Technical Problems And Troubleshooting In Histopathology Technique

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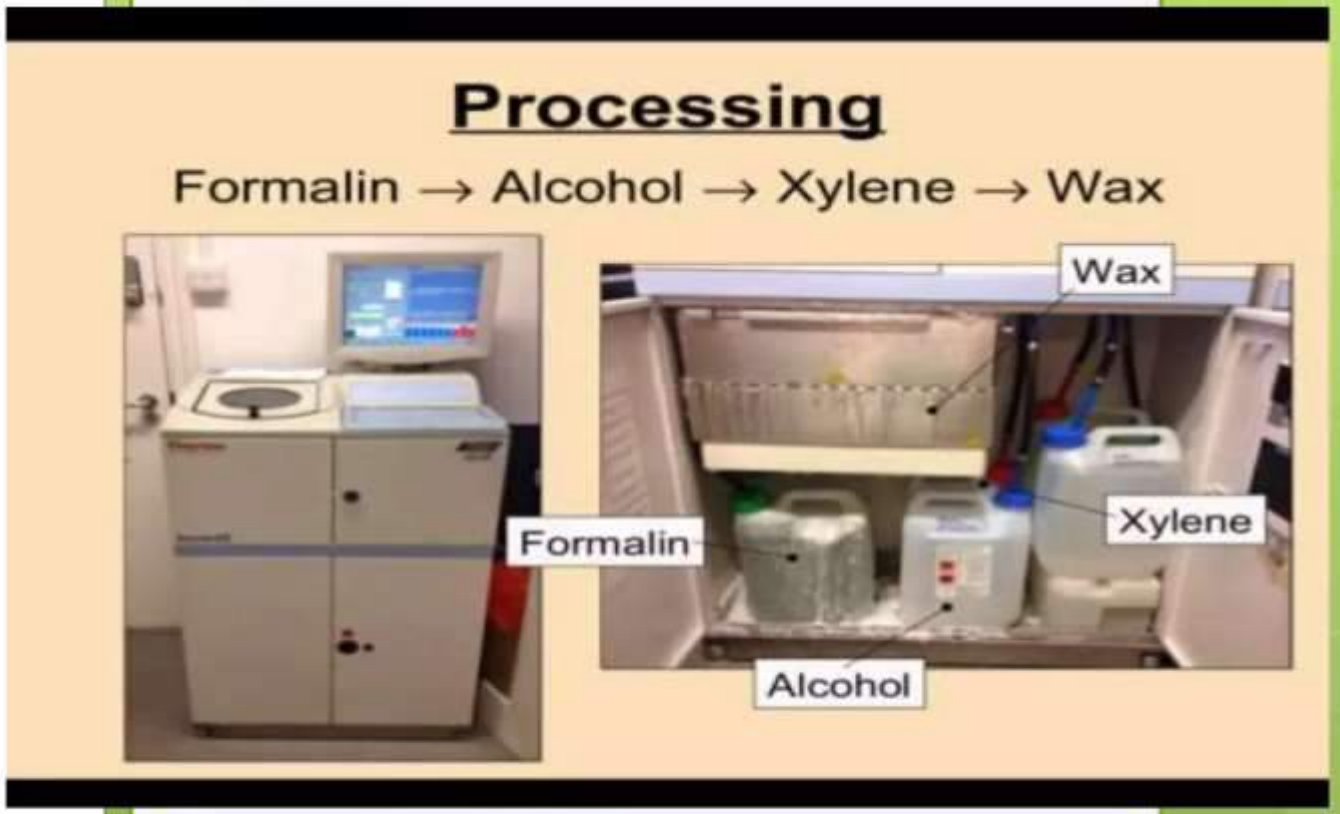
Protocols followed in Tissue Processing

- Tissue procurement
- Labeling and Storage
- Fixation
- Dehydration
- Clearing
- Impregnation
- Embedding
- Section Cutting
- Staining
- Mounting



Specimen receive and registration

- The specimen name is identical to that written on the request form
- All the patient details are properly written mentioned
- Date of biopsy/procedure
- Time of specimen receive
- Register the specimen with a lab number

