Cellular Pathology: Samples, Stains, and Cell Structures

This PDF was prepared by Stephan Grundy for ReviewPathology.com

What is Cellular Pathology?

Cellular pathology includes histopathology and cytology and examines tissue specimens from patients, taken by surgical operation, tissue smears, or post-mortem examination. Examinations of the tissues and organs are made to determine a diagnosis and provide information on further treatment. (Wikipedia)

Rudolf Virchow

Robert Remak

Theodor Schwann

Rudolf Virchow is "father" of cell theory and cellular pathology (founded on the research of Theodor Schwann and Robert Remak)

What is Histology?

- Histology is the microscopic study of tissue.
- It is one of the main tools in the pathologist's armament of detecting pathological conditions.
- It includes traditional techniques such as fixing and staining slices of tissue, and newer immunohistochemical techniques such as immunofluorescence and immunohistochemical testing.

What are the purposes of direct tissue evaluation (fix and stain)?

- 1) The choice of stain reveals substances and structures that are not normally visible; e.g., Perls iron stain reveals the presence of iron in tissues, making it possible to diagnose and evaluate conditions such as haemosiderosis (iron overload).
- 2) Tissue slides are crucial for revealing abnormal cells or infiltration/proliferation of otherwise normal cells (may be specifically diagnostic or suggest a type of condition); and abnormal tissue structure, which is often diagnostic.

How and when do we get tissue samples?

- Tissue samples are gotten through biopsy. This may be performed on a living patient, or post-mortem.
- Types of biopsy include:
- Excisional
- Incisional
- Endoscopic
- Punch
- Needle biopsies (fine needle aspiration, Tru-Cut, bone marrow aspiration)

What are excisional and incisional biopsies?

- **Excisional biopsy – performed as a follow-up to autopsy or surgical removal of a whole organ or whole lump. Most often done with lymph nodes, breast lumps, and spleen (which cannot safely be cut into: it must be removed whole or left alone).**
- **Incisional biopsy – a portion of an organ or suspected tumour is removed. Most often done in tumours of connective tissue origin to definitively distinguish between a benign tumour and a sarcoma.**

What is an endoscopic biopsy?

- An endoscopic biopsy is used to sample abnormal tissue on the interior of a hollow viscus.
- Through the endoscope, it is possible to visualize abnormal areas and pinch a small sample off with an endoscope-directed forceps.
- This is a very common practice in, e.g., colonoscopy. If a polyp or lesion is detected during the investigation, it can immediately be sampled without requiring a second procedure.

What is a punch biopsy?

- **A punch biopsy is used for taking a small sample of skin or small surface mass.**
- **This is useful in ascertaining a diagnosis for suspicious skin lesions.**
- **It is also used in some cases of inflammatory skin disease to establish a diagnosis.**
- **Punch biopsies are performed with local anaesthetic, and care must be taken with patients on anti-coagulation or known to have bleeding disorders.**

What is a Fine Needle Aspiration Biopsy?

- **Fine Needle Aspiration Biopsy (FNAB) is the least invasive form of biopsy. A fine needle (22-25 gauge) is inserted into the suspect lesion or gathers a sample of fluid to draw out cells.**
- **It is used to detect the presence of either general histological indications of cancer (such as excess and/or abnormal mitotic figures), particular cell types, or inclusions typical for the suspected cancer(s), for instance:**

  - “Little Orphan Annie eye” cells (papillary thyroid cancer)

  - Reed-Sternberg cells (Hodgkin’s lymphoma)

  - Psammoma bodies (a range of cancers, depending on location)
- **The FNAB does not retain cellular architecture: it is limited to cell type. This may limit its effectiveness in grading some cancers.**

What is a Trucut biopsy?

- The Trucut biopsy uses a thicker-gauge needle than the FNAB, with an opening near the point and a slot inside to hold the specimen.
- Unlike FNAB, the small piece of tissue obtained preserves tissue architecture.
- Such a sample is more useful in grading a tumour than are the loose cells obtained by FNAB.
- It is something of a cross between a punch biopsy and an FNAB.

