# Introduction

Histology is the branch of anatomy that focuses on the study of tissues of animals and plants. The term tissue refers typically to a collection of cells. In humans, organs comprise two or more tissue types, including epithelial, connective tissue, nervous, and muscular. The word “histology” stems from the Greek word “histos,” meaning web or tissue, and “logia,” meaning branch of learning. In brief, histological processing involves obtaining fresh tissue, preserving it (i.e., fixing it) in order to allow it to remain in as life-like a state as possible, cutting it into very thin sections (3–8 microns), mounting it on glass microscopic slides, and then staining the sections so that they can be observed under a microscope to identify different histological components within the tissue.

Histopathology is a branch of pathology concerned with the tissue changes characteristic of disease. It involves the examination of sampled whole tissues under the microscope. Three main types of specimen are received by the pathology laboratory. Specimens received by the pathology laboratory require tissue preparation then are treated and analyzed using techniques appropriate to the type of tissue and the investigation required. For immediate diagnosis during a surgical procedure a frozen section is performed.

1- Larger specimens include whole organs or parts thereof, which are removed during surgical operations. Examples include a uterus after a hysterectomy, the large bowel after a colectomy or tonsils after a tonsillectomy.

2- Pieces of tissue rather than whole organs are removed as biopsies, which often require smaller surgical procedures that can be performed whilst the patient is still awake but sedated. Biopsies include excision biopsies, in which tissue is removed with a scalpel (e.g. a skin excision for a suspicious mole) or a core biopsy, in which a needle is inserted into a suspicious mass to remove a slither or core of tissue that can be examined under the microscope (e.g. to investigate a breast lump).

3- Fluid and very small pieces of tissue (individual cells rather than groups of cells, e.g. within fluid from around the lung) can be obtained via a fine needle aspiration (FNA). This is performed using a thinner needle than that used in a core biopsy, but with a similar technique. This type of material is usually liquid rather than solid, and is submitted for cytology rather than histology (see Cytopathology).

# Techniques

## Preparing the tissue

For tissue removal, it is necessary to gather first the informed consent of the patient, as tissue taken from a live individual for diagnosis or treatment requires his/her consent. In other words, the patient must know at the time the purpose of tissue removal (e.g., diagnosis, research purposes, etc.). harvesting tissue from an animal requires approval of the procedure by the institutional review board (Institutional Animal Care and Use Committee, IACUC).

An important first step in the histological process is tissue acquisition. This step can be achieved by means of traditional tissue dissection or endoscopic ultrasound (EUS)-guided fine needle aspiration. If the former dissection . method is chosen, it is important to ensure that sharp dissecting tools are used to minimize crushing the tissue while cutting for removal. The tissue should be kept moist (e.g., 0.85% saline, isotonic) while dissecting and trimming. The tissue should be trimmed 1–2 cm in width/length (but should not be more than 5 mm thick). There should be at least one to two cut sides for easy penetration of the fixative. It is important, at this stage, to determine the desired orientation of the tissue and that all tissue components are represented during this trimming stage, if possible.

## Fixation

It is important to maintain cells in as life-like a state as possible and to prevent post-mortem changes as a result of putrefaction (destruction of tissue by bacteria or fungi) and autolysis (destruction of tissue by its own enzymes). In the latter case, as cells die, they release enzymes from

their lysosomes and other intracellular organelles, which start to hydrolyze (i.e., break down or decompose by reacting with water) components of the tissue, such as proteins and nucleic acids with the help of proteases and nucleases, respectively. Cases of autolysis are most severe in tissues rich in enzymes (e.g., liver, brain, kidney, etc.) and are less rapid in tissues such as elastic fibers and collagen. Therefore, it is critical that fixation be carried out as soon as possible after removal of the tissues to prevent autolysis and putrefaction, as well as to prevent the tissue from undergoing osmotic shock, distortion, and shrinkage. Unfortunately, fixatives may, unintentionally, introduce artifacts which can interfere with interpretation of cellular ultrastructure.

As fixation is typically the first step to prepare the tissue for microscopic, or other, analysis, the choice of fixative and fixation protocol is very important. The fixative acts to denature proteins by :

1- coagulation (of secondary and tertiary protein structures to form insoluble gels).
2- forming additive compounds (cross-linking end-groups of amino acids).
3- a combination of coagulative and additive processes.
4- promote the attachment of dyes to particular cell components by opening up protein side groups to which dyes may attach.
5- remove bound water to increase tissue refractive index to improve optical differentiation.

Prolonged fixation may result in the chemical masking of specific protein targets and prevention of antibody binding during immunohistochemistry protocols. In such cases, alternative fixation methods may be incorporated depending on the biological material.