Technical problems and troubleshooting

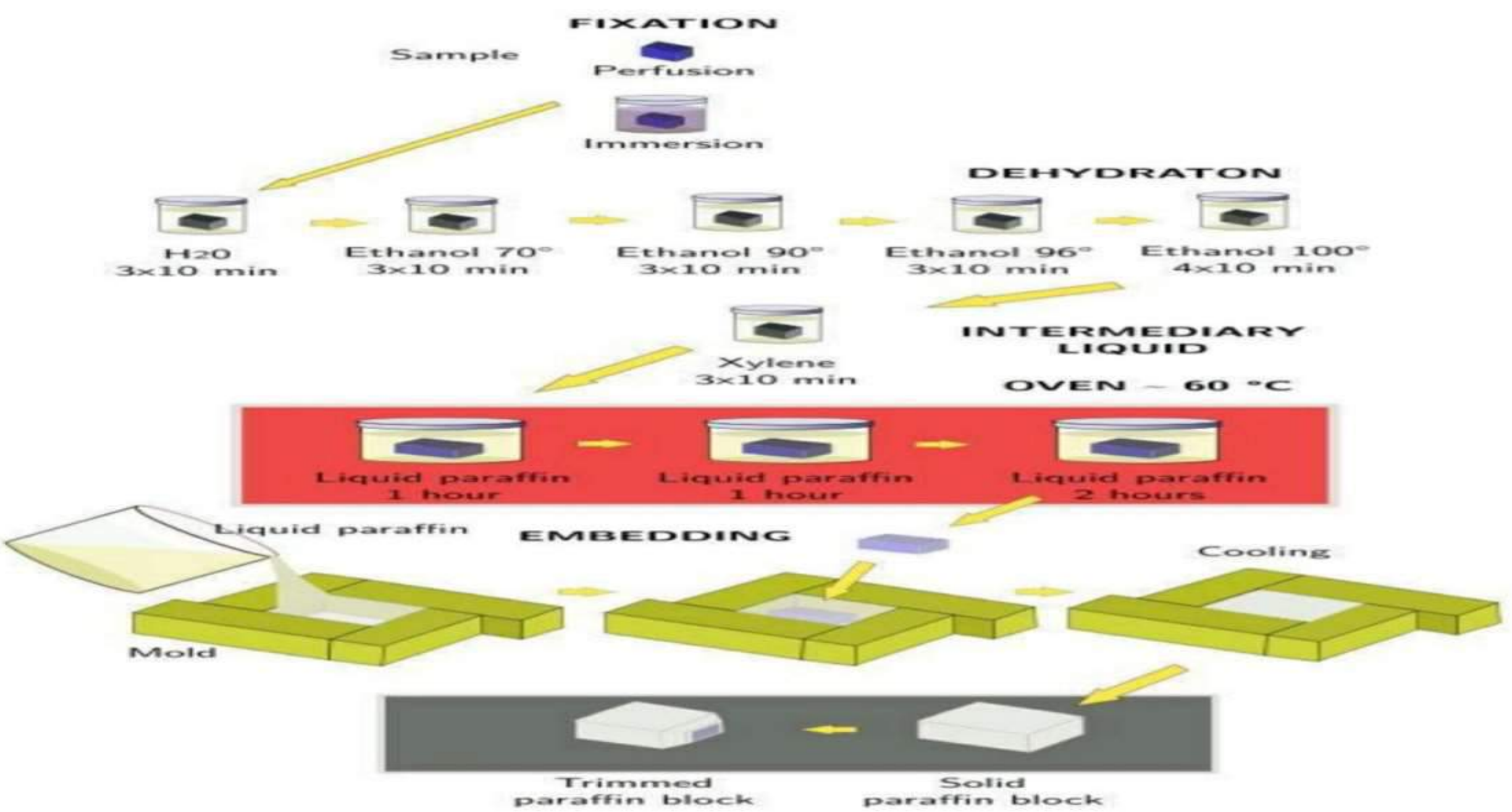
- Specimen receive and registration
- Grossing
- Fixation
- Processing
- embedding
- staining

Grossing

The specimen should be completely covered with the fixation fluid.

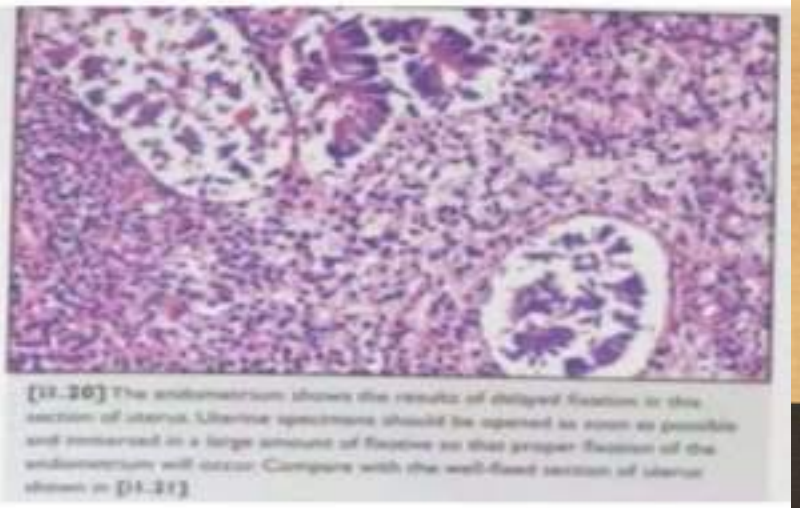
If not , change with 10 % formalin solution. Or other fixative according to your lab protocol.