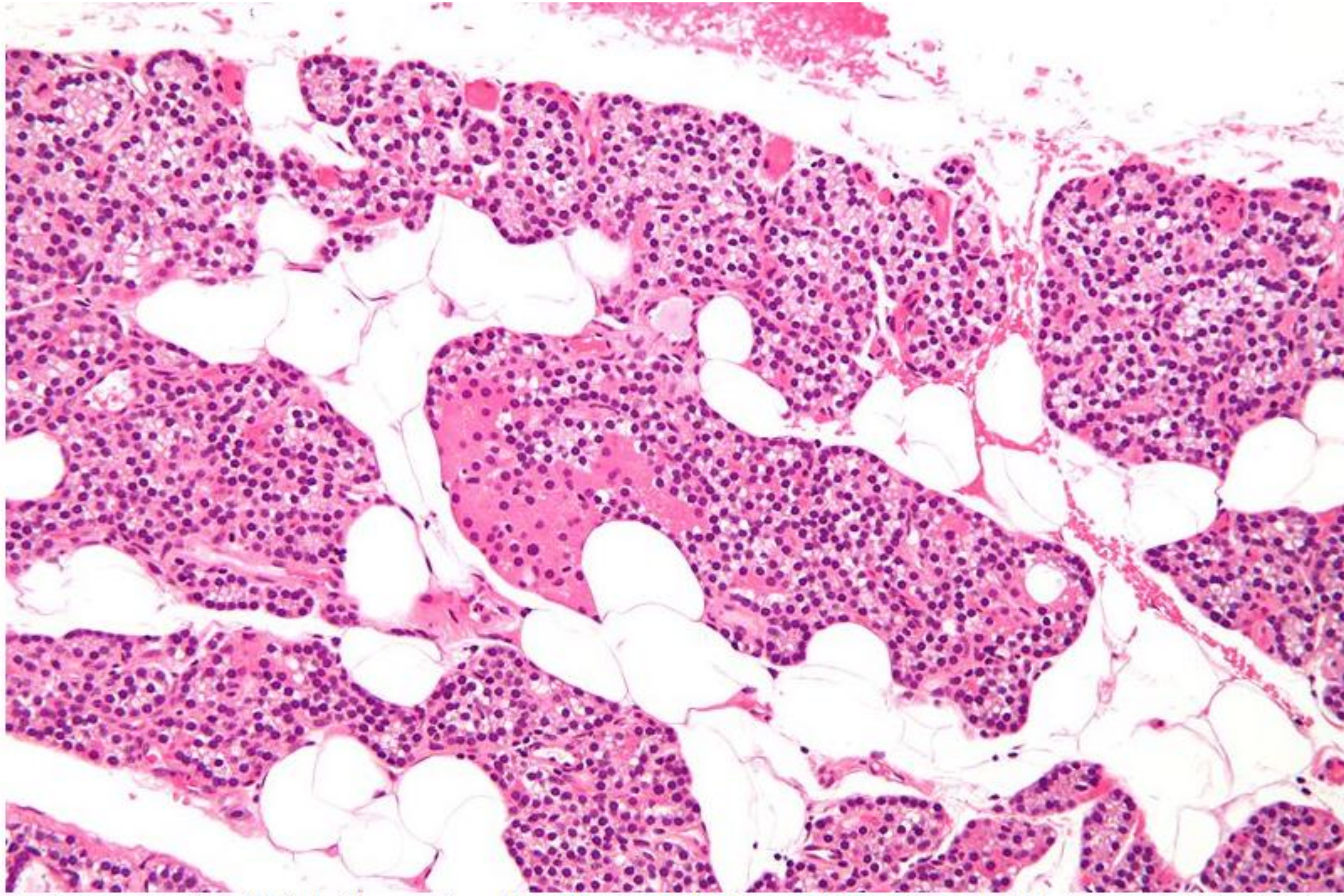
How is a bone marrow biopsy performed?

- A local anesthetic is first infiltrated into skin, then periosteum, over the biopsy site. The posterior iliac crest is preferred for safety reasons, but the anterior iliac crest and the sternum may be used; the tibia is used for infants > 1 year.
- A large needle is introduced into the marrow space and a syringe is attached to suction the marrow through needle.
- Since there is no way to anaesthetize the inside of the bone, this procedure is notoriously painful. Either conscious sedation or general anaesthesia are occasionally used, particularly in children.
- Usually a core biopsy, taking a sample of the bone core, is done immediately following the marrow biopsy.

What is the most common stain used?

- **The most common stain used is H&E – Haematoxylin and Eosin. These two dyes stain, respectively, basophilic ("base-loving") structures purplish-blue, and eosinophilic ("acid-loving") structures pink.**
- **Hence the colour structure of the vast majority of histological slides (to quote a student colleague, "purple blobs in pink stuff"). Prior to staining, most samples are colourless and transparent.**
- **Cell nuclei and other DNA/RNA containing elements are generally basophilic; most cytoplasmic proteins are eosinophilic. Thus, the classical cell image with a purple nucleus and pink cytoplasm appears.**

Parathyroid gland with H&E staining. Note that the adipocytes (fat cells) do not take up the stain at all, showing up as white shapes with only faint membrane definition. From [Wikicommons]



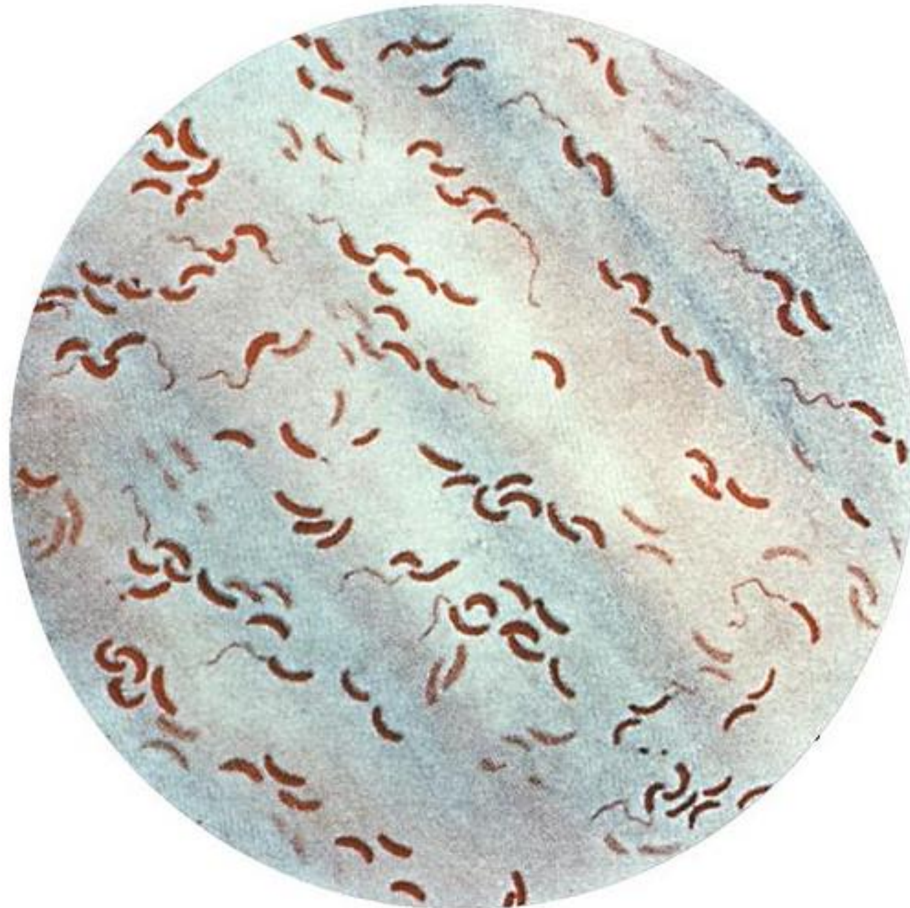
What else do stains show us?