Therefore, there is no universal fixative which will serve all requirements. Each fixative has specific properties and disadvantages. There is no single fixative, or combination of fixatives, that has/have the ability to preserve and allow the demonstration of every tissue component. Some fixatives have only special and limited applications, while mixtures of two or more reagents may be necessary to employ the special properties of each. So, it is important to identify specifically which histological structures one is trying to demonstrate, as well as the effects of short-term and long-term storage of the tissues.

**Types of fixatives**

1. **Aldehydes** include formaldehyde (formalin, when in its liquid form), paraformaldehyde, and glutaraldehyde. Tissues are fixed through cross-linking agents that react with proteins and nucleic acids in the cell (particularly lysine residues). Formaldehyde is a good choice for immunohistochemical studies, while formalin (10% neutral buffered formalin or NBF) is standard. The buffer prevents acidity in the tissues. Formaldehyde offers low levels of shrinkage and good preservation of cellular detail. This fixative is used routinely for surgical pathology and autopsy tissues requiring hematoxylin and eosin (H and E) staining . Since formalin is toxic, carcinogenic, and a poor preserver of nucleic acids, there have been attempts to find a more suitable substitute; however, this has proved difficult.

**Buffer:**

A buffer is a solution containing either a weak acid and its salt or a weak base and its salt, which is resistant to changes in pH. In other words, a buffer is an aqueous solution of either a weak acid and its conjugate base or a weak base and its conjugate acid. A buffer may also be called a pH buffer, hydrogen ion buffer, or buffer solution. Buffers are used to maintain a stable pH in a solution, as they can neutralize small quantities of additional acid of base. For a given buffer solution, there is a working pH range and a set amount of acid or base that can be neutralized before the pH will change. The amount of acid or base that can be added to a buffer before changing its pH is called its buffer capacity.

**Examples of Buffers**

blood - contains a bicarbonate buffer system
TRIS buffer
phosphate buffer

**How Buffers Work**

In order to understand how a buffer works, consider the example of a buffer solution made by dissolving sodium acetate into acetic acid. Acetic acid is (as you can tell from the name) an acid: CH3COOH, while the sodium acetate dissociates in solution to yield the conjugate base, acetate ions of CH3COO-. The equation for the reaction is:

$$CH3COOH(aq) + OH\text{-}(aq) \leftrightarrows CH3COO\text{-}(aq) + H2O(aq)$$

If a strong acid is added to this solution, the acetate ion neutralizes it:

$CH_3COO^-(aq) + H^+(aq) \leftrightarrows CH_3COOH(aq)$

This shifts the equilibrium of the initial buffer reaction, keeping the pH stable. A strong base, on the other hand, would react with the acetic acid.

**10% NEUTRAL BUFFERED FORMALIN**

**PURPOSE:** The fixative is a good routine fixative. This solution is hypotonic in buffer ions and has a pH of 6.8

**REAGENTS:**

Sodium phosphate, monobasic 4.0 gm

Sodium phosphate, dibasic 6.5 gm

Formaldehyde, 37% 100.0 ml

Distilled water 900.0 ml

2. Glutaraldehyde causes deformation of the alpha-helix structure in proteins, so it should not be used for immunohistochemistry staining. While it fixes very quickly, which makes it an excellent choice for electron microscopic studies, it provides poor penetration. It gives very good overall cytoplasmic and nuclear detail and is prepared as a buffered solution (e.g., 2% buffered glutaraldehyde). This fixative works best when it is cold and buffered and not more than 3 months old .

3. Oxidizing agents include permanganate fixatives, such as potassium permanganate, dichromate fixatives (potassium dichromate), osmium tetroxide, and chromic acid. While these fixatives cross-link proteins, they cause extensive denaturation .

4. Alcohols, including methanol and ethanol, and protein denaturants (acetic acid) are not used routinely as they cause brittleness and hardness to tissues. They are useful for cytologic smears, as they act quickly and provide good nuclear detail. Alcohols are used primarily for cytologic smears. They are fast acting, cheap, and preserve cells through a process of dehydration and precipitation of proteins. Methanol has been shown to be effective during immunostaining .

5. Mercurials fix tissues by an unknown mechanism. They contain mercuric chloride which is a known component in fixatives such as B-5 and Zenker's. These fixatives offer poor penetration and tissue hardness, but are fast and provide excellent nuclear detail, such as for visualization of hematopoietic and reticuloendothelial tissues (i.e., lymph nodes, spleen, thymus, and bone marrow). These fixatives must be disposed of carefully. Mercury deposits must be removed (dezenkerized) prior to staining, otherwise black deposits will occur in tissue sections .

6. Picrates include fixatives with picric acid, such as Bouin's solution. These fixatives have unknown modes of action. The most common is Bouin's alcoholic fixative. This fixative provides good nuclear detail and does not cause much hardness. It is recommended for fixation of testis, gastrointestinal tract, and endocrine tissues. This fixative has an explosion hazard in dry form, so it must be kept submerged in alcohol at all times.

