MUSCLE

Muscle tissue is composed of cells specialized for contraction. Muscle is classified into three types according to their structure and function:

- **Skeletal muscle cells** - striated, voluntary control
- **Cardiac muscle cells** - striated, involuntary control
- **Smooth muscle cells** - nonstriated, involuntary control

Motion, as a reaction of multicellular organisms to changes in the internal and external environment, is mediated by muscle cells.

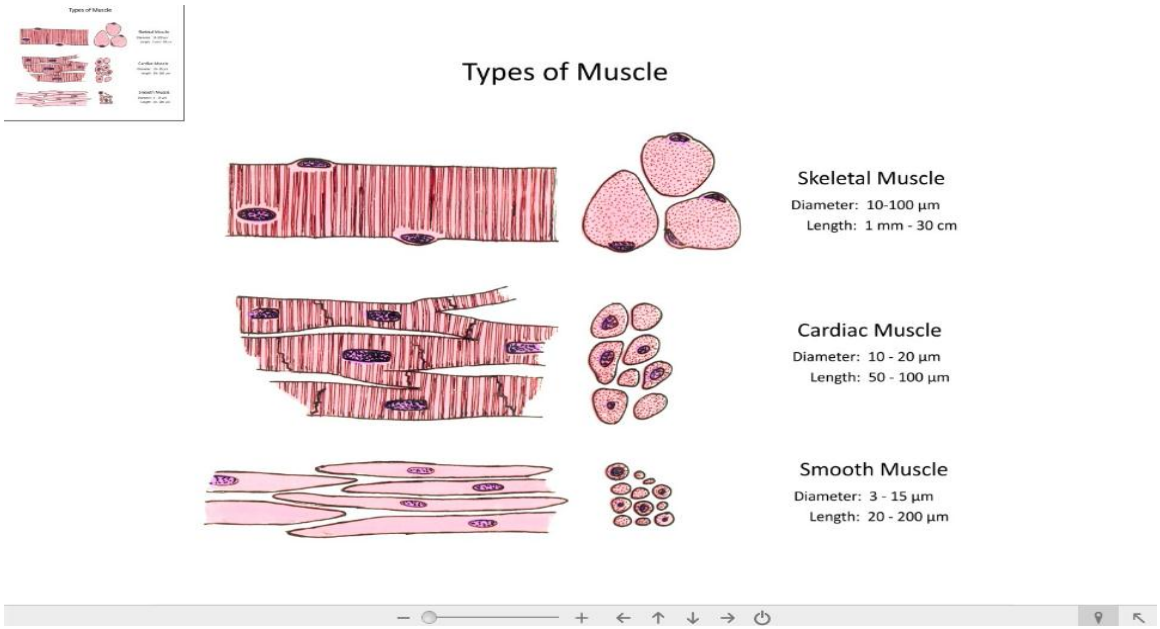
The basis for motion mediated by muscle cells is the conversion of chemical energy (ATP) into mechanical energy by the contractile apparatus of muscle cells. The proteins actin and myosin are part of the contractile apparatus. The interaction of these two proteins mediates the contraction of muscle cells. Actin and myosin form myofilaments arranged parallel to the direction of cellular contraction.

A further specialisation of muscle cells is an excitable cell membrane which propagates the stimuli which initiate cellular contraction.

Skeletal and cardiac muscle cells are called **striated** because they show an alternating series of bands. The repeating arrangement of their basic contractile unit, the **sarcomere**, produces these striations.

In all types of muscle, contraction is caused by the movement of **myosin filaments** along **actin filaments**.

The terms **muscle cell** and **muscle fiber** are synonymous.



Skeletal Muscle

Skeletal muscles are connected to bone (or cartilage) by way of ligaments and produce all movements of parts of the body.

- Long cylindrical cells

- Multinucleated cells with many peripheral nuclei
- Striated muscle - exhibit cross-striations
- Voluntary control - innervated by the somatic nervous system (motor neurons)
- Respond quickly to stimuli
- Satellite cells - skeletal muscle stem cells

Cardiac Muscle

Cardiac muscle makes up the walls of the heart and pumps blood throughout the body.

- Short, branched cells that form a syncytium (coupled by gap junctions)
- Generally single centrally located nucleus (occasionally binucleate)
- Striated muscle - exhibit cross-striations
- Intercalated discs join cells end-to-end
- Involuntary control - innervated by the autonomic nervous system
- Automatic rhythmic contractions for life
- Long-lived cells
- Lipofuscin pigment - end-stage lysosomes containing undigested material
- Poor capacity for regeneration

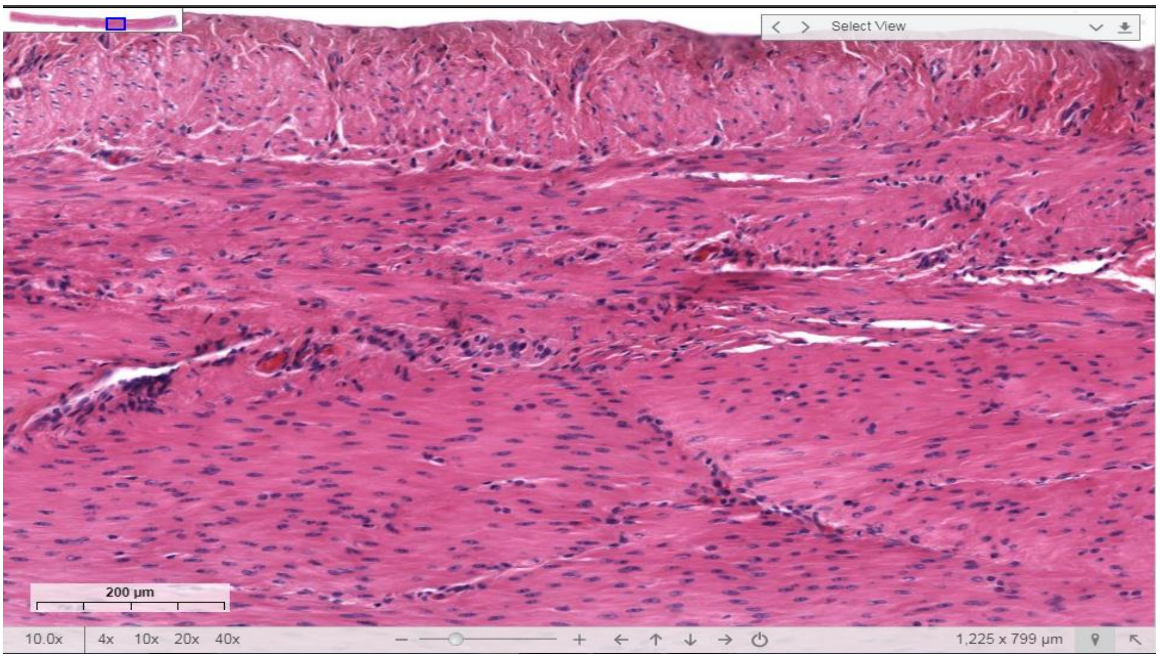
Smooth Muscle

Smooth muscle is used by various systems to apply pressure to organs (such as the stomach, intestines and uterus) and blood vessels.

- Spindle-shaped cells of variable size
- Single centrally located nucleus
- Non-striated muscle - do not exhibit cross-striations
- Involuntary control - innervated by the autonomic nervous system
- Responds slowly to stimuli and are capable of long-time sustained contractions
- Retain the ability to divide

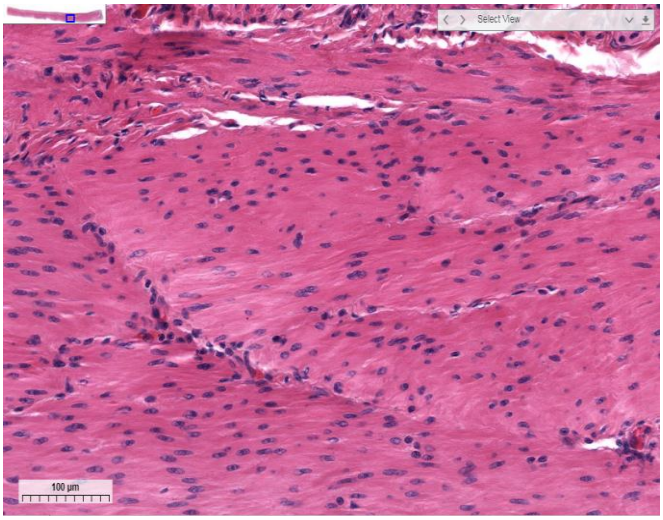
Smooth Muscle

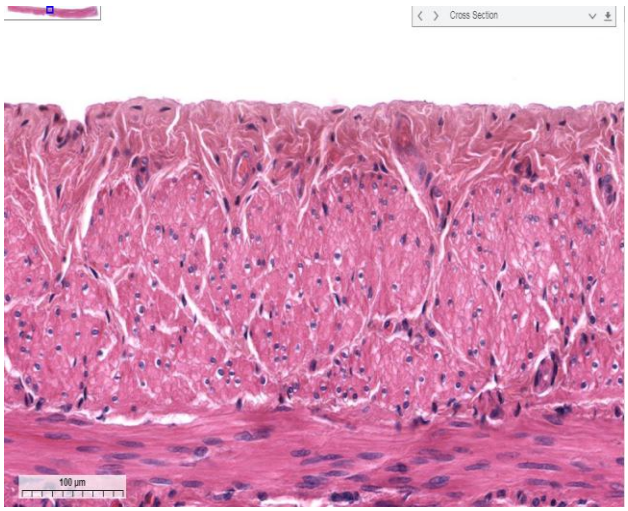
- Smooth muscle consists of spindle shaped cells of variable size. The largest smooth muscle cells occur in the uterus during pregnancy (12x600 µm). The smallest are found around small arterioles (1x10 µm).
- Smooth muscle cells contain one centrally placed nucleus. The chromatin is finely granular and the nucleus contains 2-5 nucleoli.
- The innervation of smooth muscle is provided by the autonomic nervous system.
- Smooth muscle makes up the visceral or involuntary muscle.



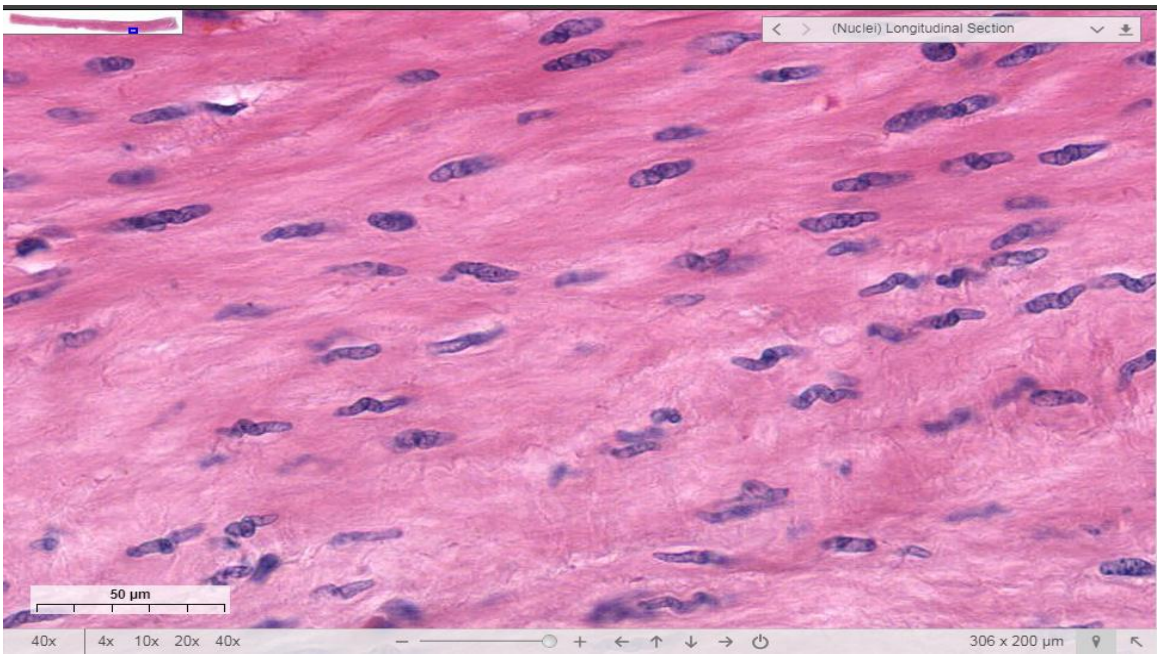
Smooth Muscle

Smooth muscle is non-striated, involuntary muscle found in many locations. Smooth muscle cells are spindle-shaped with a centrally located nucleus.





- Longitudinal Section: in relaxed smooth muscle, the nuclei are elongated with rounded ends. When contracted, the nuclei spiral, kink, or twist. The cytoplasm is pink, non-striated and with little detail.
- Cross-Section: individual cells vary in diameter depending on their location within the cell. Cross-sections through the middle of cells have centrally located nuclei, usually surrounded by an unstained region.



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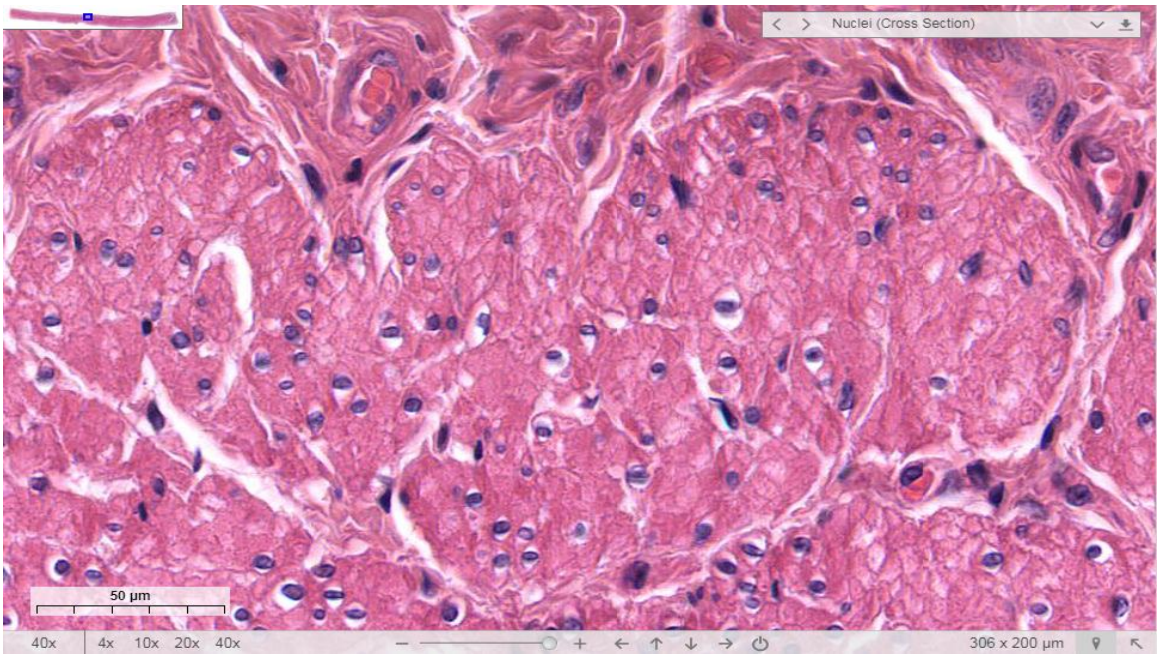
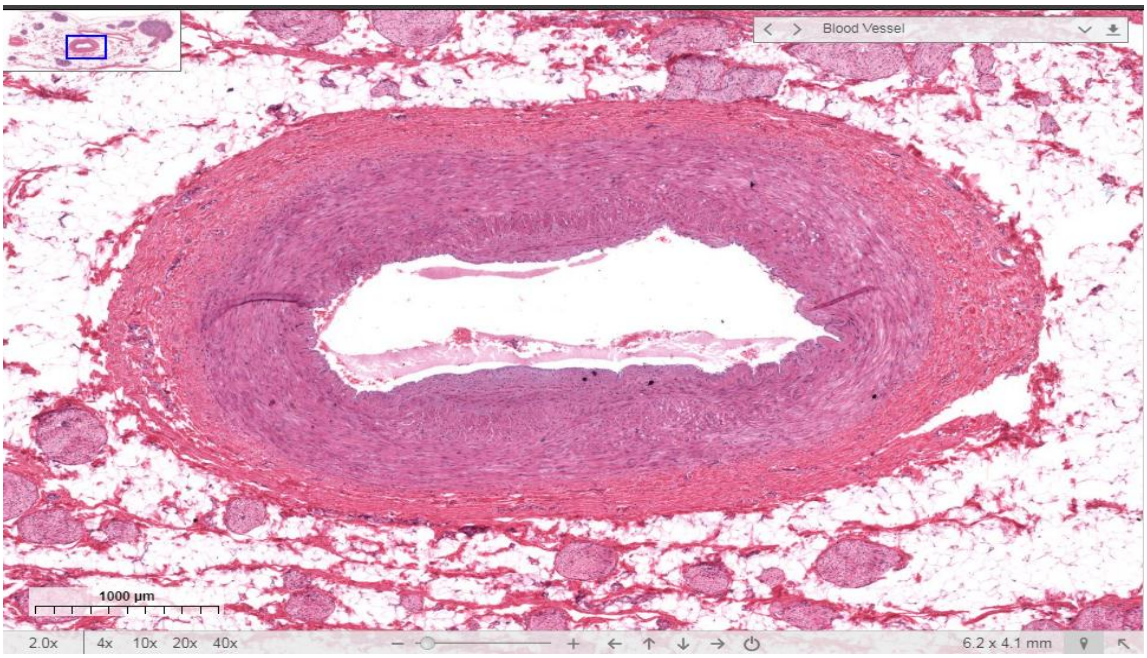
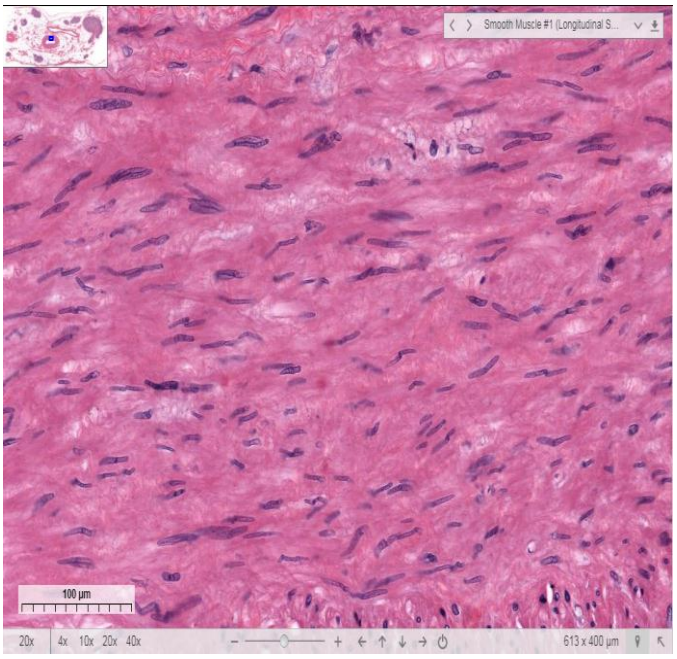


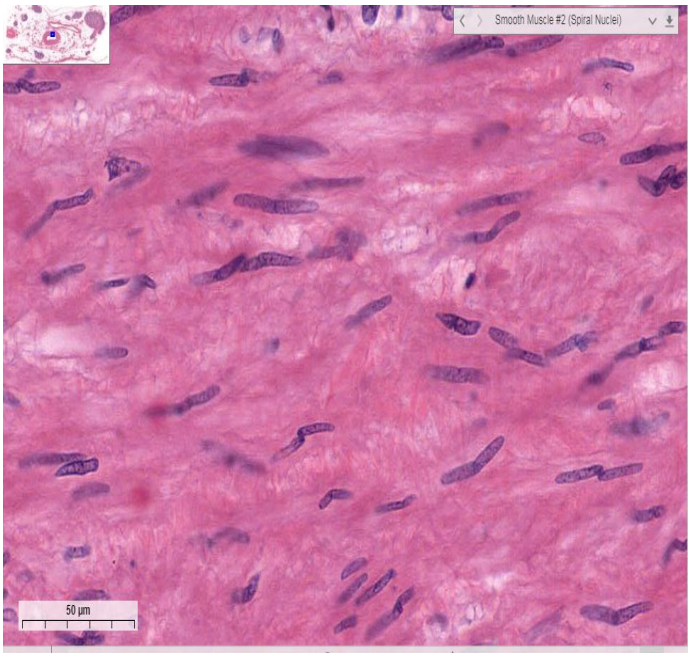
Cross-sections through the middle of cells have centrally located nuclei, usually surrounded by an unstained region.



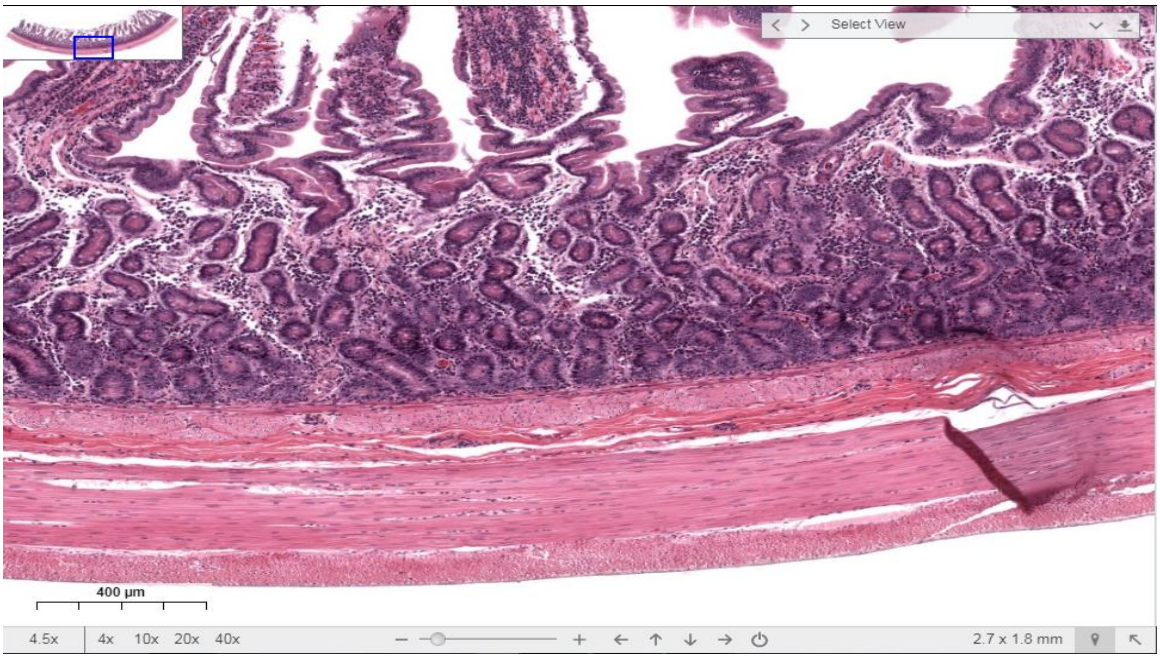
Smooth Muscle

Examine the large blood vessel in this section of mesentery. The middle layer contains smooth muscle. It appears in longitudinal section because they encircle the blood vessel. In relaxed cells, the nuclei are elongated with rounded ends. When contracted, the nuclei spiral, kink, or twist. The cytoplasm is pink, unstriated and with little detail.



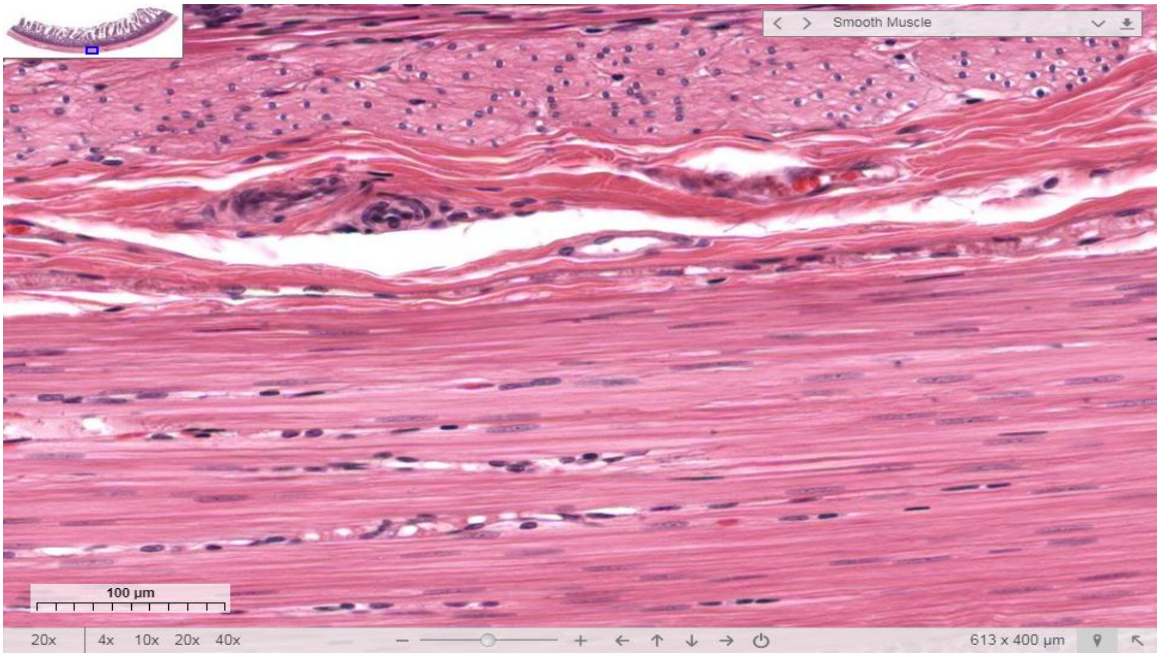


When contracted, the nuclei spiral, kink, or twist. The cytoplasm is pink, unstriated and with little detail.



