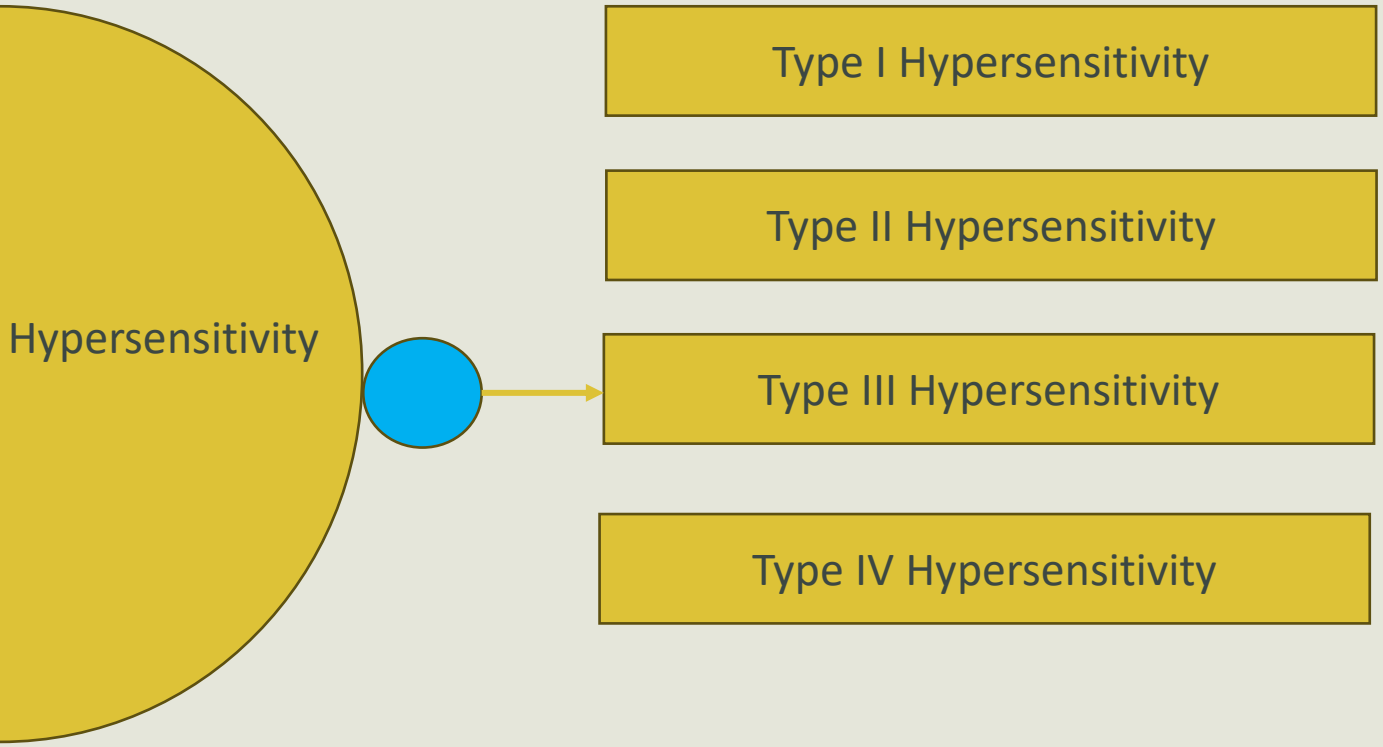
Inhalation of spores of fungi (moldy hay) leading to complement activation and interstitial pneumonia

Farmer's lung (hay sickness): *Sacchyropolyspora rectivirgula* spores chronic exposure

Pigeon breeder's lung – dust from pigeon feces

Mushroom grower's lung, Librarian's lung

Hypersensitivity



Serum Sickness:

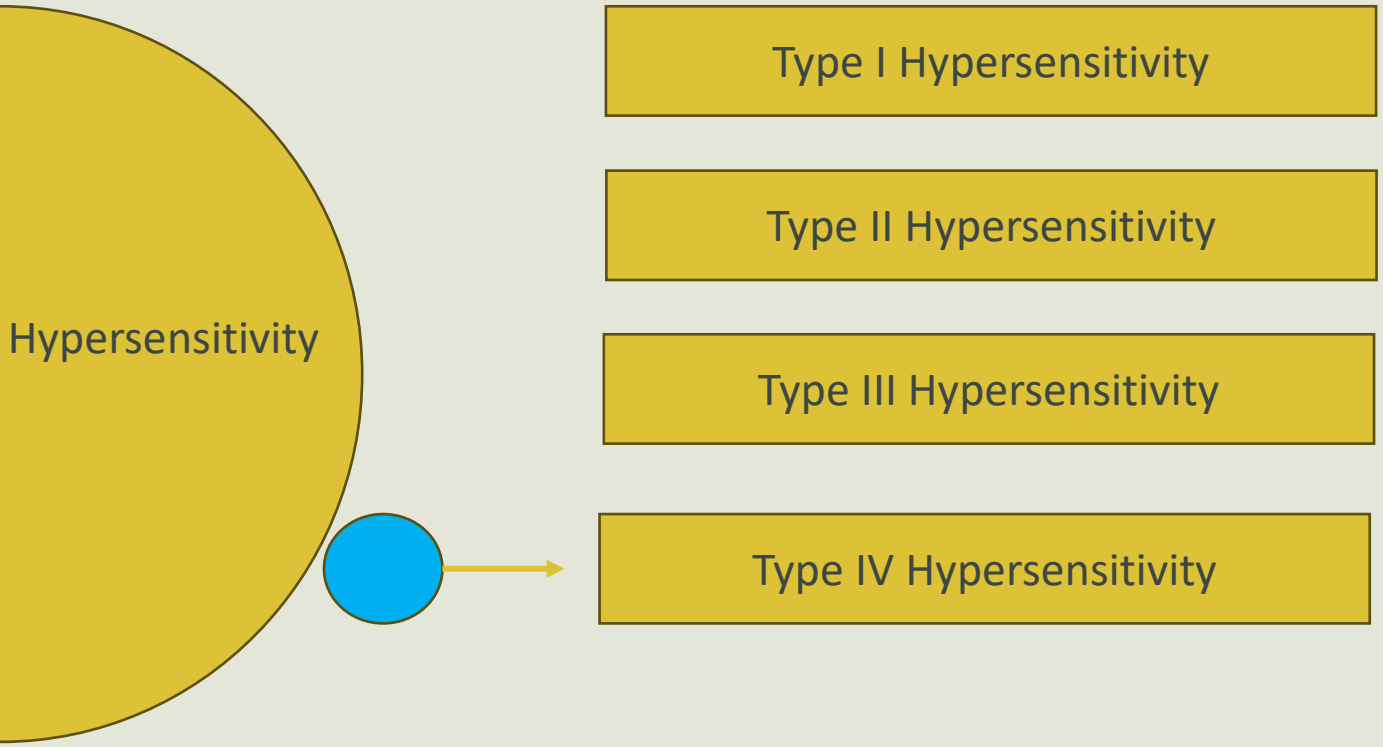
Large amounts of antigen enter blood and bind to antibody –