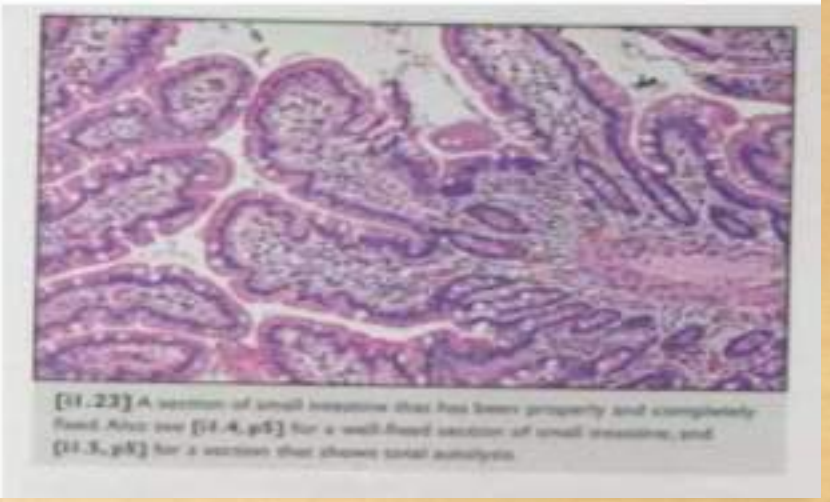
Hint:


FIXATION
Sample
Perfusion
Immersion
DEHYDRATON
H20
3x10 min
Ethanol 70°
3x10 min
Ethanol 90°
3x10 min
Ethanol 96°
3x10 min
Ethanol 100°
4x10 min
INTERMEDIARY LIQUID
Xylene
3x10 min
OVEN – 60 °C
Liquid paraffin
1 hour
Liquid paraffin
1 hour
Liquid paraffin
2 hours
Liquid paraffin
EMBEDDING
Cooling
Mold
Trimmed
paraffin block
Solid
paraffin block

Fixation

The major problems related to fixation are delayed or incomplete fixation
Autolysis is caused by delayed fixation •
.
In H& E section, the tissue may show • loss or total disappearance of nuclear chromatin. Some cells may disappear (intestinal mucosa), shrink and leave artifactual space around.
Prevention:•
add fixative to the specimen as soon as possible
Open uterus for endometrial fixation•
Open GIT specimen for mucosal • fixation
Slice solid organ eg breast kidney, • thyroid
Bisect lymph nodes•





Fixation - factors affecting fixation

There are a number of factors that will affect the fixation process:

- **Buffering**
- **Penetration**
- **Volume**
- **Temperature**
- **Concentration**
- **Time interval**

Problem: Tissue Shrinkage
Symptoms: Tissue samples appear smaller than expected or display irregular shapes.

Causes: Hyperosmolar fixatives or prolonged fixation.

Solution: Opt for isotonic fixatives when possible and limit the fixation time. If shrinkage has already occurred, try rehydrating the tissue with distilled water before further processing.

Problem: Tissue Swelling

Symptoms: Tissue samples appear swollen, and cell morphology is disrupted.

