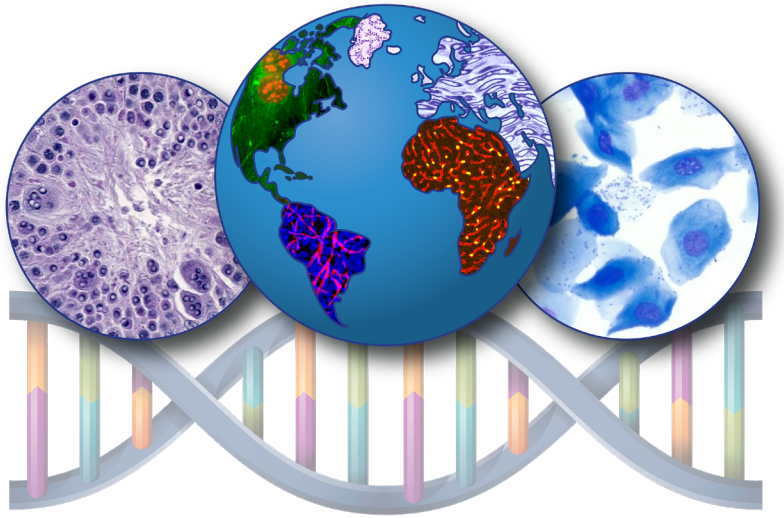
Artifacts in Histopathology



Division of Translational Toxicology Global Toxicologic Pathology Training Program

National Institutes of Health • U.S. Department of Health and Human Services

Artifacts: Overview

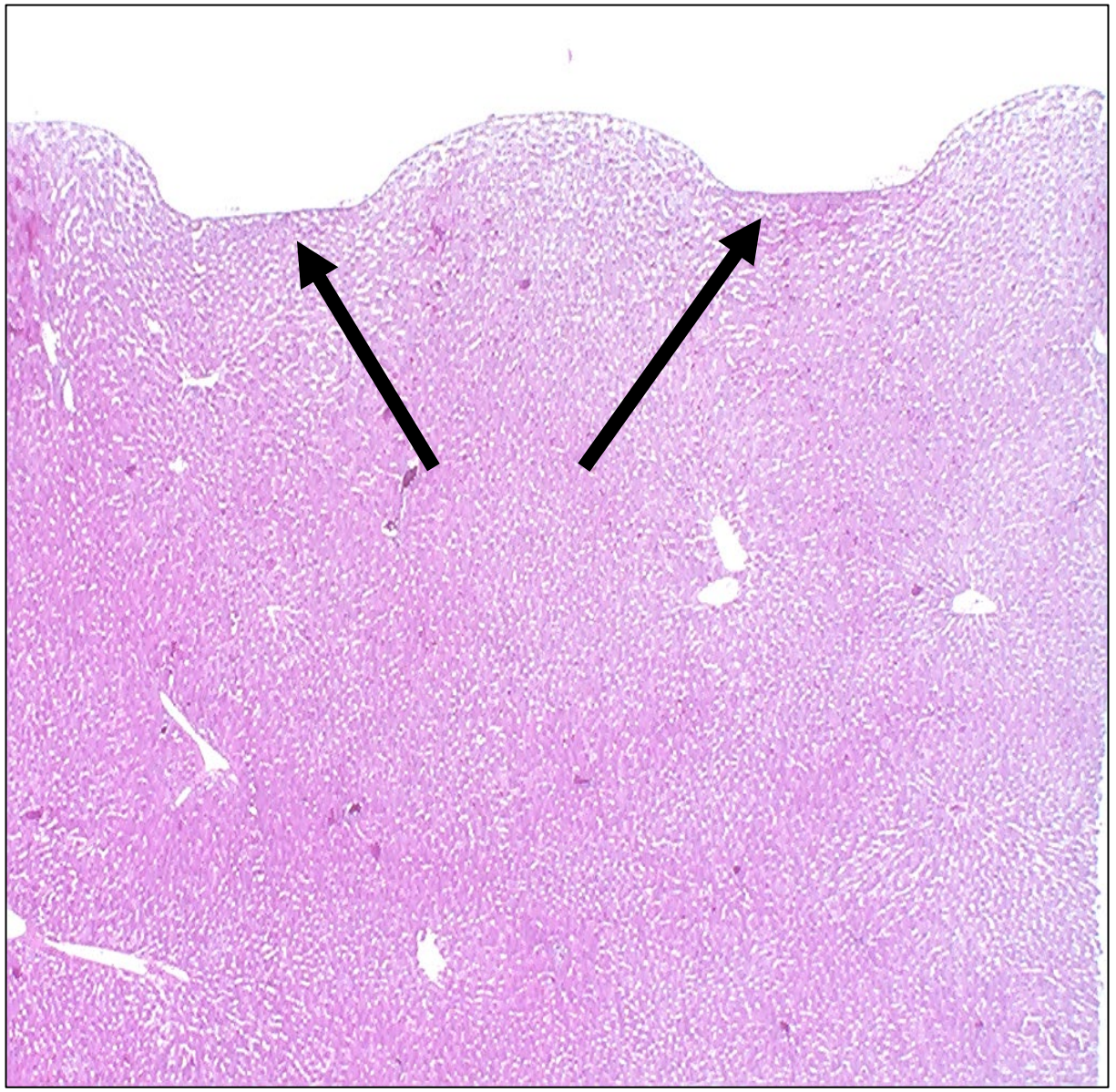
- Artifacts associated with Necropsy
  - Tissue manipulation
  - Autolysis
  - Fixation artifact
  - Over/under-inflation of tissue
  - Others
- Artifacts associated with Tissue Processing
  - Vacuolation
  - Tangential cut
  - Tissue tears

Artifacts Associated with Necropsy: Introduction

- Necropsy is the examination of a dead animal (postmortem) and is performed in toxicologic pathology to observe macroscopic (also known as "gross") and microscopic changes to the tissues caused by the test article during life, or antemortem (before death). Depending on the study, at the time the animal is euthanized, a list of tissues is observed for changes (macroscopic), then collected and further processed to a slide for microscopic examination.
- Tissues should be placed in a fixative, commonly formalin, as quickly as possible following death, at the proper ratio. For formalin, that ratio is generally 10 parts formalin to 1 part tissue. Typically, eyes are preserved in Davidson’s fixative, and testes are preserved in modified Davidson’s fixative. If tissues are not placed in the correct fixative using the proper procedures, this process can induce artifact on the slide.
- Autolysis occurs when the tissues are not preserved with sufficient preservative (fixative) in a timely manner. Autolysis means self-destruction and is the breakdown of cellular structures due to external factors, such as digestive enzymes of the gut.
- Some tissues, such as lung and gastrointestinal tract, can be infused or inflated with fixative to enhance fixation. Improperly performing this step (known as under- or over-inflation) can induce artifact.

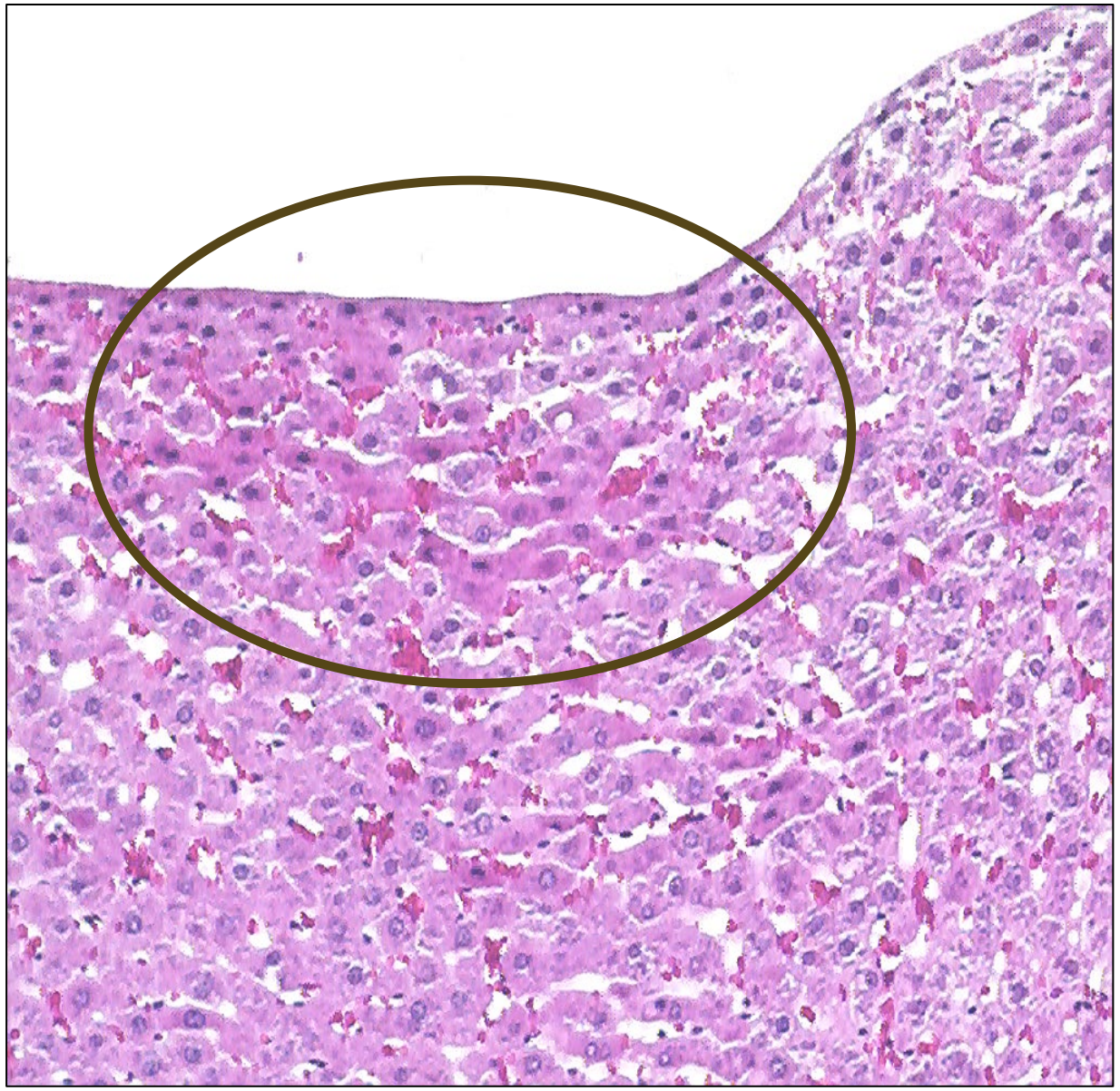
Crush Artifact: Liver

- Excessive force at necropsy can result in the tissue being crushed and, as a result, artifactually distorted. This artifact most often happens with aggressive handling of tissues at necropsy when tissues are harvested from the animal or when an excess amount of tissue is forced into a tissue embedding cassette.
- In the image, indentations at the surface of the liver (arrows) were caused by the liver sample being too large for the tissue cassette. When the cassette was closed, the liver surface was crushed inward.



Crush Artifact: Liver

- The image on the right is a higher magnification of the crushed area from the previous image. Note the hepatocytes appear hypereosinophilic (increased pink color; circle) and could be misinterpreted as necrotic.
- Rule out necrosis by noting the indentation (crush artifact) and the lack of cellular response, namely inflammation and edema.



Handling Artifact: Testis

- Rough handling or squeezing of the testis at necropsy can result in artifactual sloughing of clumps of germinal epithelial cells.
- In the testis, this rough handling or squeezing can lead to a misdiagnosis of degeneration or germ cell exfoliation.
- With true germ cell exfoliation, cells often retain relatively normal morphology but are rounded and individualized, rather than in clumps in the lumen (as seen in most tubules in the image on right), and exfoliated germ cells will be seen in the epididymis.