**factors affecting fixation**

i. Buffering: Fixation is best performed at close to neutral pH (pH 6–8; formalin is buffered with phosphate at pH 7). Common buffers include: phosphate, bicarbonate, cacodylate, and veronal .

ii. Penetration: Each fixative has its own penetration rate in tissues. While formalin and alcohol penetration are superior, glutaraldehyde is the worst. Mercurial fixatives are in between. The thinner the sections are cut, the better the penetration .

iii. Volume: The volume of the fixative should be in at least a ratio of 10:1. Fixation can be enhanced if the fixative solution is changed at regular intervals and the specimen is agitated .

iv. Temperature: If the temperature at which fixation is carried out is increased, it will yield an increased speed of fixation. Of course, too much heating of the fixative can result in cooking or creating tissue artifacts .

v. Concentration: The concentration of the fixative should be as low as possible, because too high a concentration may adversely affect the tissue and provide artifacts (formalin is best at 10%, while glutaraldehyde is best at 0.25–4%) .

vi. Time interval: The faster the fresh tissue can be acquired and fixed, the better, as to minimize cellular organelle degradation and nuclear shrinkage, resulting in artifacts. The tissue should always be kept moist with saline.

**Decalcifying agents**

Some animal tissues contain deposits of calcium salts which may interfere with sectioning, resulting in torn sections and damaged blades. Calcium compounds must be chemically removed (usually with an acid) before typical histological techniques can be used for the study of softer components. Tissues requiring decalcification include bone, teeth, and calcified cartilage . Pathological states include arteriosclerosis, tuberculosis, and several tumor types. Such tissues should be fixed prior to decalcification and washed for 12 hours in running water between fixation and decalcification. While decalcification agents remove typically calcium salts and do not interfere with staining reactions, they can cause minimal distortion to cells and connective tissue. The decalcifying agent should have a volume of 30–50 times that of the tissue and occasional agitation may be required to expedite this process. Heating should not be employed. The process is complete typically when bubbling has ceased. Over decalcification can cause a severe reduction (of what) in subsequent sectioning of the tissue. Some typical decalcifying agents include, nitric acid, Gooding and Stewart's fluid, Rapid Bone Decalcifier (RDO), and chelating agents. More recently, new methods have been discovered to allow hard tissues to be decalcified faster.

**Dehydration**

After fixation, and to begin the dehydration step (i.e., removal of water), tissues are placed in progressively increasing concentrations of a dehydrating agent (e.g., 70, 85, 95, and 100%) which is typically ethanol. Methanol, isopropanol, and acetone are alternative options, depending on the tissue being processed. It is important to include two absolute alcohol (i.e., 100%) steps to ensure that all remaining water has been removed. The dehydration step is critical, as water is immiscible with most embedding media (i.e., paraffin wax). Therefore, the tissue must be exchanged between polar (e.g., water) and non-polar (e.g., organic reagents, such as xylene) agents. If the tissue is incompletely dehydrated, it is not possible to "clear" the tissue. When it is exposed to a subsequent clearing agent (e.g., xylene) the tissue remains opaque and appears milky. This will necessitate re-dehydration of the tissue. Dehydration will also remove some of the lipoidal material in the tissue. If the lipids are supposed to be visible, it will be necessary to use an appropriate fixative that will preserve the lipids prior to the dehydration step (e.g., osmium tetroxide).

## Clearing

The term "clearing" is related to the appearance of the tissue after it has been treated with a dehydrating agent. Many agents have a similar refractive index to that of the tissue, rendering the tissue "clear" or translucent. In this step, the dehydrating agent must be removed from the tissue and replaced with a solvent of wax. A clearing agent should be used when the dehydrating agent (e.g., ethanol) is not miscible with the impregnating medium/ embedding agent (i.e., paraffin wax). It is a wax solvent and must be miscible with both the dehydrating and embedding

agents. The selection of a suitable clearing agent should be based on the speed and ease of removal from the embedding media (i.e., the lower the boiling point the more rapid the removal), interaction with the tissue, flammability, toxicity, and cost. The clearing step can be more effective with the use of a vacuum system and should be carried out in a fume hood. Typical clearing agents include xylene, chloroform, toluene, benzene, dioxane, carbon tetrachloride, cedarwood oil, isoamyl acetate, methyl benzoate, methyl salicylate, and clove oil. Due to the potential hazards of some of these chemicals, others have been proposed, such as some vegetable oils, terpenes, and alkanes. Some histological protocols have the potential option of processing the tissue without the use of a clearing agent (e.g., xylene) as a safe alternative to exposure to the hazardous effects of these chemicals. One such protocol includes the use of isopropanol as a safer alternative.