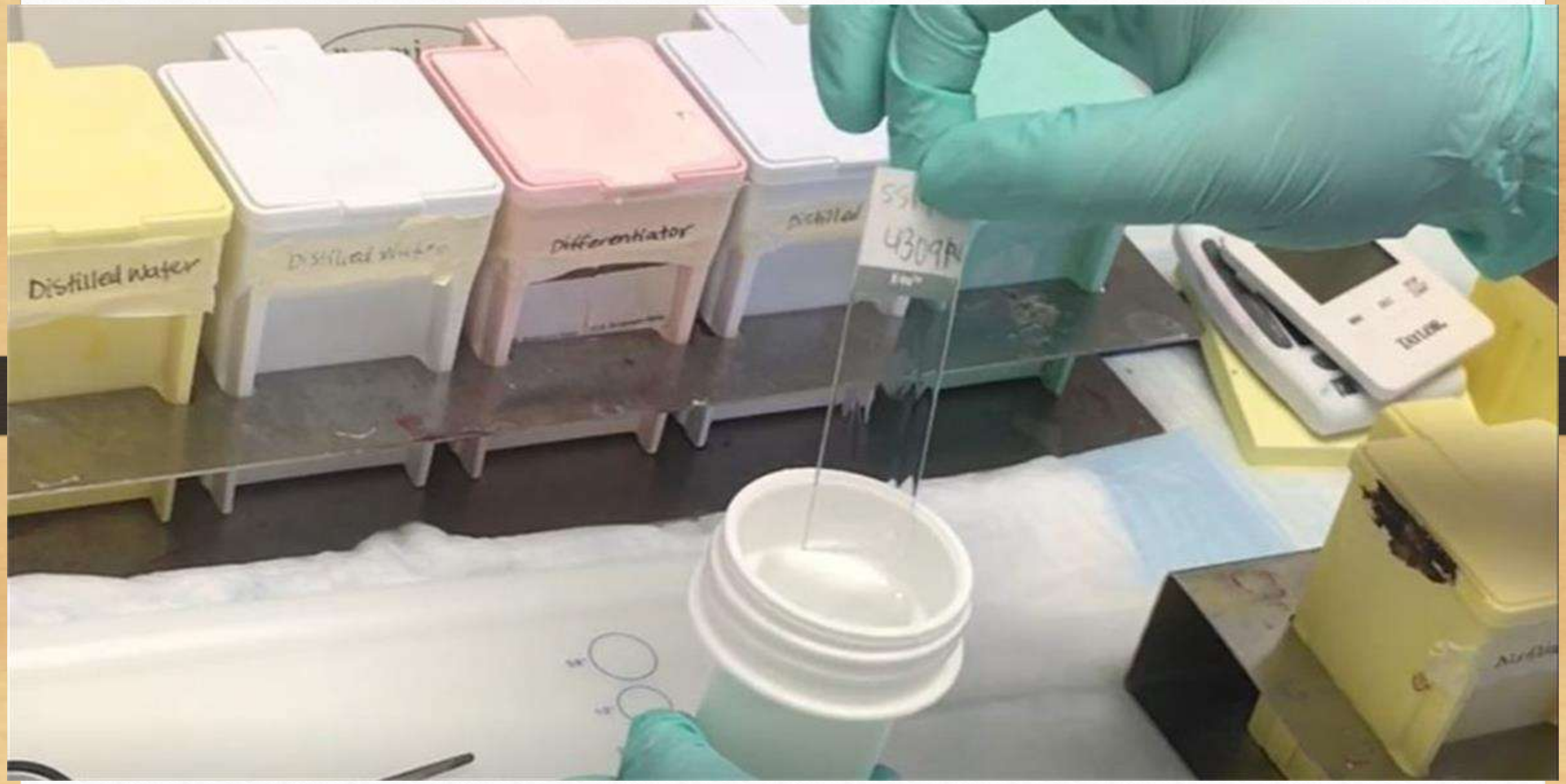
Causes: Hypotonic fixatives or overhydration during processing.

Solution: Use hypertonic fixatives to prevent tissue swelling. If you're dealing with swollen specimens, gently blot them on absorbent paper to reduce excess moisture.

Problem: Inadequate Penetration
Symptoms: Deep layers of tissue display poor fixation, resulting in artifacts.

Causes: Large or thick tissue specimens that weren't adequately trimmed, or rapid fixation methods.

Solution: For larger specimens, slice or trim them to facilitate fixative penetration. Also, ensure you're following the recommended protocols for fixing larger or thicker samples.


Distilled water
Differentiator

processing

Poor processing:

due to improper dehydration (water in tissue), impaired clearing, clearing agent in paraffin, too much heat during processing.

Prevention:

No cassettes condensation

Absolute alcohol is fresh, free of Water

Fluid are changed according to the schedule

Heat is used only for paraffin& E Stain.

processing

Most problem encountered in processing is related to either over processing or under processing