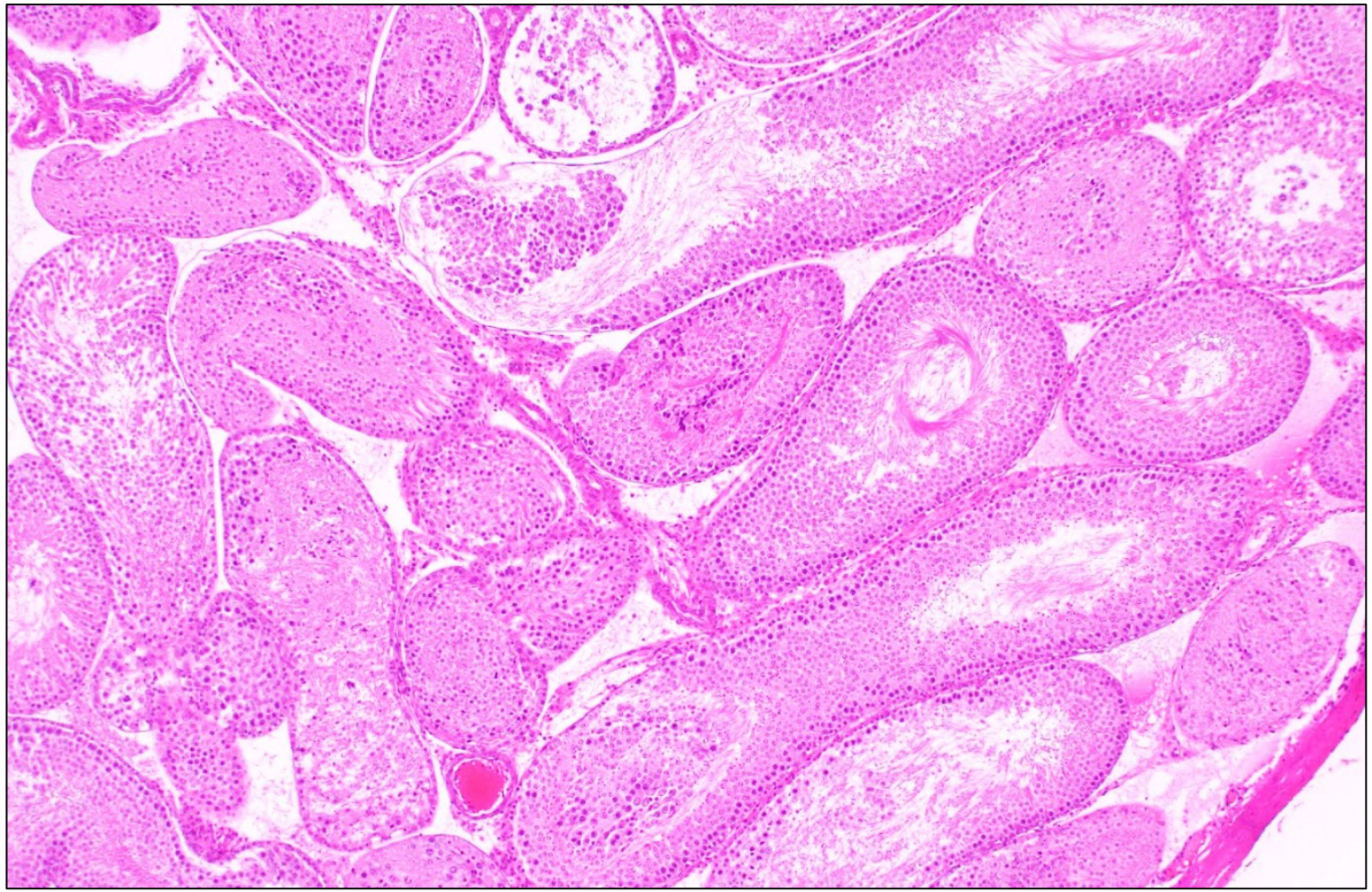
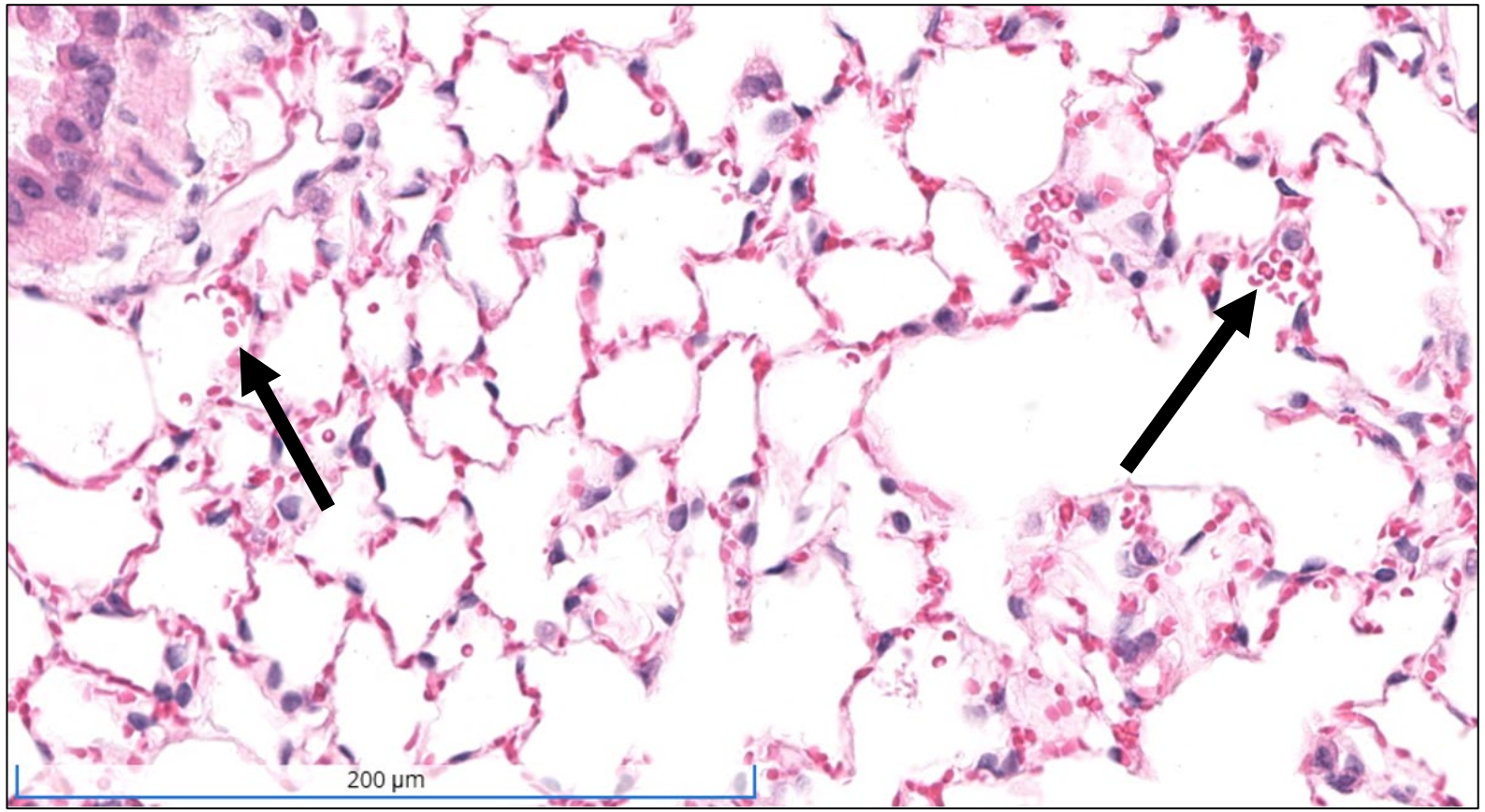


Image courtesy of Justin Vidal

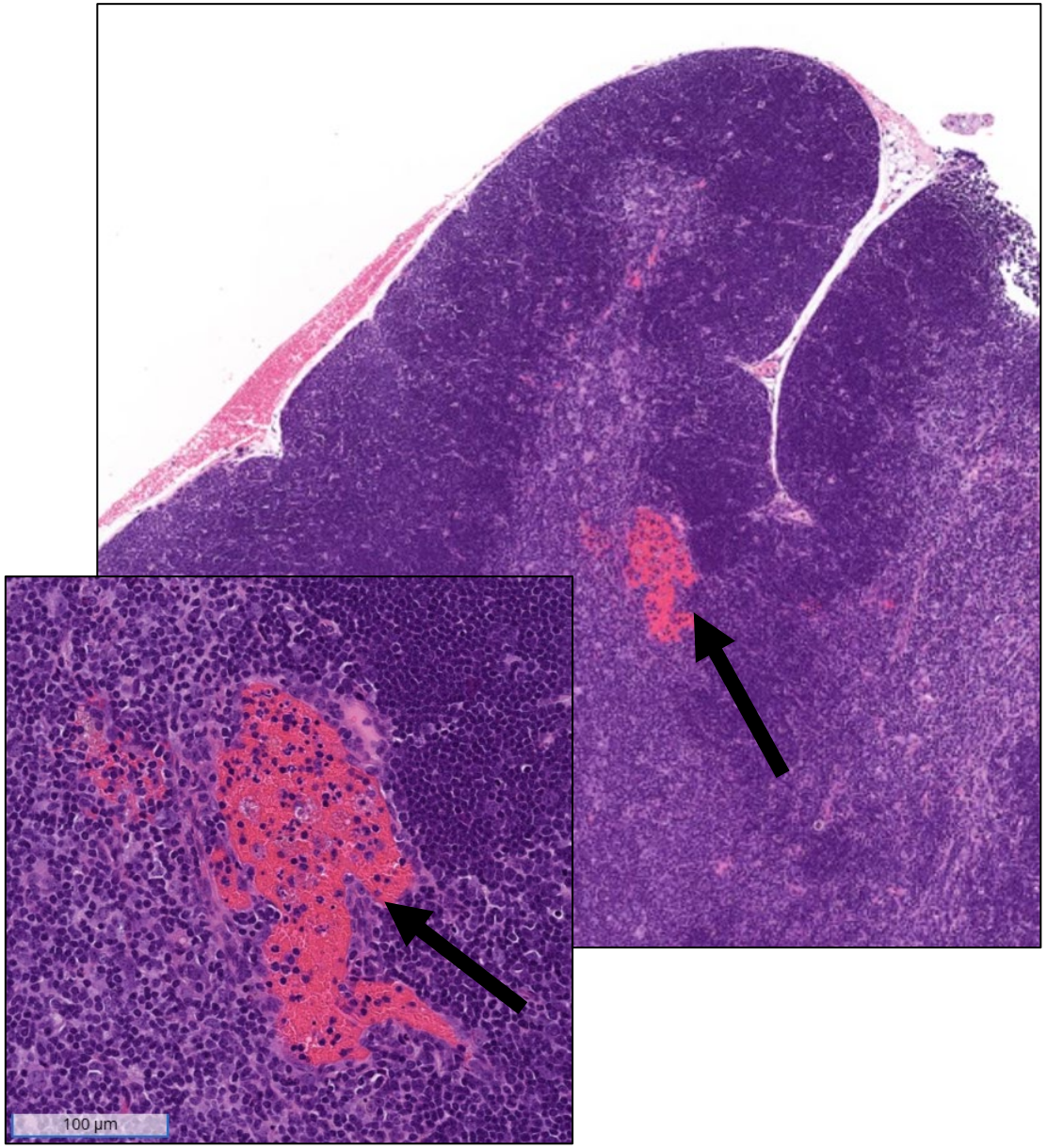
Hemorrhage Artifact: Lung

- Small amounts of extravasated erythrocytes (outside of blood vessels; arrows) can be seen in delicate tissues, like the lung.
- Occurs from rough handling of tissues at necropsy or can be part of the process of microtoming when erythrocytes get “dragged” into alveolar space.
- Differentiate from real hemorrhage by looking for edema, inflammation, and/or hemosiderin.
- Should not be diagnosed as antemortem hemorrhage.



Hemorrhage Artifact: Thymus

- Small amounts of extravasated erythrocytes (arrows) can be observed in and around the thymus.
- Occurs from rough handling of tissues at necropsy or may be due to recent venipuncture.
- Generally, should not be diagnosed as true antemortem hemorrhage unless it is related to treatment/test article or to corresponding macroscopic findings.
- Rule out by no other evidence of hemorrhage (hemosiderin, edema, inflammation).