Formation of small circulating immune complexes which are not cleared from the system

Accumulation in blood vessels – glomeruli, synoviae, choroid plexus – tissue damaging Type III reactions

Vasculitis, erythema, edema, urticaria, neutropenia, LN enlargement, Joint swelling, Proteinuria

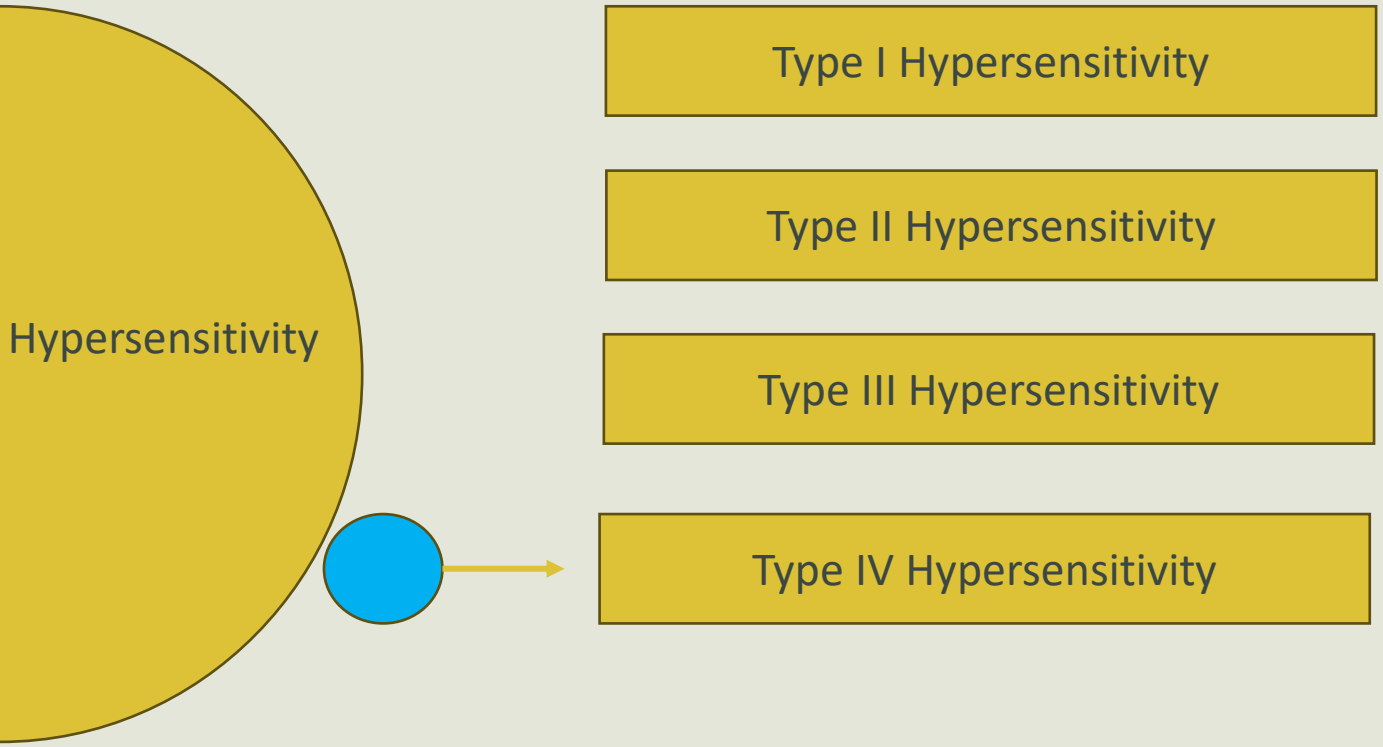
Hypersensitivity



Delayed Type of Hypersensitivity

- Cell-mediated immune response (no antibody involvement).
- Induced by **sensitized T cells** interacting with antigen-presenting cells (APCs) and antigens.
- **T cells** release **lymphokines** upon antigen contact → recruit/activate immune cells (lymphocytes, macrophages).

Hypersensitivity



Delayed Type of Hypersensitivity

- Causes localized inflammation and tissue damage.
- **Delayed onset**: Symptoms appear 48–72 hours post-exposure.
- **Cannot be transferred by serum** (antibodies) → requires **lymphocytes** for passive transfer.

Thank you