- **As well as native cells and tissue structure, stains can also reveal the presence and form of bacteria, often a crucial element of diagnosis and antibiotic selection.**
- **The Gram stain works by staining the thick peptidoglycan walls of some bacteria ("Gram-positive") purple, while leaving the bacteria that lack those walls ("Gram-negative") pink. As the cell wall structure of bacteria is an important element in antibiotic penetration and activity, this allows the choice of the most effective antibiotics.**

What else does the Gram stain reveal?

- **In addition to Gram+ and Gram+ character, by making it possible to visualize the bacteria, many types of bacteria can be identified by shape (round or “cocci” vs. rods) and by aggregation. For instance:**
- **Gram+ cocci: staphylococcus show up in grapelike clusters, streptococcus in chains.**
- **Gram– cocci: Neisseria gonorrheae and N. menigitidis are diplococci, meaning that they are round and seen in pairs.**
- **Some bacteria have characteristic shapes: for instance, the comma-shaped rods of Vibrio cholerae with their long flagella.**

Vibrio cholerae – Gram negative, comma-shaped, visible flagella



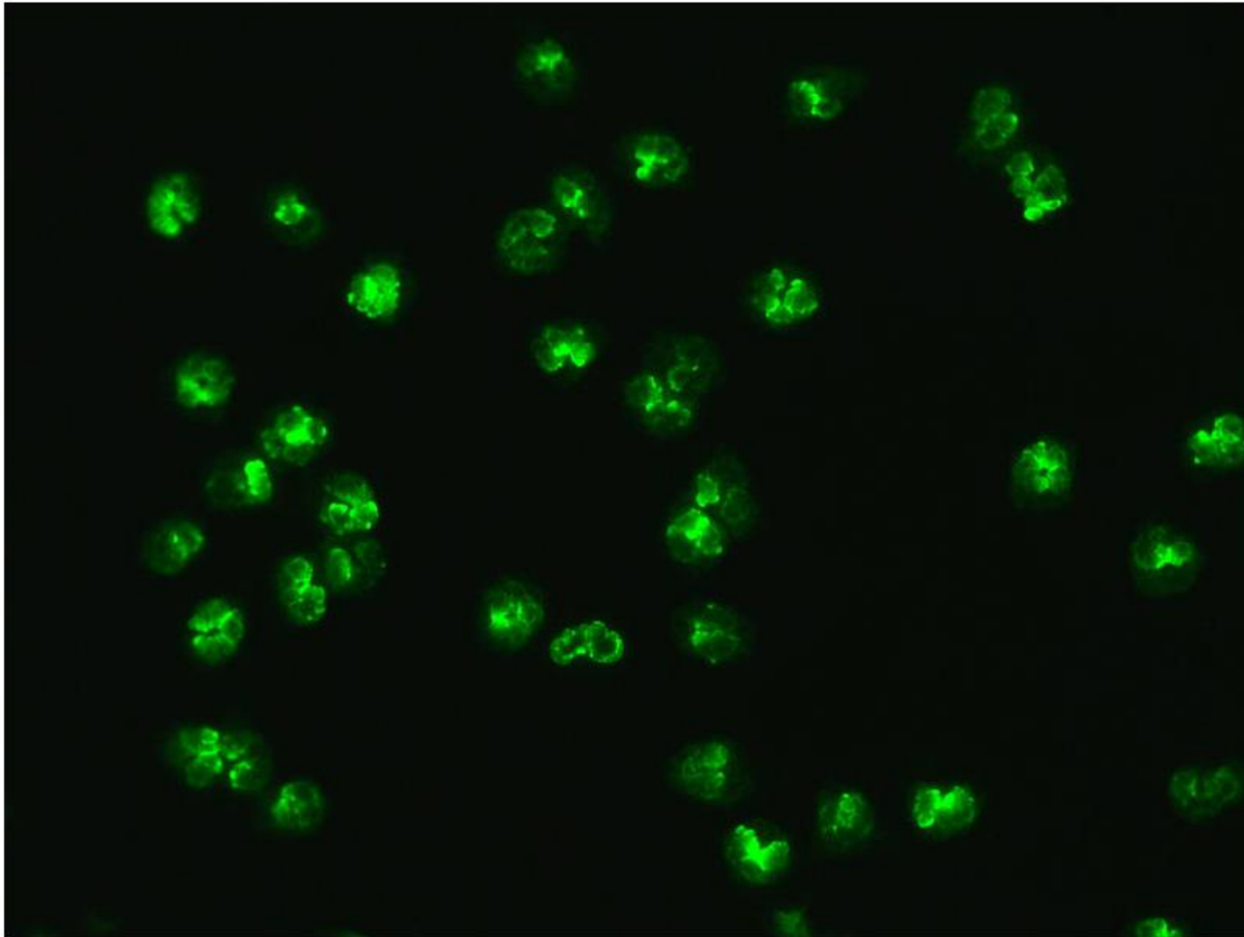
What are some other stains?

- Ziehl-Nielsen or "acid-fast bacillus" (AFB) stain. Most notably used to reveal mycobacteria, particularly M. tuberculosis (the "Dread Red Bug" – note case 76D on linked page).
- PAS (periodic acid-Schiff) – commonly used for tissue structures and a variety of other things with high carbohydrate content.
- This website provides an excellent list of special stains and their uses.