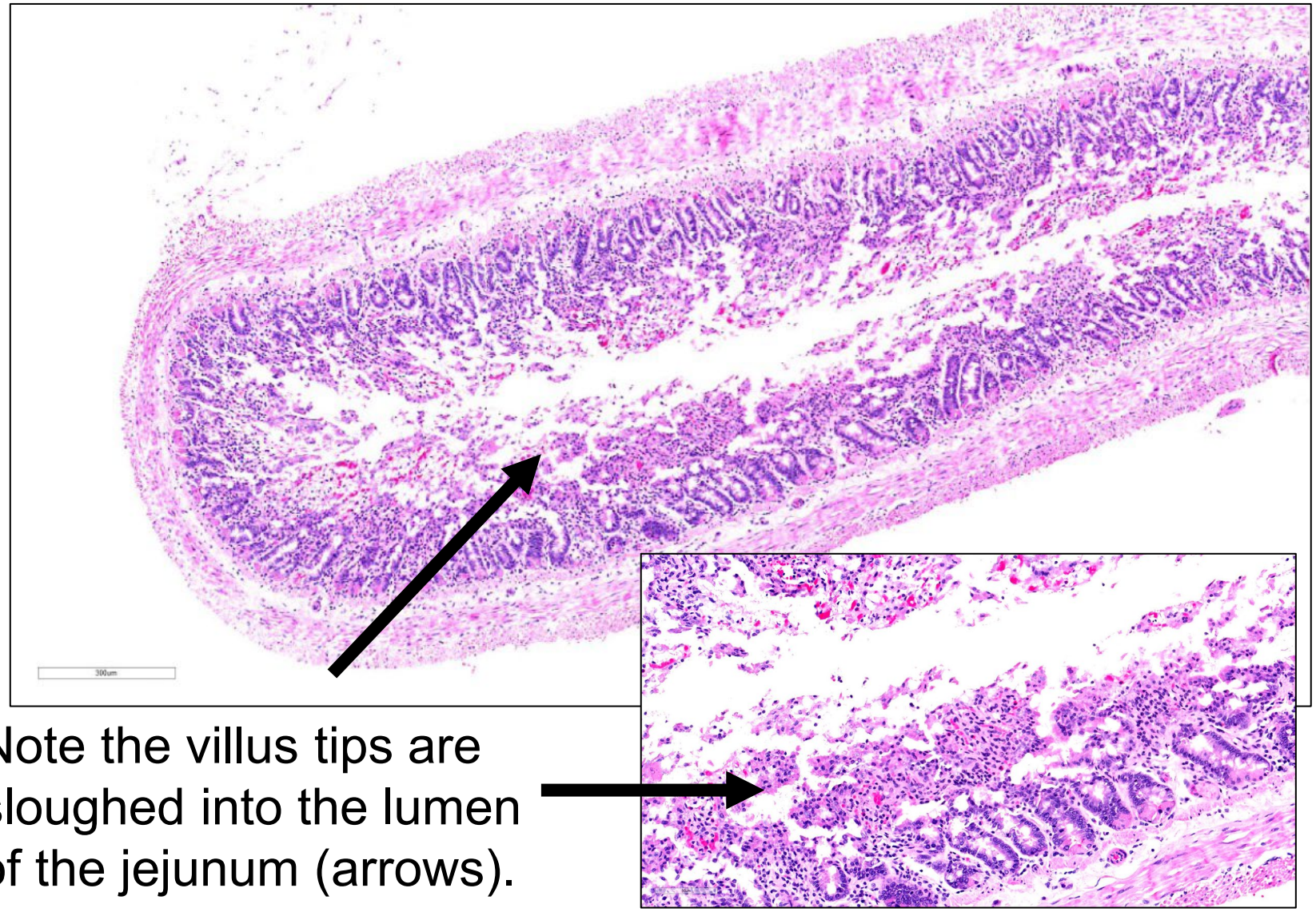
Autolysis: Gastrointestinal (GI) Tract

- Autolysis is destruction of the cell through the leakage of lysosomal enzymes after death.
- Occurs when tissues are not rapidly fixed in preservative.
- Generally, should not be diagnosed but can be noted to account for tissues being difficult or impossible to interpret.
- Cell structure and/or staining are lost but there is a lack of an inflammatory response.
- Tissue structures are separated by clear space due to fluid released from vasculature, but this occurs postmortem.

Villus architecture (arrows) is lost or difficult to stain because cells are autolyzed. Clear space (fluid) separates tissue structures (stars).

Autolysis: Gastrointestinal (GI) Tract

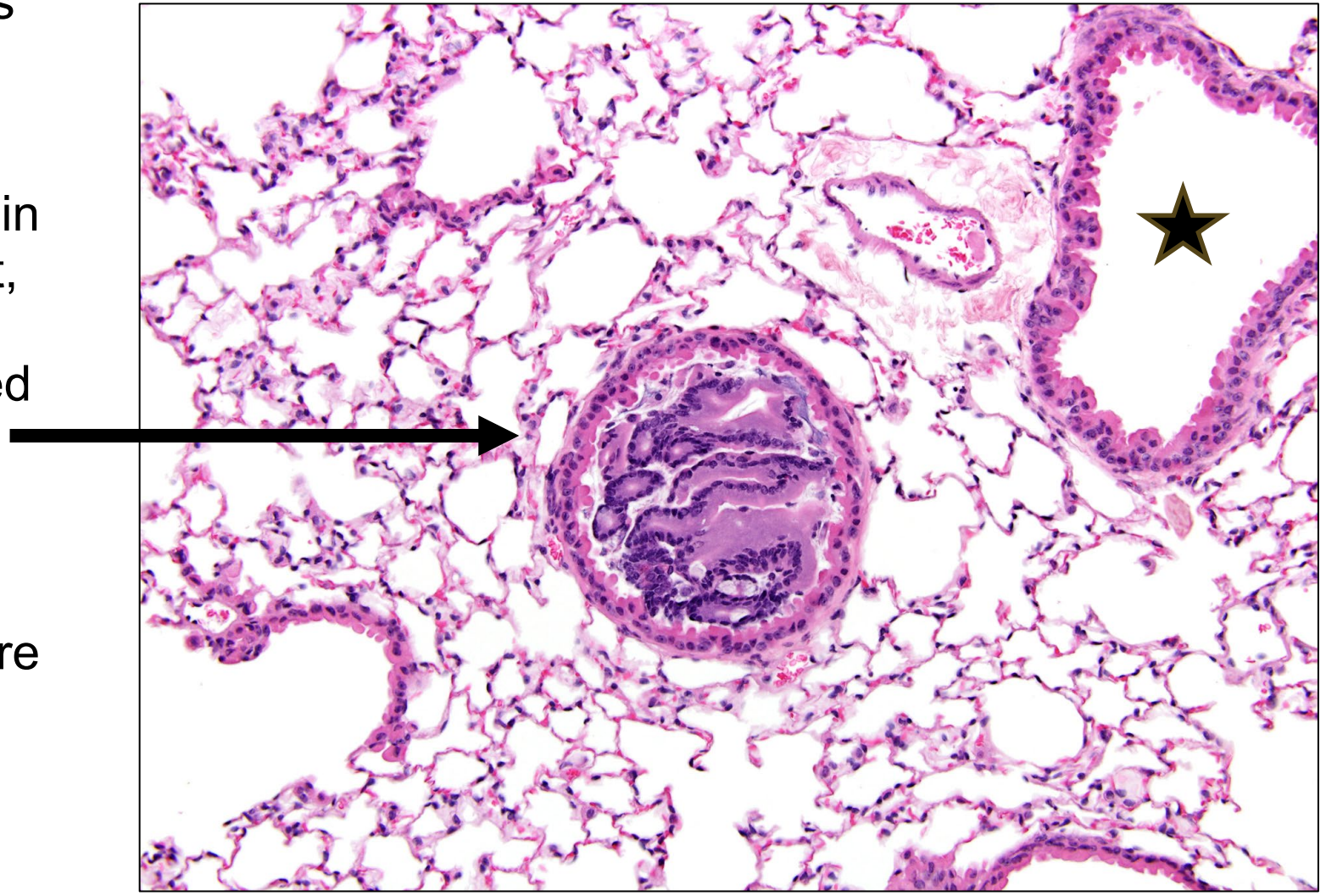
- Less severe autolysis results in the sloughing of epithelial cells from the distal villi. These cells are at the center of the lumen and are bathed in digestive enzymes of the GI tract.
- Distinguish from necrosis by lack of inflammation and the location of sloughing (limited to distal villi).



Note the villus tips are sloughed into the lumen of the jejunum (arrows).

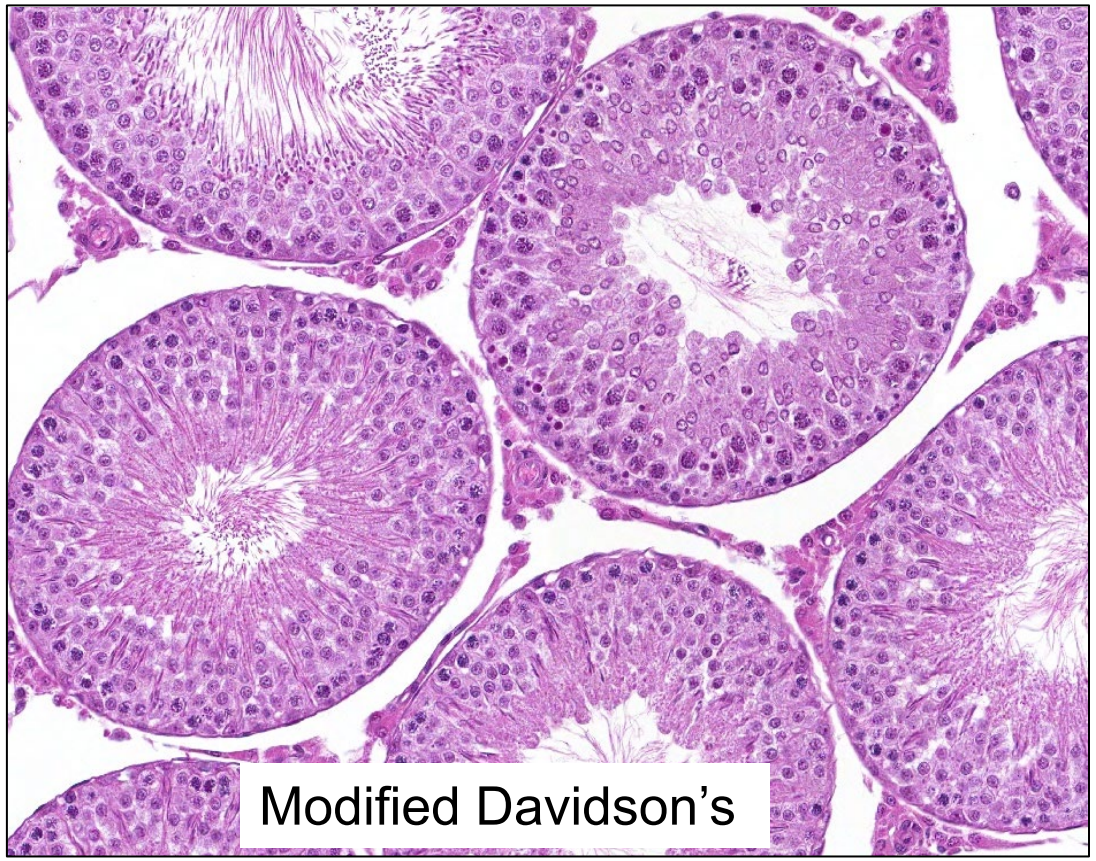
Autolysis: Lung

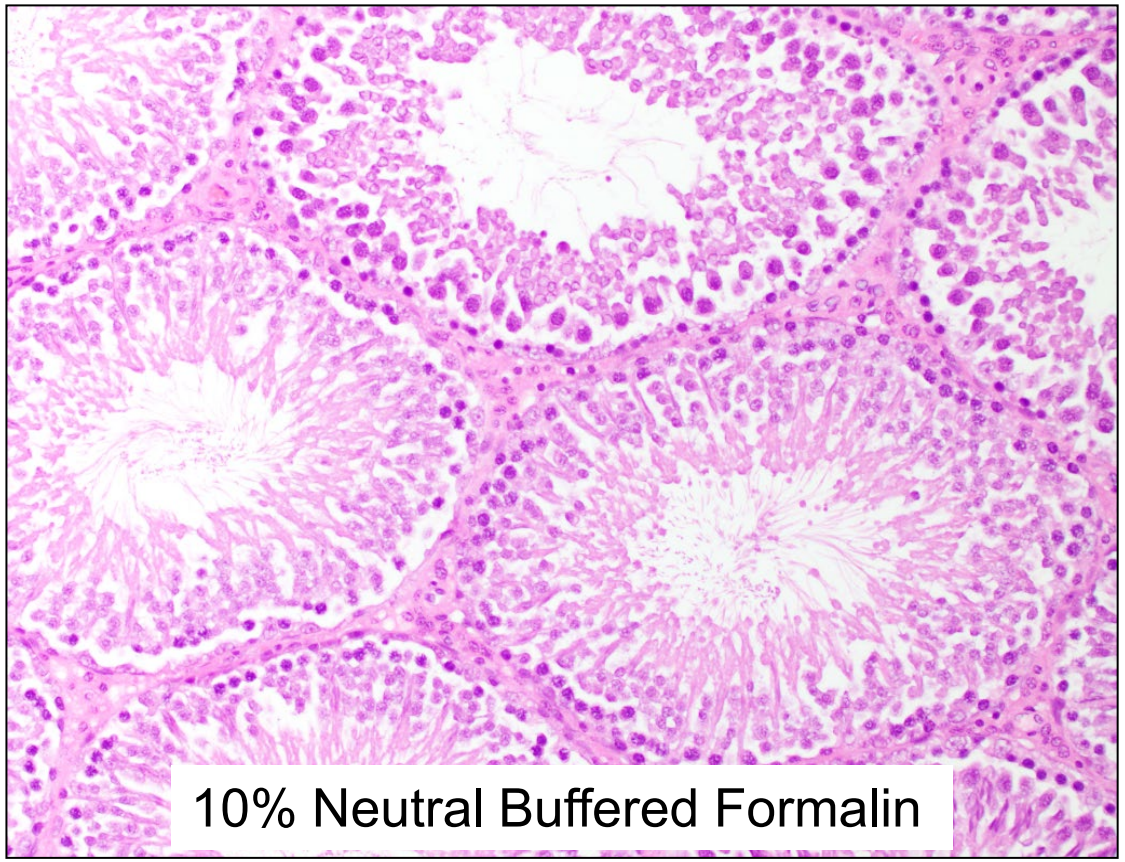
- Autolysis in the lung often results in epithelial sloughing (arrow) from bronchi and bronchioles.
- Note the lining of the bronchiole in the center (arrow) remains intact, but the lumen is filled with epithelial cells that have sloughed from elsewhere. Compare to unaffected bronchiole (star).
- The architecture of the alveoli remain relatively normal and there are no other signs, such as inflammation or edema, to suggest a true, antemortem response.



Fixation Artifact: Testis

Formalin fixation of some tissues, such as testes and eyes, is less than ideal as cellular detail is lost. The testis fixed with Modified Davidson's fixative (left) retains cellular detail, but the testis fixed in formalin (right) shows artifactual cytoplasmic shrinkage and loss of cellular detail.