**Infiltration/impregnation**

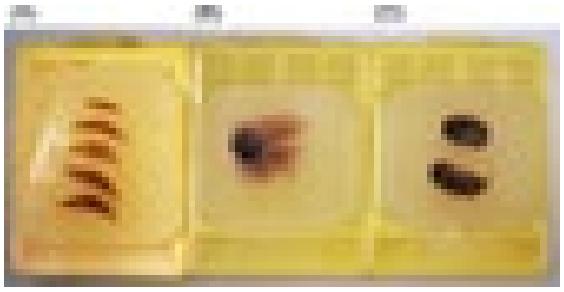
The role of the infiltration agent is to remove the clearing agent from the tissue and to completely permeate the tissue with paraffin wax. This will allow the tissue to harden and produce a wax block from which thin histological sections can be cut. Ideally, the consistency of any solidified embedding medium should be the same as the specimen it encloses. Unfortunately, this rarely happens due to the wide variation in consistency of tissue and the large variety in embedding media. Paraffin wax is commonly used and heated to a temperature that is 2–3°C above its melting point. Any higher temperature will result in tissue hardening. The paraffin wax should be 20–25 times the volume of the tissue. Generally, the tissues are transferred directly from the clearing agent to

pure paraffin, but sometimes with fragile specimens, it is necessary to use graded mixtures of clearing agent and paraffin. The duration and number of changes of paraffin necessary for impregnation vary with the size and consistency of the tissue. As exposure of the tissue to paraffin increases, it is more likely that shrinkage and hardening will occur. Complete infiltration is only possible after complete dehydration and complete clearing. The selection of paraffin depends on the nature of the tissue to be embedded and thickness of section required. A high melting point of the wax (e.g., 55–60°C) increases the hardness and decreases the thickness to which the tissue may be sectioned (e.g., 45–50°C is considered soft). Paraffin wax can be purchased in the form of tablets, pellets, or granules. Numerous substances can be added to the molten paraffin to modify its consistency and melting point. Typically, the process of infiltration occurs with the use of a tissue processing machine, although this can be carried out using a heated container maintained 2–3°C above the melting point of wax. If residual clearing agents remain in tissue or improper processing of the tissue has occurred, this will lead to difficulties with sectioning. Evaporation of the clearing agent, infiltration with paraffin wax, and removal of any air bubbles trapped in the specimen will be more completely alleviated if clearing and infiltration procedures are carried out at reduced pressure (under vacuum).

**Embedding**

After the infiltration process has been completed, it is necessary to obtain a solid block containing the tissue. To accomplish this, it is necessary to first coat a stainless steel histological base mold of suitable

size to fit the tissue with glycerol or “mold release” to prevent adherence of the wax block containing the tissue to the metal mold upon solidification. Pre-warming of the metal block is advised to prevent premature solidification of the wax block. In addition, using warmed forceps to help press the tissue against the base of the metal mold, in addition to reducing the chance of premature solidification, helps with this process. Prior to beginning the infiltration process, an embedding cassette should be placed on top of the mold and labeled with the name of the tissue, fixative, and date. If an embedding unit (machine) is being used, the combined unit should be dispensed two-thirds full with molten paraffin. The specimen should be oriented in the metal mold to ensure that the tissue will be cut in the correct plane of section. Alternatively, the mold can be filled slightly and the tissue can then be placed in the mold and positioned in the desired orientation at the base of the mold. The combined unit should then be set out on the cooling tray of the embedding unit (machine) and not disturbed until the wax has cooled and solidified completely. After sufficient time, the cassette and mold should be separated and the paraffin block should be placed in the microtome in preparation for sectioning. If the tissue has been thoroughly fixed, dehydrated, cleared, and infiltrated, tissues embedded in paraffin wax provide good cutting qualities. On average, paraffin blocks remain durable and retain their good cutting qualities and staining characteristics indefinitely.