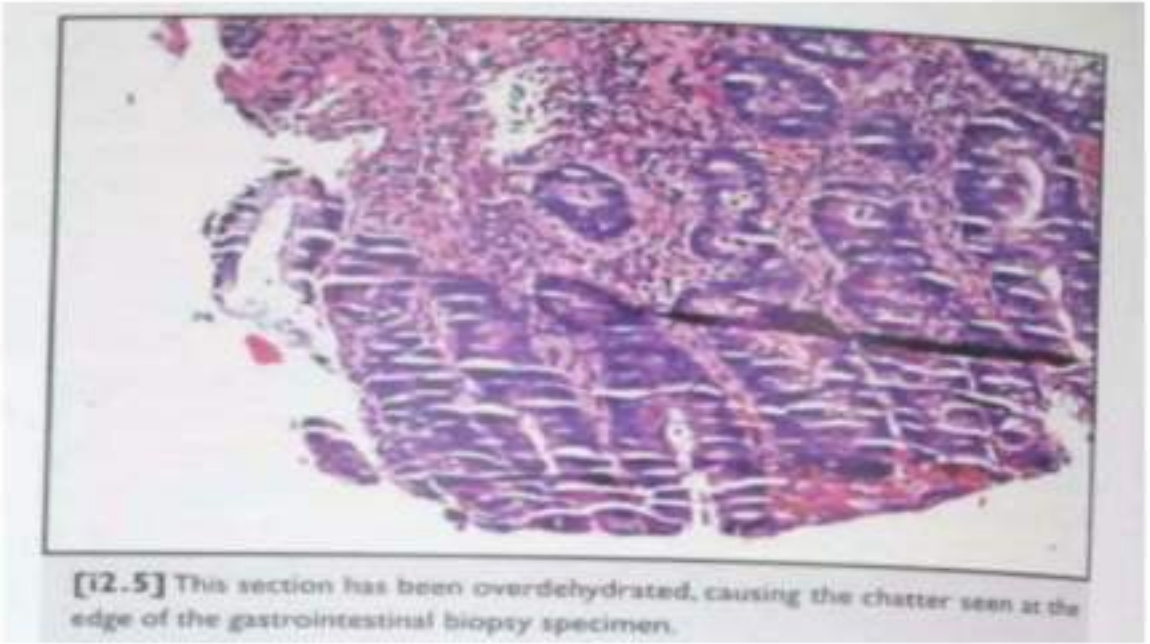
Overdehydration

Due to excessive dehydration, which results in microchatter around the edges of the tissue on H & Stain.

Prevention:

Small biopsies precessed separetely

Shorten the dehydration time



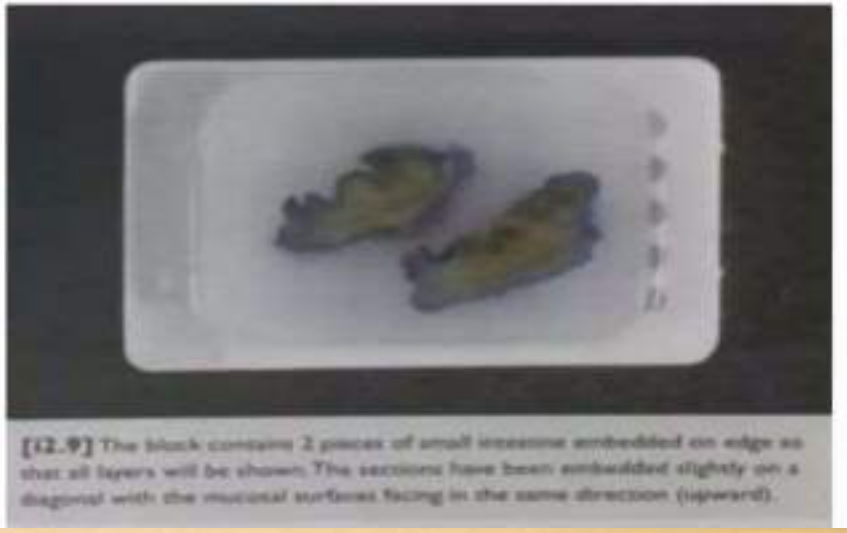
[i2.5] This section has been overdehydrated, causing the chatter seen at the edge of the gastrointestinal biopsy specimen.

Embedding and specimen orientation

Hints:

- Hard tissue such as bone, can will section more easily if they are embedded diagonally

- Tissue with wall, such as cyst, gall bladder, and GIT must be embedded on the edge.