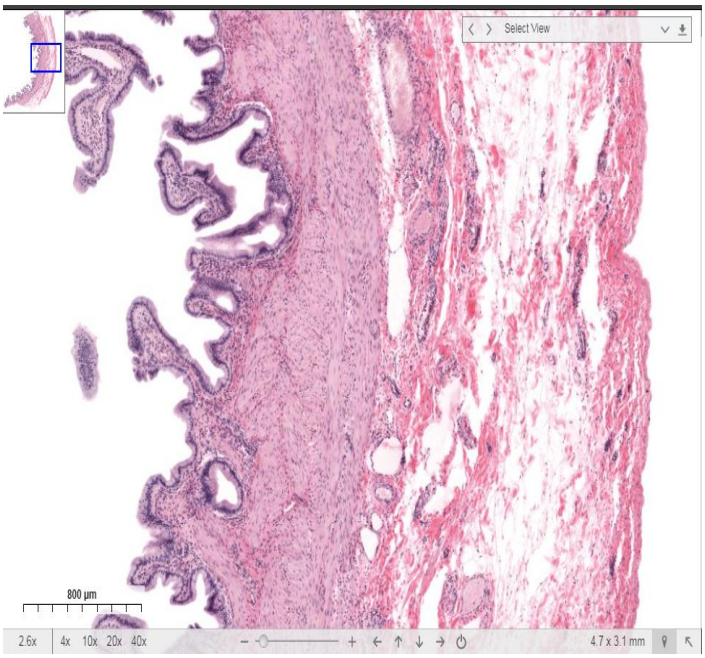
Small Intestine

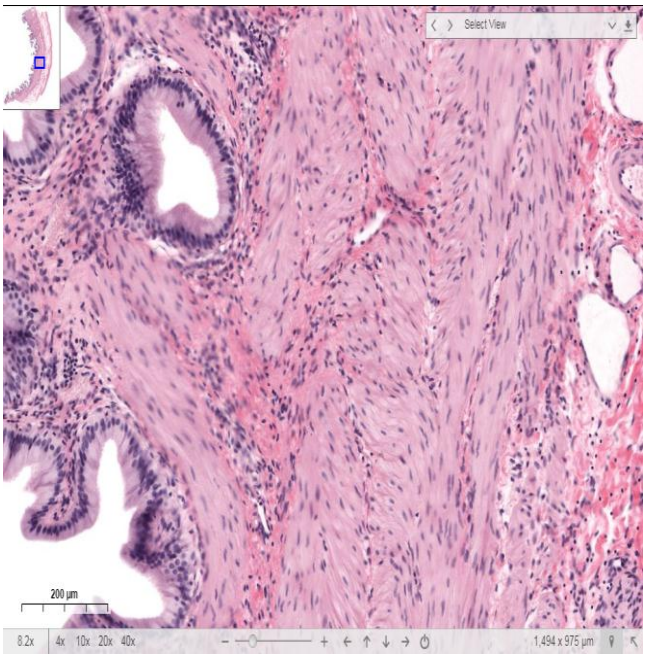
The wall of the small intestine contains two orthogonal layers of smooth muscle.



- Inner Layer - individual cells vary in diameter depending on their location within the cell. Cross-sections through the middle of cells have centrally located nuclei, usually surrounded by an unstained region.
- Outer Layer - in relaxed smooth muscle, the nuclei are elongated with rounded ends. When contracted, the nuclei spiral, kink, or twist. The cytoplasm is pink, unstriated and with little detail.

Compare the smooth muscle to the adjacent layers of collagen fibers.

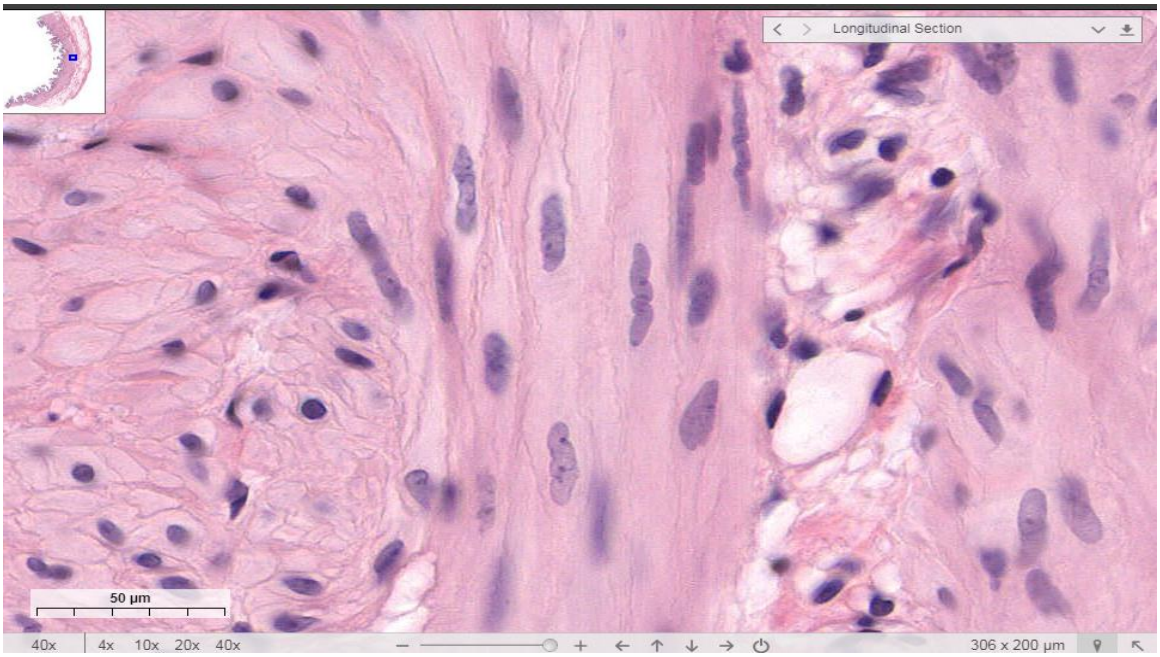




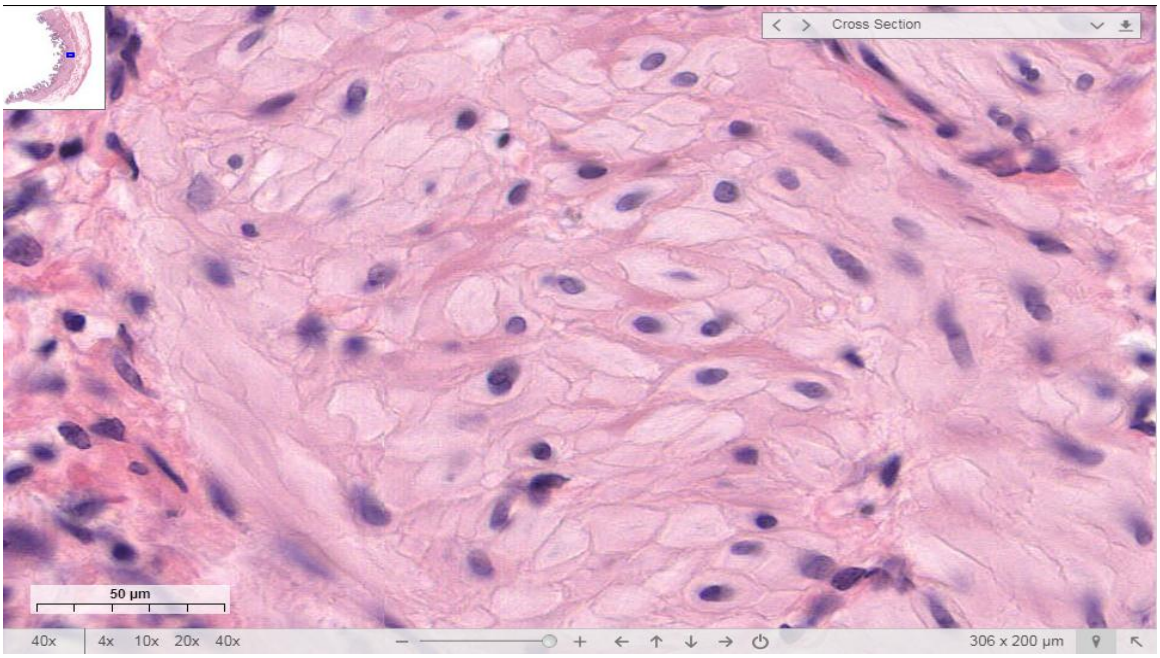
Gallbladder

The wall of the gallbladder contains interwoven sheets of smooth muscle. Both cross and longitudinal profiles of smooth muscle are seen.

Cell boundaries are readily seen in this specimen.



- Longitudinal Sections: The spindle-shape of cells cut through their centers is apparent. In relaxed smooth muscle, the nuclei are elongated with rounded ends. When contracted, the nuclei spiral, kink, or twist. The cytoplasm is pink, unstriated and with little detail.



- Cross-Sections: individual cells vary in diameter depending on their location within the cell. Cross-sections through the middle of cells have a centrally located nuclei, usually surrounded by an unstained region.

Structure of smooth muscle

In the cytoplasm, we find longitudinally oriented bundles of the myofilaments actin and myosin. Actin filaments insert into attachment plaques located on the cytoplasmic surface of the plasma membrane. From here, they extend into the cytoplasm and interact with myosin filaments. The myosin filaments interact with a second set of actin filaments which insert into intracytoplasmatic dense bodies. From these dense bodies further actin filaments extend to interact with yet another set of myosin filaments. This sequence is repeated until the last actin filaments of the bundle insert into attachment plaques .

In principle, this organisation of bundles of myofilaments, or myofibrils, into repeating units corresponds to that in other muscle types. The repeating units of different myofibrils are however not aligned with each other, and myofibrils do not run exactly longitudinally or parallel to each other through the smooth muscle cells. Striations, which reflect the alignment of myofibrils in other muscle types, are therefore not visible in smooth muscle.

Smooth endoplasmatic reticulum is found close to the cytoplasmatic surface of the plasma membrane. Most of the other organelles tend to accumulate in the cytoplasmic regions around the poles of the nucleus. The plasma membrane, cytoplasm and endoplasmatic reticulum of muscle cells are often referred to as sarcolemma, sarcoplasm, and sarcoplasmatic reticulum.

During contraction, the tensile force generated by individual muscle cells is conveyed to the surrounding connective tissue by the sheath of reticular fibres. These fibres are part of a basal lamina which surrounds muscle cells of all muscle types. Smooth muscle cells can remain in a state of contraction for long periods. Contraction is usually slow and may take minutes to develop.

Origin of smooth muscle

Smooth muscle cells arise from undifferentiated mesenchymal cells. These cells differentiate first into mitotically active cells, myoblasts, which contain a few myofilaments. Myoblasts give rise to the cells which will differentiate into mature smooth muscle cells.

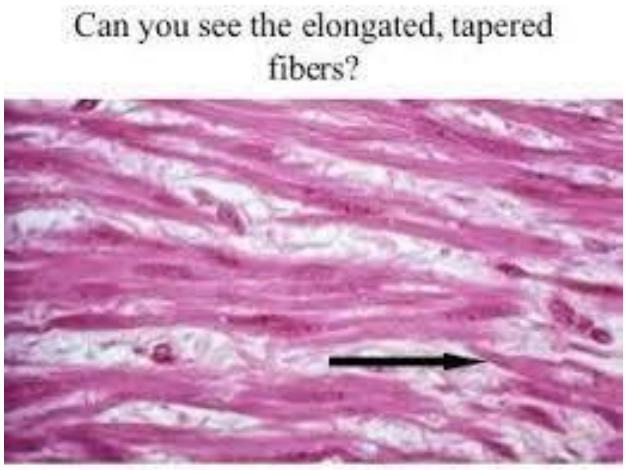
Types of smooth muscle

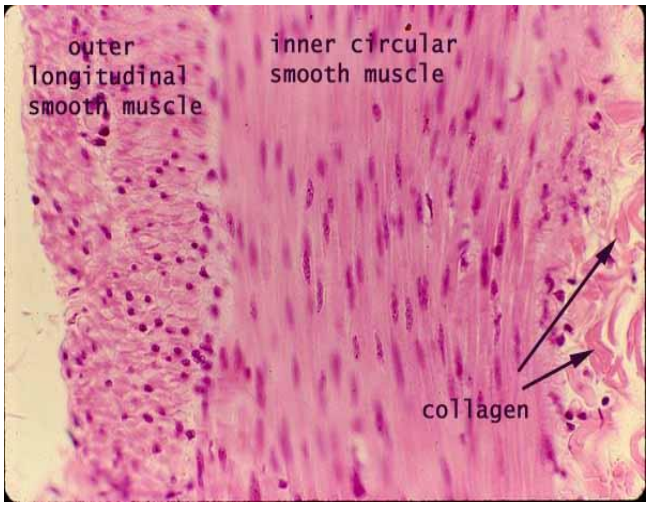
Two broad types of smooth muscle can be distinguished on the basis of the type of stimulus which results in contraction and the specificity with which individual smooth muscle cells react to the stimulus:

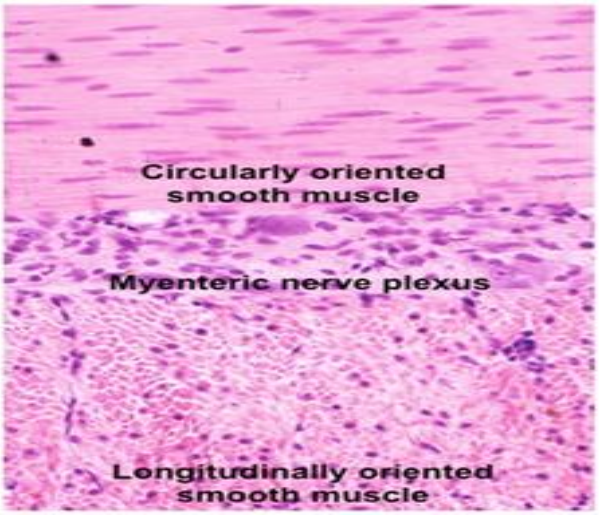
1. The multiunit type represents functionally independent smooth muscle cells which are often innervated by a single nerve terminal and which never contract spontaneously (e.g. smooth muscle in the walls of blood vessels).
2. The visceral type represents bundles of smooth muscle cells connected by GAP junctions, which contract spontaneously if stretched beyond a certain limit (e.g. smooth muscle in the walls of the intestines).

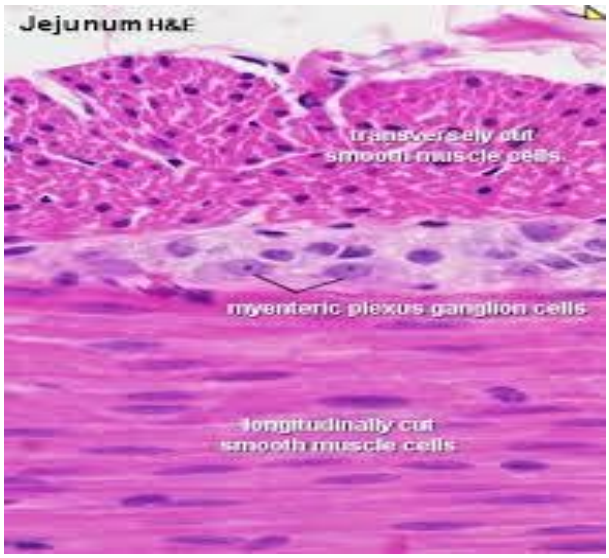
Suitable Slides

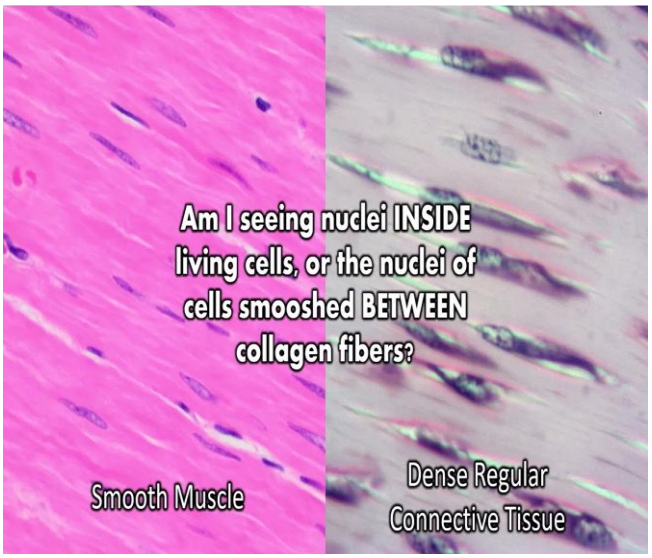
Sections of the intestines (duodenum, jejunum, ileum or colon) - H&E Jejunum, baboon - H&E The outer part of the tube forming the intestines consists of two layers of smooth muscle - one circular layer and one longitudinal layer. If you look at the tissue close to the border between the two layers of smooth muscle, you will be able to see both longitudinally sectioned smooth muscle cells and transversely sectioned smooth muscle cells. The smooth muscle cells are much longer than their nuclei. Transversely

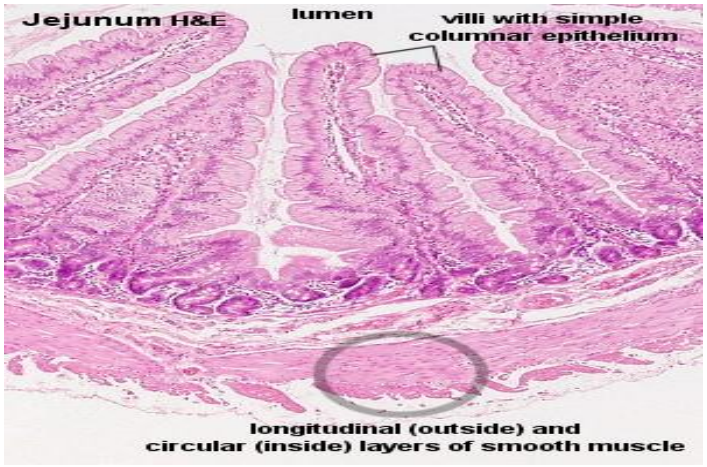


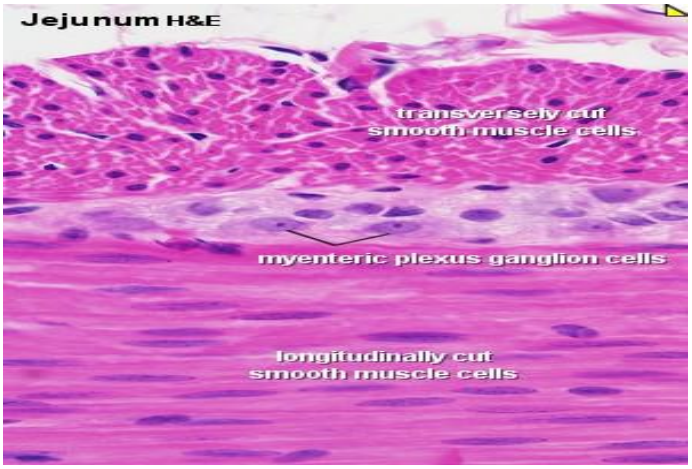












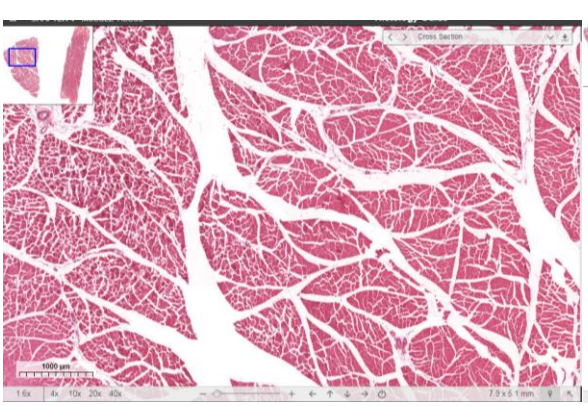
sectioned smooth muscle cells may not have their nuclei in the plane of the section. Occasionally you will find small nerves between the two muscle layers, and, if you are lucky and/or patient,

you will also see some very large nuclei in this region. These nuclei belong to peripheral nerve cells (ganglion cells of the myenteric plexus), which regulate the contraction of the muscle around the gastrointestinal tract. **Draw a small area which contains both longitudinally sectioned and transversely sectioned smooth muscle at high magnification.**

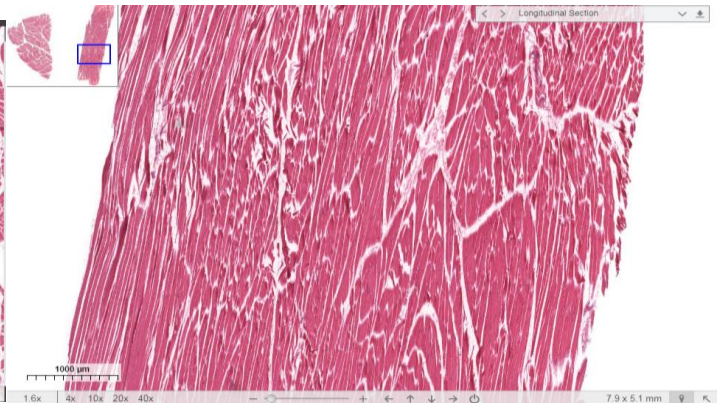
The only tissues which perhaps could be confused with smooth muscle are dense regular connective tissues and peripheral nerves. Both the number of nuclei and their shapes clearly distinguish smooth muscle from dense regular connective tissues. Nuclei are much more frequent and larger in smooth muscle, and they are very elongated if cut longitudinally. Peripheral nerves will be surrounded by a capsule of cells and connective tissue - the perineurium. The thickness of longitudinally cut nerve fibres is constant while smooth muscle cells are spindle shaped. Also, axon and nodes of Ranvier should be visible in peripheral nerves

Skeletal Muscle

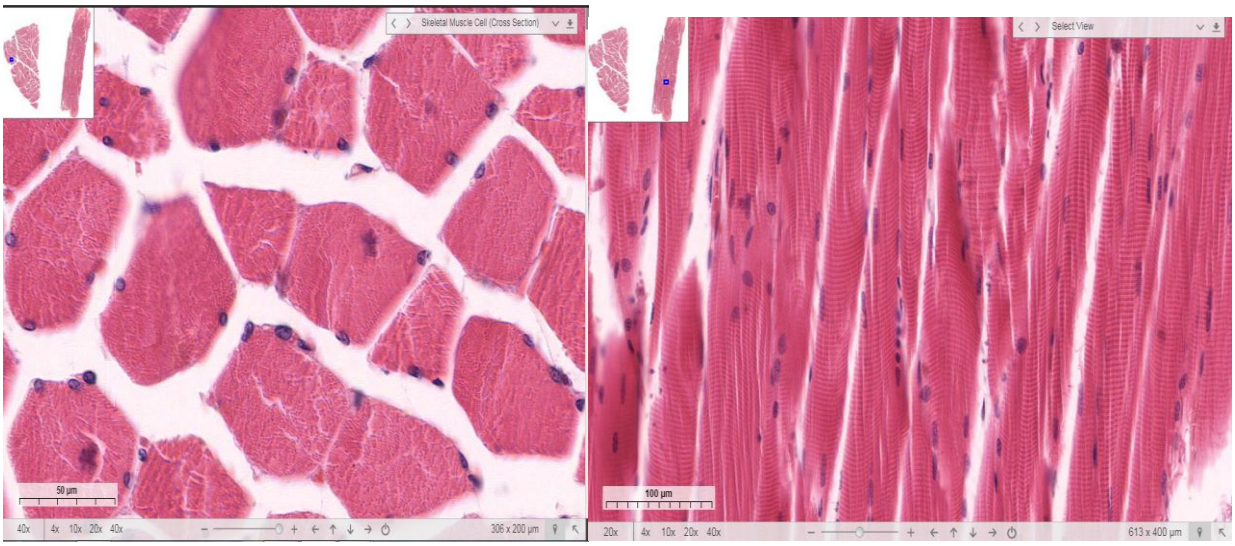
- Skeletal muscle consists of very long tubular cells (also called muscle fibres). The average length of skeletal muscle cells in humans is about 3 cm (sartorius muscle up to 30 cm, stapedius muscle only about 1 mm). Their diameters vary from 10 to 100 µm.
- Skeletal muscle fibres contain many peripherally placed nuclei. Up to several hundred rather small nuclei with 1 or 2 nucleoli are located just beneath the plasma membrane.
- Skeletal muscle fibres show in many preparations characteristic cross-striations. It is therefore also called striated muscle.
- Skeletal muscle is innervated by the somatic nervous system.
- Skeletal muscle makes up the voluntary muscle.