How does immunofluorescence work?

- Antibodies are labelled with a fluorescent dye and introduced to the sample, where they bind to cellular antigens.
- Exposing the bound sample to a short-wavelength, high-energy light causes the antigen-antibody complexes to glow.
- This makes it possible to determine both the presence and distribution of the selected cellular antigen in the sample under light microscopy.

Perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) under immunofluorescence/light microscopy.



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What is the difference between direct and indirect immunofluorescence?

- **With direct immunofluorescence, a single antibody is conjugated to a fluorophore and binds to a single target antigen.**
- **With indirect immunofluorescence, the primary antibody binds to the target antigen, while a secondary antibody, conjugated to a fluorophore, binds to the primary antibody.**
- **Direct immunofluorescence is faster and simpler; indirect immunofluorescence is more sensitive.**

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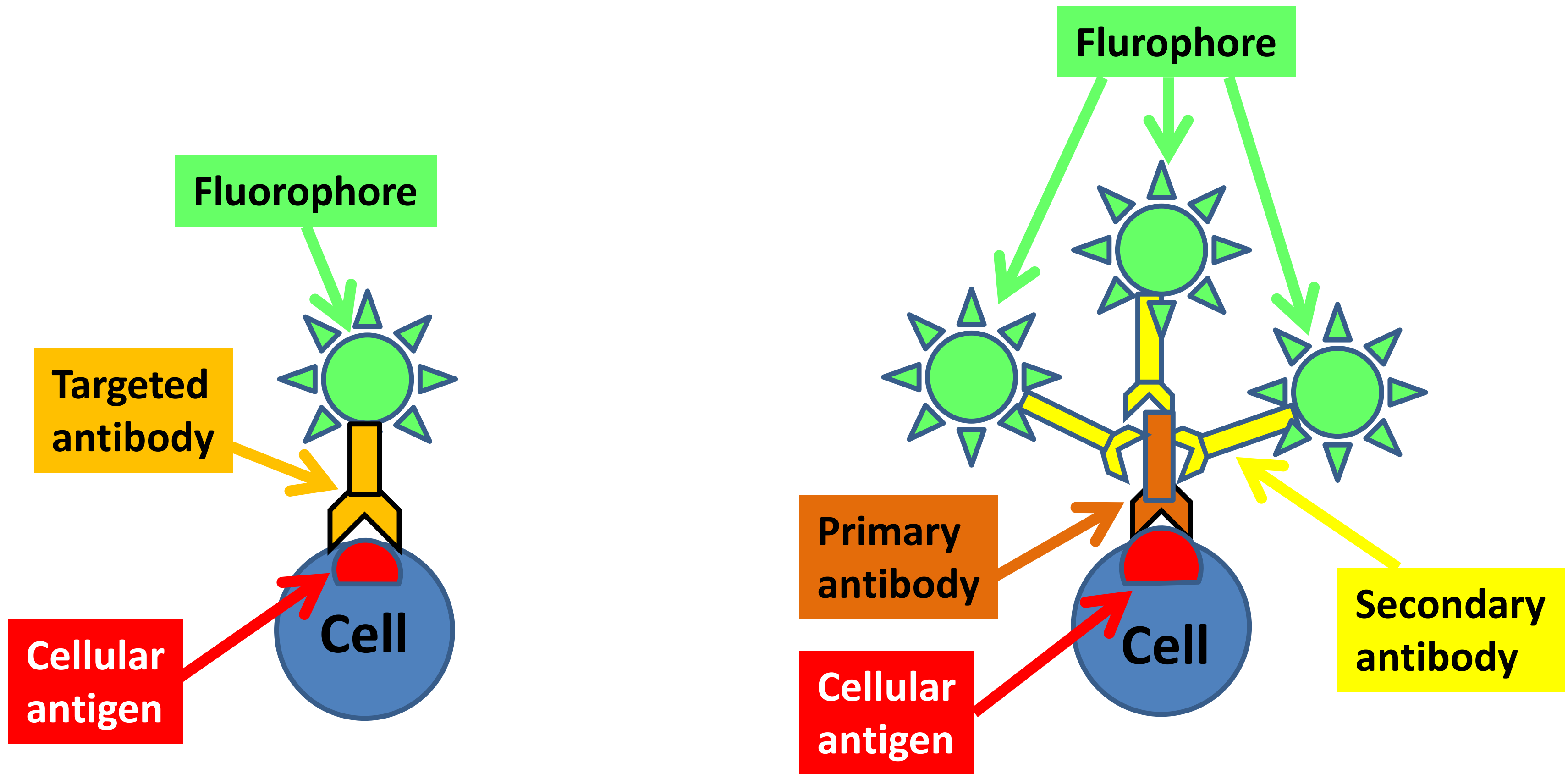


Direct Immunofluorescence | Indirect Immunofluorescence

Note that indirect immunofluorescence allows greater fluorophore binding to a singular cellular antigen, explaining its advantage of sensitivity compared to direct immunofluorescence.

What other immunofluorescence techniques are used?

- **Indirect immunofluorescence complement fixation – antibody/antigen binding produces high levels of complement protein C3, amplifying the findings to increase sensitivity.**
- **Double immunofluorescence – can be done by either direct or indirect technique. Two antibodies are labelled with different fluorophores. Extremely sensitive when done by the indirect method.**

What is immunofluorescence used to diagnose?