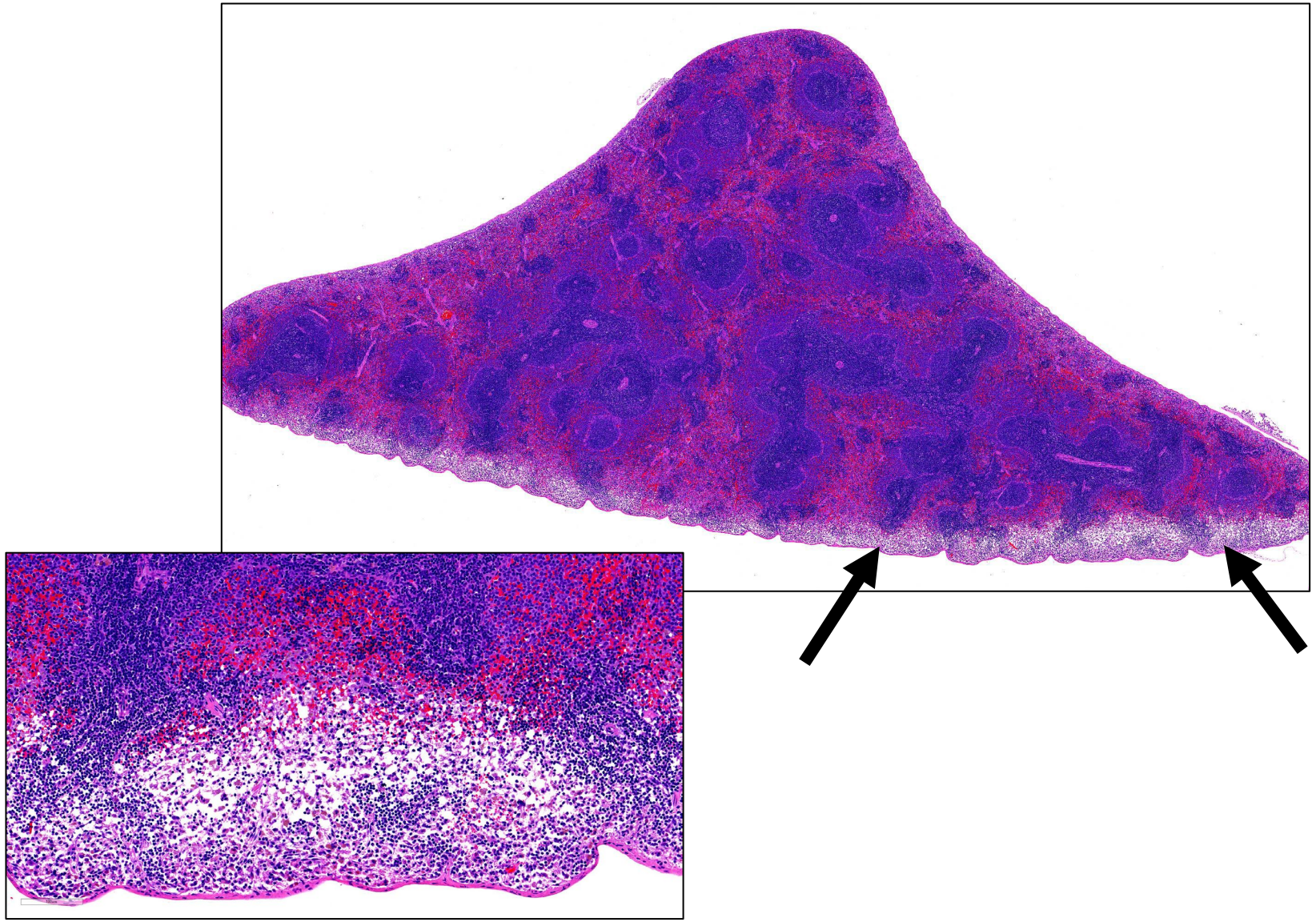




Images courtesy of Justin Vidal

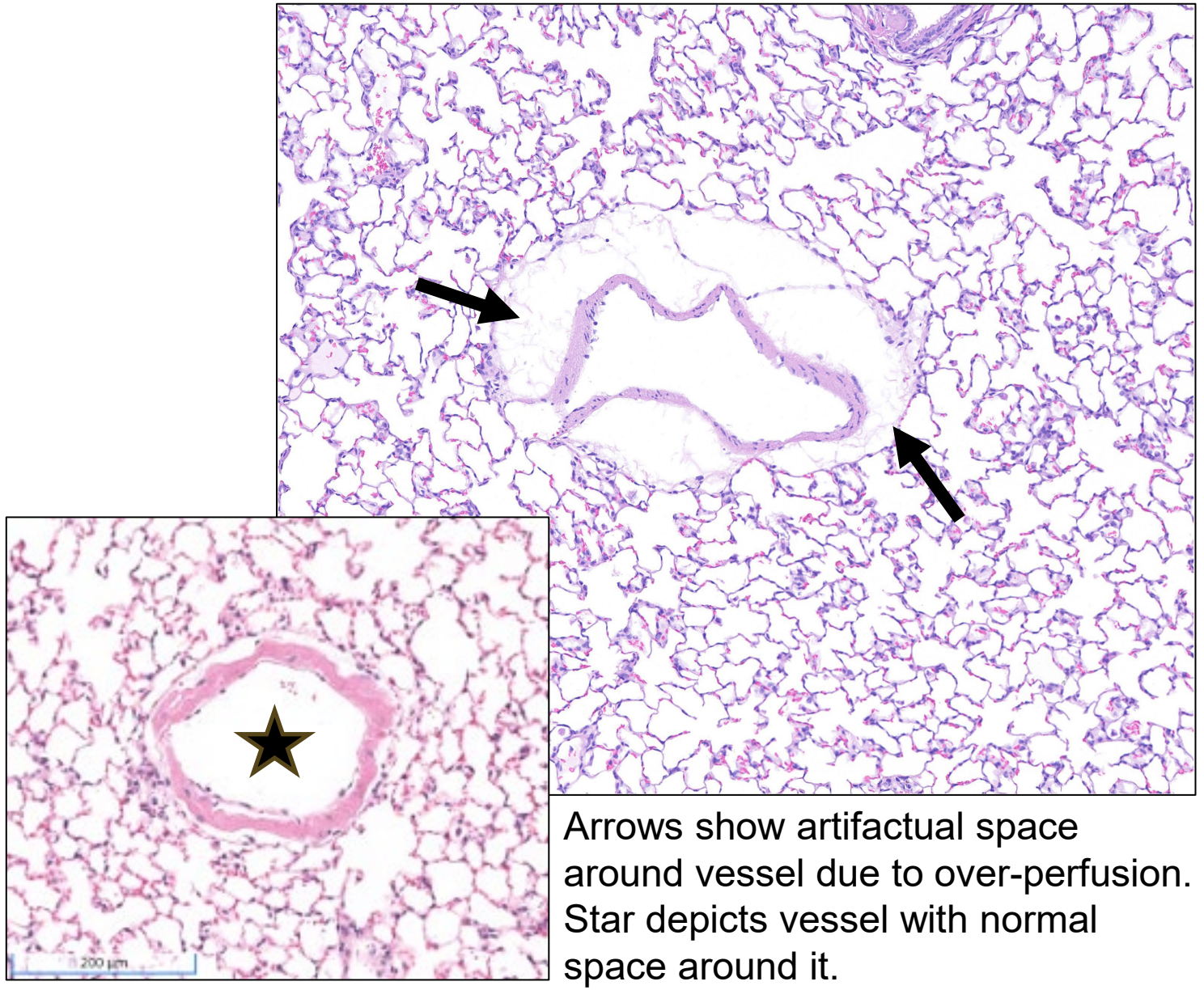
Euthanasia Solution (Intraperitoneal Injection): Spleen

- IP injection of some solutions, such as euthanasia solution, can artifactually distort tissues.
- Note the broad range of decreased cellularity or clear space along one border of the spleen (arrows and inset). This comes from IP injection that either entered or was in close proximity to the spleen.



Over-inflation of Tissue: Lung

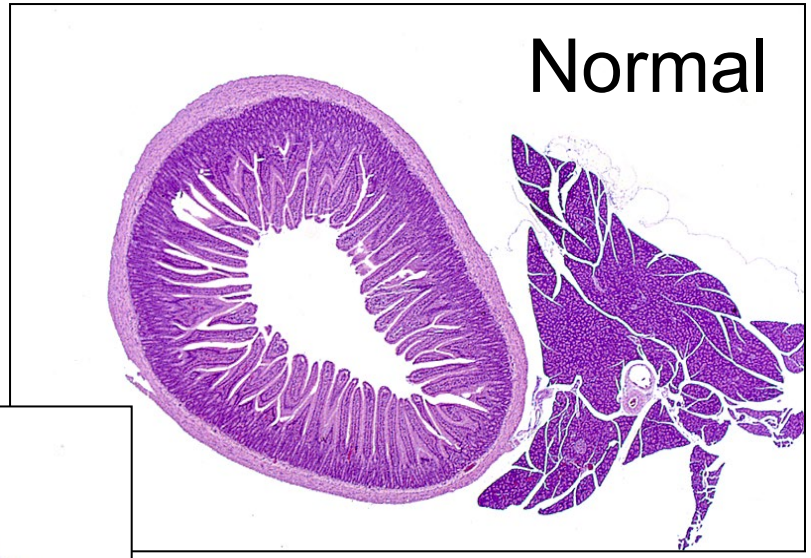
- At necropsy, formalin is infused into the lung to enhance fixation and preserve normal alveolar architecture (to prevent collapse).
- Aggressive infusion can result in overpressure and induce artifactual space (arrows), which mimics edema. Rule out by noting no evidence of edema in other locations.



Arrows show artifactual space around vessel due to over-perfusion. Star depicts vessel with normal space around it.