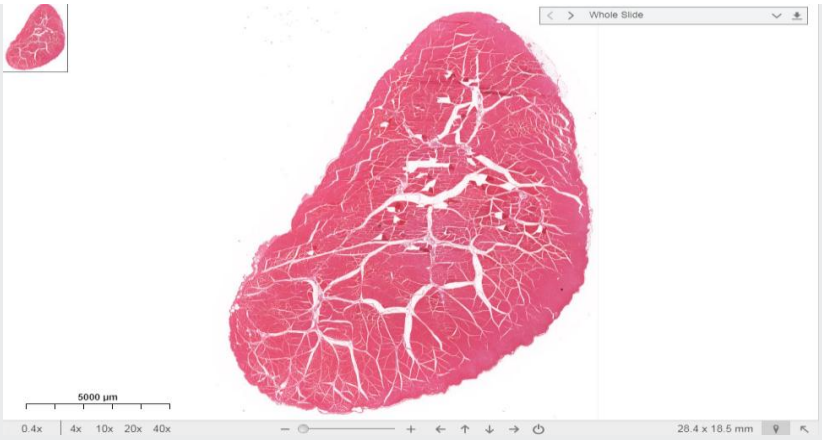


Cross-Section



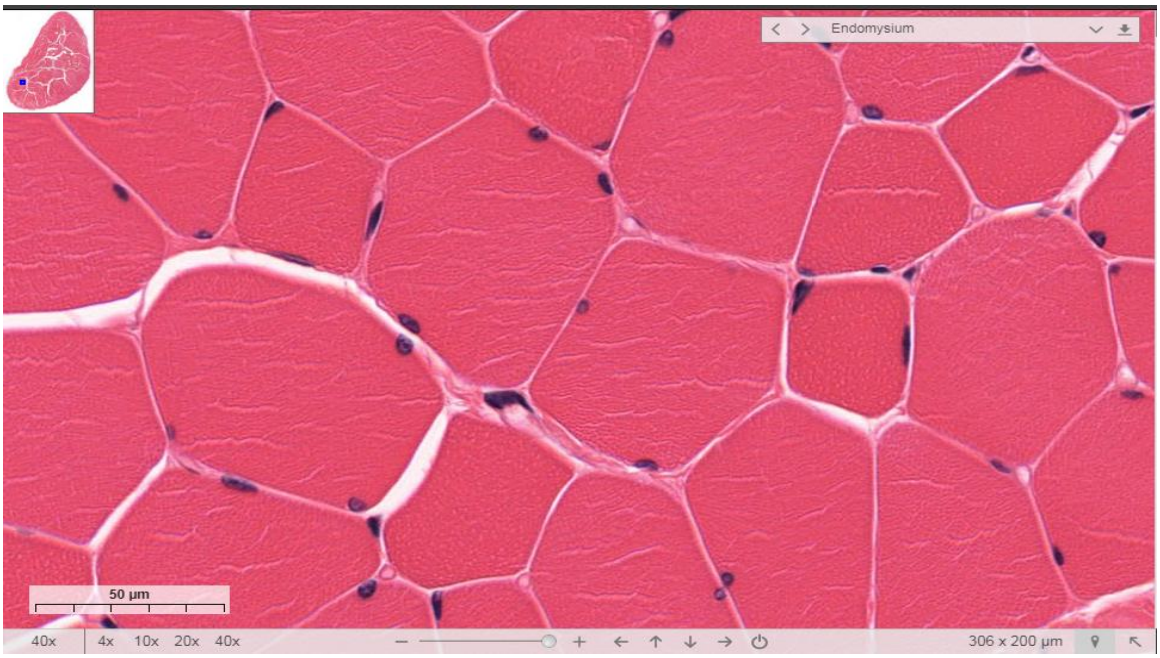
Longitudinal Section



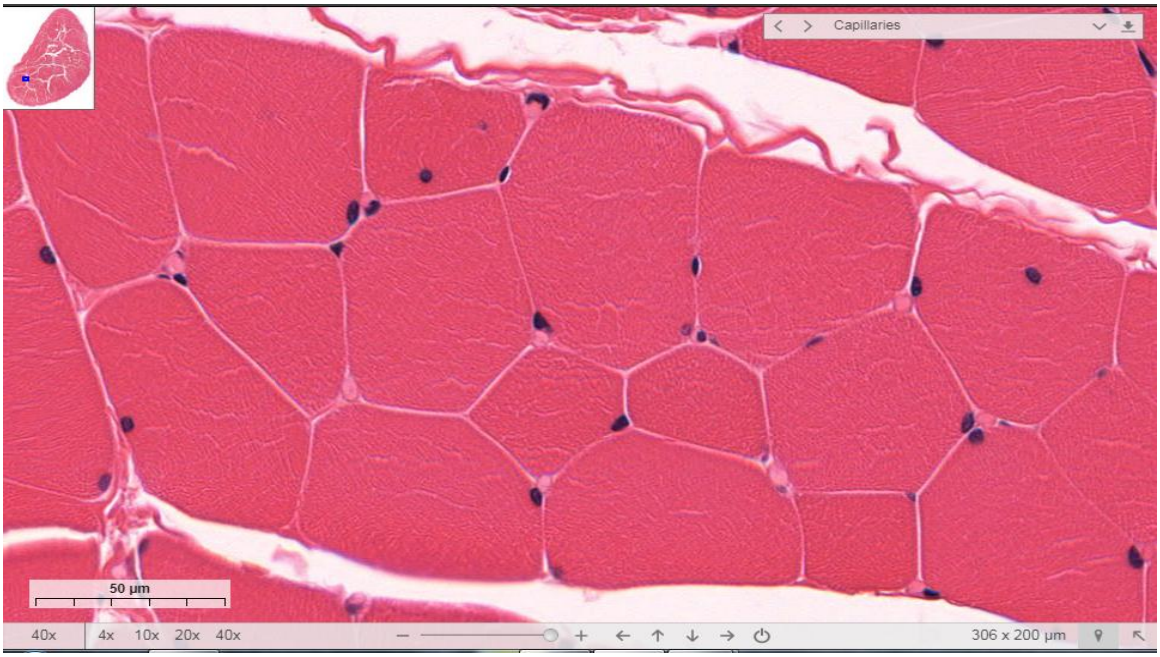


Skeletal Muscle

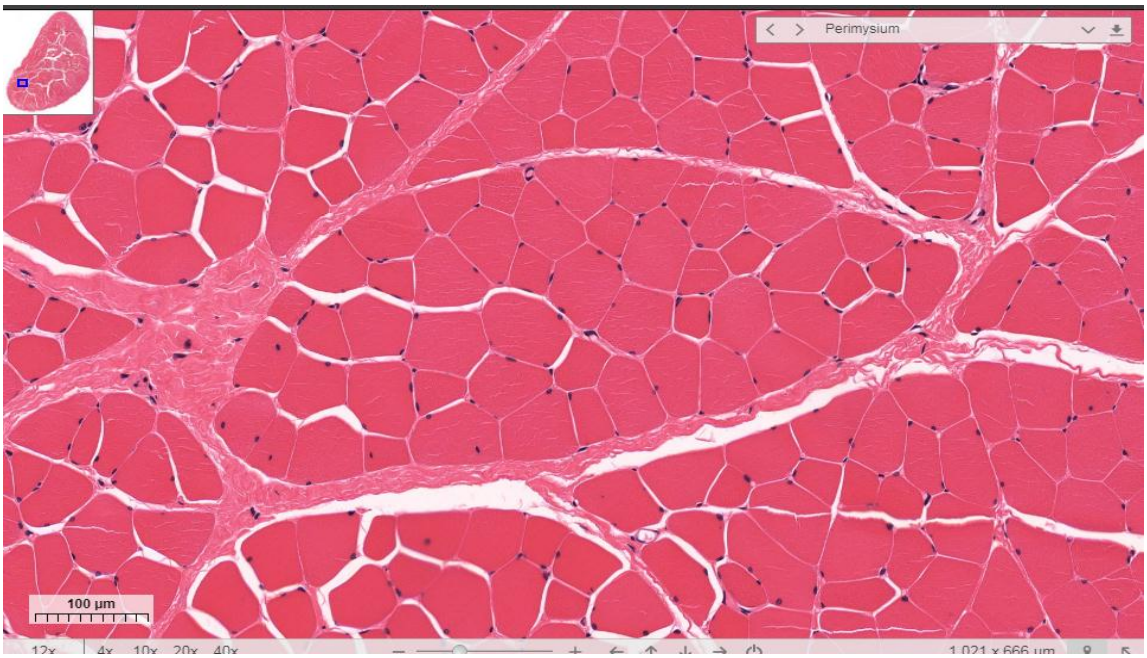
This cross-section of skeletal muscle shows the connective tissue sheaths that organize muscle fibers into muscles. These sheaths usually conduct forces generated by muscle contraction through tendons to bone.



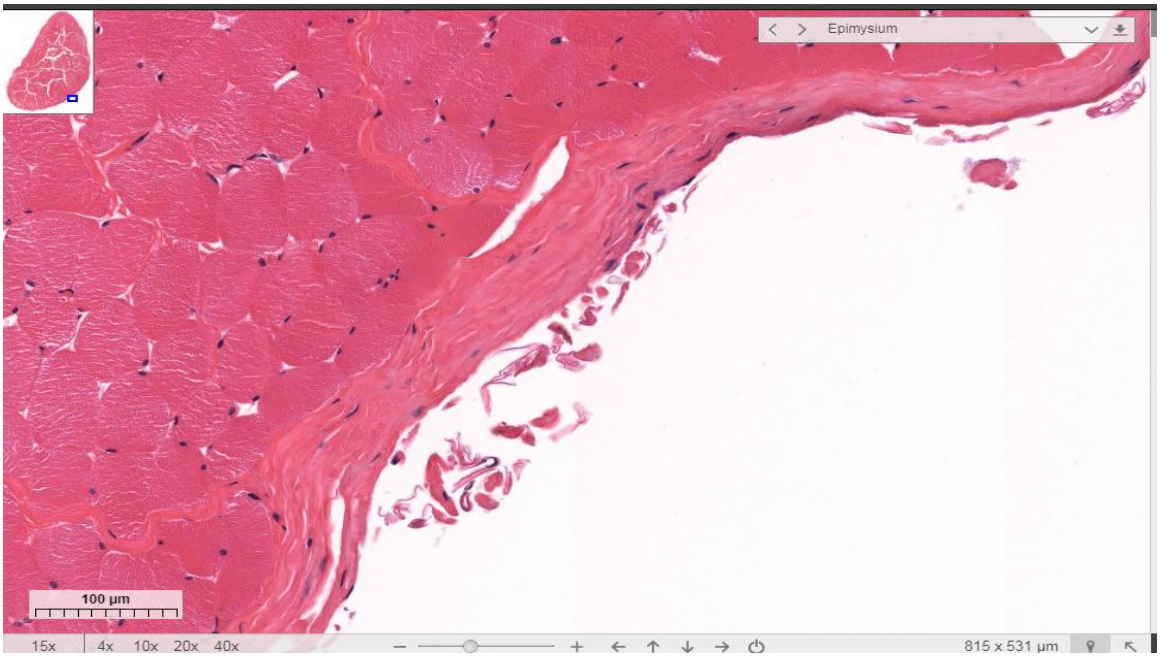
Endomysium - thin layer of connective tissue that surrounds each muscle cell.



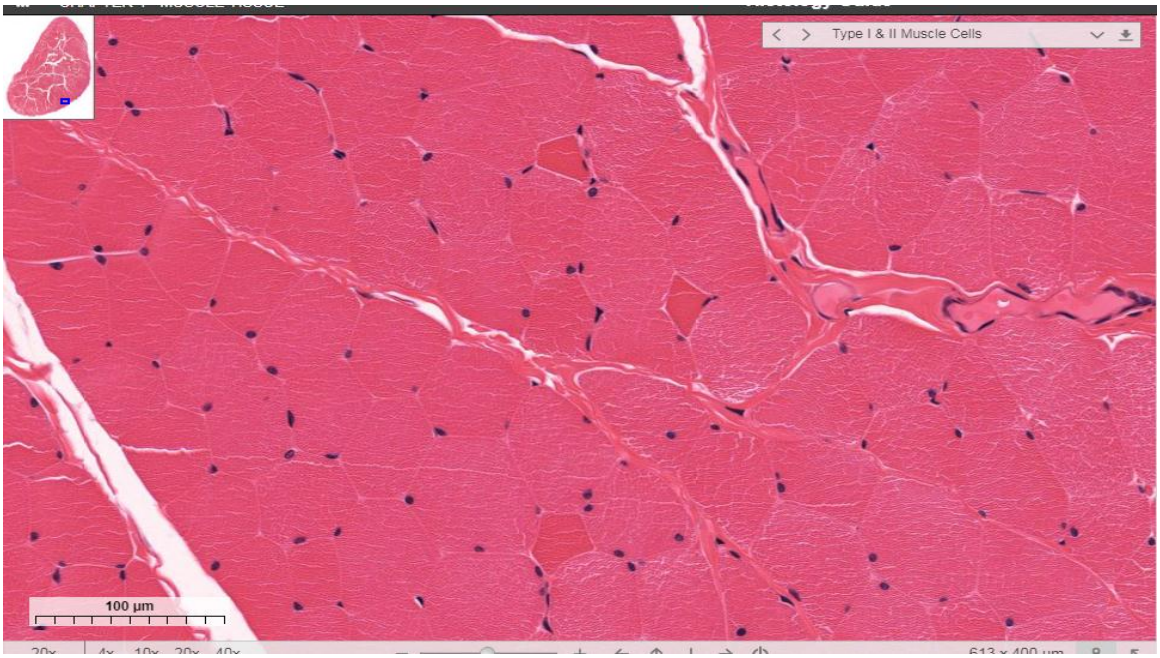
- Capillaries travel through this layer and provide a rich blood supply. The capillaries are found at the corners of the muscle cells.
- Small nerve fibers are also found in this layer



- Perimysium - thick layer of connective tissue that surrounds a group of muscle cells to form fascicles.
  - Blood vessels travel through this layer
    - Nerves are found in this layer

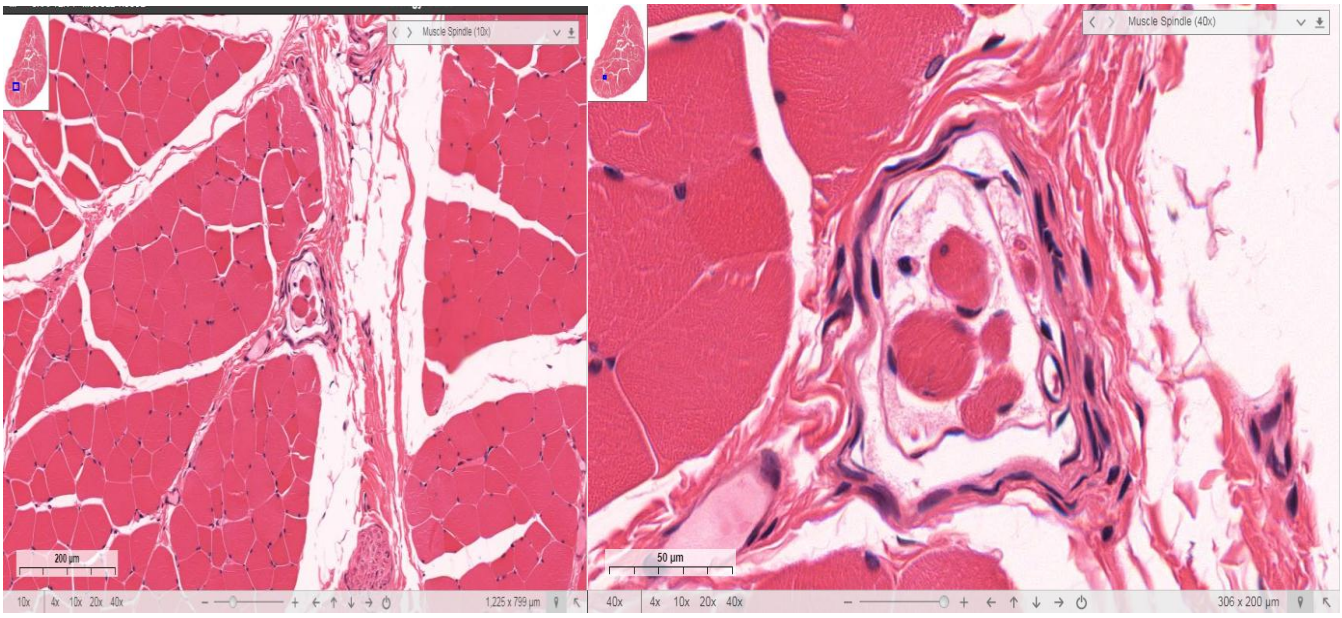


- Epimysium - dense connective tissue that surrounds the entire muscle and is usually continuous with a tendon.



Skeletal muscle cells (or fibers) are classified based on contractile speed and metabolic activity. Two types of fibers can be distinguished in this specimen.

- Type I - smaller muscle cells that specialize in long, slow contractions. They stain darker than type II fibers.
- Type II - larger muscle cells that specialize in fast contractions. The majority of muscle cells in this specimen.



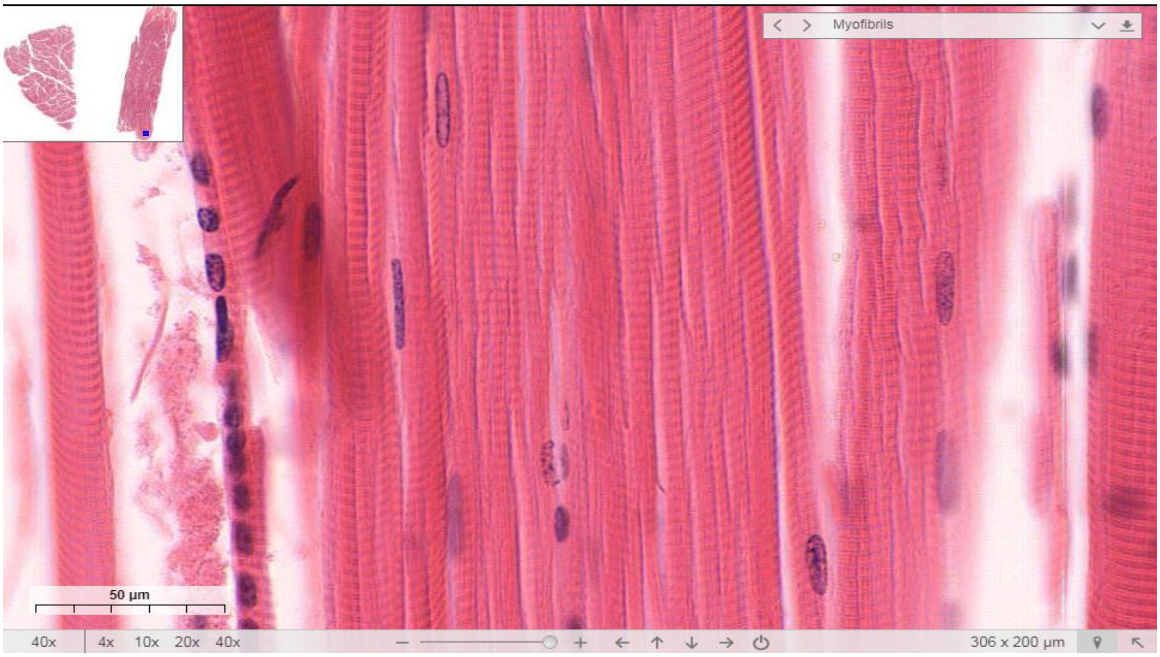
The cross section of **a muscle spindle** is present in this section. **Muscle spindles are sensory receptors** within a muscle that primarily detect changes in length of a muscle.

Cross-Section

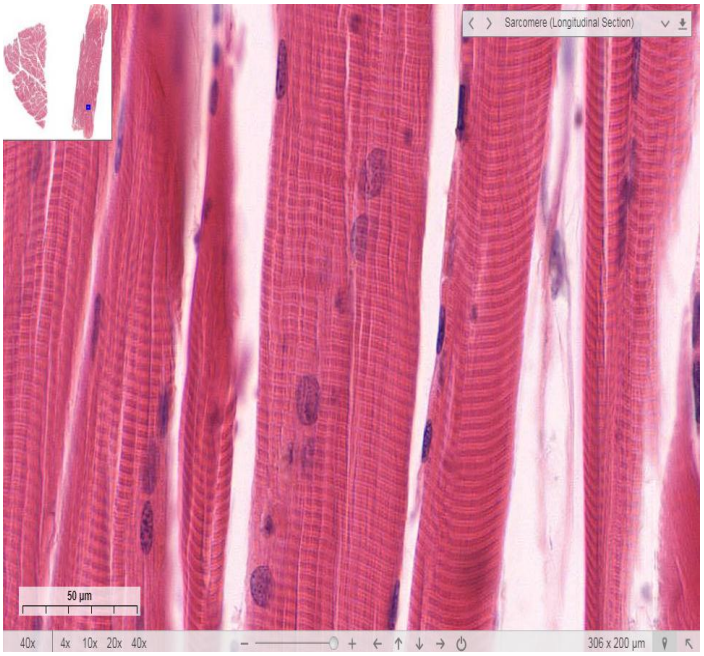
- Skeletal Muscle Cells - have polygonal cross-sections (50 to 100 µm in diameter) with nuclei at the periphery

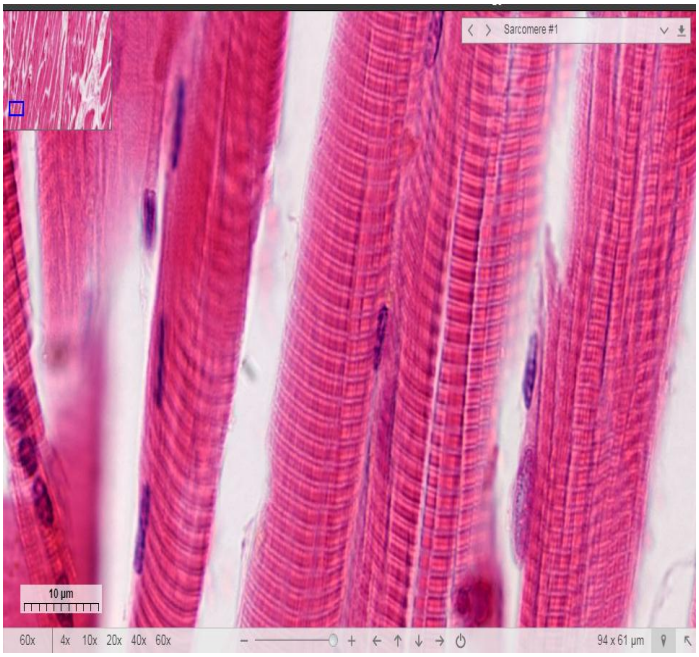
Longitudinal Section

- Skeletal Muscle Cells - can vary in length from a few millimeters to almost a meter



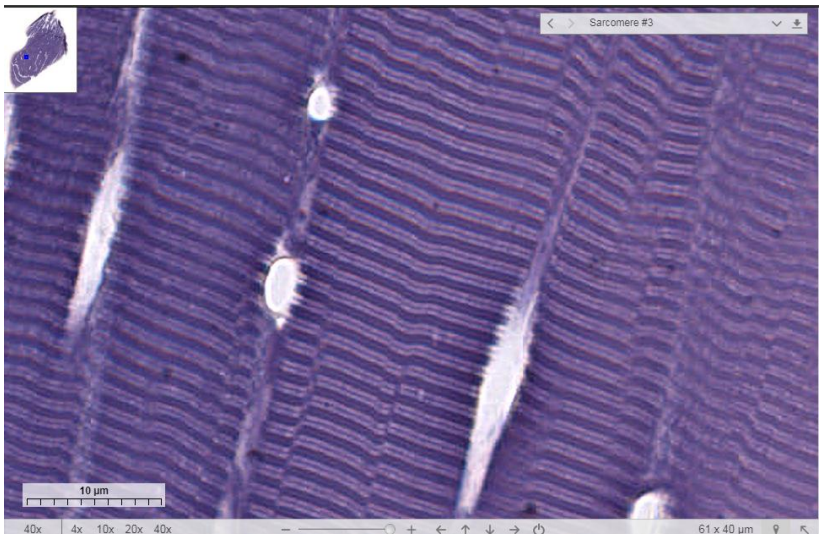
- Myofibrils - the cytoplasm is filled with myofibrils that extend the entire length of the cell. Individual myofibrils are only seen where they are slightly separated.





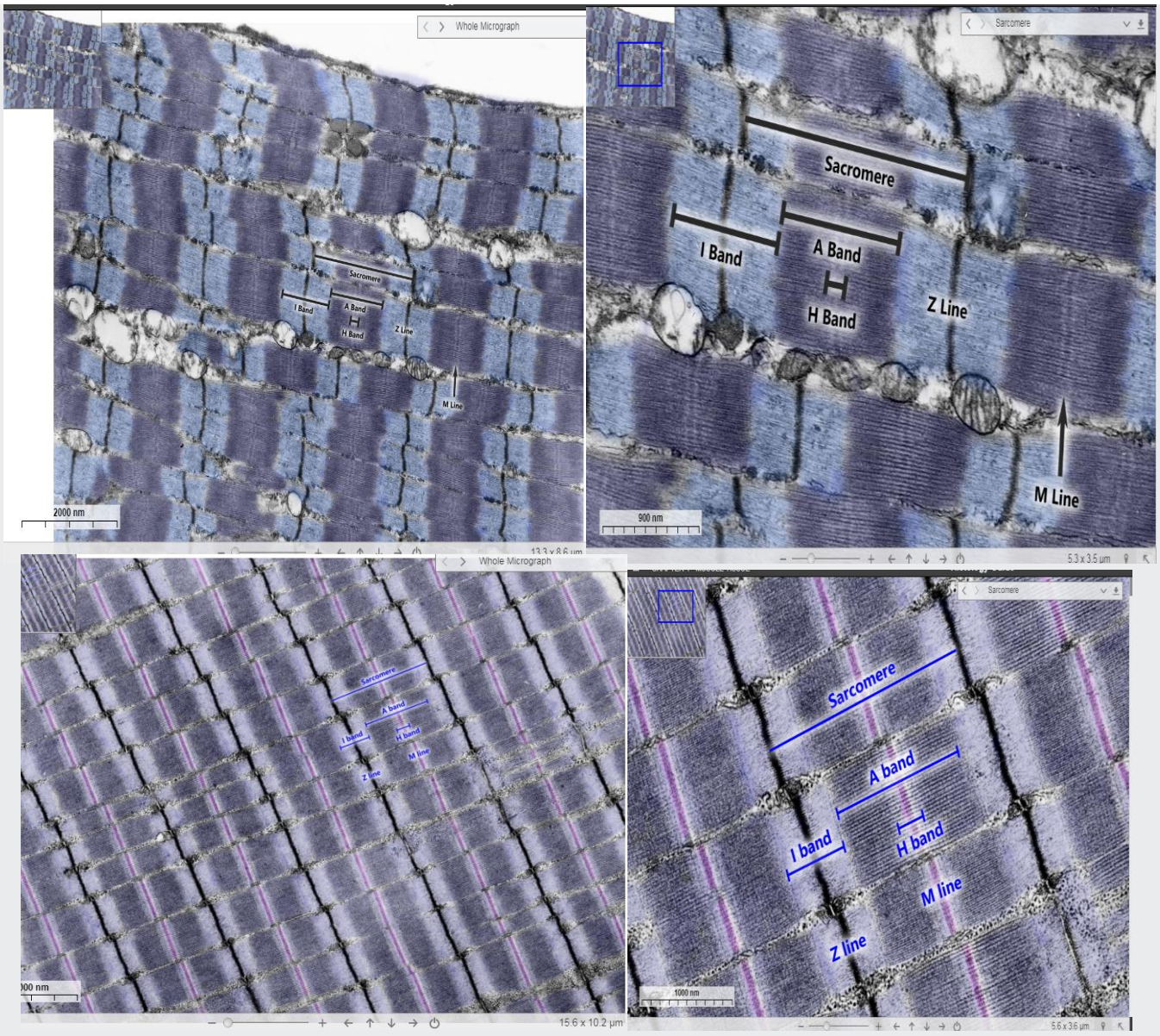
- Sarcomere - myofibrils show an alternating series of striations due to the repeating sarcomeres. (Shown better in the next two slides.)
  - A band - the main dark band
  - I band - the main light band
  - H band - thin light band in the middle of the A band

- Z band - thin dark line in the middle of the I band



Skeletal Muscle
(Phosphotungstic Acid/Hematoxylin)

This specimen was stained with phosphotungstic acid/hematoxylin (PTAH) to show the muscle cross-striations.