- **Conditions characterized by the presence or build-up of specific antibodies. For instance:**
- **Anti-neutrophil cytoplasmic antibodies – Wegener's granulomatosis (perinuclear or p-ANCA); Churg-Strauss disease (cytoplasmic or c-ANCA).**
- **Immunoglobulin or complement deposits, notably in nephropathies.**
- **Auto-antibodies against specific tissue types (e.g., thyroid, pancreas, gastric mucosa).**
- **Autoimmune diseases such as systemic lupus erythematosis.**
- **Viral infections.**
- **…and many others.**

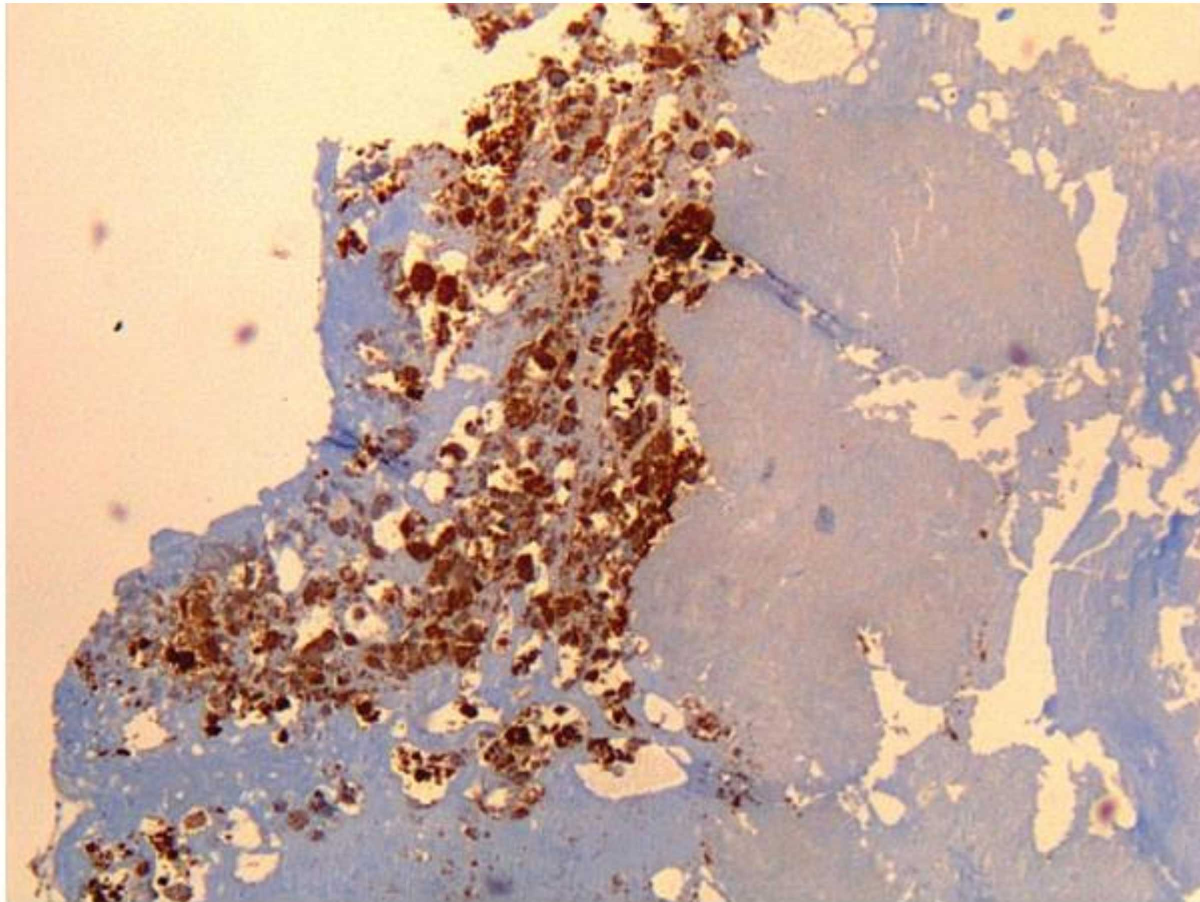
What is immunohistochemical staining and why is it used?

- Technically this term includes the methods of immunofluorescence. Most often, it refers to a technique by which enzymes, antigens, specific genes, and cell proliferation markers may all be identified.
- It is particularly useful in evaluating suspected tumours for stage, grade, tissue of origin, and prognosis.
- IHC staining is also valuable in diagnosing viruses and evaluating specific gene expression.