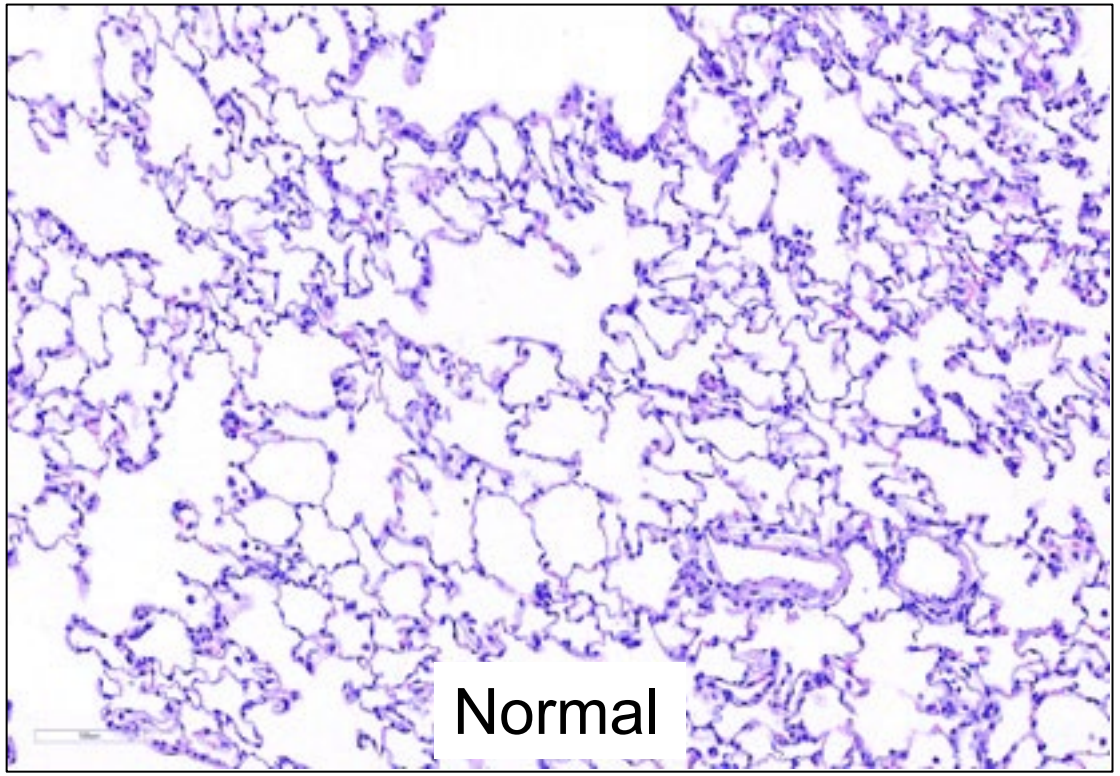
Over-inflation of Tissue: GI Tract

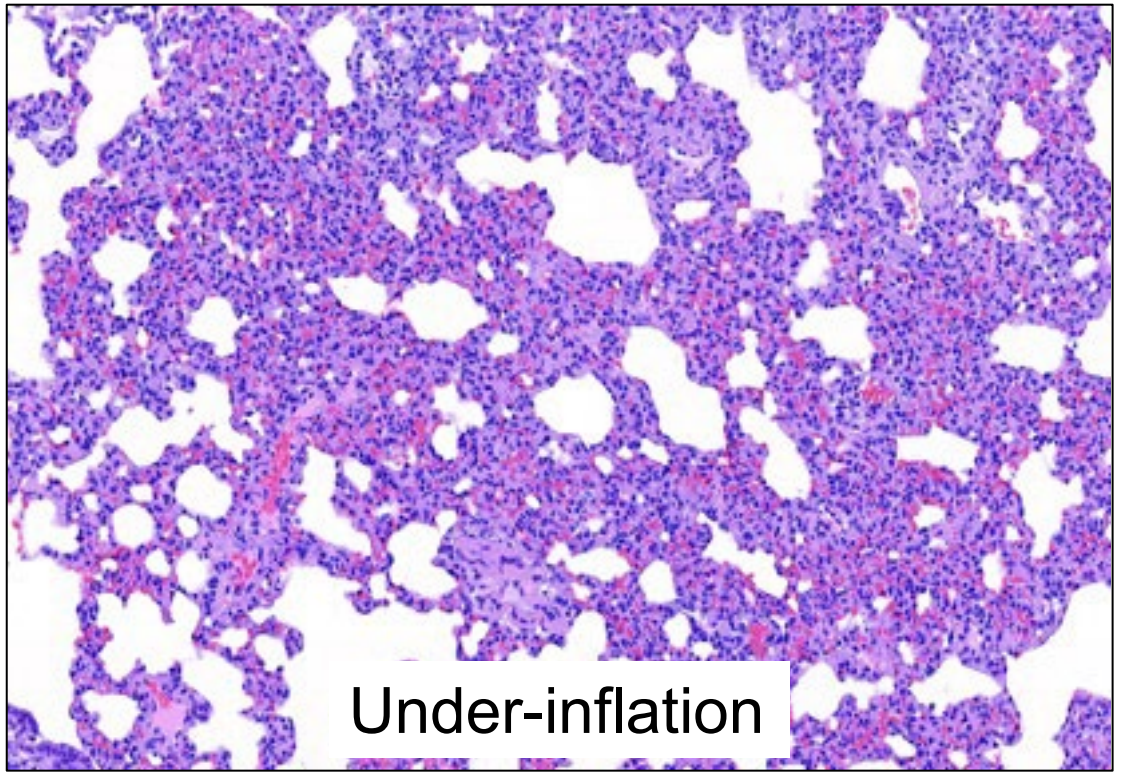
- At necropsy, formalin is infused into the GI tract to prevent premature autolysis.
- Aggressive infusion can result in overpressure and induce artifactual separation of villi (left image).
- This could be misinterpreted as villous atrophy or loss.



Under-Inflation of Tissue: Lung

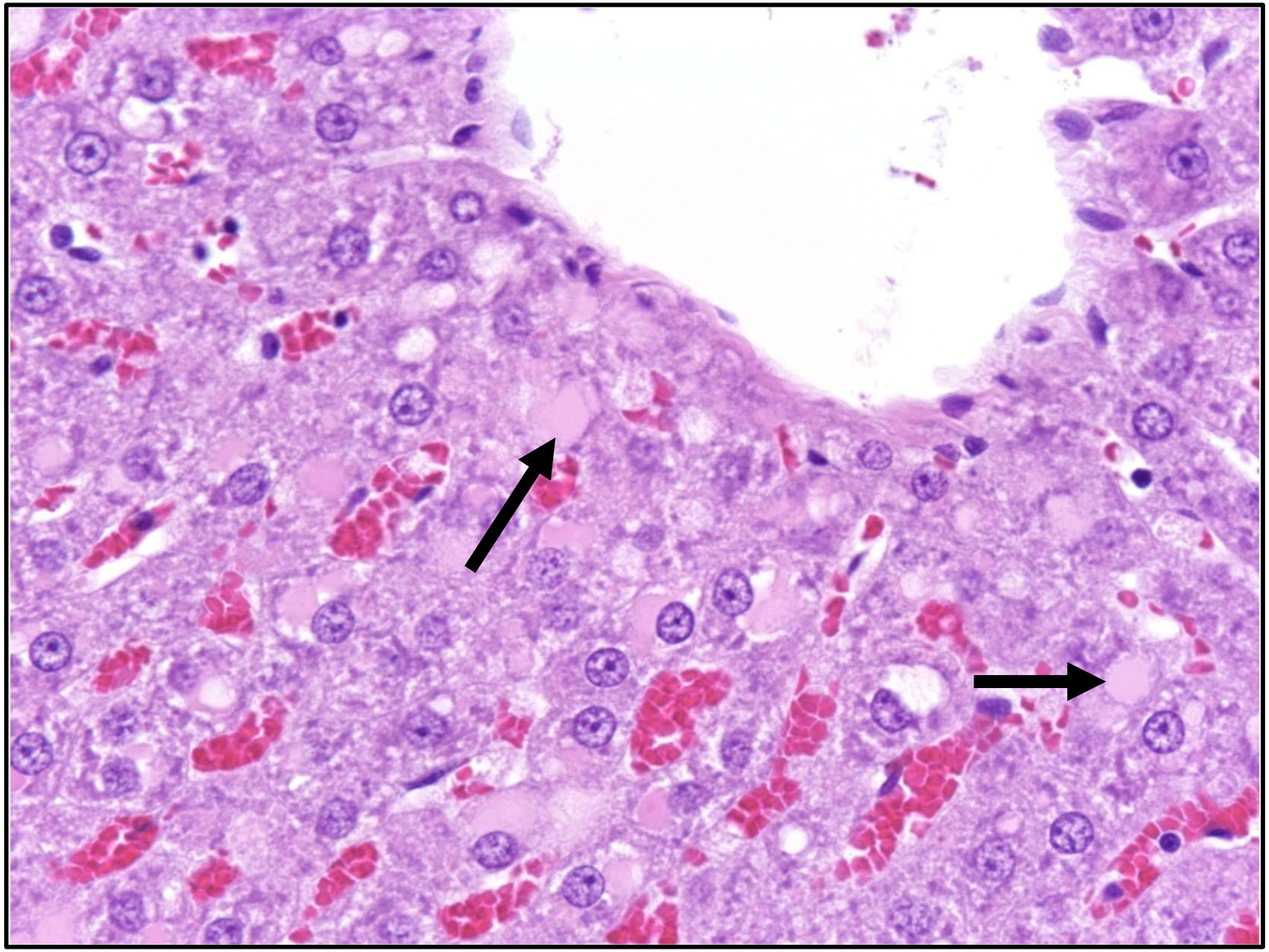
The lung on the left is properly infused with formalin and alveolar space is preserved. When not properly performed (right), the alveolar space collapses and the lung appears to have increased numbers of cells, mimicking inflammation or neoplasia, and, in general, is more difficult to assess.





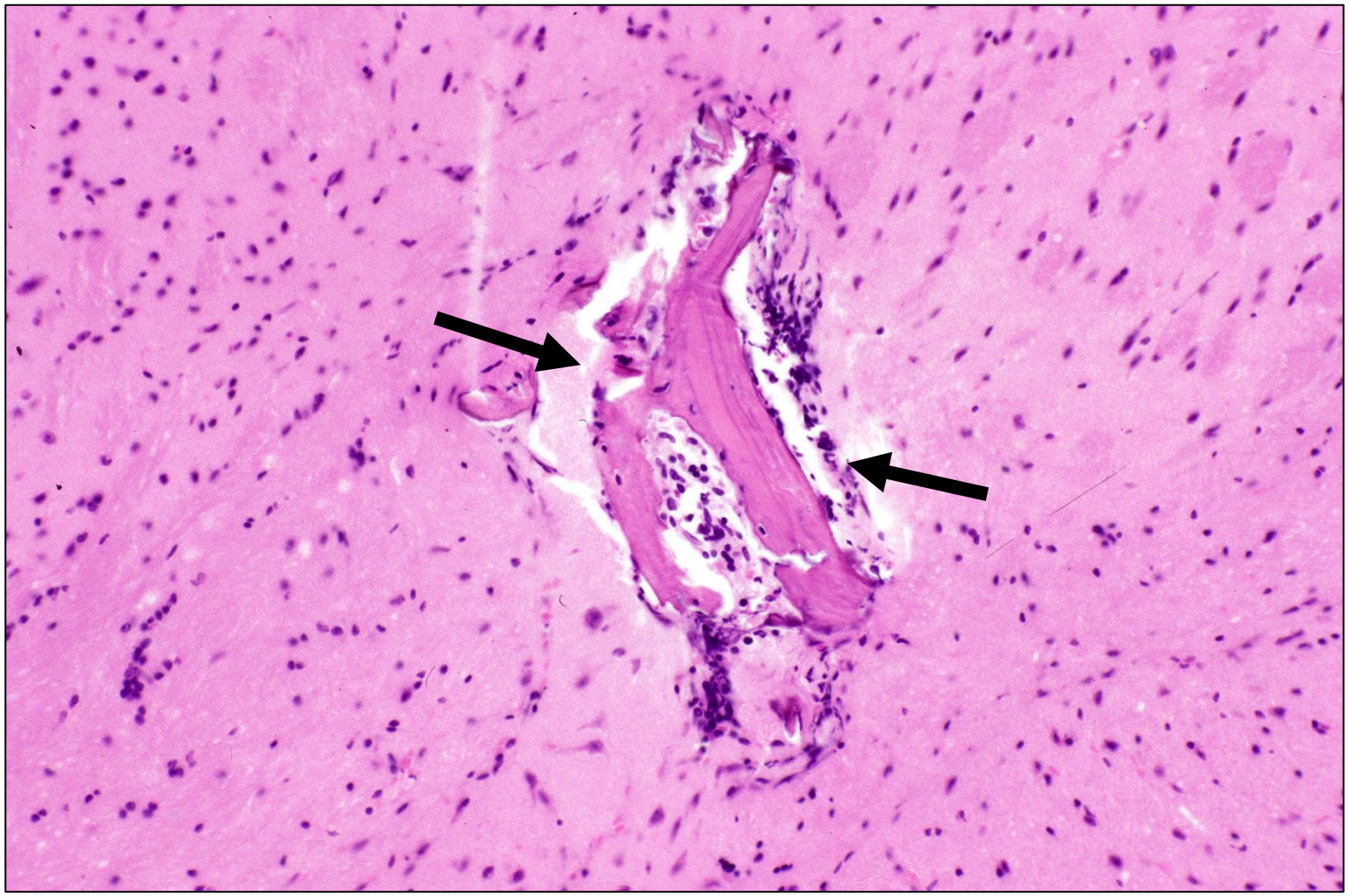
Cytoplasmic Inclusions: Liver

- Postmortem changes in the liver often present as vacuoles or inclusions that accumulate in the cytoplasm (arrows).
- These changes are the result of lysosomal swelling or plasma influx into the cell and are postmortem changes that should not be diagnosed.
- Differentiate from real inclusions by inconsistent size and shape and evidence of autolysis in the liver or other tissues of the animal.



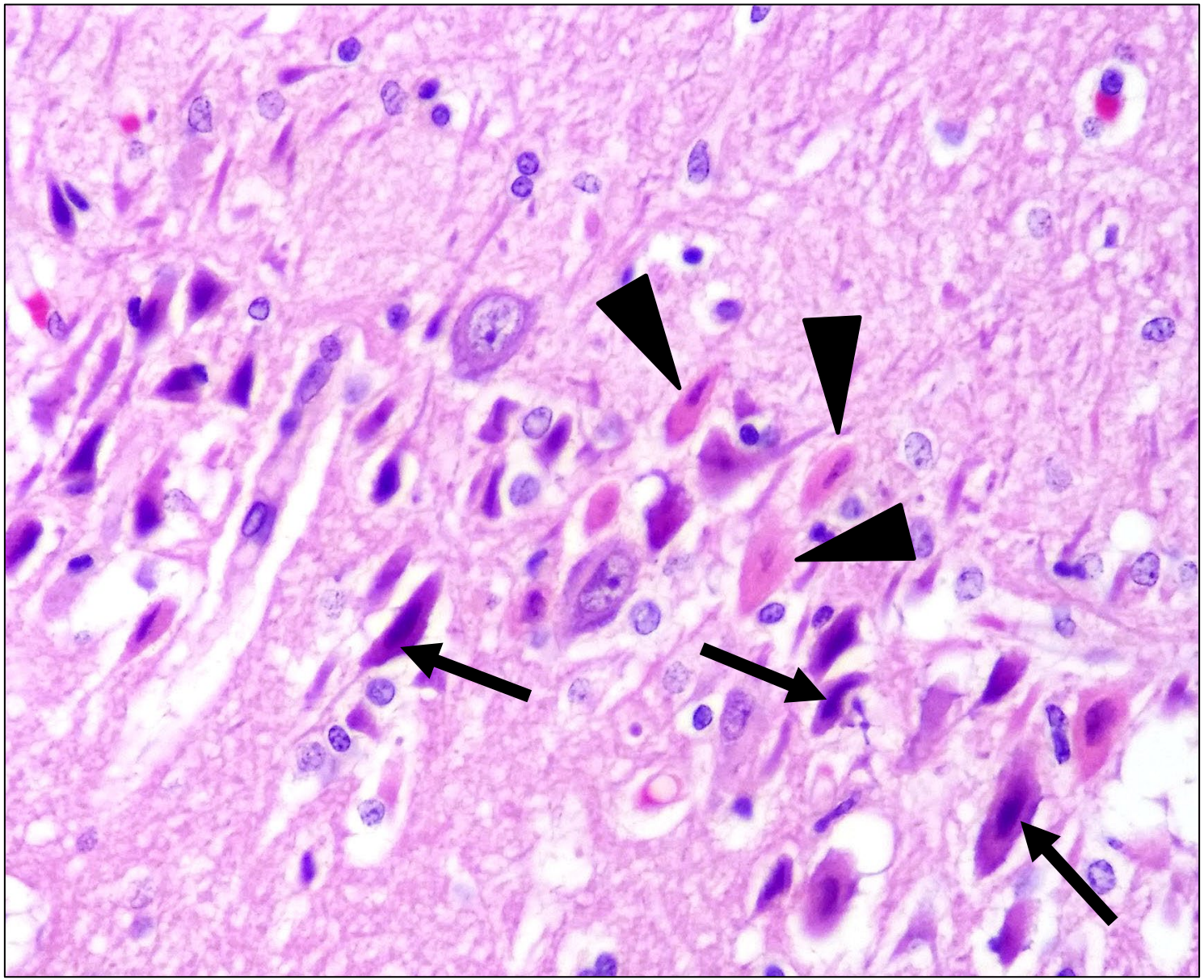
Bone Fragments: Brain

- Bone fragments (arrows) can often appear in sections of the brain, especially in larger animals such as dog and nonhuman primate.
- This is primarily due to the use of a bone saw at necropsy to extract the brain. Contact with brain tissue transfers bone fragments from the saw blade to the tissue and is generally confined to the superficial layers of the brain.