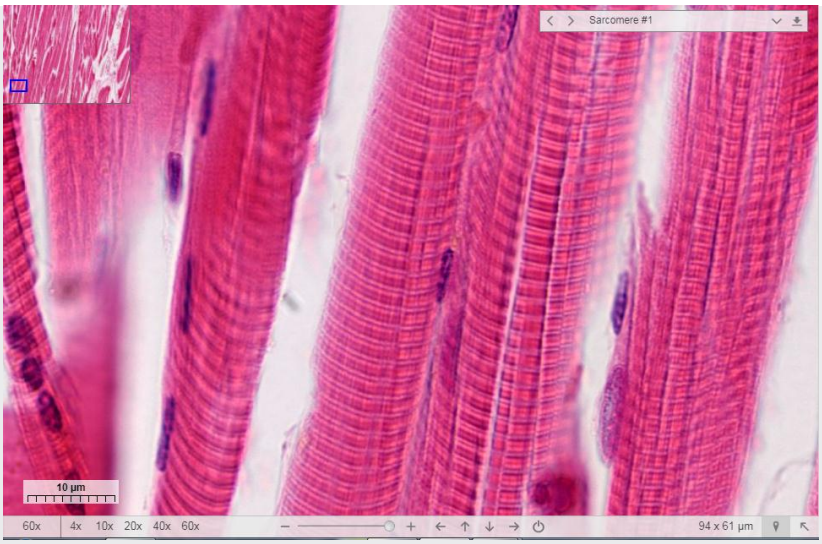


EM Skeletal Muscle

Skeletal muscle cells contain myofibrils composed of longitudinal arrays of sarcomeres.

Try to identify the following in these sarcomeres. Switch to the color image for a sarcomere with labels.

- **I band** - parallel arrays of thin (actin) filaments that point in opposite directions from the Z line into the A band. Narrows with contraction.
- **Z line** - dark region in the center of the I band. Thin filaments anchor to the Z line.
- **A band** - parallel arrays of thick (myosin) filaments that point in opposite directions from the M line.
- **H band** - light region in the center of the A band. Thin filaments do not extend into this region.
- **M line** - dark region in the center of the H band. Thick filaments anchor to the M line.



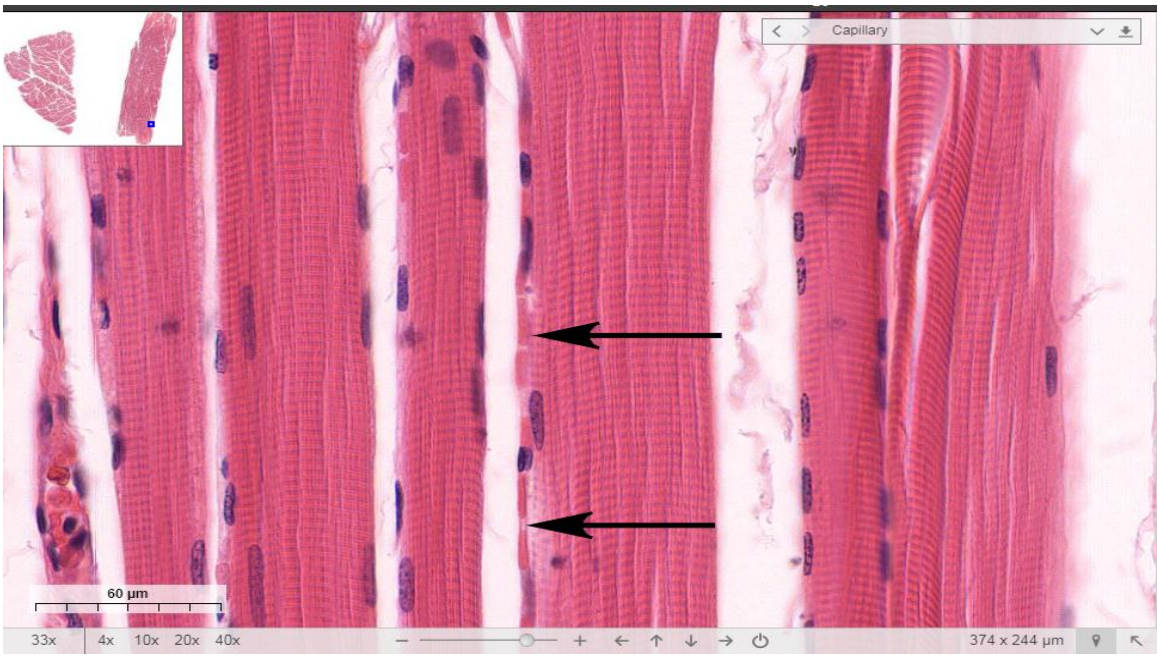
Skeletal Muscle

This image was obtained from a longitudinal section of skeletal muscle using a 60x oil immersion objective. The increased resolution is helpful in viewing sarcomeres.

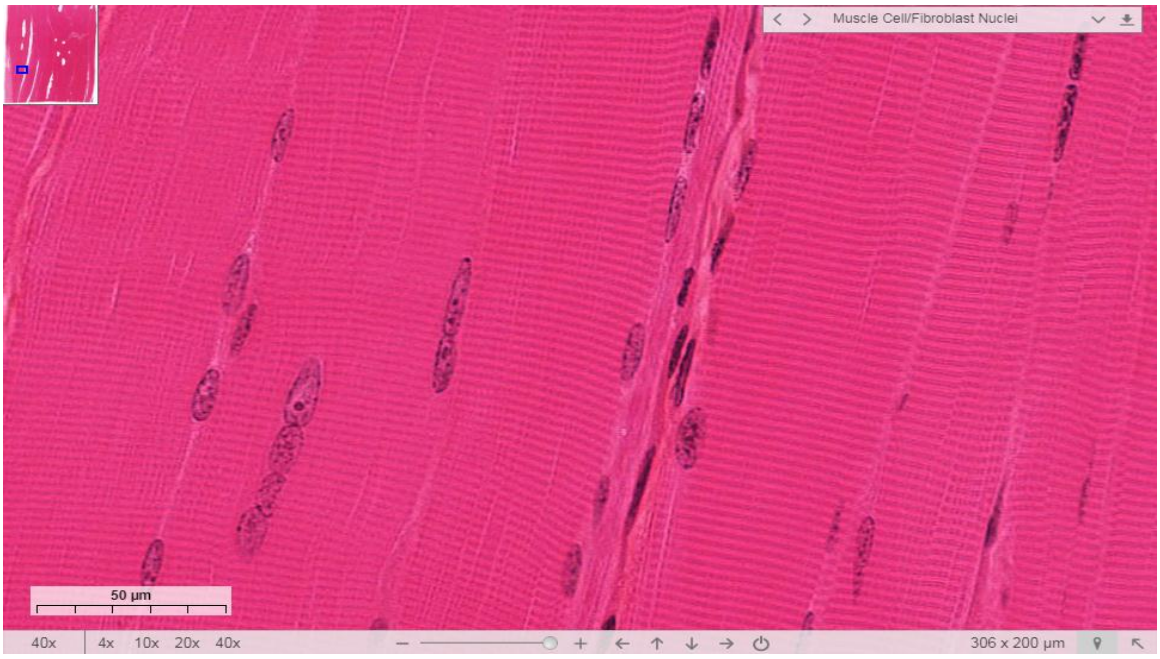
Skeletal muscle cells (or fibers) are filled with tightly packed myofibrils. Myofibrils show an alternating series of striations due to the repeating sarcomeres. Each sarcomere is 2 to 3 µm long and extends from one Z line to the next.

. Identify the bands in this higher magnification image of several sarcomeres.

- Satellite Cells - occur on the surface of muscle cells. Only their small nuclei of mostly heterochromatin can be identified



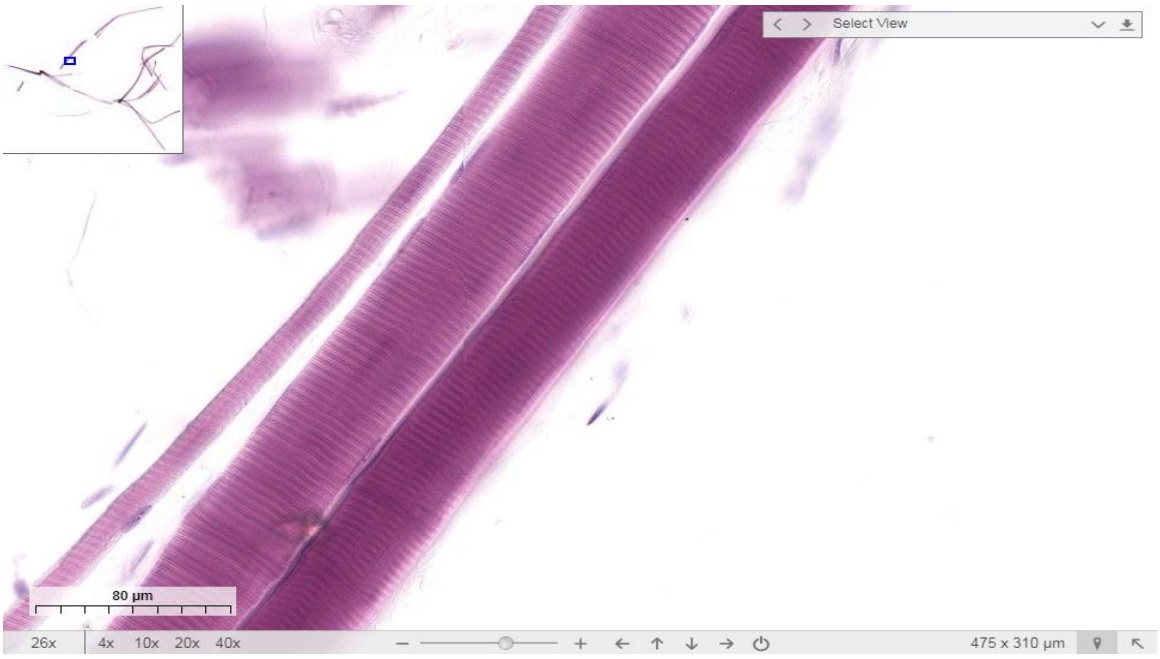
- Capillary - a collapsed capillary containing flattened red blood cells is present on the left edge of the center muscle cell



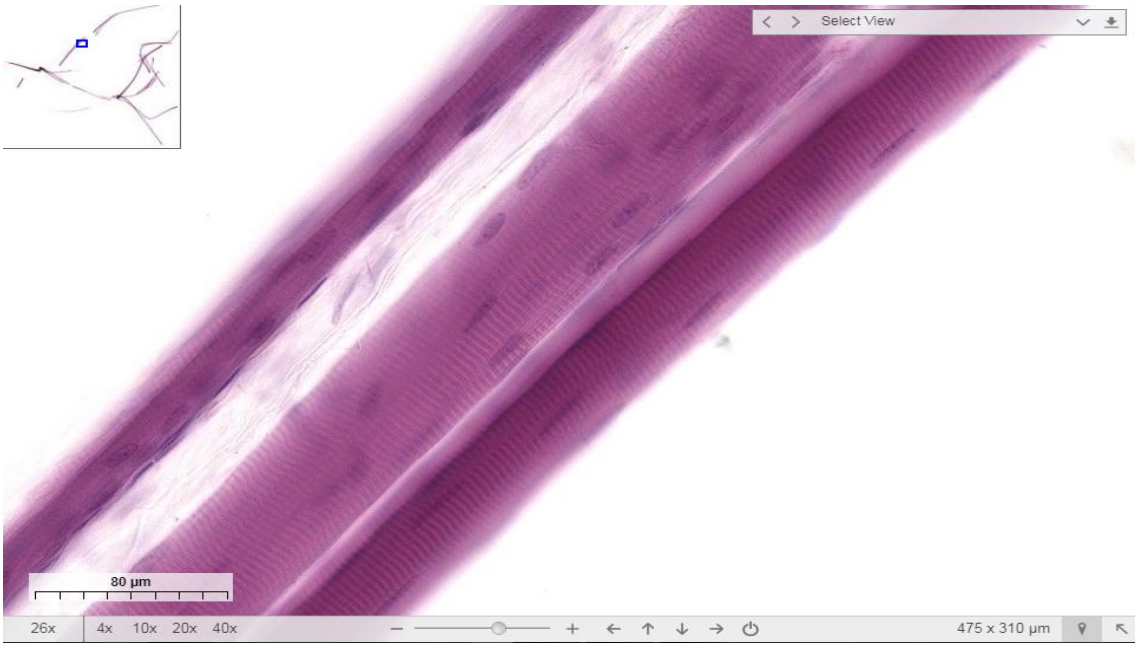
- Muscle Cell / Fibroblast Nuclei - muscle cell nuclei are mostly euchromatic and have a flattened, oval shape. Fibroblast nuclei are located between muscle cells and are more elongated and darker.

Skeletal Muscle Cells

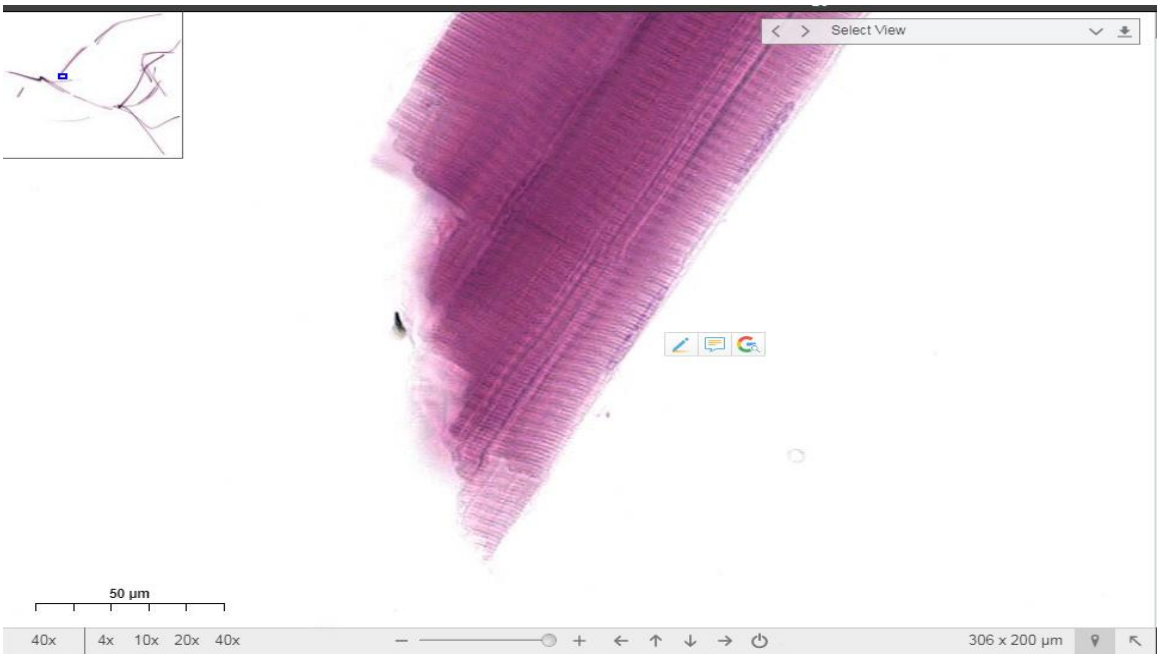
Whole mount of skeletal muscle cells (or fibers) that have been teased apart.
Human muscle cells are 10 to 100 µm in diameter and range from a few mm to 30 cm in length.



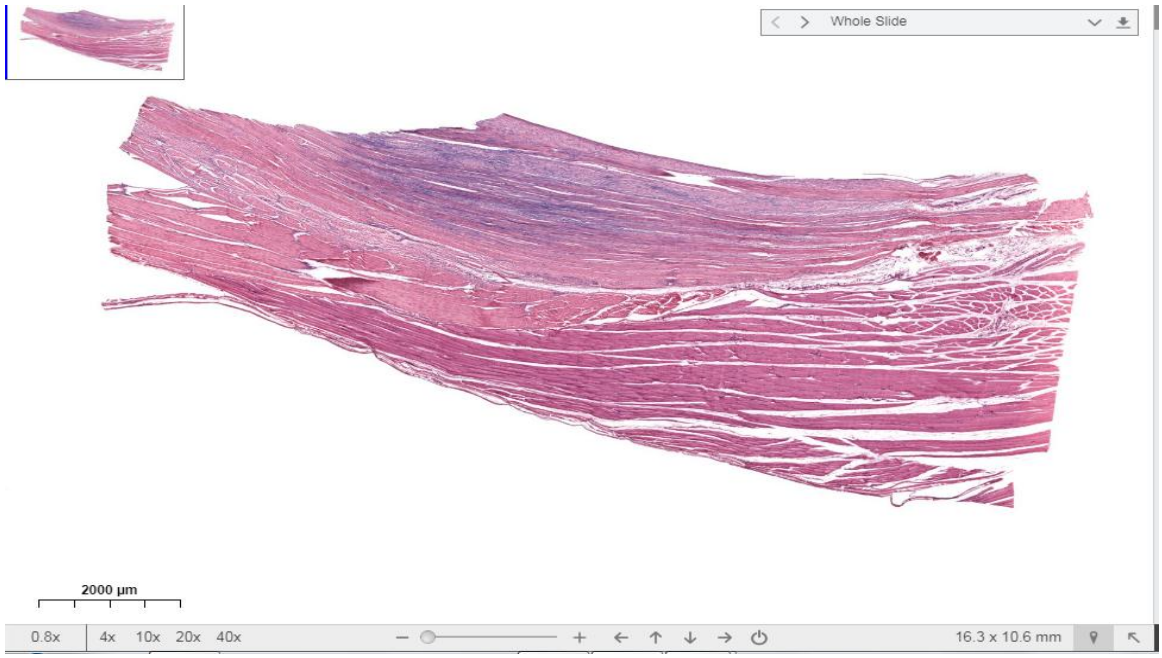
In this preparation, individual muscle cells are about 50 to 100 µm in diameter and are upto 5 mm in length.



- Nuclei - the large number of nuclei in a single cell can be better appreciated in this whole mount compared to tissue sections.

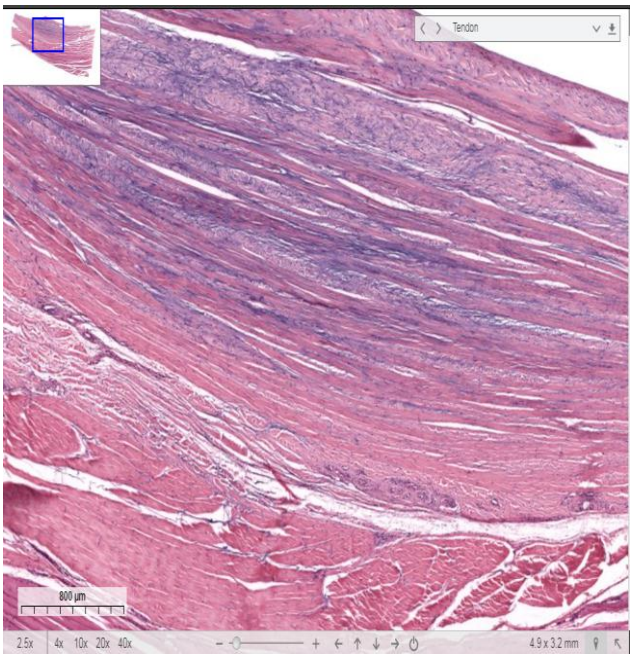


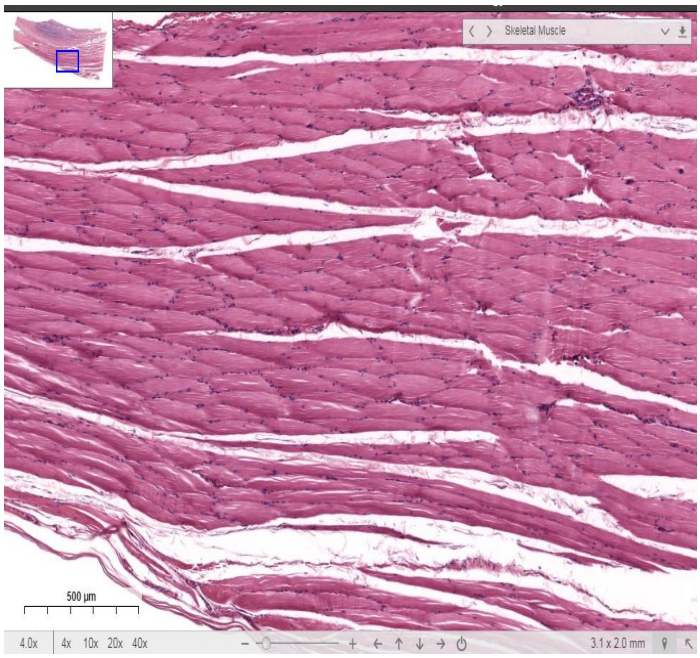
- Myofibrils - the individual myofibrils can be seen where cells are broken open at their ends.
- Striations - are visible in this whole mount because individual myofibrils in skeletal muscle are aligned with each other.



Muscle Tendon Insertion

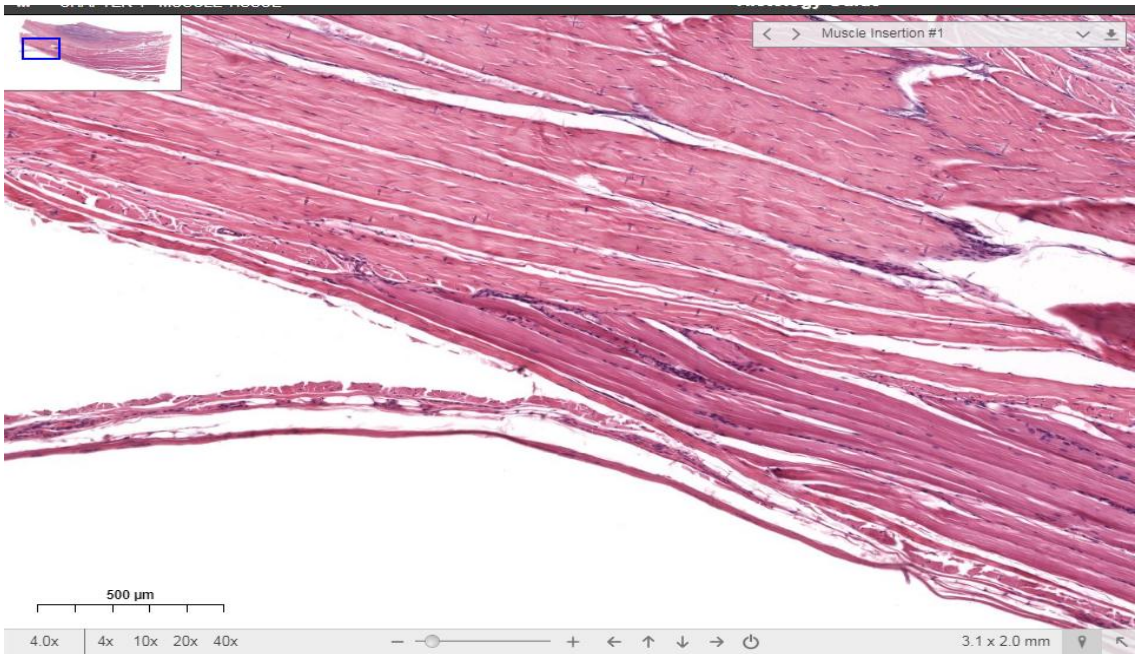
At the end of muscles, the connective tissue that surrounds muscle cells usually continues as a band of connective tissue that forms a tendon which attaches the muscle to bone.



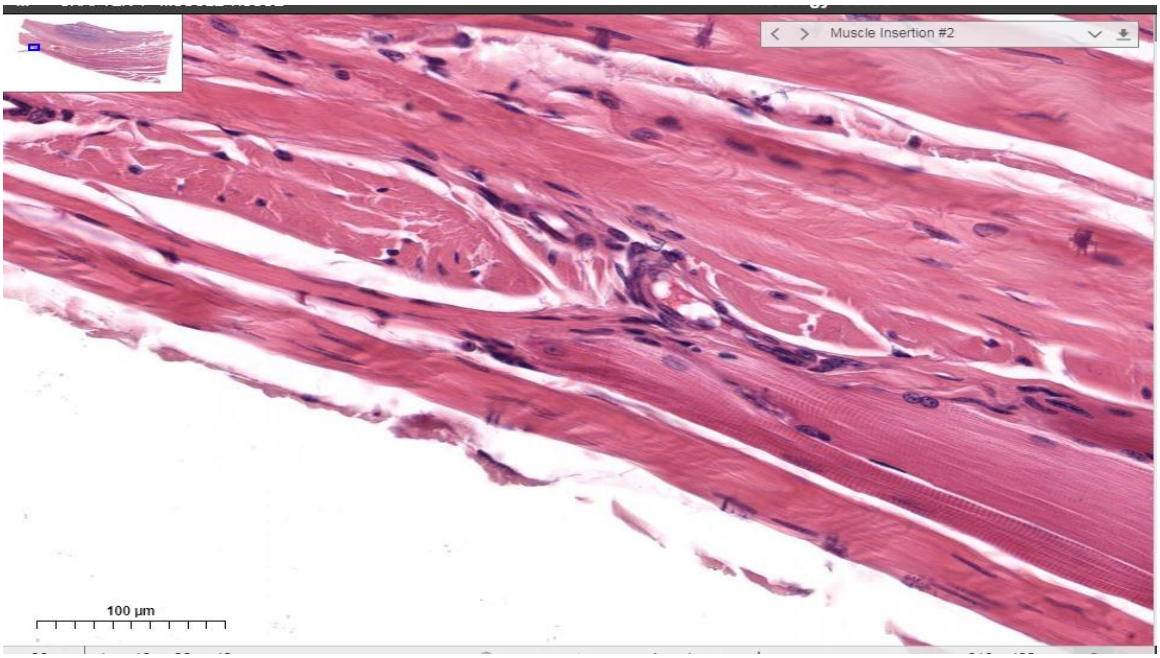


At the end of muscles, the connective tissue that surrounds muscle cells usually continues as a band of connective tissue that forms a tendon which attaches the muscle to bone.