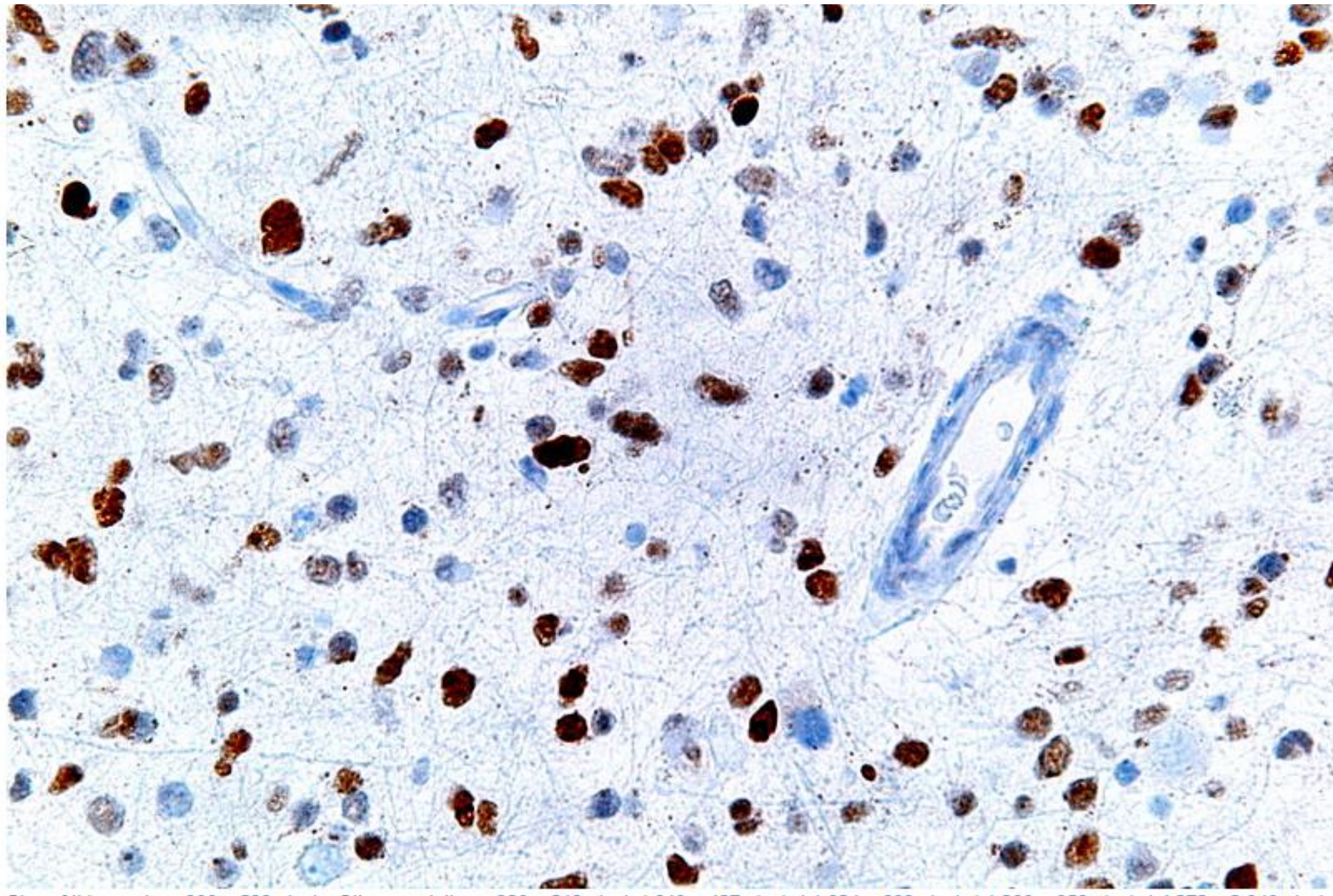
How does chromogenic immunohistochemical staining work?

- Instead of being conjugated to a fluorophore, as in immunofluorescent IHC staining, the antibody is conjugated to an enzyme, causing the deposition of insoluble, coloured precipitates where the antigen is localized. The principle is otherwise the same as immunofluorescent staining, and both direct and indirect methods may be used in this manner.
- The result is visible by light microscopy.
- For a detailed description of the process, see: http://kromat.hu/UserFiles/files/patologia/Dako_IHC_metodikai_k%C3%A9zik%C3%B6nyv_I.pdf

Coxiella burnetti in the heart valve of a 60 year-old man, displayed by chromogenic IHC staining.