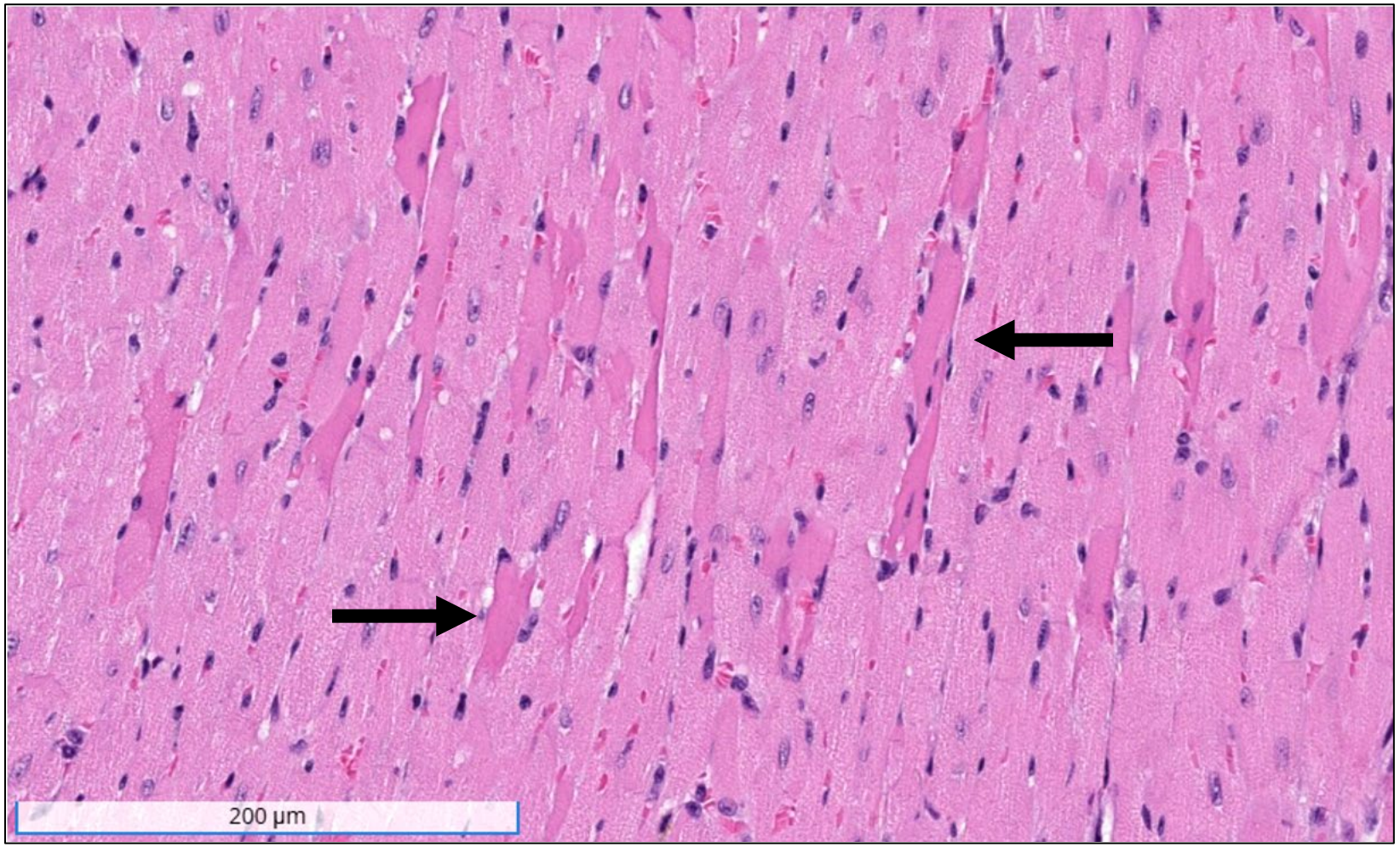
Dark Neuron: Brain

- Processing artifact can often lead to neurons being shrunken and staining dark purple (arrows).
- This can be confused as degeneration or necrosis.
- In the CNS, true necrotic neurons are hypereosinophilic and appear reddish to deep pink (arrowheads). Think “Red is Dead.”



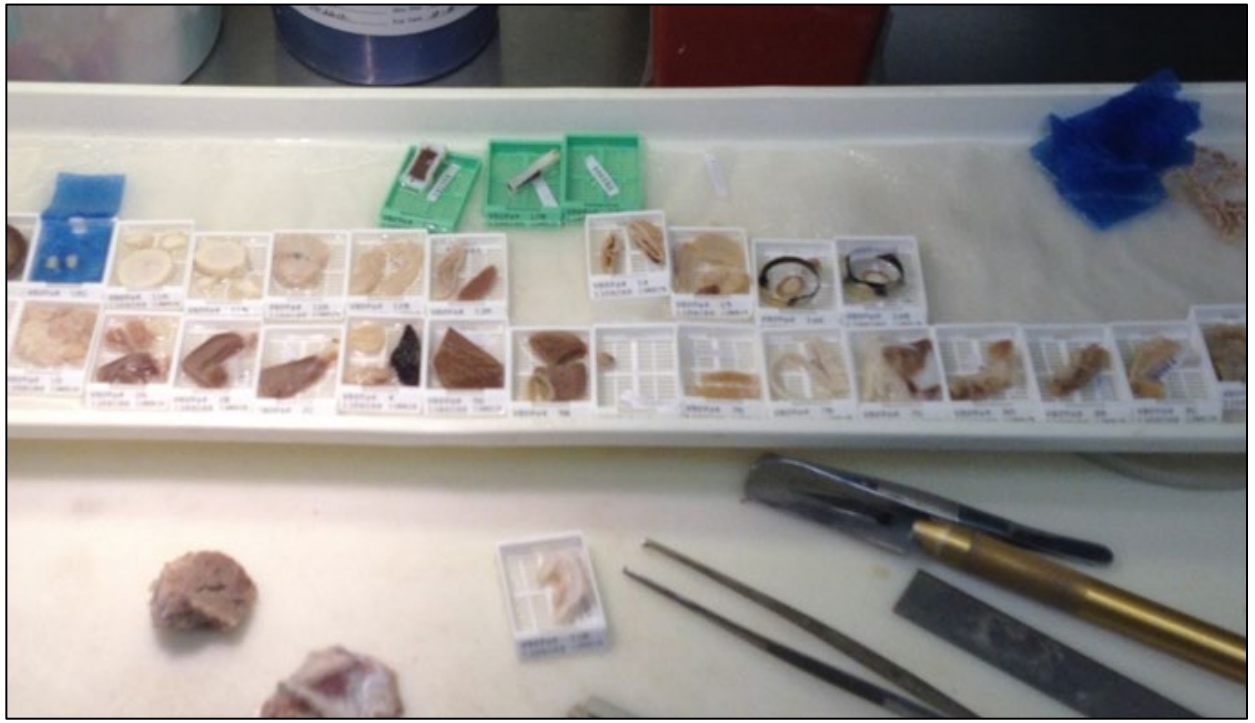
Muscle Fiber Contraction: Heart

- Myofibers in the heart and skeletal muscle are prone to contraction following tissue harvesting and processing.
- Myofibers are hypereosinophilic and shrunken (arrows) and could be misinterpreted as necrotic.
- Note there is no inflammation or other cellular response to support necrosis.



Artifacts with Tissue Processing: Introduction

- Tissue processing begins with removing the tissue from the initial fixative (usually formalin) and then trimming it down to a smaller size to fit in a tissue cassette.
- Tissue cassettes are placed into an automatic processer (usually overnight) that dehydrates the tissue and injects paraffin to maintain the tissue architecture.
- Tissues are placed in a paraffin bath to produce a block (commonly known as FFPE – Formalin-Fixed Paraffin-Embedded).

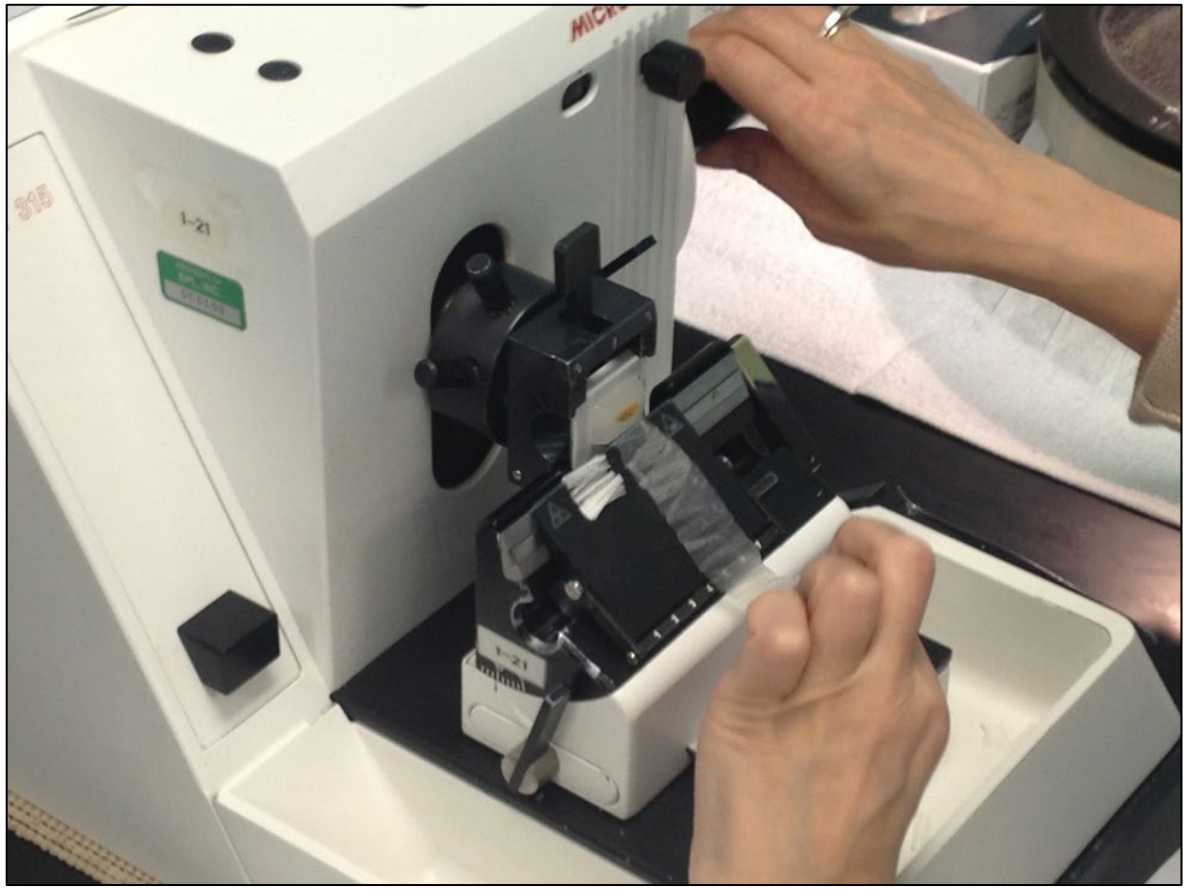


Tissue trimming station where tissues are trimmed down to a size that fits into the white tissue cassette.

- Artifacts can occur with a misfunctioning processor or when tissues are not trimmed properly, such as not properly orienting the tissue in the block.