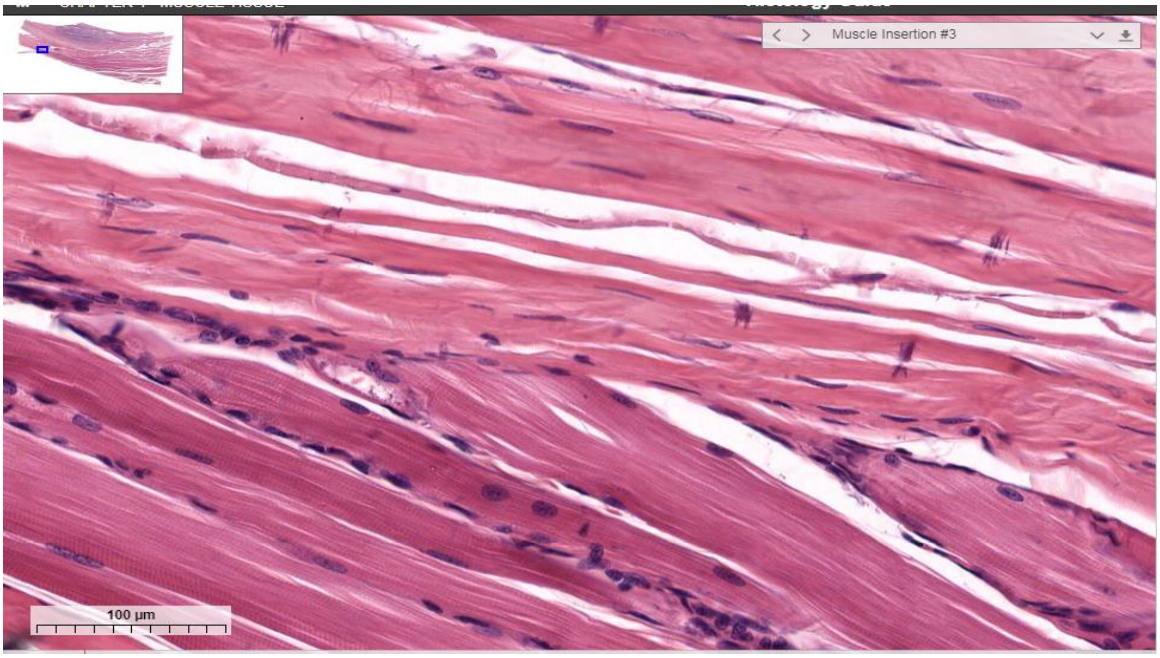
In this specimen, the tendon (dense regular connective tissue) is across the top, while the skeletal muscle is at the bottom.



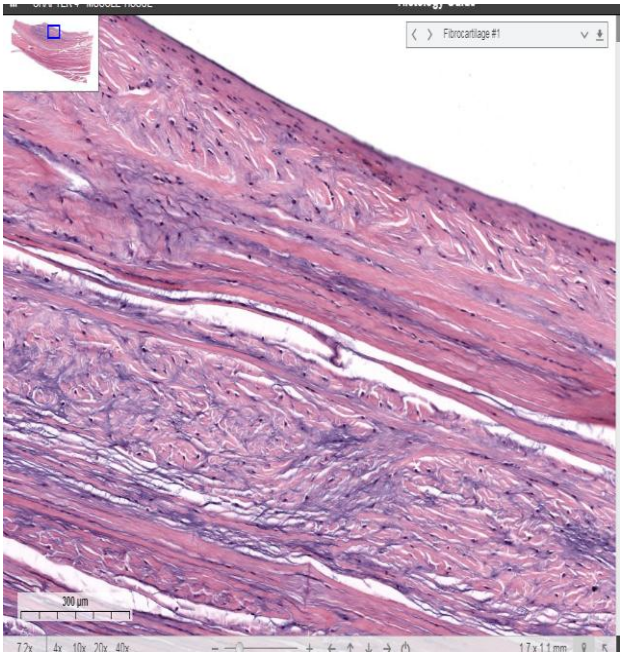
The muscle insertion into the tendon is best seen near the tip of the muscle.

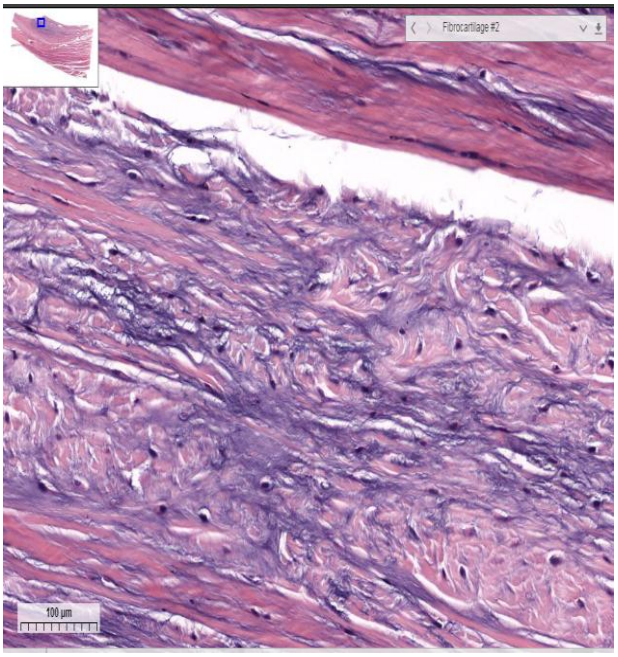


- Some muscle fibers end in collagen fibers that continue into the tendon.

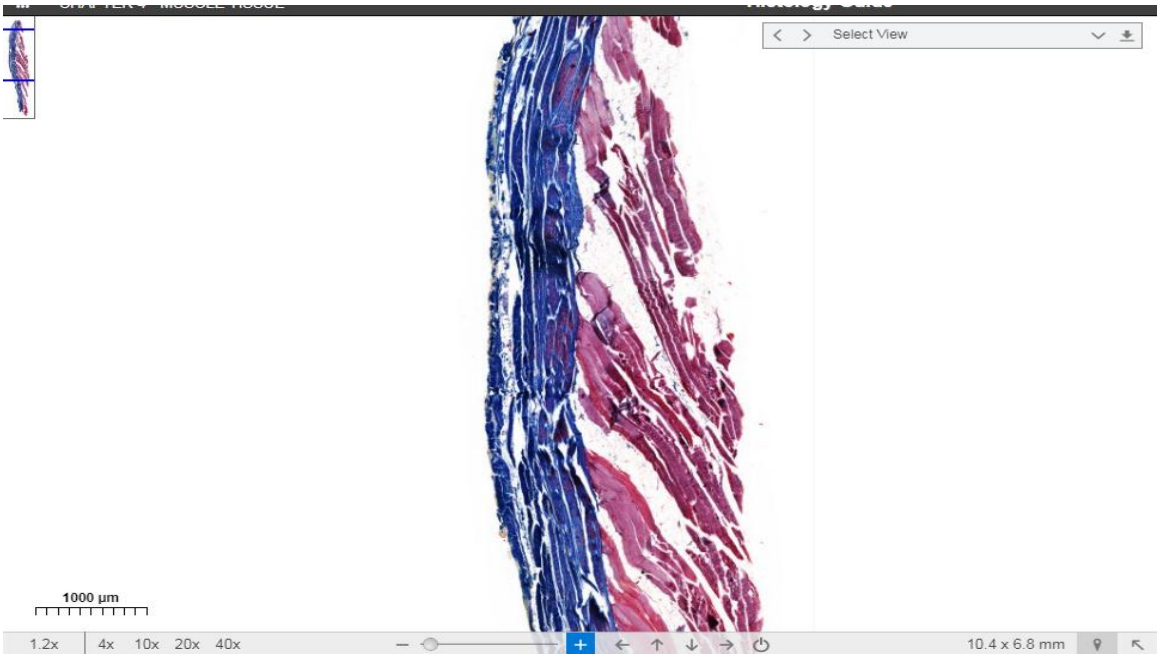


- The endomysium of other muscle fibers merge with collagen fibers of the tendon.





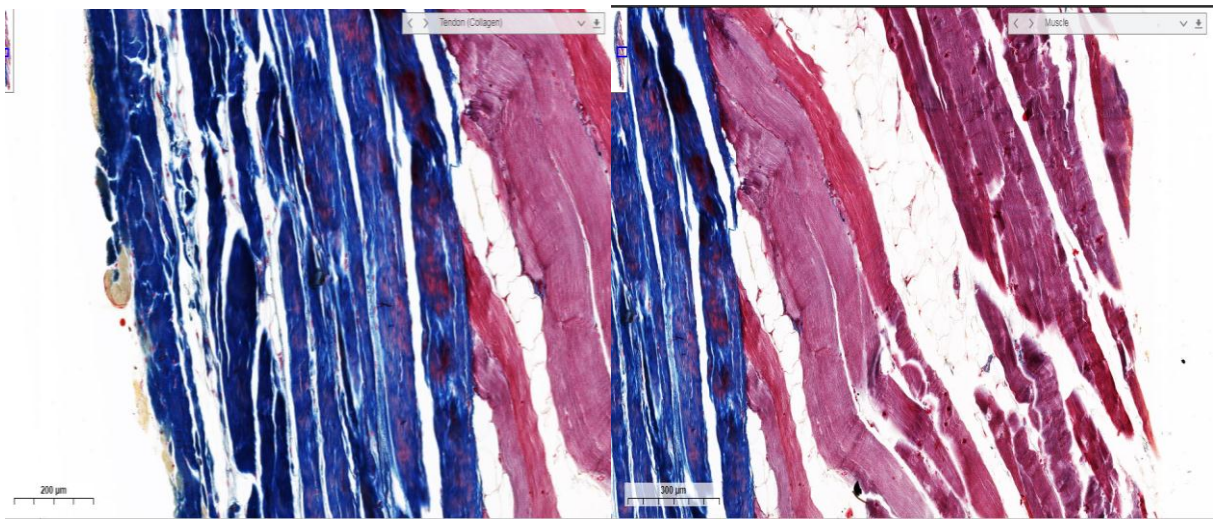
Tendons may also contain areas of fibrocartilage (#1 and #2) as seen in this specimen. The collagen fibers in fibrocartilage have an irregular arrangement. While those in the dense regular connective tissue of the tendon have a uniform, parallel arrangement. Fibrocartilage is made by chondrocytes that are round to oval with a clear space surrounding the nuclei. In contrast, the rest of the tendon is made by fibroblasts that are thin, spindle shaped cells tightly applied to the collagen bundles. Both fibrocartilage and dense regular connective tissue contain type I collagen.



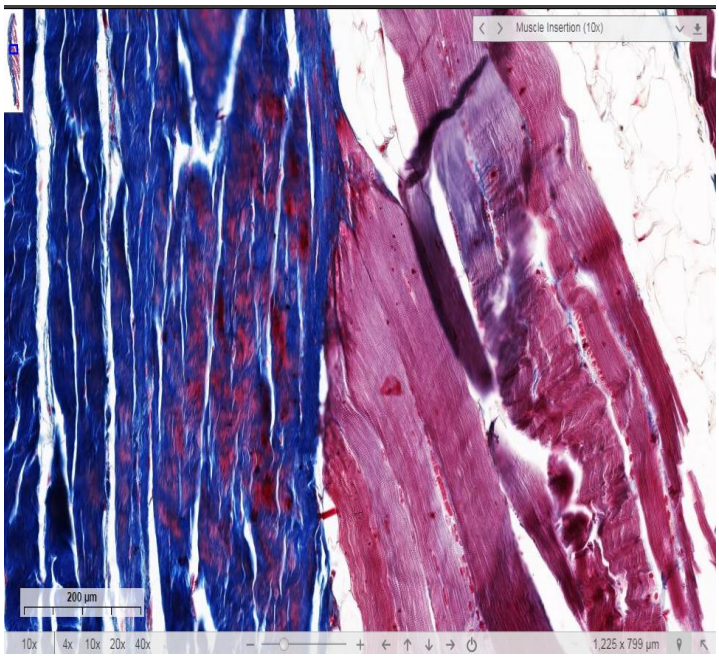
Muscle Tendon Insertion (Azan)

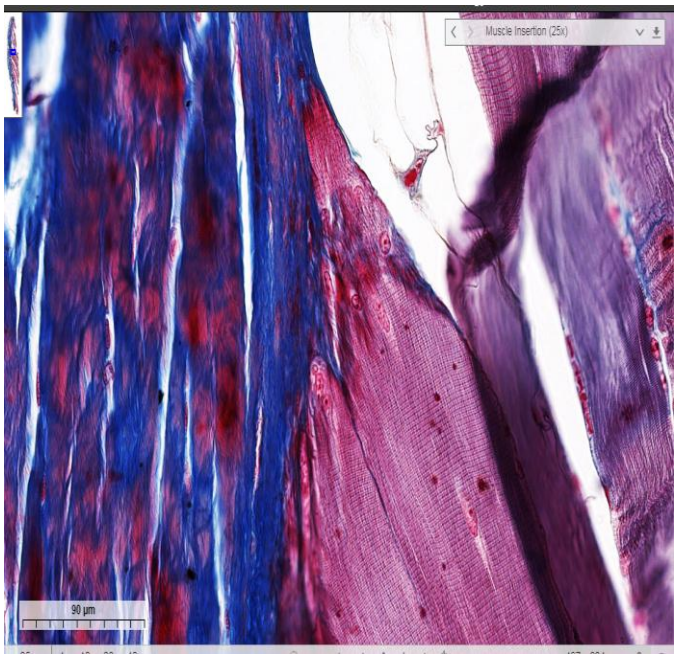
At the end of muscles, the connective tissue that surrounds muscle cells usually continues as a tendon. This fascia lata is an example of a muscle insertion in to a tendon.

In MH 030 Tendon, the fine structure of this insertion can be difficult to see because both collagen and muscle stain pink with H&E.

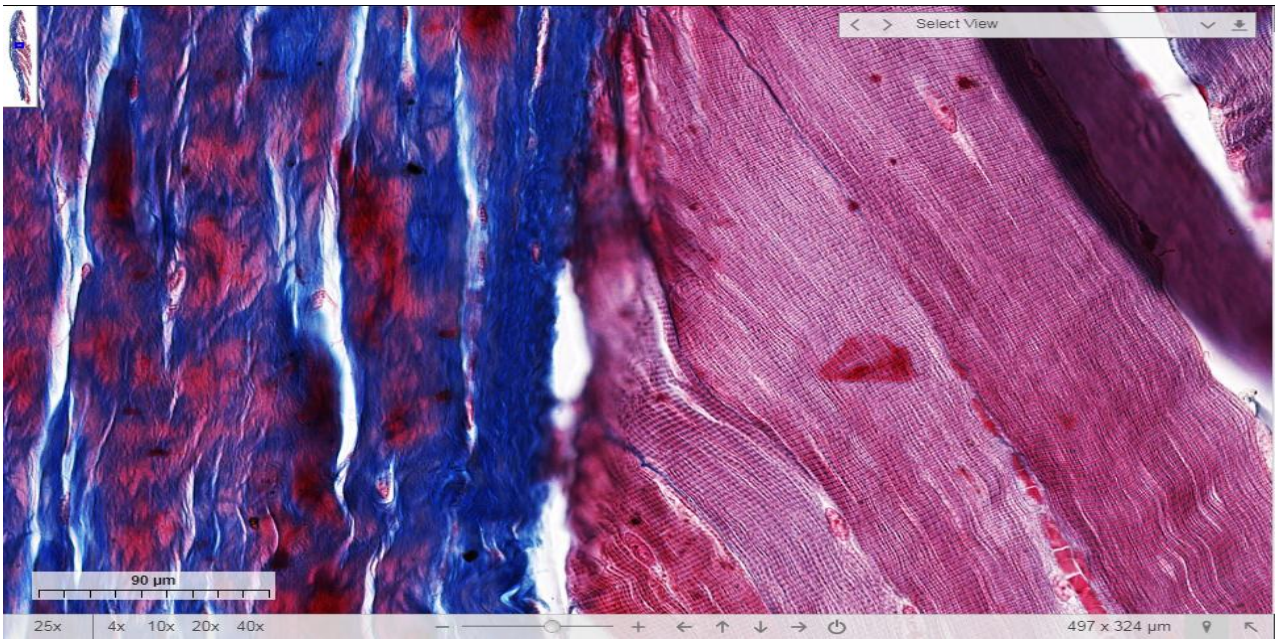


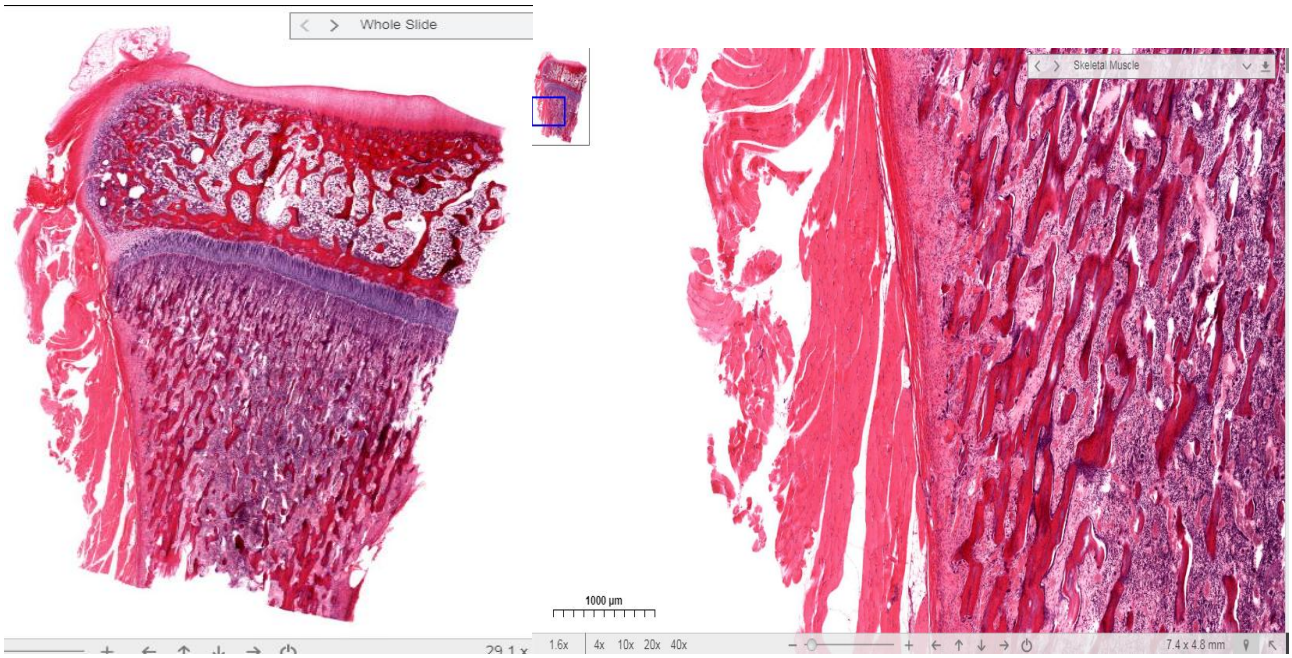
This specimen is stained with azan to distinguish collagen (blue) from muscle (pink/red).





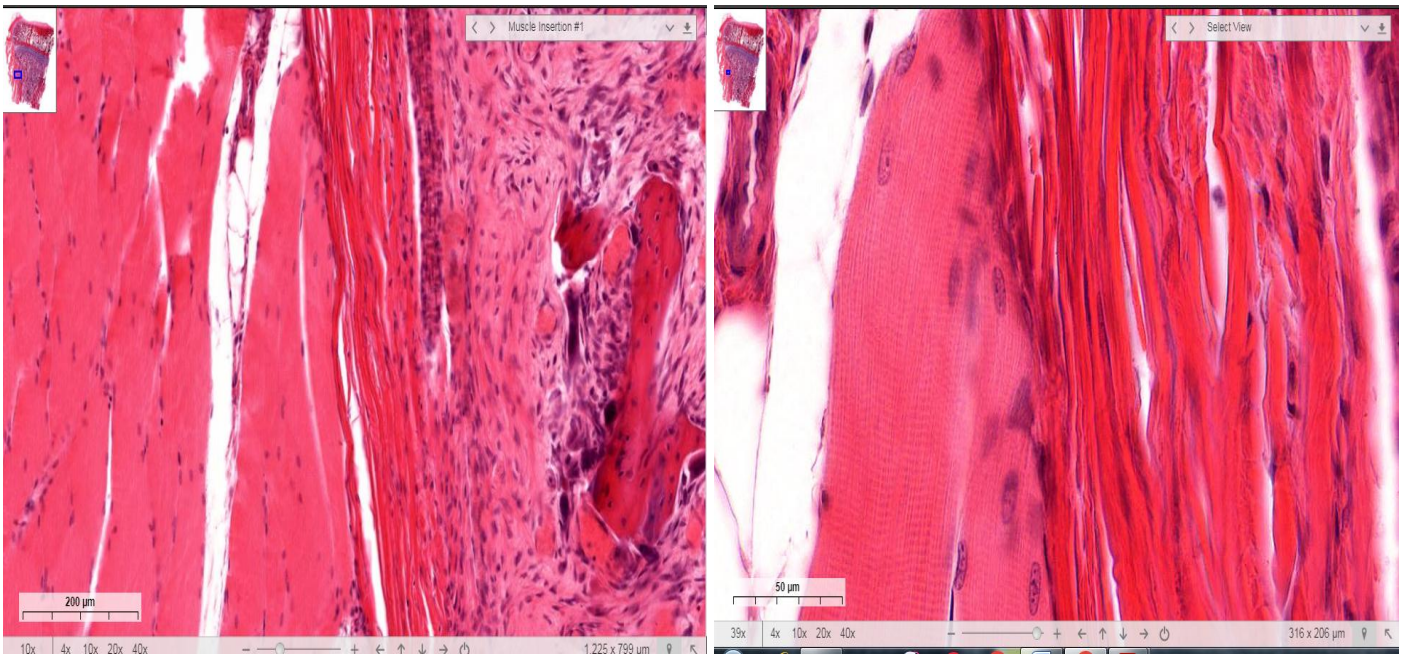
An excellent example of a muscle insertion occurs in the middle of the specimen. At higher magnification, the collagen fibers of the endomysium are seen to be continuous with those in the tendon.





Muscle Tendon Insertion

Besides ending in tendons, muscle can also attach to the periosteum (dense regular connective tissue) that covers bone. Most of this specimen is bone except for skeletal muscle along the left edge of the bone.



Many examples of muscle insertions are seen. The endomysium is a thin layer of connective tissue that surround each muscle. These fibers merge with collagen fibers of the periosteum.

Structure of skeletal muscle

Muscle fibres in skeletal muscle occur in bundles, fascicles, which make up the muscle. The muscle is surrounded by a layer of connective tissue, the epimysium, which is continuous with the muscle fascia. Connective tissue from the epimysium extends into the muscle to surround individual fascicles (perimysium) from which a delicate network of reticular fibres surrounds each individual muscle fibre (endomysium). The connective tissue transduces the force generated by the muscle fibres to the tendons.

The insertion into the tendon of the connective tissue fibres surrounding the muscle fibres, i.e. the muscle-tendon junction, is shown in one of the Lab sections on the connective tissue page. It may be a good idea to take another look at the section.

Origin of skeletal muscle

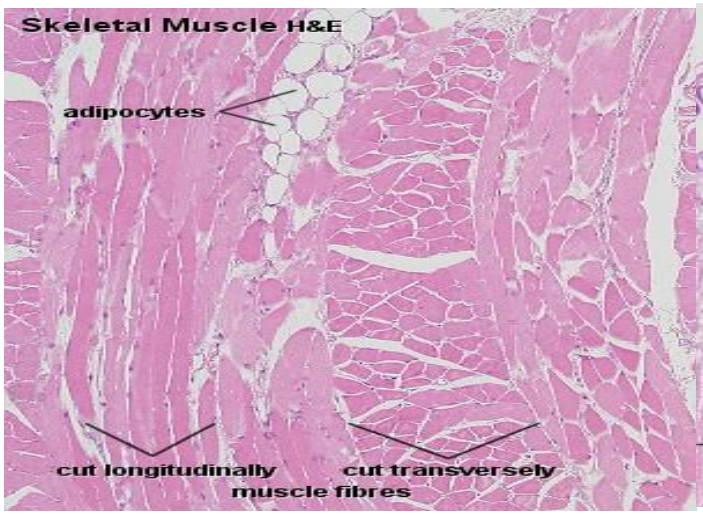
The myoblasts of all skeletal muscle fibres originate from the paraxial mesoderm. Myoblasts undergo frequent divisions and coalesce with the formation of a multinucleated, syncytial muscle fibre or myotube. The nuclei of the myotube are still located centrally in the muscle fibre. In the course of the synthesis of the myofilaments/myofibrils, the nuclei are gradually displaced to the periphery of the cell.

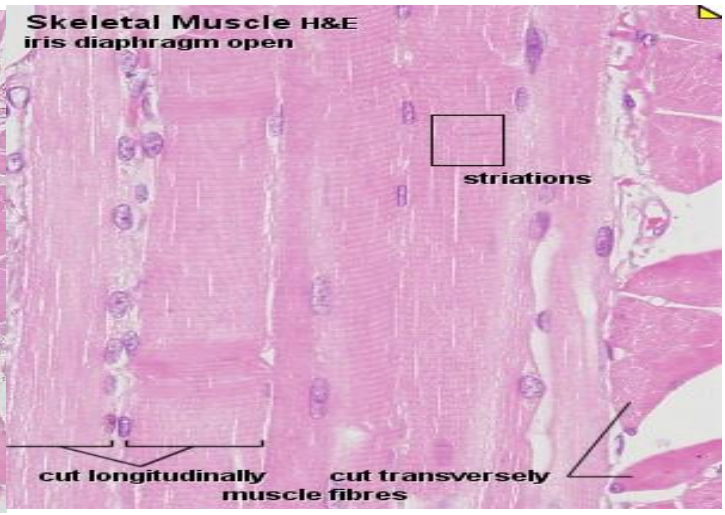
Satellite cells are small cells which are closely apposed to muscle fibres within the basal lamina which surrounds the muscle fibre. Their nuclei are slightly darker than those of the muscle fibre. Satellite cells are believed to represent persistent myoblasts. They may regenerate muscle fibres in case of damage.