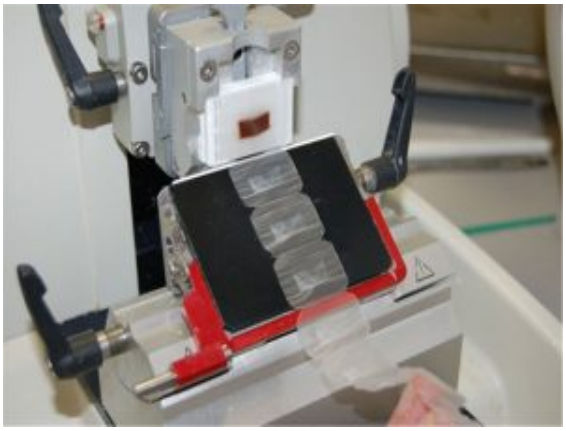


**Embedding media**

The most common infiltrating agent and embedding medium is paraffin wax. Ester wax offers a lower melting point than paraffin wax and tends to be harder when solid, allowing this medium to be suitable for cutting thinner (i.e., 2–3 μm) sections with minimal tissue shrinkage. When water-soluble waxes (i.e., polyethylene glycol waxes) are used, tissues are transferred directly from aqueous fixatives to wax for infiltration without dehydration or clearing. This results in less tissue shrinkage, but sectioning is more difficult than with paraffin wax. Tissue blocks must be kept in a dry atmosphere. If cellulose nitrate (i.e., celloidin/low-viscosity nitrocellulose) is chosen as an embedding medium, tissues must be dehydrated and embedded with solutions of cellulose nitrate dissolved in an alcohol/ether mixture. The solvent is allowed to evaporate to produce a tissue block of required consistency. No heat is applied using this method. This medium is used typically for large pieces of, for example, bone and brain tissues. Synthetic resins are used for preparing sections most typically for electron microscopy and light microscopy (0.5–2 μm sections), such as for undecalcified bone. Freezedrying protocols can be applied when special staining techniques are used.

## Microtomy

Microtomes are used to cut the tissue into thin sections for microscopic viewing. The type of specimen will determine the type of microtome to be used. Rotary microtomes are the most common microtomy instrument. The feed mechanism is achieved by turning a wheel at one side of the machine. While the knife is fixed and is secured in a knife holder, the object moves against the cutting surface of the knife, according to the thickness of section required. The knife holder allows the knife to be set at an oblique angle to the specimen. One complete rotation of the operating wheel is equivalent to one complete cycle. The downward motion of the knife reflects the cutting stroke, while the upward stroke reflects the return stroke and activation of the advance mechanism. The feed mechanism is activated by turning a wheel located on the side or top of the microtome. The tissue block is passed across the knife at every stroke to produce a section. Microtomes have a feed mechanism to advance the specimen (or knife) to a predetermined thickness for sectioning (i.e., typically 5–10 µm) and can produce serial sections.



**Cryostat**

A cryostat or freezing microtome is used for obtaining thin sections of unfixed tissues. It can be used, additionally, for observing fatty tissues. The microtome is maintained at −15 to −20°C in a refrigerated chamber. The cabinet is designed to operate at −5 to −30°C. The tissue block can be mounted in a high-viscosity water-soluble gel, such as 1% glucose, gelatin, or cellulose on the platform and must be frozen immediately. An anti-roll plate is used to keep sections flat on the knife blade for direct

mounting onto the slide. Sections are cut one at a time. When a section is cut, the anti-roll plate is lifted and a section is picked up from the surface of the knife and placed onto a slide using a camel hair brush. Sections are fixed in 5% acetic acid in absolute alcohol and then subsequently stained (e.g., with hematoxylin and eosin). Frozen sectioning is typically used for rapid preparation and diagnosis by a pathologist.

**Microtome knives**

There are many different types of microtome knives (e.g., stainless steel, carbide, diamond, glass, or disposable blades). Wedge-shaped stainless steel knives are used for most paraffinembedded specimens. They must be kept clean and well-oiled or lubricated. The knife's edge should be cleaned with a clearing agent with a soft, moistened cloth in a fume hood. As an alternative to wedge-shaped stainless steel knives, disposable blades provide an excellent cutting edge for paraffin sectioning and are available in different sizes and thicknesses. Glass, sapphire, and diamond knives are used for specimens embedded in hard resin plastic (e.g., epoxy, glycolmethacrylate). Diamond and sapphire knives tend to function better than glass knives, but are much more expensive. If a wedge-shaped stainless steel knife is used, it must be free of nicks and sharpened with a carborundum stone (manual sharpening) or by an automatic knife sharpener (with a glass wheel and with an abrasive). A process called stropping produces a finely polished, smooth, and even knife edge. The knife is secured at the desired angle place by adjusting holder screws.

**Section thickness and rough cutting**

A thickness of 6 µm is standard for histological tissue sections. For highly cellular tissues (e.g., lymph nodes), 4 µm is used most often. For thicker sections, 10 µm is used. For neurological tissues and myelinated nerves, 6–20 and15–20 µm is used, respectively. The tissue block will be examined to establish how it needs to be oriented in the block holder. Excess paraffin should be trimmed away from each side of the tissue block to create a trapezoid shape. The longer edge should be parallel with the knife edge. The tissue block should be roughly cut by advancing the block manually and sectioning until the entire surface of the tissue is exposed.