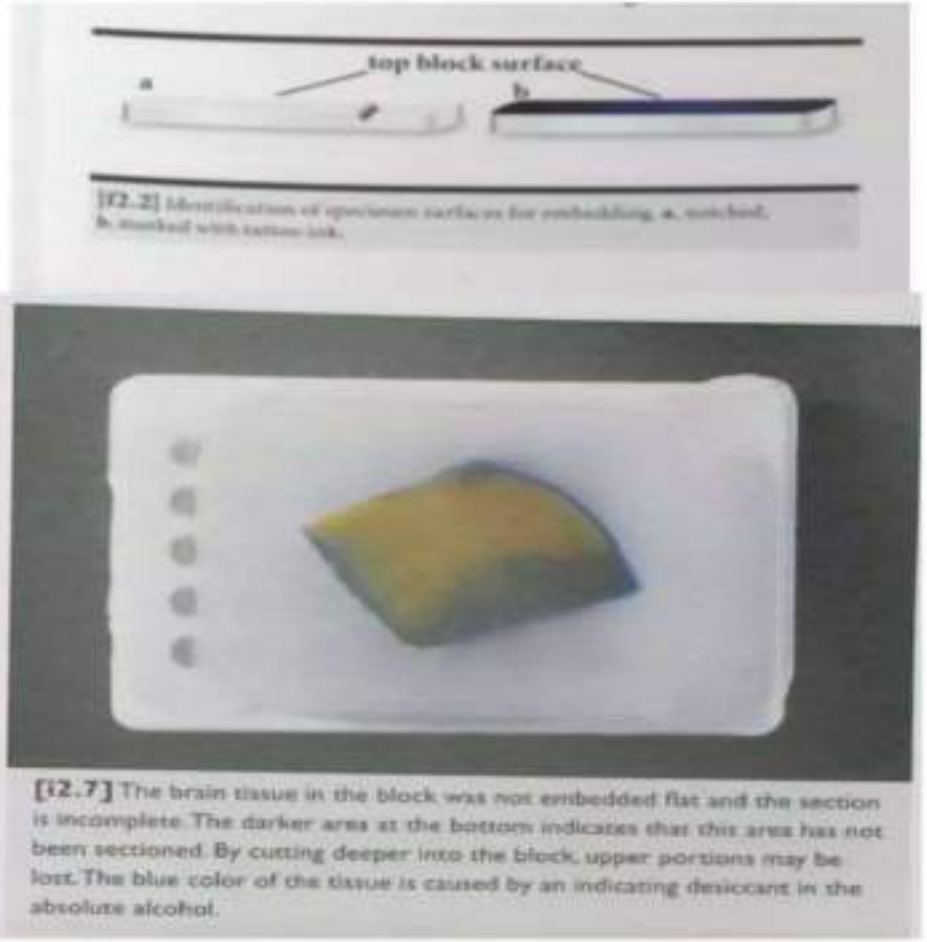
Embedding and specimen orientation

It refers to casting or blocking (paraffin blocking)

Hints:

Specimen orientation •

Proper pressure force applied to the entire specimen during orientation and initial solidification to obtain flat tissue



[12.2] Identification of specimen surfaces for embedding. a. notched. b. marked with tattoo ink.

[12.7] The brain tissue in the block was not embedded flat and the section is incomplete. The darker area at the bottom indicates that this area has not been sectioned. By cutting deeper into the block, upper portions may be lost. The blue color of the tissue is caused by an indicating desiccant in the absolute alcohol.

Improper embedding causes air pockets to appear in the paraffin wax mold as white dots, this phenomenon is called **Crystallization**. The air can be removed by melting the top of the mold with a slightly hot tool. Crystallized molds have problems when cutting and are treated by embedding. paraffin molds can be stored in a cool place for a long time

Troubleshooting in Embedding and specimen orientation

Soft Mushy Tissue

- The most common cause is thick sections at gross examination and have been compressed between the top and bottom of the cassette.

Prevention:

- The tissue section should be thin
- Adequate time for fixation
- Fluid change according to schedule

Incorrect orientation

- Section may be incorrectly oriented at the embedding station if the correct method was not indicated.

Prevention:

- Marking the side of tissue to be embedded facing up with ink

Troubleshooting in Embedding and specimen orientation

Tissue carryover

- Small pieces or fragments of tissue may be carried from 1 tissue to the next at the embedding table, resulting in cross-contamination

Prevention:

- Carefully clean forceps used at the embedding table between specimens
- Open only the cassette with the tissue to be embedded

Tissue not embedded at the same level

- If the tissue is not properly flattened by pressing it down uniformly when it is placed in the embedding mold, or multiple tissues to be palced in the same mold are embedded at different levels

Prevention:

- Press the tissee uniformly
- Keep the paraffin molten enough to get all pieces embedded at the same level.
- Work very fast when embedding multiple pieces

Troubleshooting in microtomy

Block face unevenly sectioned

- Occur when the block holder is not parallel to the blade. One side of the block is exhausted while attempting to get a complete section of the block face

Prevention:

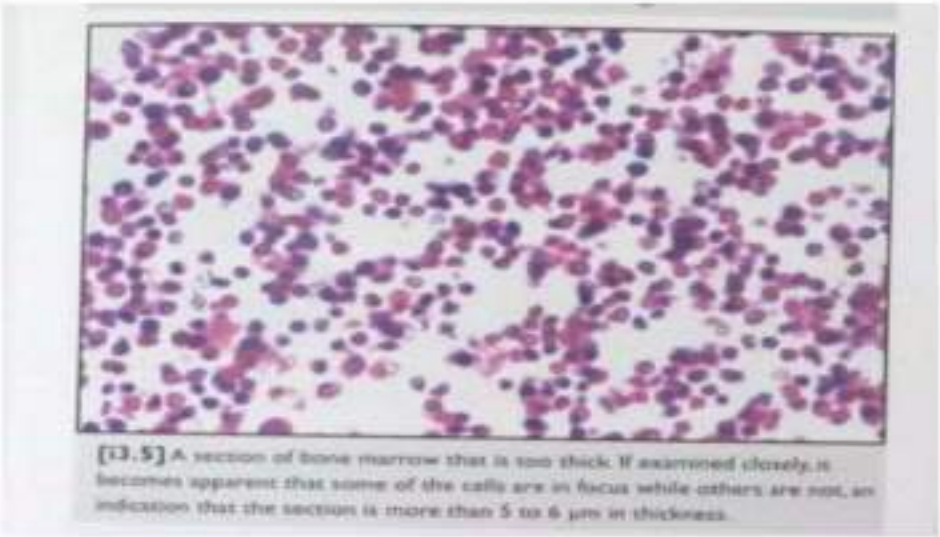
- Ensure at the beginning of the sectioning that the block holder is adjusted so that the block face and the blade are perfectly parallel.