Positive p53 IHC staining of an anaplastic astrocytoma



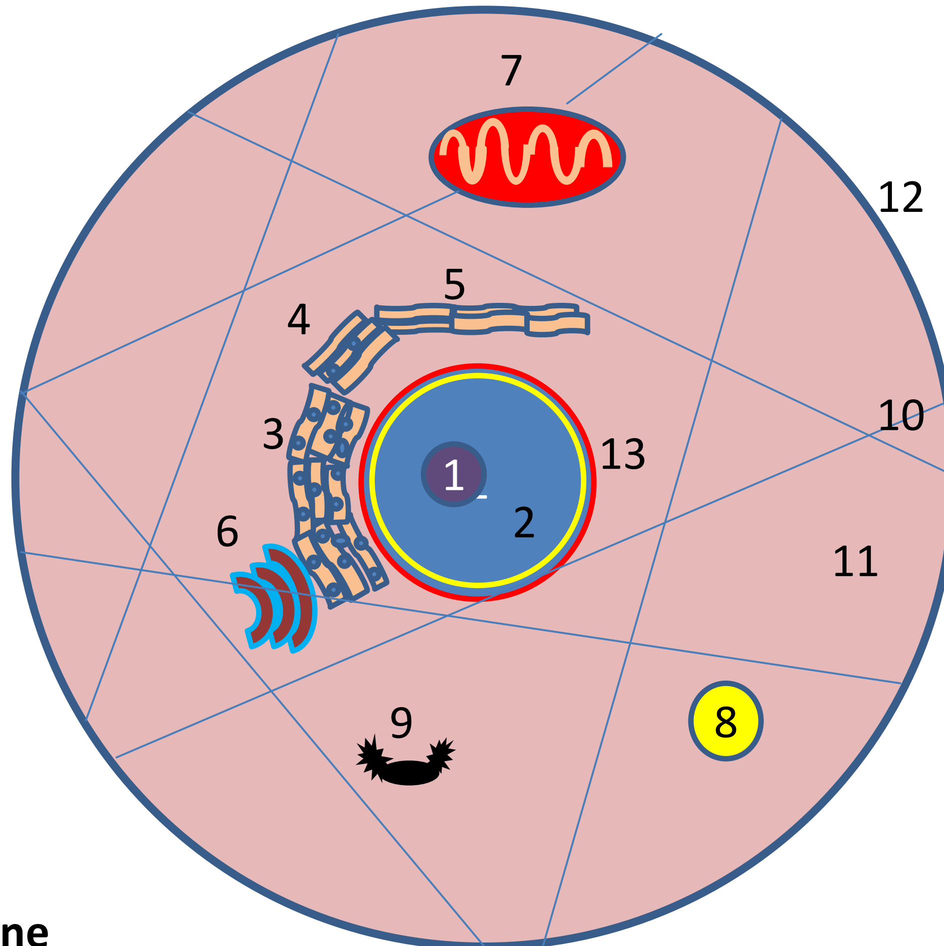
Cellular Anatomy and Basics

Basic cellular anatomy

- **The cell is a complex structure including a number of organelles with various functions.**
- **Cytoskeleton – a network of filaments and tubules which maintain the cell's shape.**
- **Nucleus – the repository of the majority of the cell's DNA.**
- **Nucleolus – a mini-organelle within the nucleus which generates ribosomes (translate messenger RNA for protein synthesis).**
- **Golgi apparatus – packages proteins into vesicles for transport.**
- **Rough and smooth endoplasmic reticulum (ER) – sites of synthesis for, respectively, proteins and lipids/phospholipids/steroids.**
- **Mitochondria – generate cellular energy; also involved with cell signalling and regulation of apoptosis. Contain mitochondrial DNA (maternally inherited only).**
- **Lysosomes – vesicles containing lytic enzymes, which break down foreign materials and cellular debris.**
- **Centrosomes – organize microtubules for cellular reproduction.**
- **Cell membrane – contains and protects the cell's contents; passage through the membrane is regulated by various means.**

Cell organelles (most require electron microscopy to visualize)

1. **Nucleolus**
2. **Nucleus**
3. **Rough ER (with ribosomes)**
4. **Transitional ER (mixed smooth and rough)**
5. **Smooth ER (no ribosomes)**
6. **Golgi apparatus**
7. **Mitochondrion**
8. **Lysosome**
9. **Centrosome**
10. **Cytoskeleton**
11. **Cytosol**
12. **Cell membrane**
13. **Nuclear membrane (two layers)**



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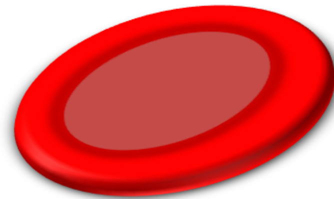
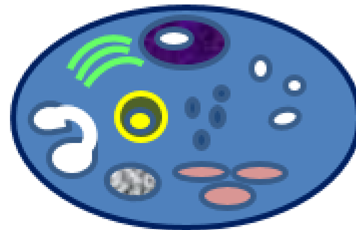
Not all Cells have all Organelles

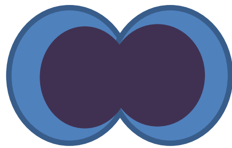
The mature red blood cell lacks organelles other than cell membrane and cytosol.

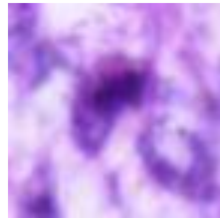


The platelet has no nucleus, but contains mitochondria,large and small vesicles, microfibrils, and microtubules, as well as several types of specialized granules.

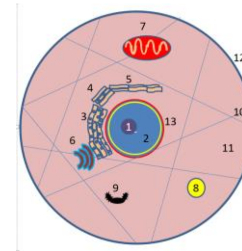


Visible stages of a cell's life

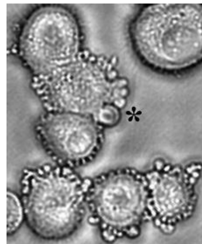




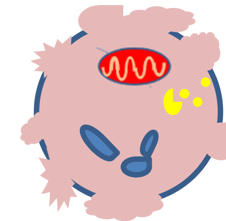
Reproduction (mitotic figure; number indicates rate of proliferation)



Quiescence – cell carries out its normal functions; no sign of proliferation, senescence, or trauma.



Apoptosis (natural death). Note membrane blebbing and increased autophagous vesicles.

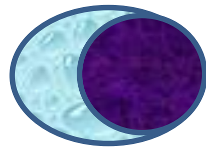


Necrosis (unnatural death). Nucleus fragments, mitochondria swell, membrane breaks down in an uncontrolled fashion (vs. blebbing), vesicles burst.

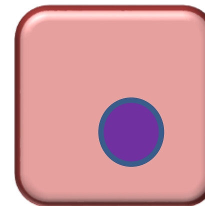
Identifying Abnormal Cells

To recognise an abnormal cell, you must know what a normal cell of that type looks like.

Some cells have characteristics that are normal for another type, but abnormal for their own. The large eccentric nucleus of a plasma cell would be markedly pathological in a cuboid squamous cell.



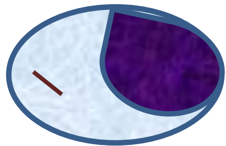
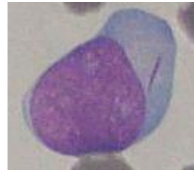
Normal plasma cell



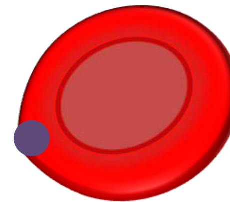
Normal squamous cell

In other cases, there are obvious abnormalities: various sorts of characteristically pathological inclusions, membrane damage, multiple or absent nuclei, replacement of the cell with necrotic material, and so forth.

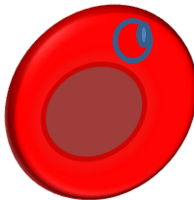
Sample Pathological Inclusions

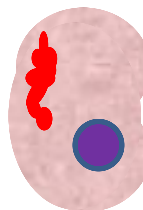
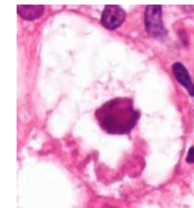
Auer rod in lymphocyte – Acute myeloid leukaemia



Heinz body – sign of oxidative stress (splenectomy, α-thalassaemia, liver damage, etc).