Suitable Slides

Sections of skeletal muscle, tongue or upper oesophagus - H&E

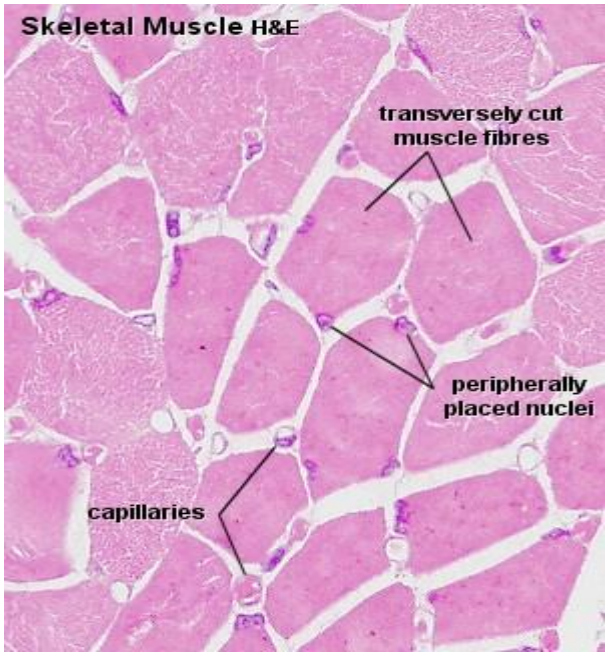
Tongue, Skeletal Muscle, human - H&E
Skeletal muscle in the tongue is arranged in bundles which typically run at right angles to each other. Both longitudinally and transversely cut skeletal muscle fibres are present. In both section planes you can see that the nuclei are located in the periphery of the muscle fibre. Striations are visible in longitudinally cut fibres. They stand out more clearly if you close the iris diaphragm of the microscope. Remember to open the diaphragm after you have seen the striations clearly ! In well-preserved tissue it is possible to identify Z-

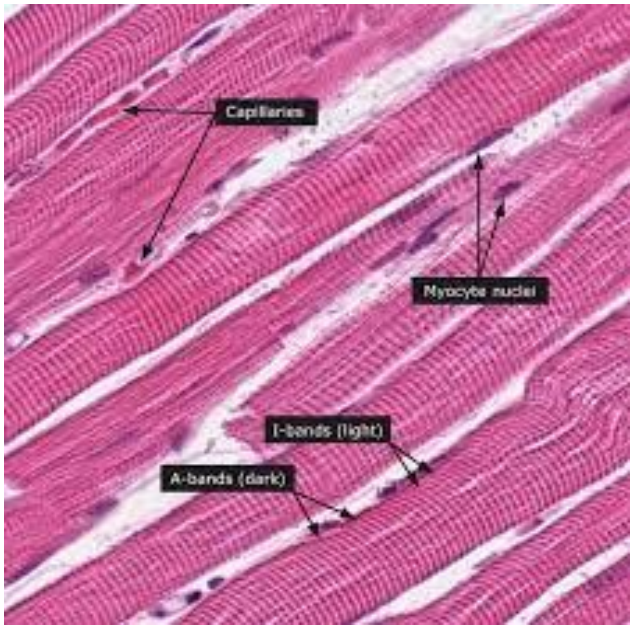


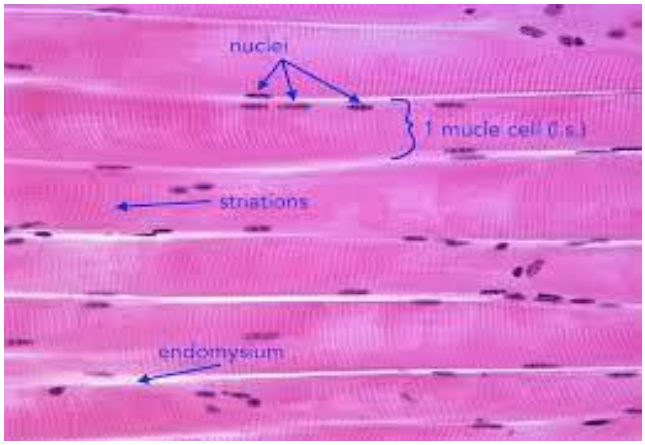


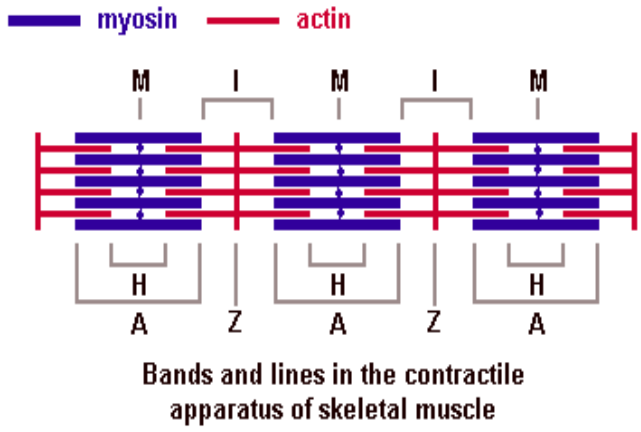
lines and H-bands in addition to A-bands and I-bands. Numerous capillaries between the muscle fibres supply the muscle with oxygen and nutrients.

Draw a small section of longitudinal and transversely cut skeletal muscle at high magnification. The muscle surrounding the upper one-third of the oesophagus is skeletal muscle. Smooth muscle surrounds its lower one-third. In section of the middle of the esophagus it is usually possible to identify both muscle types and their appearances can be compared.









Bands and lines in the contractile apparatus of skeletal muscle

The Contractile Apparatus of Skeletal Muscle

The spatial relation between the filaments that make up the myofibrils within skeletal muscle fibres is highly regular. This regular organisation of the myofibrils gives rise to the cross-striation, which characterises skeletal and cardiac muscle. Sets of individual "stria" within a myofibril correspond to the smallest contractile units of skeletal muscle, the sarcomeres.

Depending on the distribution and interconnection

of myofilaments a number of "bands" and "lines" can be distinguished in the sarcomeres e:

I-band - actin filaments,
A-band - myosin filaments which may overlap with actin filaments,
H-band - zone of myosin filaments only (no overlap with actin filaments) within the A-band,
Z-line - zone of apposition of actin filaments belonging to two neighbouring sarcomeres (mediated by a protein called alpha-actinin),
M-line - band of connections between myosin filaments (mediated by proteins, e.g. myomesin, M-protein).

The average length of a sarcomere is about 2.5 µm (contracted ~1.5 µm, stretched ~3 µm).

The protein titin extends from the Z-line to the M-line. It is attached to the Z-line and the myosin filaments. Titin has an elastic part which is located between the Z-line and the M-band. It contributes to keeping the filaments of the contractile apparatus in alignment and to the passive stretch resistance of muscle fibres. Other cytoskeletal proteins interconnect the Z-lines of neighbouring myofibrils. They also connect the Z-lines of the peripheral myofibrils to the sarcolemma.

Excitation and Contraction of Skeletal Muscle

The area of contact between the end of a motor nerve and a skeletal muscle cell is called the motor end plate. Numerous fine branches of the motor nerve make plate-like contacts (boutons) with the muscle cell. The excitatory transmitter at the motor end plate is acetylcholine. The space between the boutons and the muscle fibre is called primary synaptic cleft. Numerous infoldings of the sarcolemma in the area of the motor end plate form secondary synaptic clefts.

The spread of excitation over the sarcolemma is mediated by voltage-gated ion channels.

Invaginations of the sarcolemma form the T-tubule system which "leads" the excitation into the muscle fibre, close to the border between the A- and I-bands of the myofibrils. Here, the T-tubules are in close apposition with cisternae formed by the sarcoplasmatic reticulum. This association is called a triad.

Voltage-sensitive channels in the walls of the T-tubules (dihydropyridine (DHP) receptors) allow small amounts of calcium to enter the sarcoplasm of the muscle fibre. This influx mediates the efflux of calcium from the sarcoplasmatic reticulum through calcium-sensitive calcium channels (ryanodine receptors).

Sites of interaction between actin and myosin are in resting muscle cells "hidden" by tropomyosin. Tropomyosin is kept in place by a complex of proteins collectively called troponin. The binding of calcium to troponin-C induces a conformational change in the troponin-tropomyosin complex which permits the interaction between myosin and actin and, as a consequence of this interaction, contraction.

ATP-dependent calcium pumps in the membrane of the sarcoplasmatic reticulum typically restore the concentration of Ca to resting levels within 30 milliseconds after the activation of the muscle fibre.

Types of Skeletal Muscle

Skeletal muscle cells respond to stimulation with a brief maximal contraction - they are of the twitch type. Individual muscles fibres cannot maintain their contraction over longer periods. The sustained contraction of a muscle depends on the "averaged" activity of often many muscles fibres, which individually only contract for a brief period of time. The force generated by the muscle fibre does depend on its state of contraction at

the time of excitation. Excitation frequency and the mechanical summation of the force generated is one way to graduate the force generated by the entire muscle. Another way is the regulation of the number of muscle fibres which contract in the muscle. Additional motor units, i.e. groups of muscle fibres innervated by one nerve fibre and its branches, are recruited if their force is required. The functional properties of the muscle can be "fine-tuned" further to the tasks the muscle performs by blending functionally different types of muscle fibres:

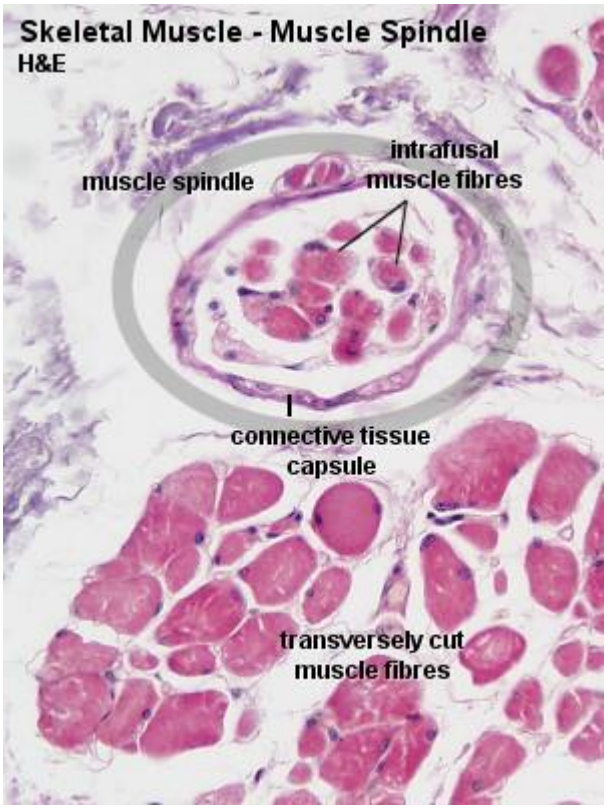
Red or type I fibres

Red muscles contain predominantly (but not exclusively) red muscle cells. Red muscle cells are comparatively thin and contain large amounts of myoglobin and mitochondria. Red fibres contain an isoform of myosin with low ATPase activity, i.e. the speed with which myosin is able to use up ATP. Contraction is therefore slow. Red muscles are used when sustained production of force is necessary, e.g. in the control of posture.

White or type II fibres

White muscle cells, which are predominantly found in white muscles, are thicker and contain less myoglobin. ATPase activity of the myosin isoform in white fibres is high, and contraction is fast. Type IIa fibres contain many mitochondria and are available for both sustained activity and short-lasting, intense contractions. Type IIb fibres contain only few mitochondria. They are recruited in the case of rapid accelerations and short lasting maximal contraction. Type IIb fibres rely on anaerobic glycolysis to generate the ATP needed for contraction.

Skeletal muscle fibres do not contract spontaneously. Skeletal muscle fibres are not interconnected via GAP junctions but depend on nervous stimulation for contraction. All muscle fibres of a motor unit are of the same type.



Fibre type is determined by the pattern of stimulation of the fibre, which, in turn, is determined by the type of neuron which innervates the muscle. If the stimulation pattern is changed experimentally, fibre type will change accordingly. This is of some clinical / pathological importance. Nerve fibres have the capacity to form new branches, i.e. to "sprout", and to re-innervate muscle fibres, which may have lost their innervation as a consequence of an acute lesion to the nerve or a neurodegenerative disorder. The type of the muscle fibre will change if the type of stimulation provided by the sprouting nerve fibre does not match with the type of muscle. The process of reinnervation and type adjustment may result in fibre type grouping within the muscle, i.e. large areas of the muscle are populated by muscle fibres of one type.

Muscle Spindles

Muscle spindles are sensory specialization of the muscular tissue. A number of small specialised intrafusal muscle fibres (nuclear bag fibres and nuclear chain fibres) are surrounded by a capsule of connective tissue. The intrafusal fibres are innervated by efferent motor nerve

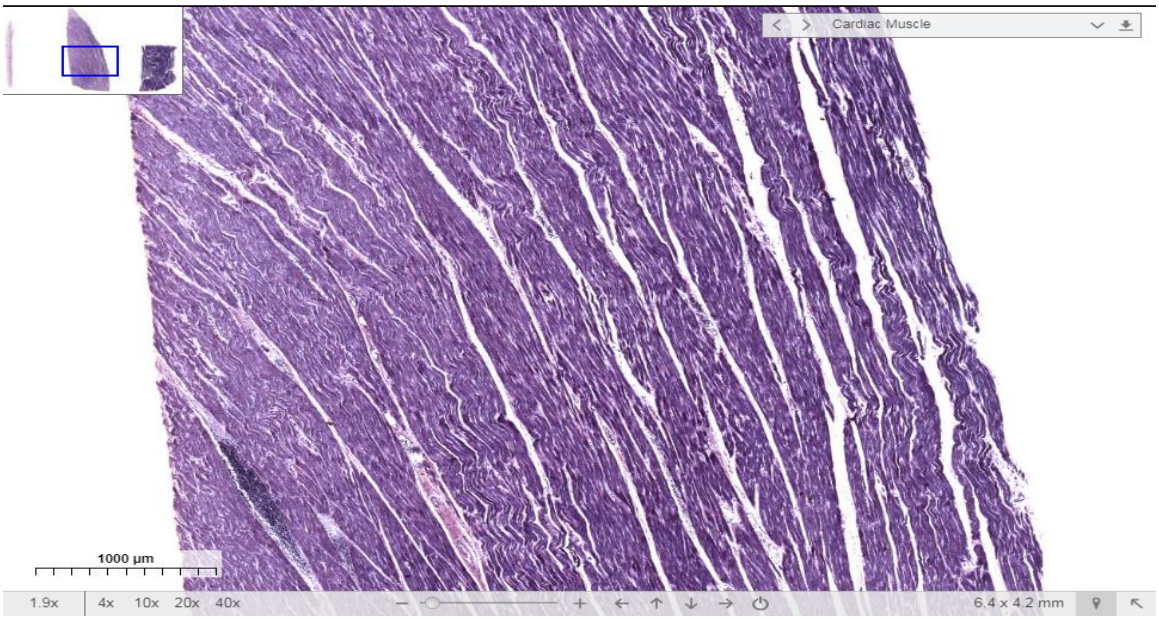
fibres. Afferent sensory nerve fibres surround the intrafusal muscle fibres. If the muscle is stretched, the muscle fibres in the muscle spindle are stretched, sensory nerves are stimulated, and a change in contraction of the muscle is perceived. Different types of intrafusal fibres and nerve endings allow the perception of position, velocity and acceleration of the contraction of the muscle. The contraction of the intrafusal fibres, after stimulation by the efferent nerve fibres, may counteract or magnify the changes imposed on the muscle spindle by the surrounding muscle. The intrafusal fibres and the efferent nerves can in this way set the sensitivity for the sensory nerve ending in the muscle spindle.

Cardiac Muscle

- Cardiac muscle consists of muscle cells with one centrally placed nucleus. Nuclei are oval, rather pale and located centrally in the muscle cell which is 10 - 15 µm wide.
- Cardiac muscle is innervated by the autonomic nervous system.
- Cardiac muscle exhibits cross-striations.
- Cardiac muscle is for these reasons also called involuntary striated muscle.

Cardiac Muscle

Cardiac muscle is striated, involuntary muscle found in the heart wall. Cardiac muscle cells (or cardiomyocytes) contain the same contractile filaments as in skeletal muscle.

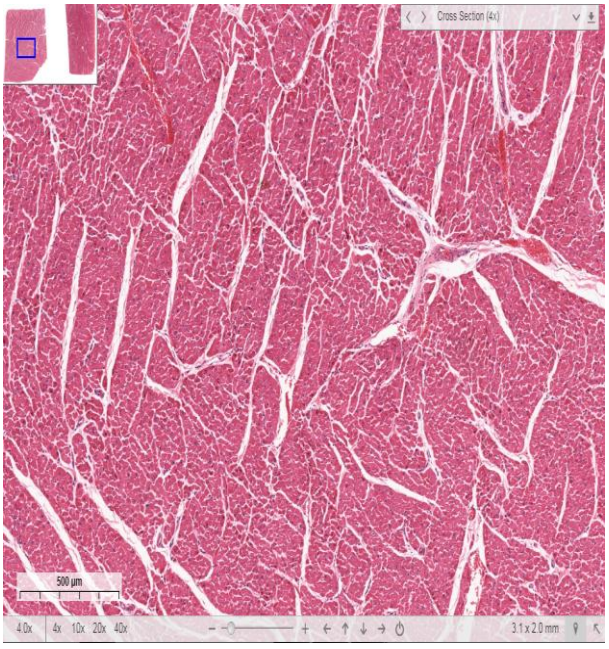


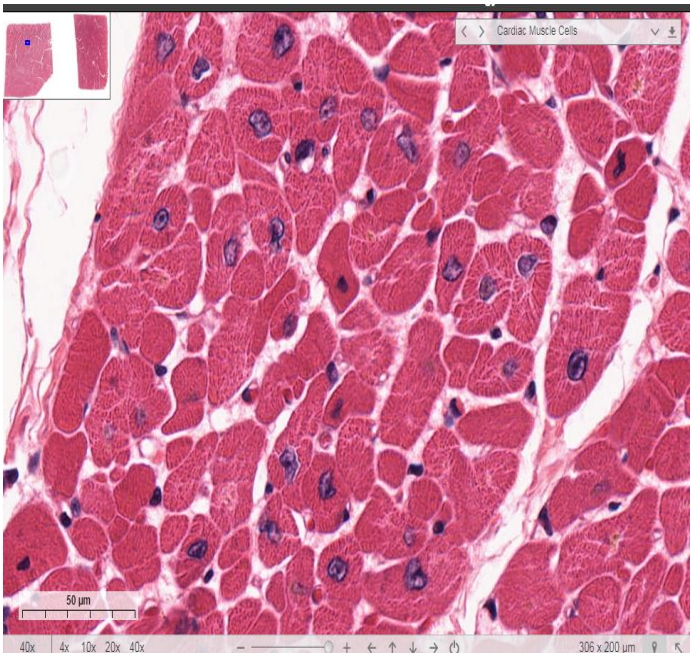
Cardiac Muscle
(Phosphotungstic Acid/Hematoxylin)

Phosphotungstic acid/hematoxylin (PTAH) is used to demonstrate muscle striations, intercalated discs, nervous tissue and fibrin.

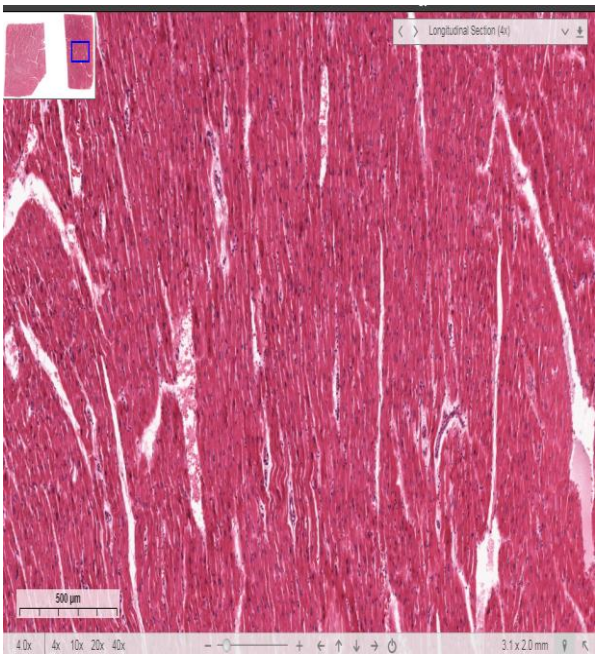
Tissue sections of cardiac and muscle are shown.

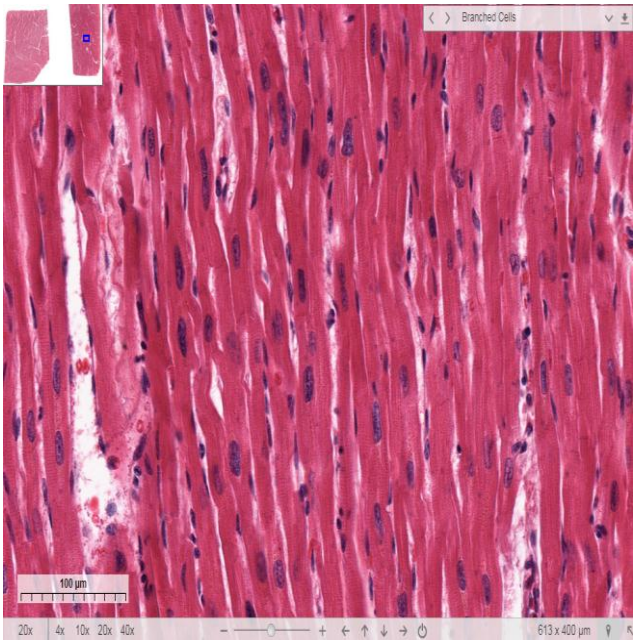
The longitudinal section of cardiac muscle stained with PTAH shows the morphology of individual cardiac muscle cells, and in particular, the intercalated discs.





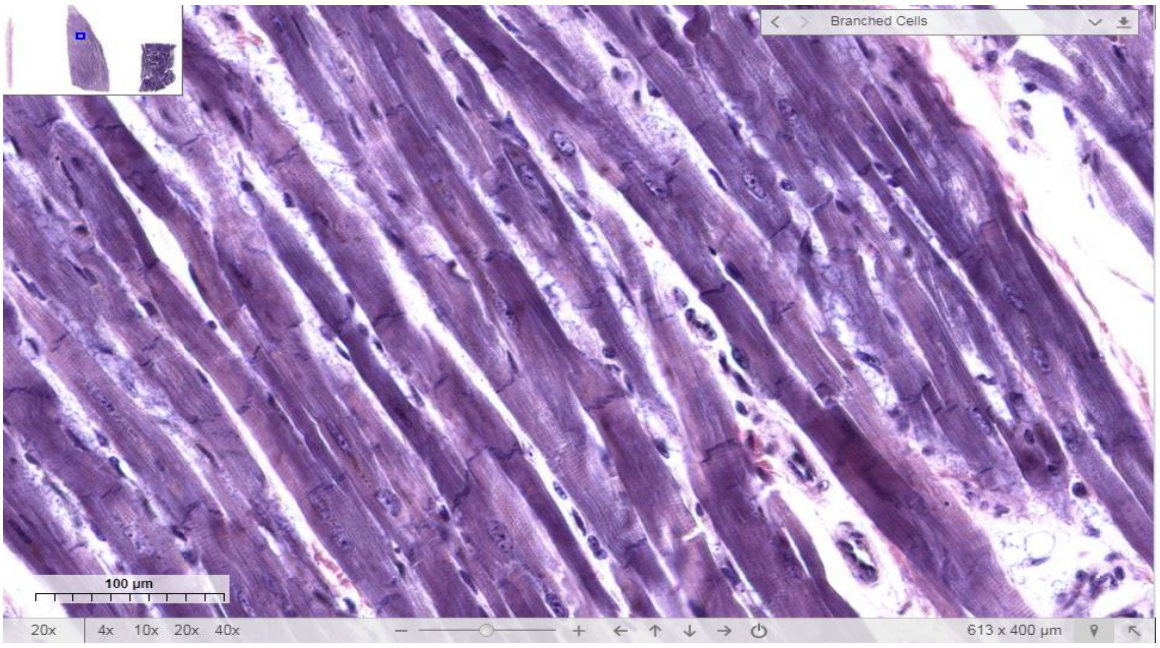
- Cross-Section
- Cardiac muscle cells - have rounded cross-sections (less than 25 µm in diameter) with a centrally located nucleus.