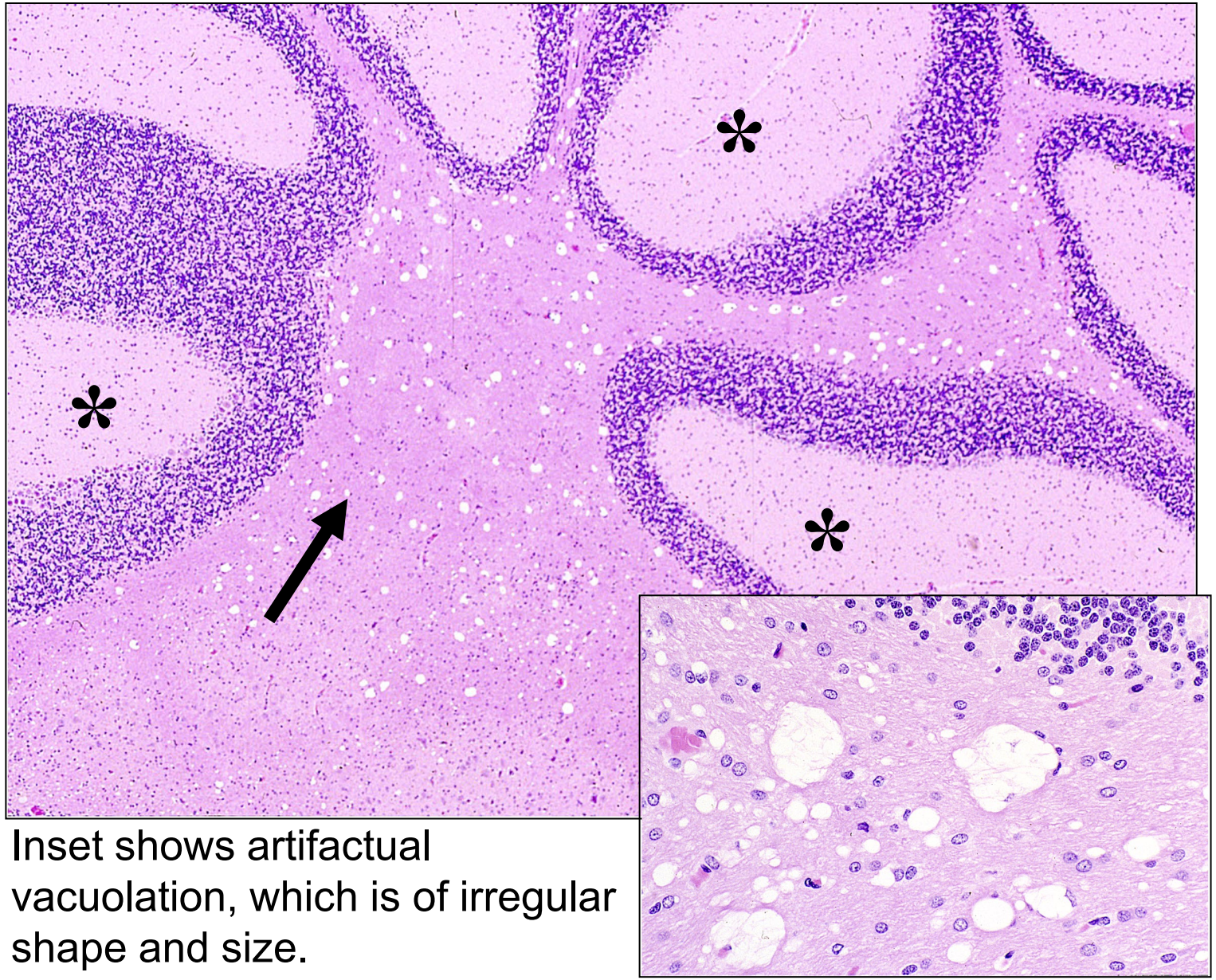
Artifacts with Tissue Processing: Introduction

- The paraffin block is cut on a microtome that slices the block to produce a thin paraffin ribbon.
- The tissue section is embedded in the ribbon.
- The ribbon is floated on a waterbath and placed onto a glass slide to be stained.
- Artifacts may occur when the microtome knife dulls or malfunctions, the tissue is cut too thickly, or poor staining techniques are used.



Vacuolation: Brain

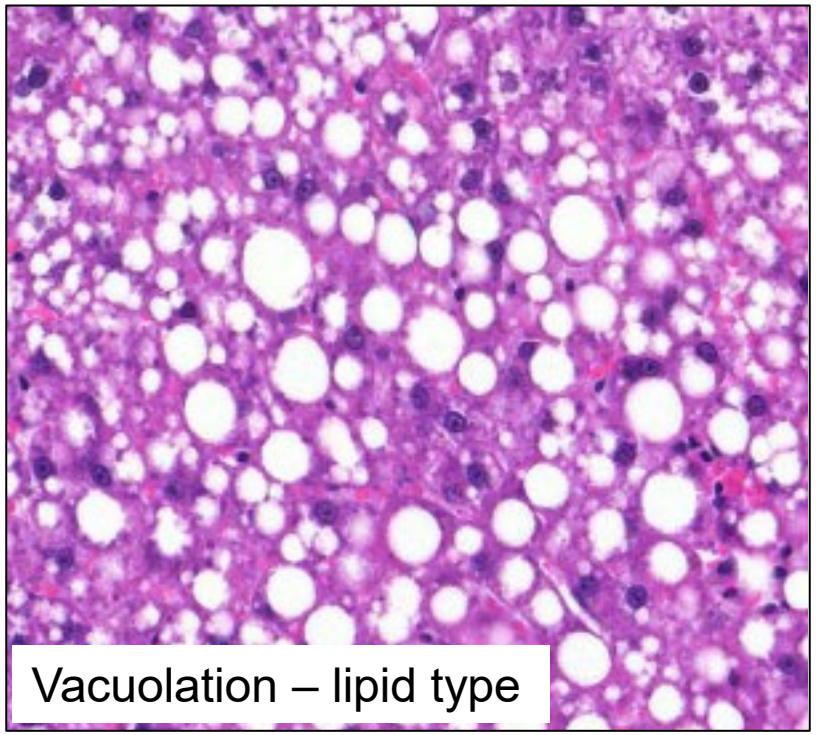
- Artifactual vacuolation can occur in the brain due to improper processing, especially within white matter tracts (arrow). Note the gray matter is unaffected (*).
- Differentiate from true vacuolation by noting that artifacts have inconsistent size and shape of vacuoles, lack of cell debris within vacuoles, and random distribution of vacuoles.
- For examples of real vacuolation of brain tissue, see the DTT Global Toxicologic Pathology Program's Neuropathology Training Module.



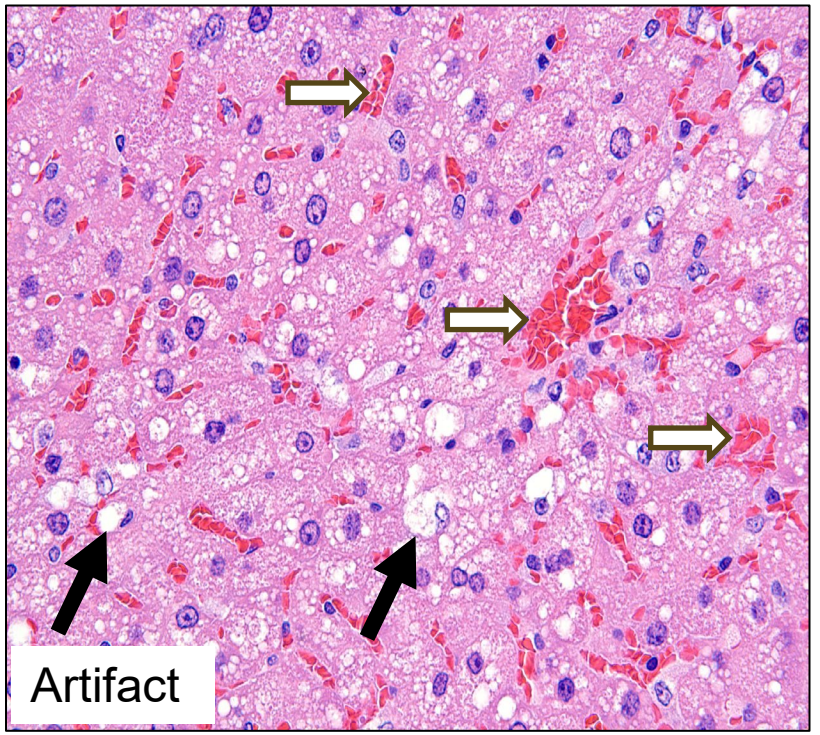
Inset shows artifactual vacuolation, which is of irregular shape and size.

Vacuolation: Liver

- Postmortem vacuolation occurs rapidly in the liver.
- Note the uneven size, shape, and distribution of the vacuolation (black arrows in right image), all of which are clues the vacuolation is due to postmortem changes.
- Additionally, the liver sinusoids (white arrows) are congested, which further supports the animal was found dead.



Vacuolation – lipid type

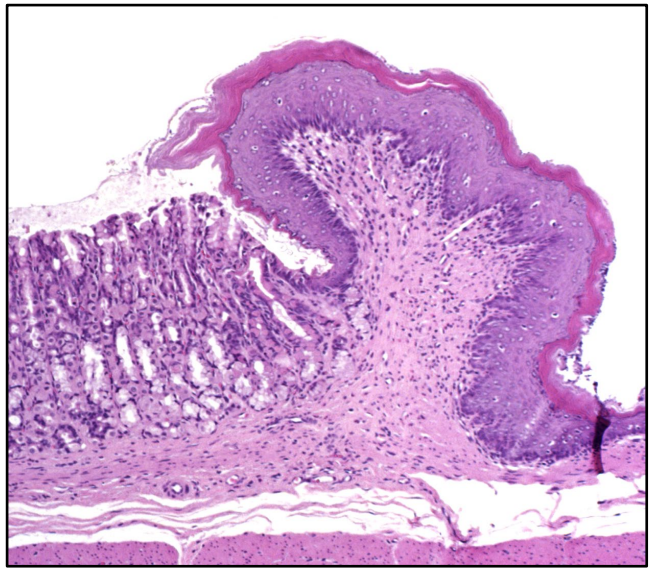


Artifact

Note the vacuoles on the left are of consistent shape and grouped together. Postmortem vacuolation (image on the right) has inconsistent shape and random distribution.

Tangential Cut: Stomach

- This section of stomach at the limiting ridge (border of glandular and non-glandular stomach) appears hyperplastic (thickening of epithelial layer - white arrow) and hyperkeratotic (thickening of top keratin layer – black arrows).
- This results from the section being cut at a tangent, rather than straight through, and gives the illusion the tissue is thicker than it really is.
- Focal hyperplasia generally would have a supporting tissue reaction, such as inflammation or other cellular changes. See Stomach, Forestomach, Epithelium – Hyperplasia, [Focal, Diffuse].

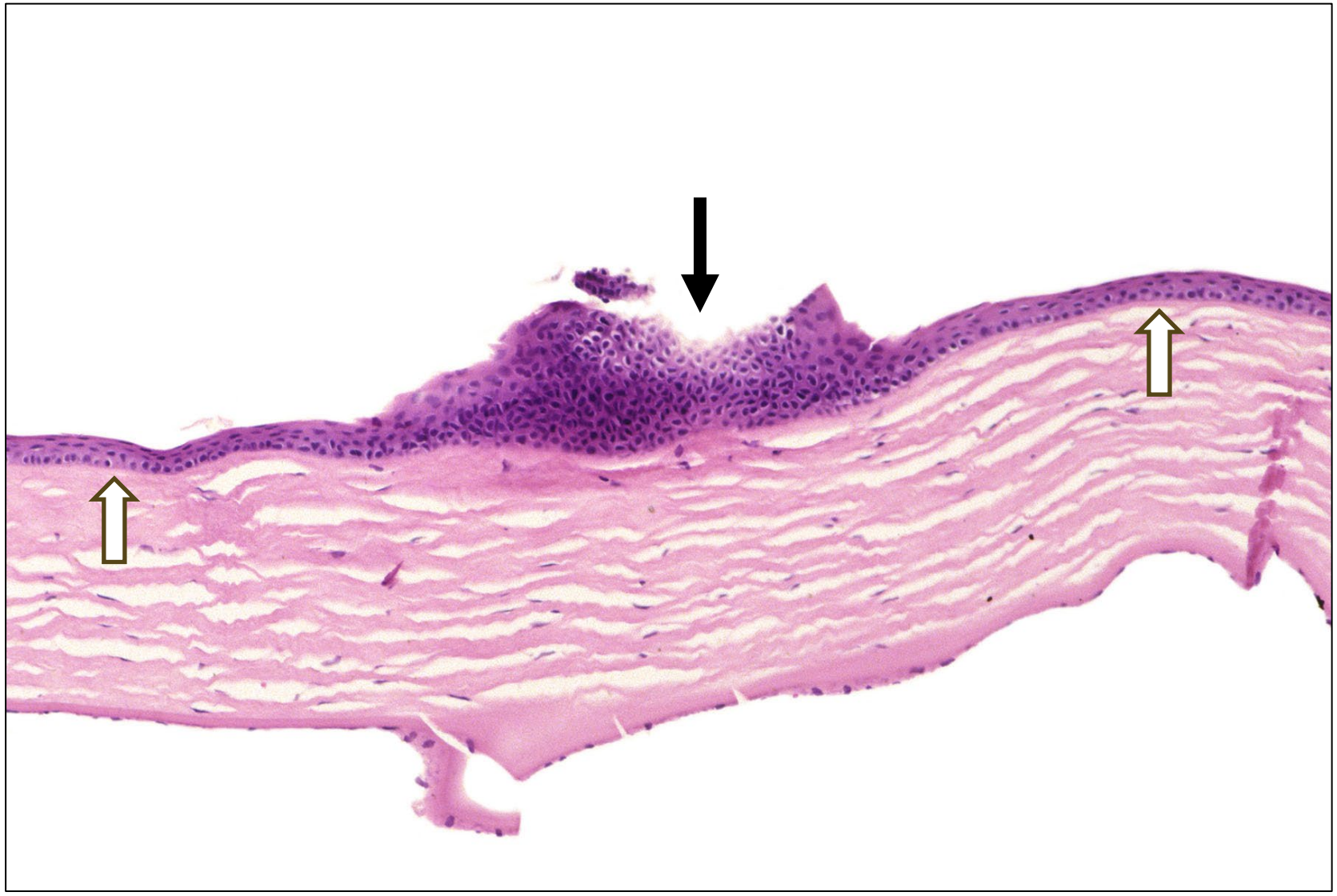


Normal limiting ridge, rat

Tangential cut, causing the limiting ridge to appear to be hyperplastic and hyperkeratotic