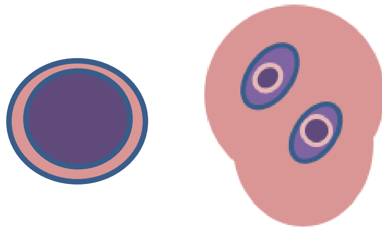


'Ring-shaped' trophozoite of *Plasmodium falciparum* malaria

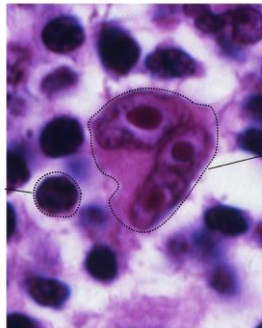



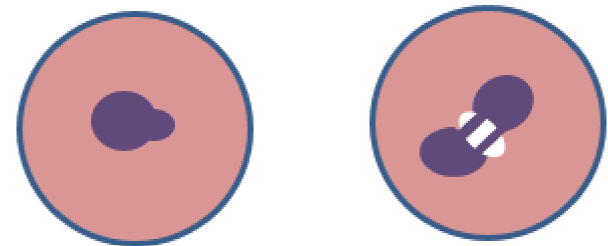
'Ropelike' Mallory body in hepatic cell – cirrhosis (particularly alcoholic), Wilson's disease, hepatocellular carcinoma.

Sample nuclear abnormalities



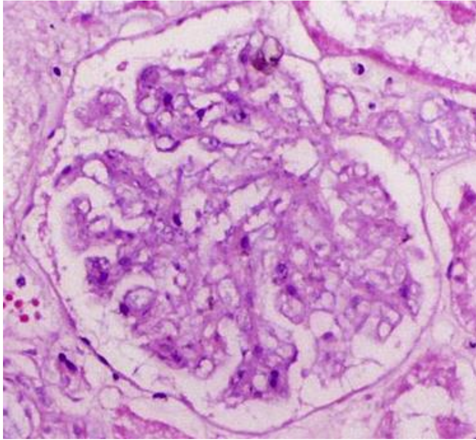
Normal lymphocyte beside Reed-Sternberg cell. The "owl-eyed" RS cell is characteristic of Hodgkin's Lymphoma. There may be more than two nuclei.



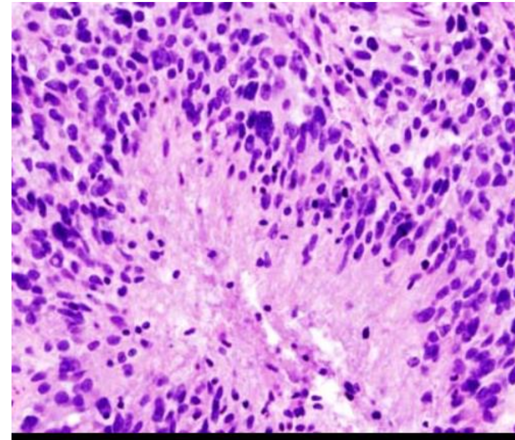


Nuclear budding and nuclear bridging (Breakage-Fusion-Breakage cycle). These are signs of chromosomal instability, often seen in tumour cells.

Examples of cell organizational abnormalities

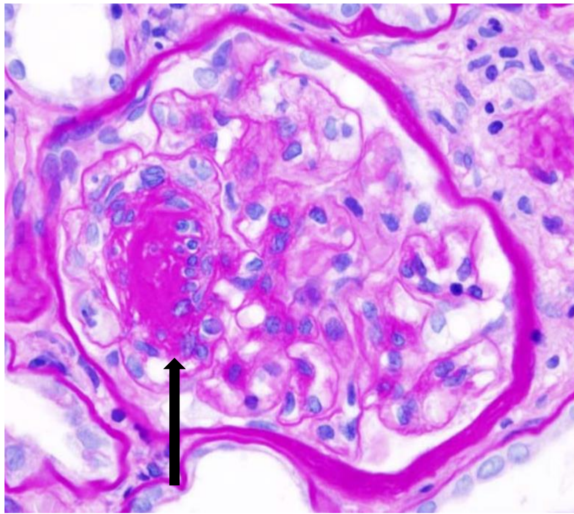


Coagulative necrosis of kidney glomerulus. Outlines are preserved, but cells lack



Palisading cells surrounding coagulative necrosis. Glioblastoma multiforme.

Examples of abnormal bodies seen in slides



Kimmelstiel-Wilson nodule in diabetic glomerulosclerosis. A nodule of pink hyaline material in the former glomerular capillary loop region.
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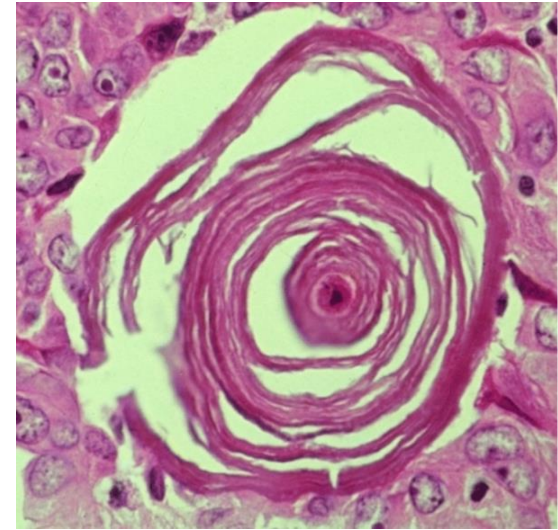

Psammoma body. A round collection of calcium. Seen in a number of pathological conditions: papillary thyroid, renal, and ovarian cancers, meningiomas, some mesotheliomas, and several endocrine tumour.