Troubleshooting in microtomy

Thick section

Prevention: •

Adjust the thickness •



[13.5] A section of bone marrow that is too thick. If examined closely, it becomes apparent that some of the cells are in focus while others are not, an indication that the section is more than 5 to 6 μm in thickness.

Problem: Tissue Adhesion
Symptoms: Sections stick to the microtome blade or slide, causing damage.

Causes: Over fixation, suboptimal tissue processing, or poor sectioning technique.

Solution: Reduce fixation time and use adequate dehydration and clearing steps during processing. Ensure your microtome and blade are clean and well-maintained. Proper sectioning technique is key to avoiding tissue adhesion.

Problem: Artifact Formation
Symptoms: Unintended distortions or imperfections in tissue morphology.

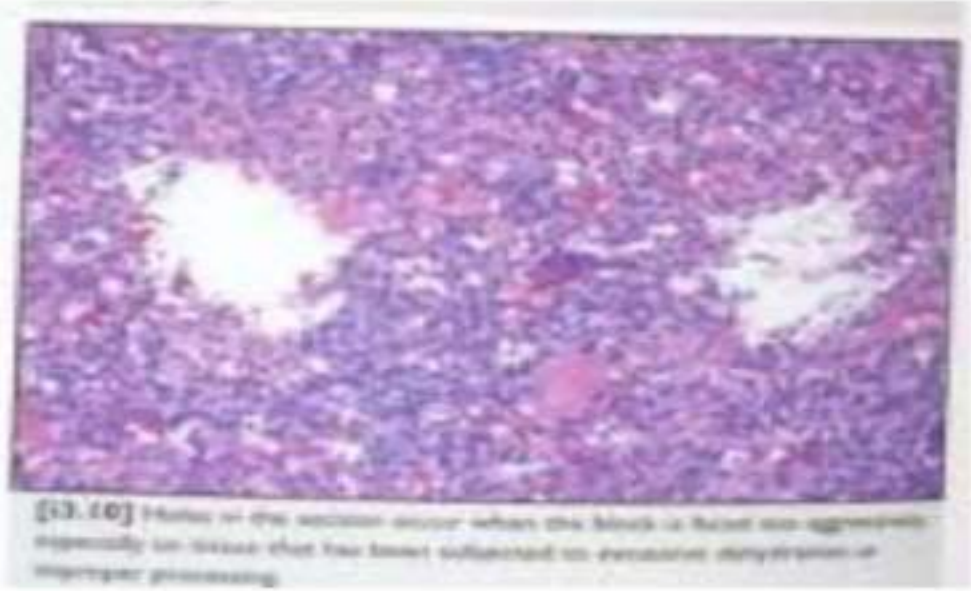
Causes: Overhandling, poor sectioning technique, or contamination during fixation.

Solution: Handle tissues with care, use a well-maintained microtome, and practice good laboratory hygiene. Contamination can often be avoided with clean equipment and meticulous sample preparation.

Troubleshooting in microtomy

Holes in the section

- Occur when block is faced too aggressively.
- The specimen is either excessively dehydrated or improperly processed.
- Prevention:
- Ensure to chill the block with ice before cutting and discard ribbons until the hole disappear
- Facing the block less aggressively, with smaller micrometer advances of the block for eaah section removed