**Section adhesives, sectioning tissues, and sealing of blocks**

Section adhesives, such as gelatin, casein glue, starch, and albumin, can be used to aid in adhering sections to the slide prior to further processing, such as staining. Gelatin can be added to the water bath. The use of adhesives in the water bath promotes bacterial and fungal growth. Daily cleaning of the water bath with sodium hypochlorite solution (e.g., Clorox soap) is necessary to prevent contamination. Alternatively, a thin coat of albumin can be applied directly to the slide by dipping it into the solution or using your fifth finger (i.e., most ulnar and smallest finger). This latter process is referred to as “subbing.” A newer idea is to use “plus” (+) slides. Treatment of the slide with a reactive silicon or polylysine compound chemically changes the glass, such that it bears abundant amino groups, which ionize to provide a positively charged surface. Sections which contain a preponderance of anionic groups, such as carboxyls and sulfate-esters adhere strongly to this modified glass. When creating a ribbon (what is a ribbon), i.e., a series of adjacent tissue

sections, the hand wheel should be turned at a slow and even speed. Rotating the wheel too rapidly will cause sections of unequal thickness. The floatation bath should be heated to a few degrees below the melting point of the paraffin wax. Tap, deionized, or distilled water can be used. The ribbon should be gradually lowered onto the flotation bath to eliminate wrinkles and entrapped air. Air bubbles may be removed with a camel's hair brush or by submerging a slide under the ribbon. If the sections are wrinkling, a 70% alcohol solution can be added to the water bath prior to section collection. If necessary, sections may be separated, depending on their sizes, and each can be placed on a clean, pre-marked glass slide. Individual sections or tissue ribbons may be picked up by submerging a clean glass slide into the water bath at a ~45° angle, directly beneath the location of the section or ribbon. The slide should be lifted out of the water slowly to ensure that the sections lay on the slide. The slides should be drained vertically on a paper towel for several minutes before placing them onto a warming table (37–40°C). The slides should remain on the warming table, overnight, for 20–30 minutes at approximately 58°C or a few degrees below the melting point of the paraffin wax. Failure to drain the slides will create air bubbles under the tissue and decrease the section's adhesion to the slide. Air bubbles produce section unevenness and staining artifacts, making the final preparation difficult to examine with the microscope. Once the desired sections have been cut, the block can be removed from the block holder and sealed with molten paraffin wax to ensure that the tissue will not dry out and become brittle (blocks can last for weeks, months, or years.

## Problems encountered with sectioning tissue blocks

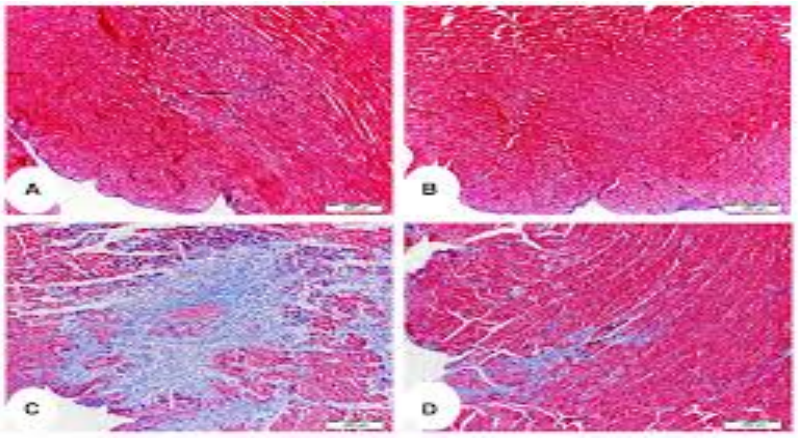
Histologists are confronted often with difficult tissue blocks that will not section easily. This may be the result of, for example, brittle or shrunken tissue, improperly infiltrated tissues, or sections with, for example, holes or scratches in them. If the tissue block appears to be brittle, a 10% diluted ammonium hydroxide solution may be applied (via soaking) to soften the tissue to prevent cracking and to more easily facilitate sectioning. If sections have holes in them, this can be indicative of incompletely infiltrated tissue. This may be alleviated by placing the tissue block back in the heated wax bath to melt it and then proceed to re-embed the block. If artifactual scratches or tears occur across the tissue sections, this may be indicative of flaws or dirt on the cutting edge of the knife and may be alleviated by repositioning or replacing the blade. Alternatively, other problems can occur if the tissue block appears to be too soft or too hard. If too soft, a remedy may be to place the block tissue side down on several sheets of Kimwipes or paper towel in the freezer (−15°C) or a refrigerator (0–4°C) (chilling times may vary), prior to sectioning. This technique will help to harden the wax so that it better matches the hardness of the infiltrated tissue and will result in more successful tissue sectioning. If too hard, a piece of wet cotton/Kimwipe may be placed in lukewarm water and then placed over the surface of the block (times may vary). This will allow the tissue to expand/swell and soften as it absorbs water. It should be noted, however, that with either

too soft or too hard tissue blocks, these solutions are temporary and may allow only a few successful sections to be cut.