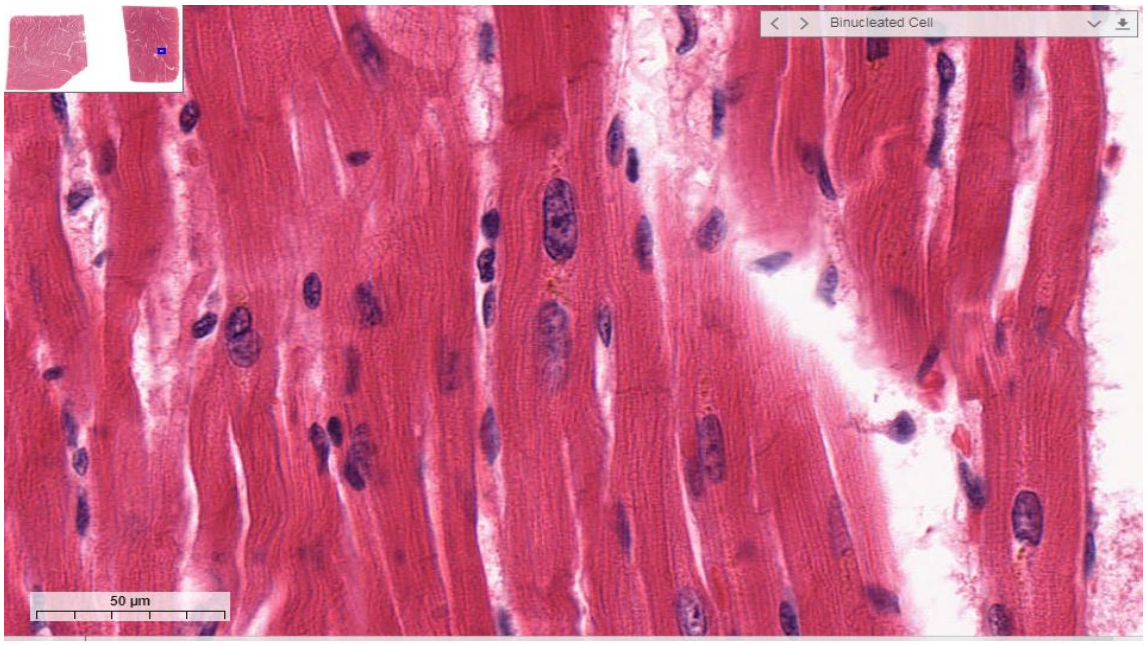
Longitudinal Section - cardiac muscle cells are smaller in size than skeletal muscle (50 to 250 µm in length)

- Branched Cells - cells are joined end-to-end and are often branched

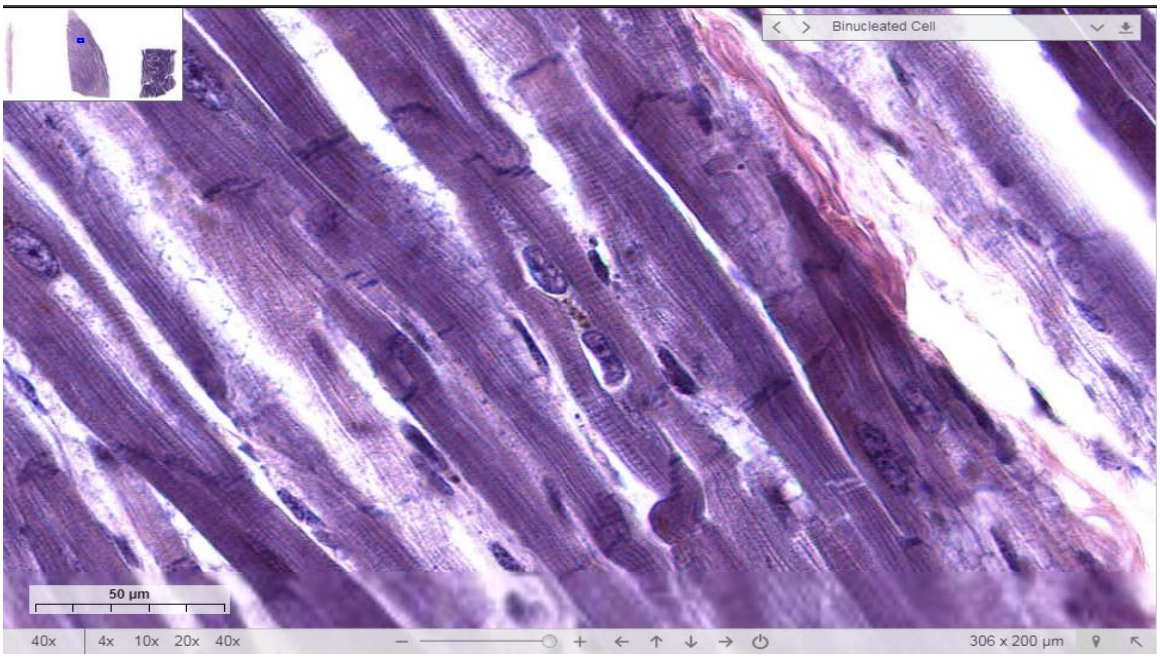


- Branched Cells - cells are joined end-to-end and are often branched. They are 50 to 250 µm in length

(Phosphotungstic Acid/Hematoxylin)

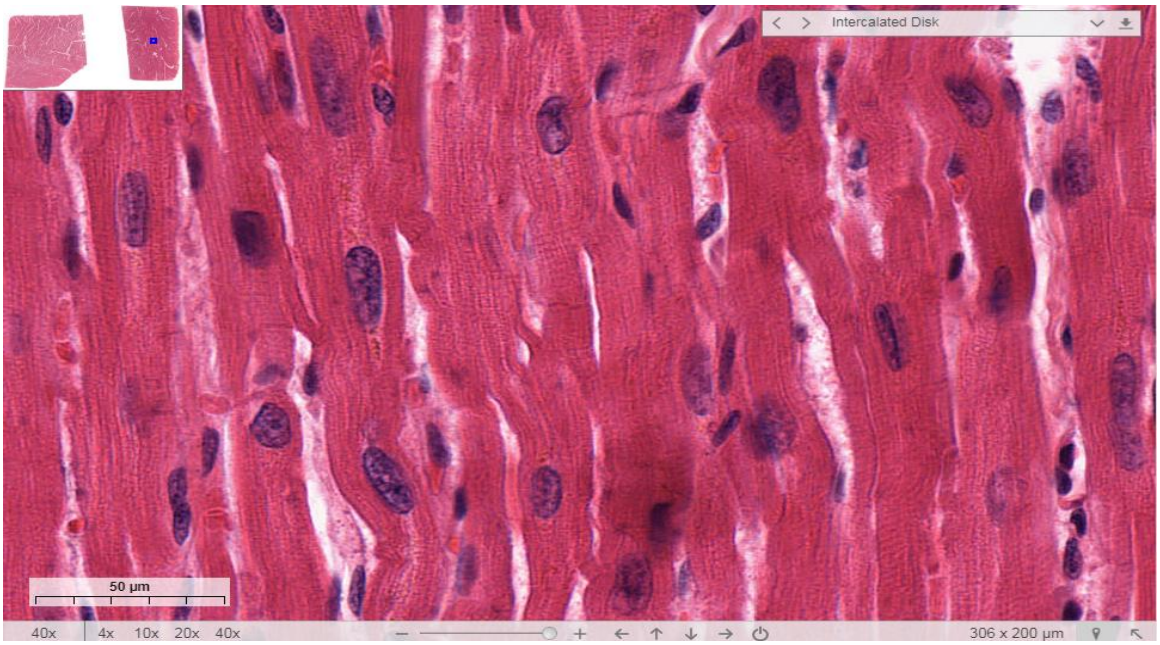


- Nuclei - cells have a single or occasionally two centrally located nuclei

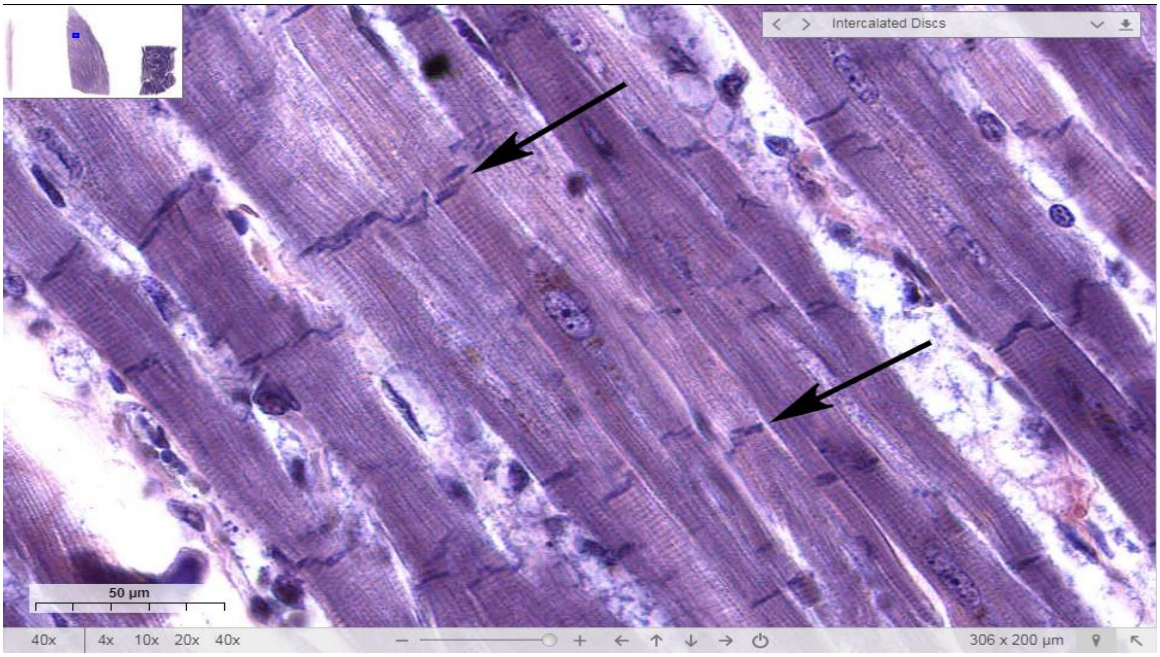


(Phosphotungstic Acid/Hematoxylin)

- Nuclei - cells have a single or occasionally two centrally located nuclei.

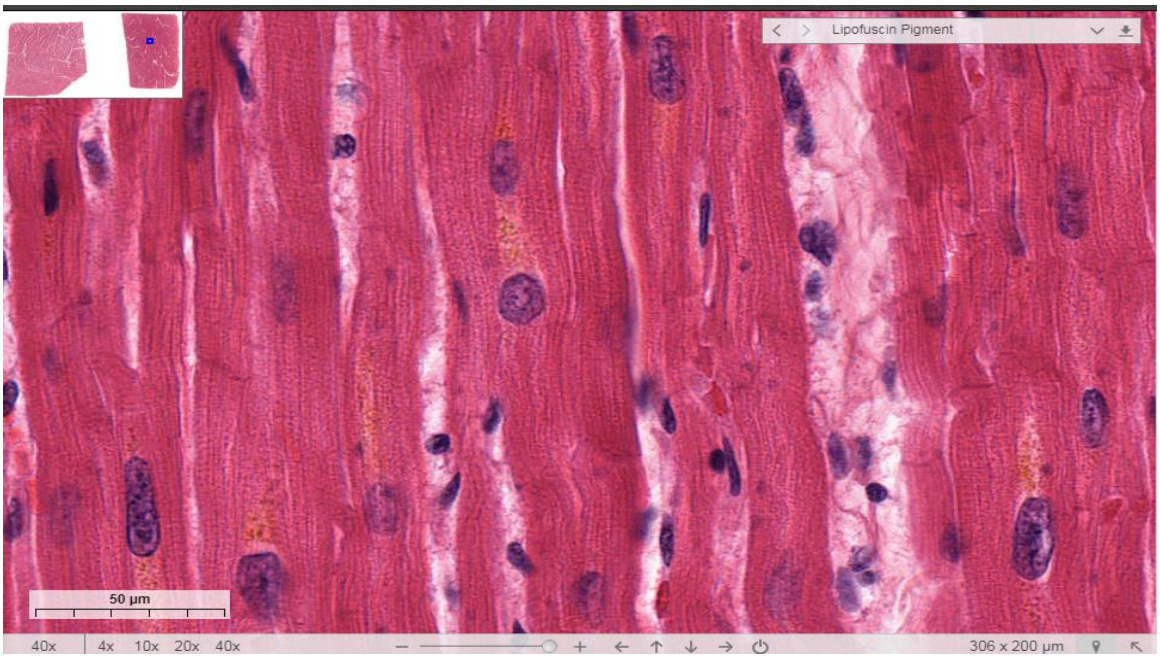


- Intercalated Discs - specialized junctions that connect the individual cells. They are usually unstained, but occasionally appear as thin, dark line between adjacent cells. The intercalated discs are perpendicular to the direction of muscle fibers. They are best seen when stained with phospho-tungstic acid (MH 056 Muscle Types).

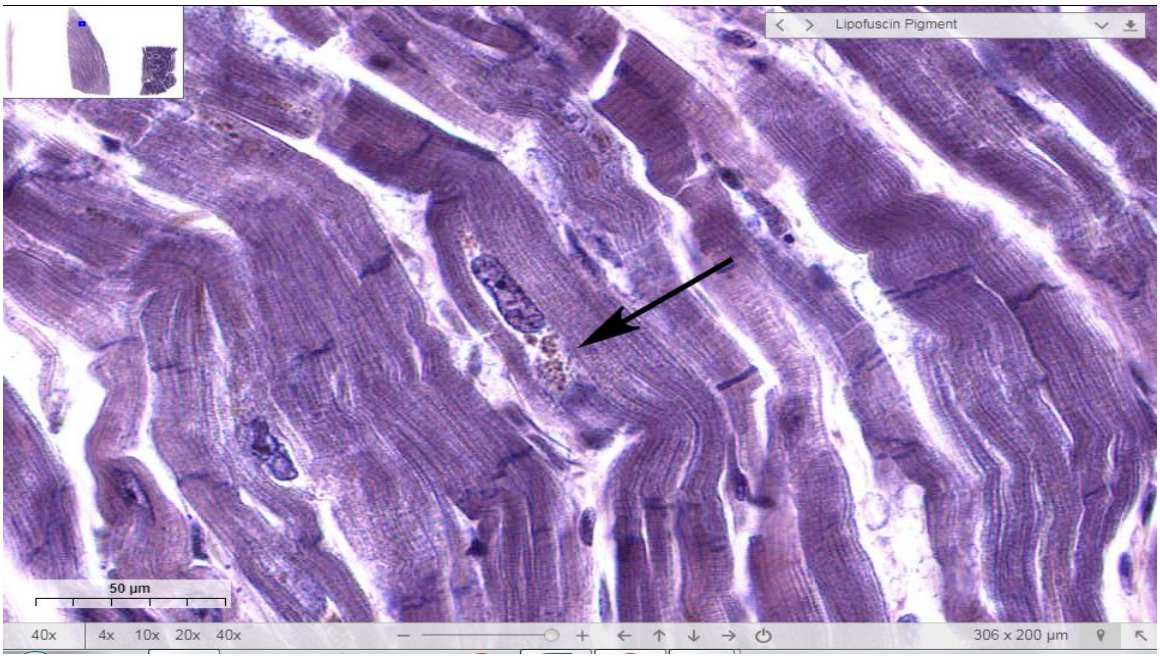


(Phosphotungstic Acid/Hematoxylin)

- Intercalated Discs - specialized junctions that connect the individual cells (arrows). They appear as dark purple bands across muscle fibers.

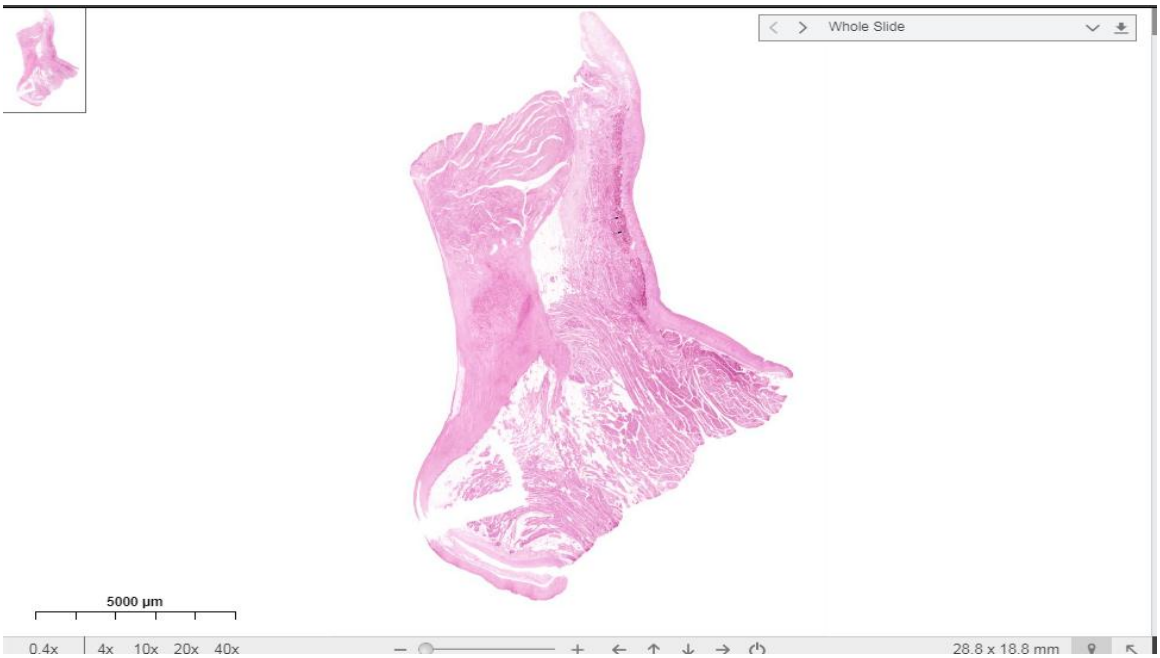


- Lipofuscin Pigment - residue of lysosomal digestion that accumulates as yellow-brown granules near the nucleus of some cells.



(Phosphotungstic Acid/Hematoxylin)

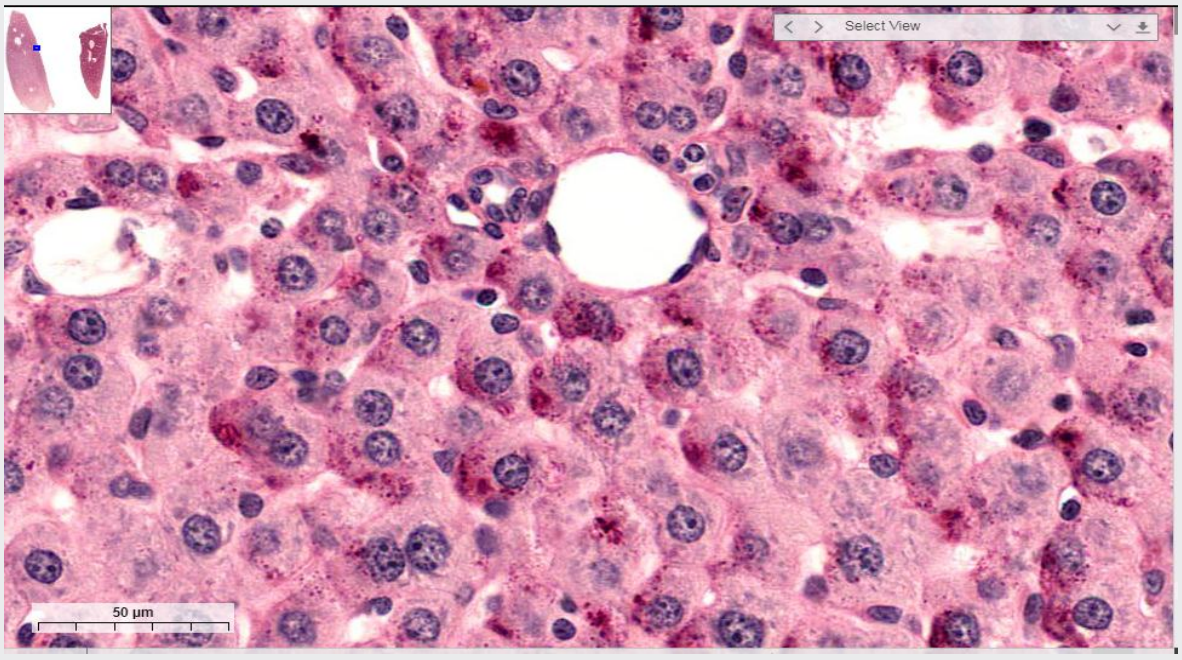
- Lipofuscin Pigment - residue of lysosomal digestion that accumulates as yellow-brown granules next to the nucleus of some cells.



Purkinje Fibers
(Periodic Acid - Schiff Stain)

Purkinje fibers are specialized cardiac muscle cells that conduct electrical impulses that allow coordinated contraction of cardiac muscle.

Purkinje fibers have fewer myofibrils, but a higher content of glycogen than normal cardiac muscle cells. This allows their identification with periodic acid-Schiff (PAS) stain which chemically converts sugars in glycogen into a magenta-colored product (see MH 128 Liver - Glycogen).

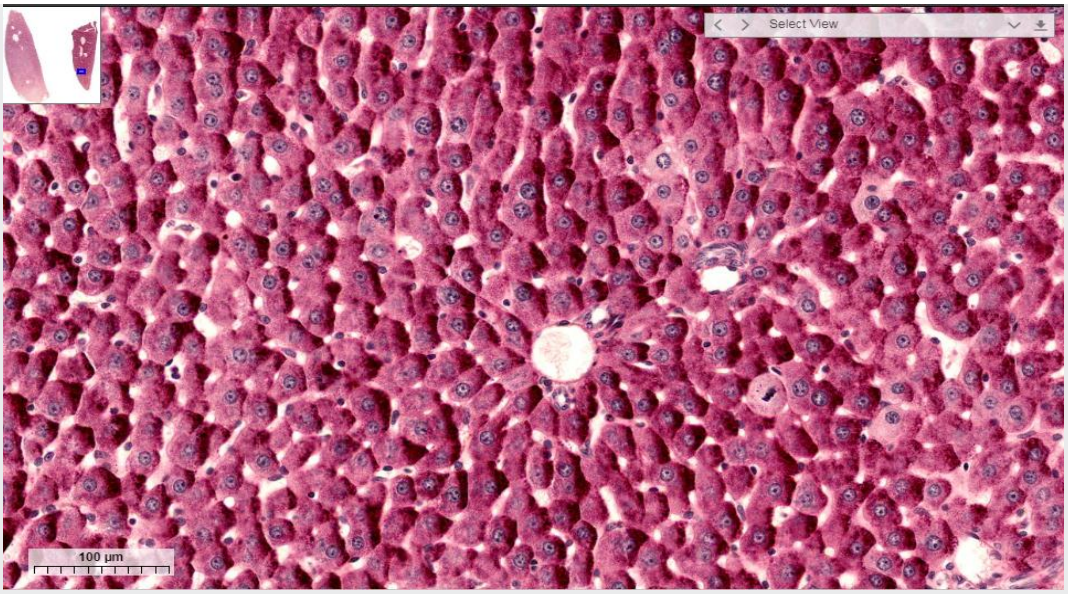


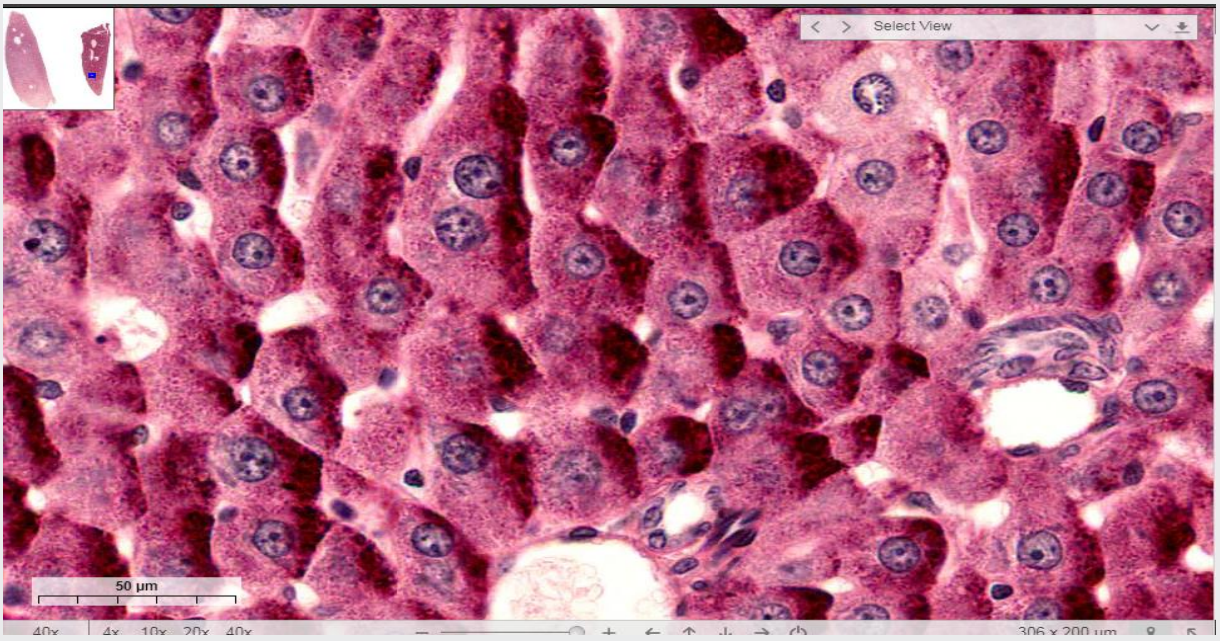
Liver (fasted)

The liver is composed primarily of hepatocytes. They store large quantities of glucose (as glycogen) after a meal and release it when fasting. **Periodic acid-Schiff reagent** (PAS) can be used to demonstrate the amount of glucose stored as glycogen.

Examine the staining of individual hepatocytes from this fasted animal.

- PAS staining appears as scattered red to magenta particles in the cytoplasm.
- The intensity of staining varies between cells from light to no staining.
- Nuclei are dark blue to purple because the section was counter stained with hematoxylin.

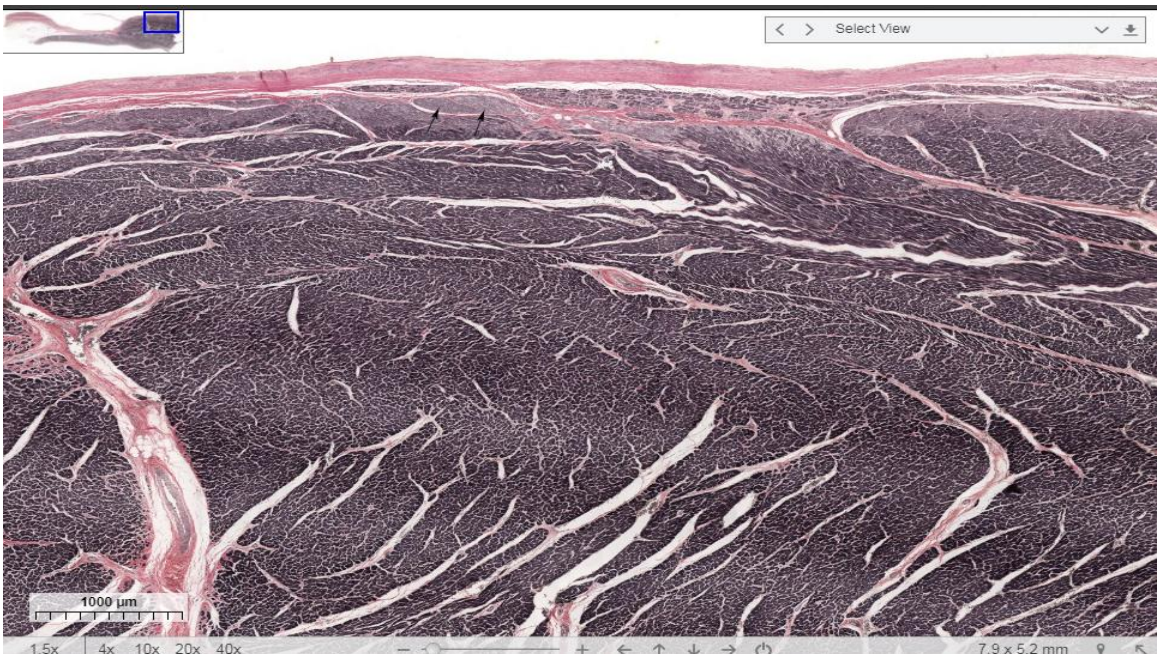




This liver is from a fed animal. At higher magnification, examine the staining of individual hepatocytes.