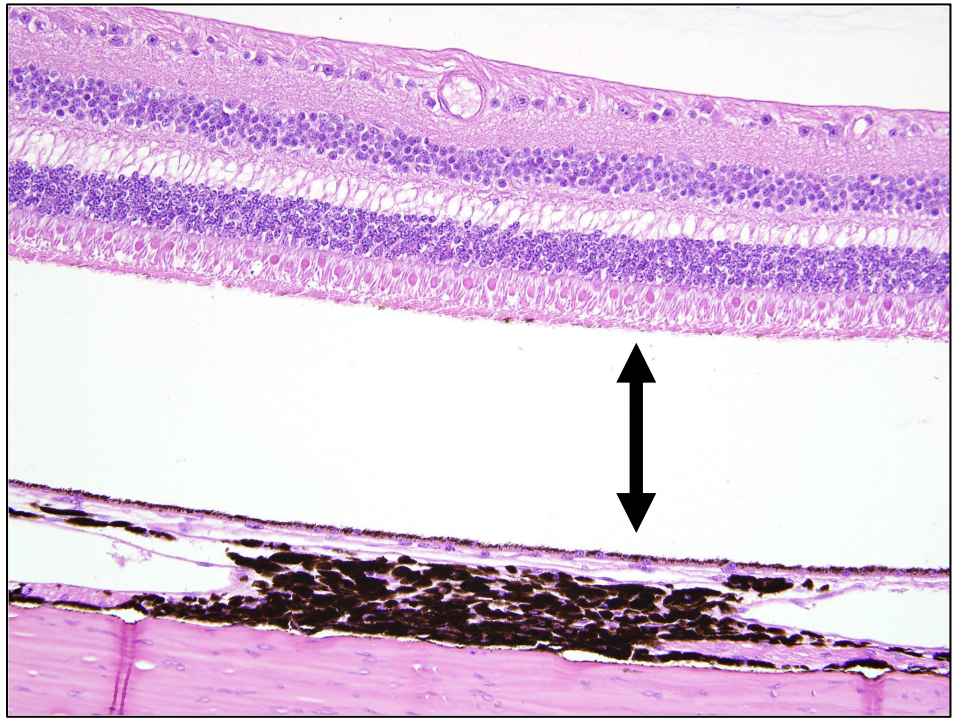
Tangential Cut: Cornea, Eye

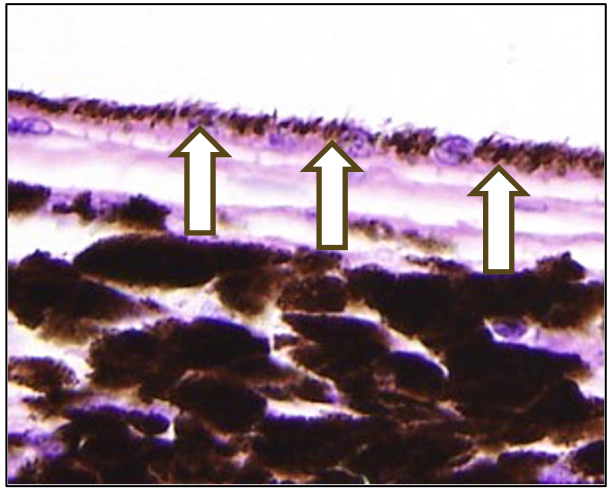
- This section of cornea appears artifactually thickened (black arrow).
- This results from internal twisting of the section of cornea and the epithelial layer being cut at a tangent as opposed to the sections on either side, which are positioned properly and appear normal (white arrows).
- True thickening would have associated changes, such as inflammation or fibrosis.

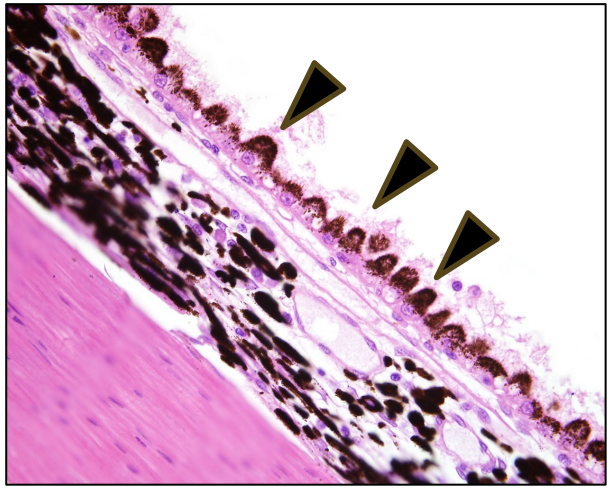


Tissue Tear: Retina, Eye

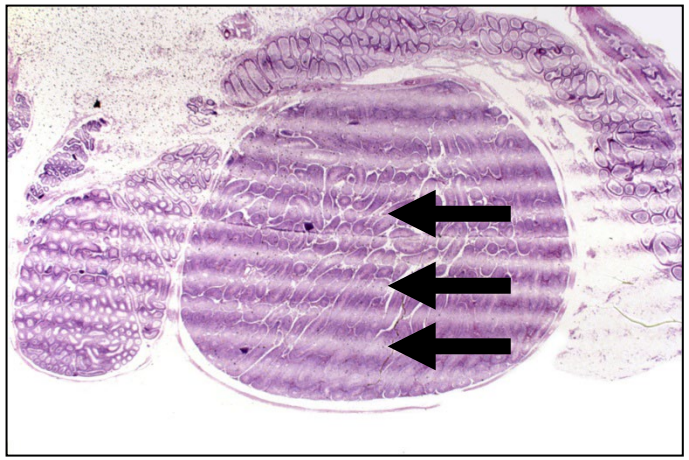
- The retina is often artifactually detached from the underlying choroid at the level of the retinal pigmented epithelium (RPE) (double black arrow).
- In the bottom left image, the RPE is relatively flat and regularly spaced (white arrows), which supports artifactual separation.
- In actual separation (detachment; bottom right), the RPE is hypertrophied and rounded (black arrowheads) or there are inflammatory cells and cellular debris within the space.
- See true detachment here: Eye, Retina – Detachment.



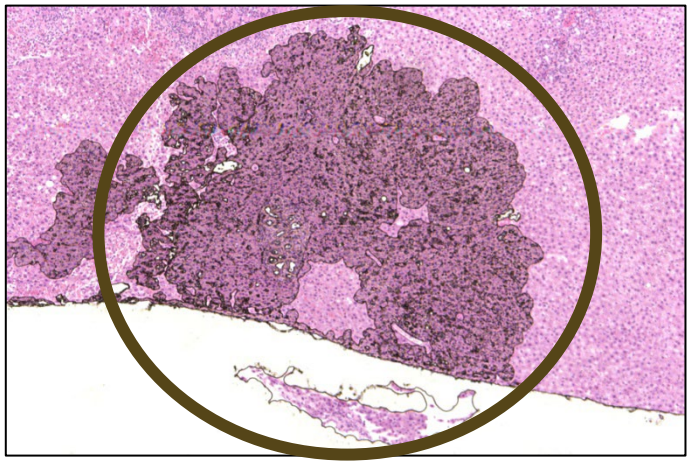




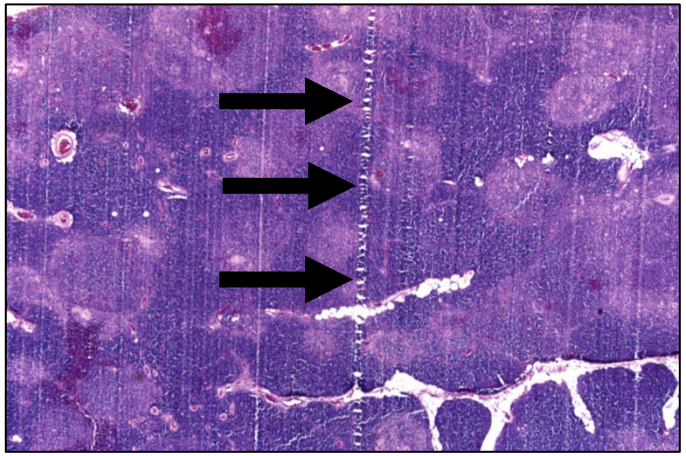
Tissue Processing Artifacts - Various



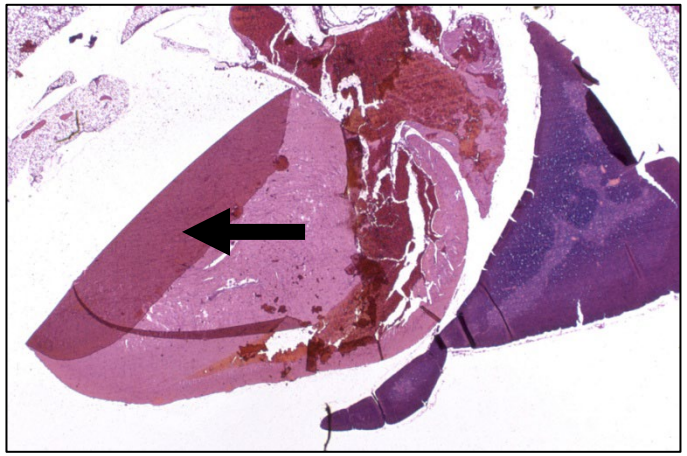
- Common processing artifacts include chattering of the blade (arrows) (Venetian blind).



- Air bubble (circle) that forms under the coverslip obscuring the tissue.



- Microtome lines that tear out some of the tissue (arrows).



- Tissues that fold onto themselves and obscure the folded section (arrow).

These artifacts can generally be read through or can be recut to remove the effect.

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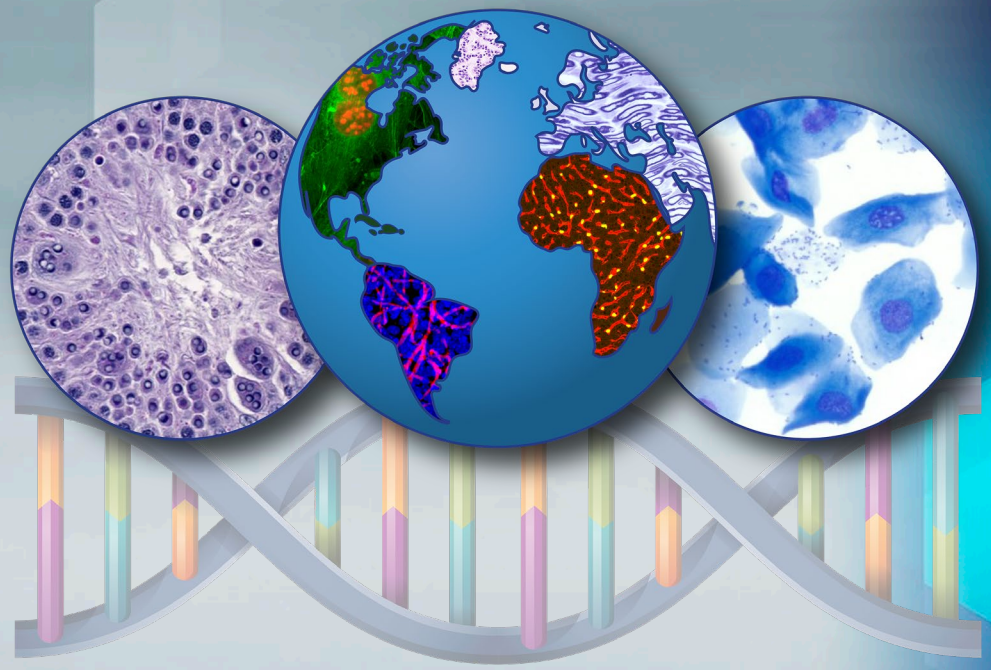

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