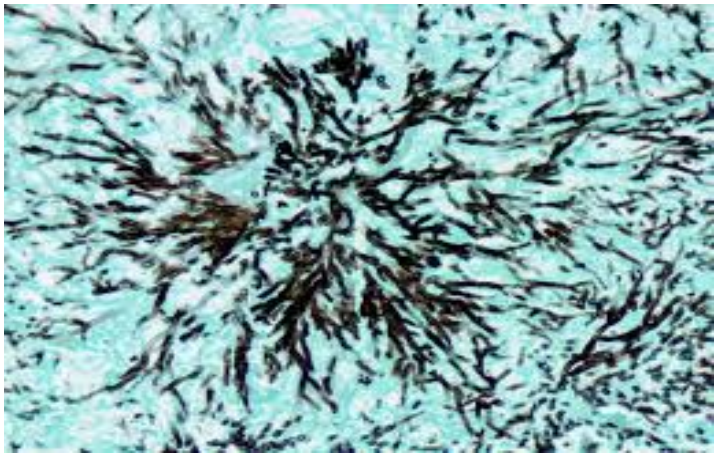
## Staining

Staining of tissue slides is carried out by reversing the embedding process in order to remove the paraffin wax from the tissue to allow water-soluble dyes to penetrate the sections. This process is referred to as “deparaffinization.” The tissue slides must be exposed to a clearing agent and subsequently taken through a descending alcohol series to water (also referred to as “bringing your slides to water”). Choosing the appropriate dye for a particular tissue slide is related to its ability to color otherwise transparent tissue sections and various cellular components of the tissue. The term “routine staining” includes the hematoxylin and eosin (i.e., H and E) stain. This stain is used routinely as it provides the pathologist or researcher with a detailed view of the tissue, clearly staining, for example, the cytoplasm, nucleus, and organelles. The term “special stains” refers to a large number of staining techniques, other than H and E, that allow the visualization of particular tissue structures, elements, or microorganisms that cannot be identified with H and E staining Examples include:

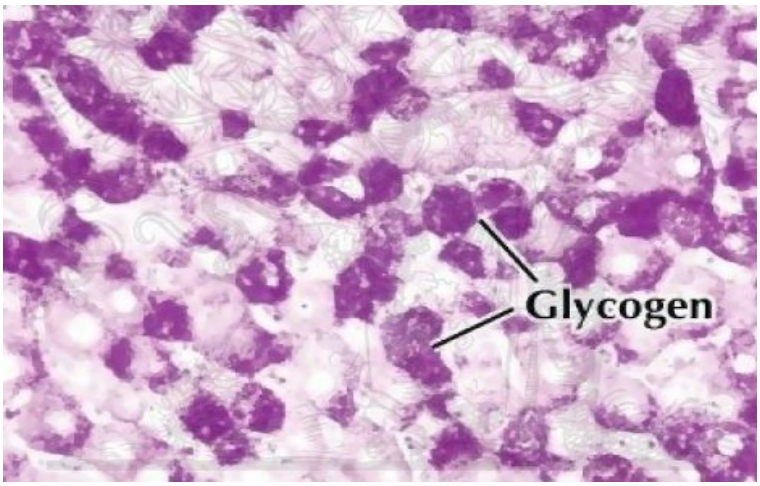
Masson’s trichrome (e.g., skin; identification of collagenous connective tissue)



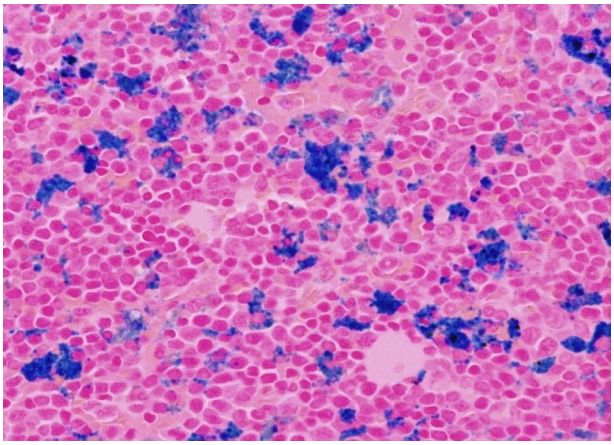

Gomori silver stain (e.g., reticulum fibers .)



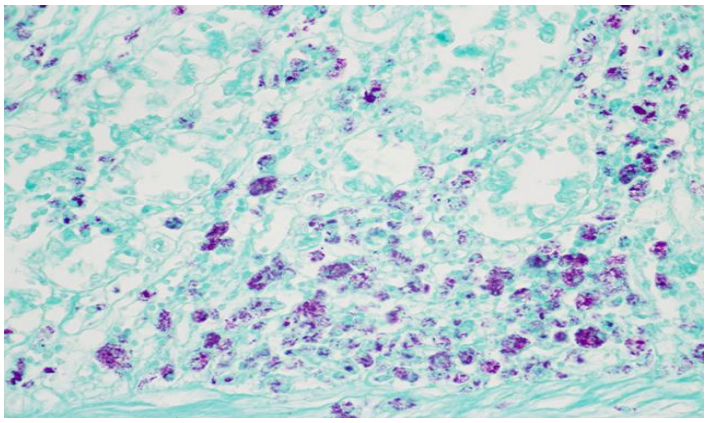
Periodic acid-Schiff (e.g., kidney; identification of high proportion of carbohydrates, such as glycogen, glycoproteins, and proteoglycans)