- PAS staining appears as course magenta-colored particules throughout the cytoplasm of most cells.
- Almost all the cells are intensely stained.
- Nuclei are dark blue to purple because the section was counter stained with hematoxylin.

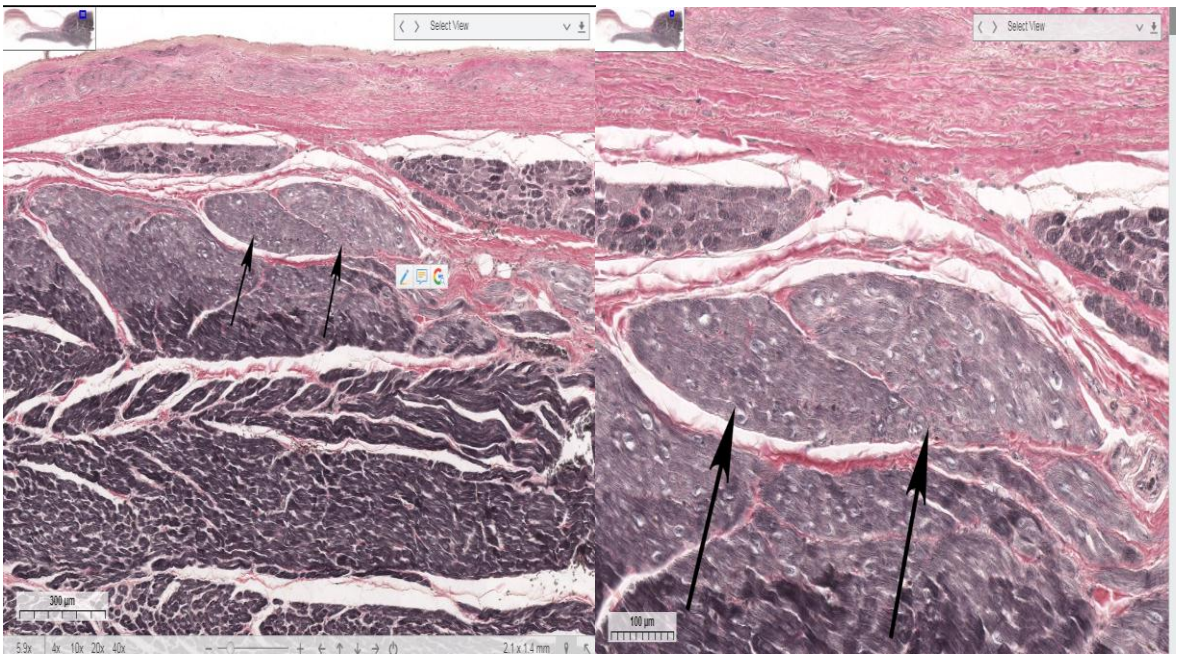
This indicates that the hepatocytes of a fed animal contain considerably more glycogen than a fasted animal.



Purkinje Fibers
(Phosphotungsitc Acid/Hematoxylin)

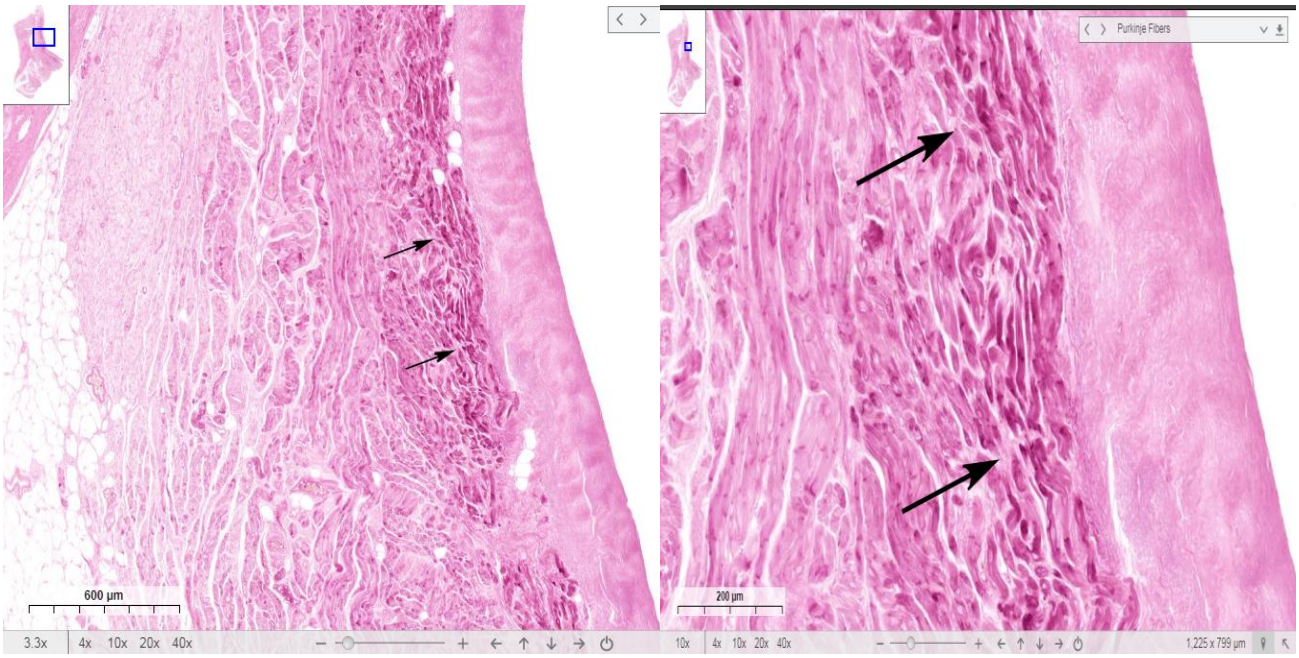
Purkinje fibers form the atrioventricular bundle (bundle of His), which is part of the specialized conduction system in the heart. This system generates the stimulus for the heart beat and conducts it to cardiac muscle

Purkinje fibers are modified muscle fibers. They have fewer myofibrils, but a higher content of glycogen. This allows their identification with phosphotungstic acid/hematoxylin (PTAH) stain which reveals cells with fewer myofibrils.



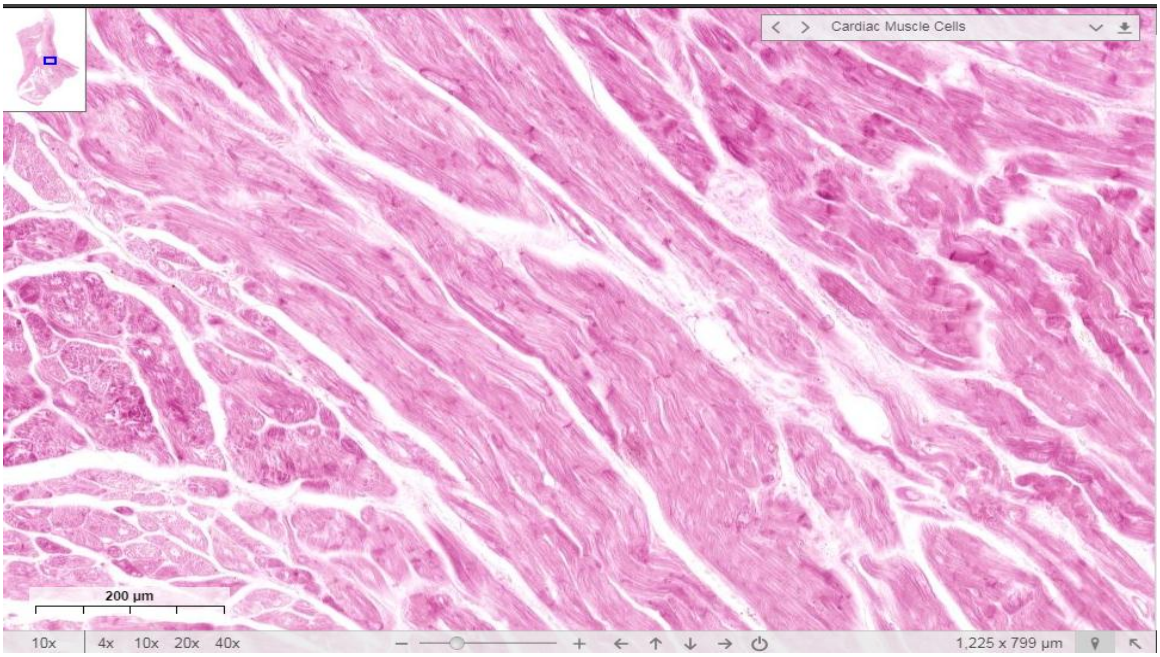
Purkinje Fibers (Phosphotungsitc Acid/Hematoxylin) The endocardium is at the top of this specimen.

Beneath the endocardium are cardiac muscle cells stained light gray. This lighter staining reflects the lower content of myofibrils in Purkinje cells.

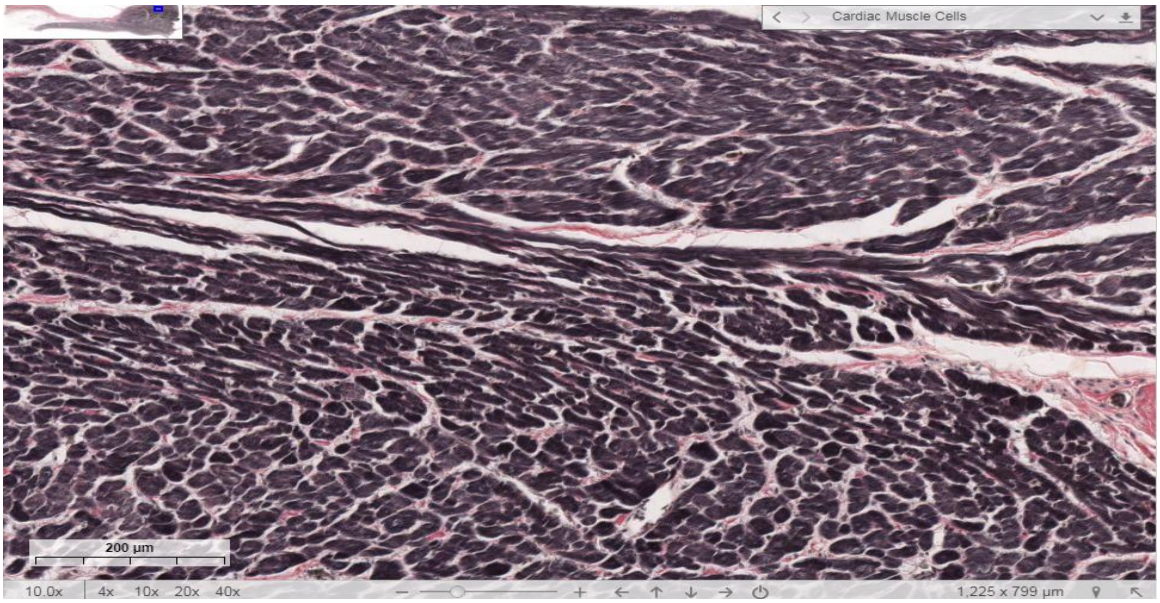


The endocardium is at the right of this specimen.

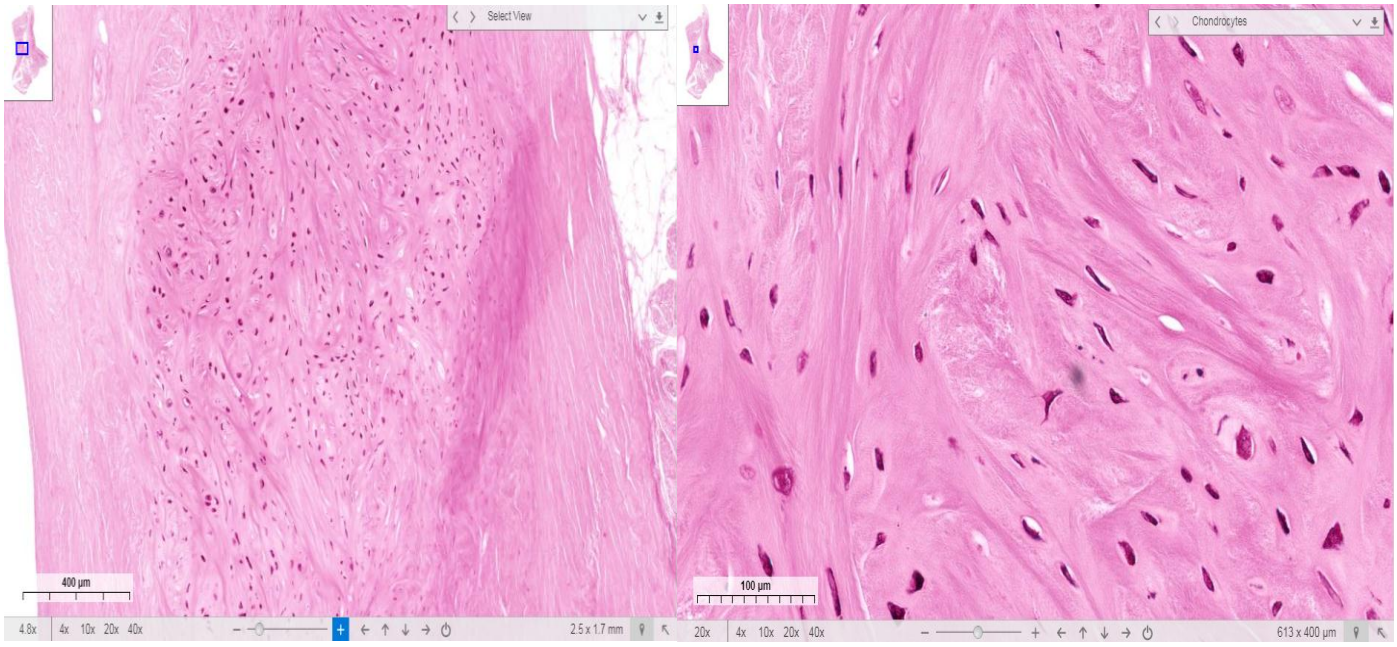
Beneath the endocardium are the strongly, PAS-stained (dark magenta) cardiac muscle cells. This high concentration of glycogen is characteristic of Purkinje cells.



Normal cardiac muscle cells which store little glycogen occur to the left of the Purkinje cells.



Purkinje Fibers (Phosphotungsitc Acid/Hematoxylin) Underneath the Purkinje cells, normal cardiac muscle cells have a darker appearance because of their higher content of myofibrils.



(The cardiac skeleton is also stained in this specimen because it contains fibrocartilage. The chondrocytes are PAS positive.)

Structure of cardiac muscle

The ultrastructure of the contractile apparatus and the mechanism of contraction largely correspond to that seen in skeletal muscle cells. Although equal in ultrastructure to skeletal muscle, the cross-striations in cardiac muscle are less distinct, in part because rows of mitochondria and many lipid and glycogen droplets are found between myofibrils.

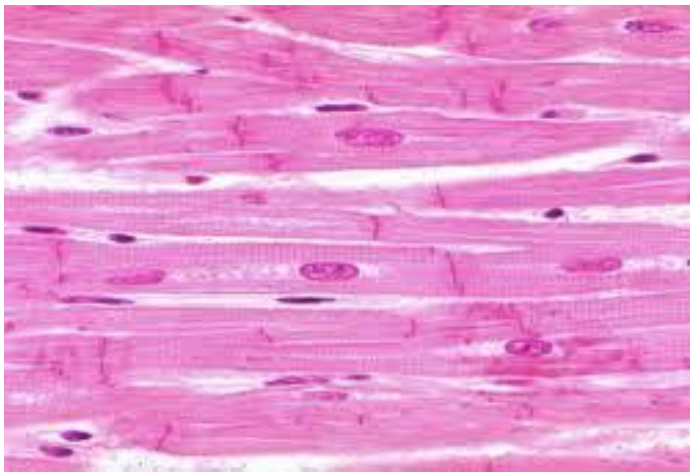
In contrast to skeletal muscle cells, cardiac muscle cells often branch at acute angles and are connected to each other by specialisations of the cell membrane in the region of the intercalated discs. Intercalated discs invariably occur at the ends of cardiac muscle cells in a region corresponding to the Z-line of the myofibrils (the last Z-line of the myofibril within the cell is "replaced" by the intercalated disk of the cell membrane). In the longitudinal part of the cell membrane, between the "steps" typically formed by the intercalated disk, we find extensive GAP junctions.

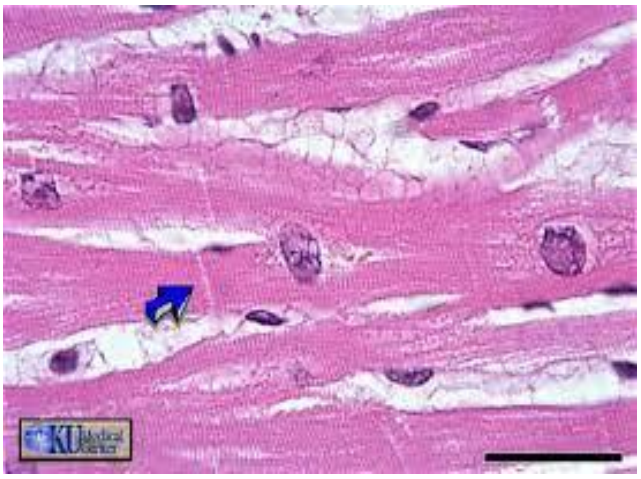
T-tubules are typically wider than in skeletal muscle, but there is only one T-tubule set for each sarcomere, which is located close to the Z-line. The associated sarcoplasmatic reticulum is organised somewhat simpler than in skeletal muscle. It does not form continuous cisternae but instead an irregular tubular network around the sarcomere with only small isolated dilations in association with the T-tubules.

Cardiac muscle does not contain cells equivalent to the satellite cells of skeletal muscle. Therefore cardiac muscle cannot regenerate.

Suitable Slides
Sections of cardiac muscle - Alizarin Blue, Whipf's polychrome, iron haematoxylin, H&E

Cardiac Muscle, primate - Alizarin blue
Use a low magnification to find a part of the tissue in which the cardiac muscle cells are cut longitudinally. At high magnification you should see striations and the large nuclei of the cardiac muscle cells. If you follow the course of individual cardiac muscle cells you will note fine, dark blue lines which seem to cross the fibres. These are the intercalated discs which connect the individual muscle cells and permit the conduction of electrical impulses between the cells ... how?
A light streak of cytoplasm is often visible extending from the poles of the nucleus. This part of the cytoplasm does not contain myofibrils, and it appears very light in transversely cut cardiac muscle cells. Myofibrils are often visible in transversely cut cells. Their visible separation reflects the large numbers of mitochondria located between them. Also, the large number of red blood cells visible in most preparations reflects the need of a good blood supply to the constantly active muscle cells.
Draw longitudinally cut cardiac muscle cells which show all the features mentioned. Label the features in your drawing, and include an suitable scale.





Excitation in cardiac muscle

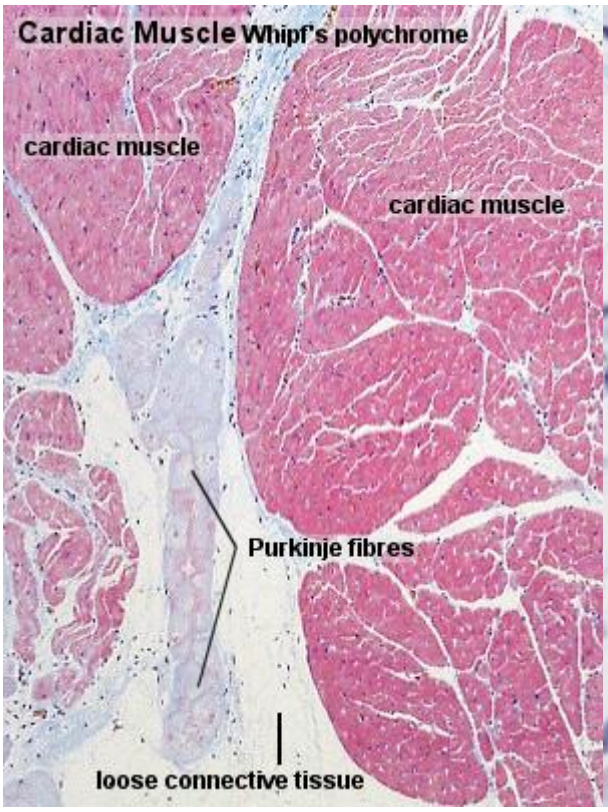
In theory, a stimulus can be propagated throughout the muscular tissue by way of the GAP junctions between individual muscle cells. A further system of modified cardiac muscle cells, Purkinje fibres, has developed, which conduct stimuli faster than ordinary cardiac muscle cells (2-3 m/s vs. 0.6 m/s), and which ensure that the contraction of the atria and ventricles takes place in the order that is most appropriate to the pumping function of the heart. Purkinje fibres contain fewer myofibrils than ordinary cardiac muscle cells. Myofibrils are mainly located in the periphery of the cell. Purkinje fibres are also thicker than ordinary cardiac muscle cells.

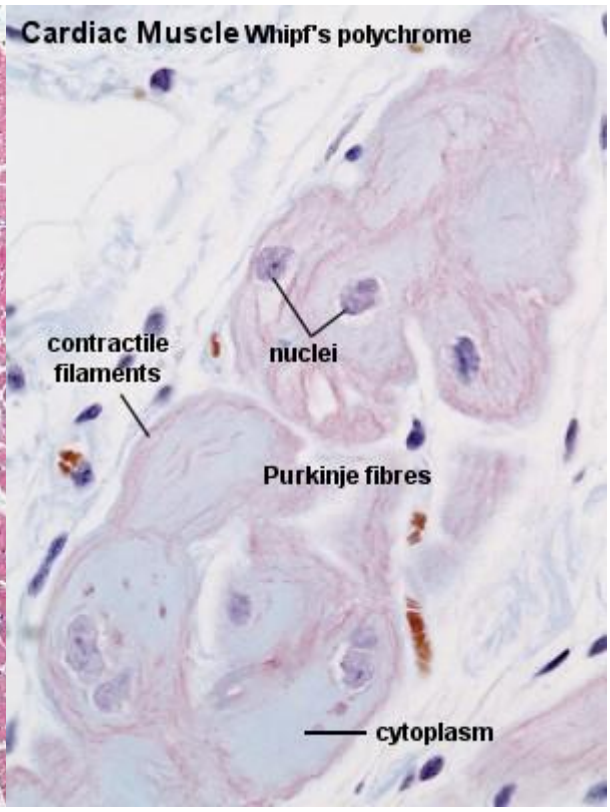
Modified muscle cells in nodal tissue (nodal muscle cells or P cells; P ~ pacemaker or pale-staining) of the heart exert the pacemaker function that drives the Purkinje cells. The rhythm generated by the nodal muscle cells can be modified by the autonomic nervous system, which innervates the nodal tissue and accelerates (sympathetic) or decelerates (parasympathetic) heart rate.

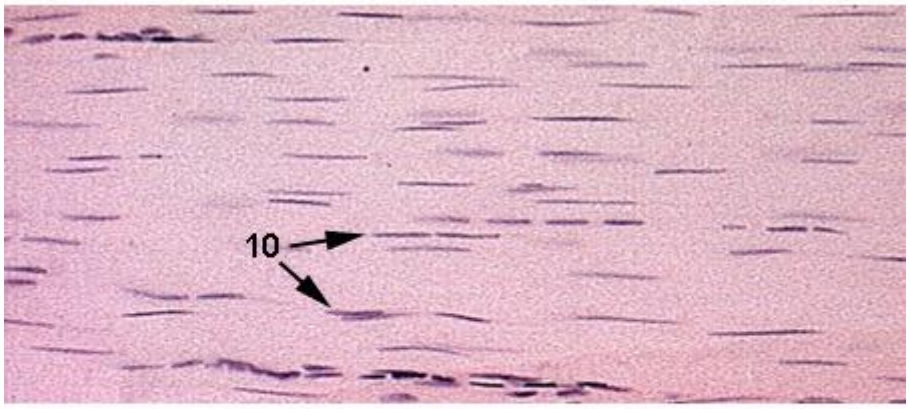
Suitable Slides

Sections of cardiac muscle (interventricular septum) - Whipf's polychrome, iron haematoxylin, H&E

Purkinje Fibre, sheep - Whipf's polychrome
Cardiac muscle cells in this preparation have a red-violet appearance. Much of the connective tissue looks light blue, striations of cardiac muscle cells are visible. Intercalated discs may be more difficult to find, but nuclei stand out very clearly. Bundles of Purkinje fibres are present in areas of connective tissue between areas of "normal" cardiac muscle tissue and beneath the endocardium. Purkinje fibres appear as a chain of light blue profiles with a red rim. Browse through the tissue at low magnification and change to high magnification when you suspect the presence of Purkinje fibres. The red rim is formed by the contractile filaments. They are displaced to the periphery of the cells and can sometimes be used to define the outline of individual cells. The nuclei are large, but the cells are even larger and you will not see a nucleus in each cell. Draw a Purkinje fibre at high magnification. Try to include a bit of "normal" cardiac muscle and a suitable scale.







9) Identify this tissue
10) Identify the cell type indicated by the arrow
11) Identify the eosinophilic material in this specimen
12) Where in the body is this tissue found?