Perl's Prussian blue iron (e.g., liver; identification of ferric (Fe3+) iron in tissue preparations or blood and bone marrow smears)



Ziehl-Neelsen (acid-fast bacillus) (e.g., lung; identification of acid fast bacilli)



## Immunohistochemistry

A major change in histopathology in recent times has been the development of immunohistochemistry. Where special stains are a relatively crude and, in most cases, relatively non-specific way of staining tissue components, immunohistochemical stains are by comparison far more specific in what they stain.

This technique involves attaching a dye to an antibody that will only bind to a certain protein type on or within a cell. Antibodies are like keys that can only open a certain lock (cell protein or antigen). Hundreds of antibodies are available which allow labelling of hundreds or even thousands of different protein types. Where a special stain may allow the pathologist to identify, for example, a cell as being cancerous,

immunohistochemistry can identify which organ in the body that cancerous cell came from and how aggressively it may behave.

The dyes that attach to the labelling antibody can be also altered, including using different coloured dyes or even fluorescent dyes that are easier to see on microscopy. Some laboratories can use fluorescence-labelled antibodies to allow for computerised slide analysis, reducing the time taken to examine large numbers of slides and identifying which slides need to be reviewed by a pathologist and which are within the normal range.

## Electron microscopy

The usual microscopes used by pathologists are not powerful enough to see the smallest parts that make up a cell. This is not usually a problem, but some diseases can only be diagnosed at this subcellular level. Examples include types of kidney disease (glomerulonephritis) or aggressive cancers which lose their normal proteins, making immunohistochemistry less useful in their identification.

In these cases a very powerful type of microscope is used called the electron microscope. This utilises beams of electrons rather than visible light to magnify the cells in a tissue sample. It can magnify up to 2 million times, whereas the maximum power of a conventional light microscope is only 1 to 2 thousand times.

## Flow cytometry

This technique is used most commonly as an adjunct in the diagnosis of cancers of the blood cells (leukaemias and myelomas). Cells are suspended in a liquid and passed through a laser beam (single wave length light beam). A detector measures how the beam is scattered and if fluorescent light is emitted from excited particles on the cells. This is interpreted by a computer as a number of cells/ particles/ proteins (whatever substance is being examined for) and is shown on a graph. This can be used to give the quantities and relative proportions of different types of cells in the blood and identify any abnormal cells (e.g. leukaemias).

## Molecular Pathology and Cytogenetics

With the explosion of information about cell DNA (the genetic coding material) and genes that has resulted since the completion of the Human Genome Project, increasing numbers of genes are being recognised that, if faulty, may be involved in the development of disease including cancers. This is shaping up to change the way that disease is thought of, diagnosed and treated.

Molecular pathology is an umbrella term for the analysis of the genetic material (chromosomes and their DNA) of cells, and is becoming an increasingly widely requested component of the pathology workup of a submitted tissue. One of the subdivisions of molecular pathology is

cytogenetics, which is the analysis of chromosomes (the form in which DNA is found in the cell nucleus). The two most commonly used techniques in molecular pathology and cytogenetics are fluorescence in situ hybridisation (FISH) and direct sequencing of DNA.

FISH is a technique used to stain chromosomes to reveal areas where genes may have been deleted, duplicated or broken. Fluorescent labels are attached to specific DNA sequences (parts of specific genes) which allow faulty genes to be seen when examining the cells under a special type of microscope.

Direct sequencing of cell DNA is a way of looking at individual genes or groups of genes, to detect and characterise which mutation is present in a particular patient's tumour. This can be done in the traditional manner (Sanger sequencing, capillary electrophoresis), or by the newer and much faster method of Next Generation Sequencing.

As an example of the usefulness of cytogenetics one can look at breast cancer. Anatomical pathology can give a diagnosis of what type of breast cancer a patient may have, how far it has spread, whether or not it is likely to be an aggressive tumour and whether it will respond to hormone and targeted therapies. Cytogenetics can add to this information by identifying whether the patient has a faulty gene(s) which predisposed them to the development of breast cancer. If present, this would mean that they have an increased chance of developing cancer in the opposite breast and of developing other specific cancer types (e.g.

ovarian cancer). It also has implications for the patient's direct relatives and offspring. Did they inherit the faulty gene(s) and what are the chances that they will develop cancer in the future? By direct sequencing of the faulty gene, the close relatives of the patient can be screened for the mutation, after appropriate consent, allowing for preventative steps to be taken to minimise their chances of developing a similar cancer in the future. There are also treatments being developed which will target the products of specific gene mutations in a patient.