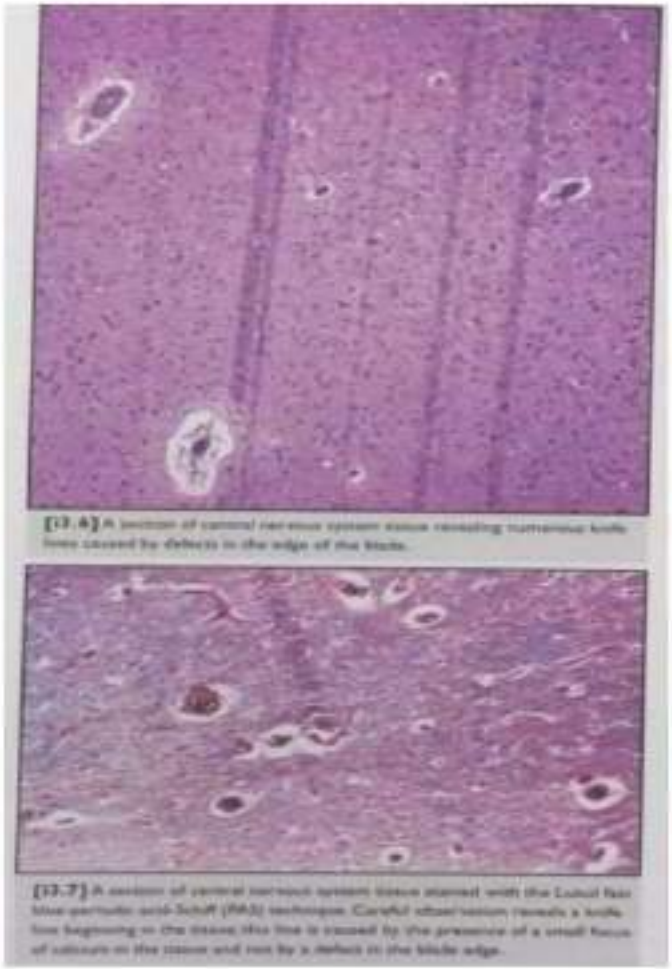
Troubleshooting in microtomy

Vertical scratches

- Caused by defect in the blade edge, calcium, bone or hard material in the specimen

Prevention:

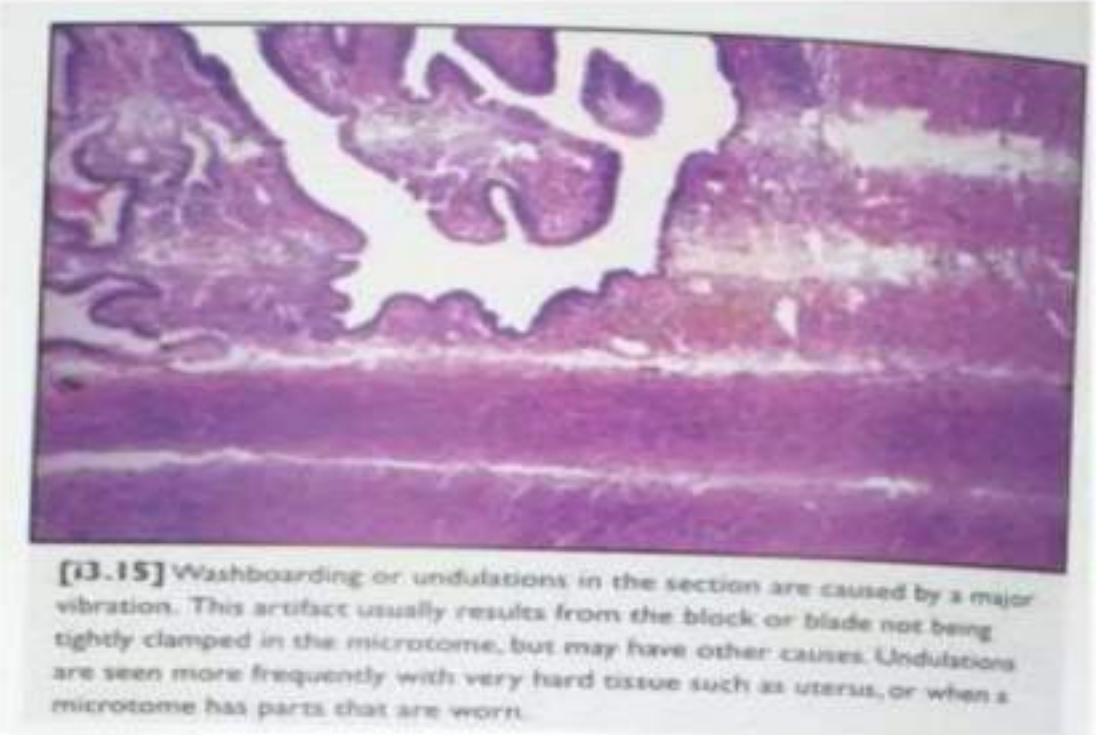
- Ensure at the beginning of the sectioning that the block holder is adjusted so that the block face and the blade are perfectly parallel.



Troubleshooting in microtomy

Washboarding or Undulation in the section

- Commonly occurs in very hard tissue such as uerus or in over fixed tissue.
- It is the macroscopic type of chatter commonly caused by loose clamping of blade or block.
- Prevention:
- Proper clamping of blade and block
- Ensure the block holder shaft is not over extended
- Ensure the microtome is in good working order
- Decrease the blade tilt



[13.15] Washboarding or undulations in the section are caused by a major vibration. This artifact usually results from the block or blade not being tightly clamped in the microtome, but may have other causes. Undulations are seen more frequently with very hard tissue such as uterus, or when a microtome has parts that are worn.

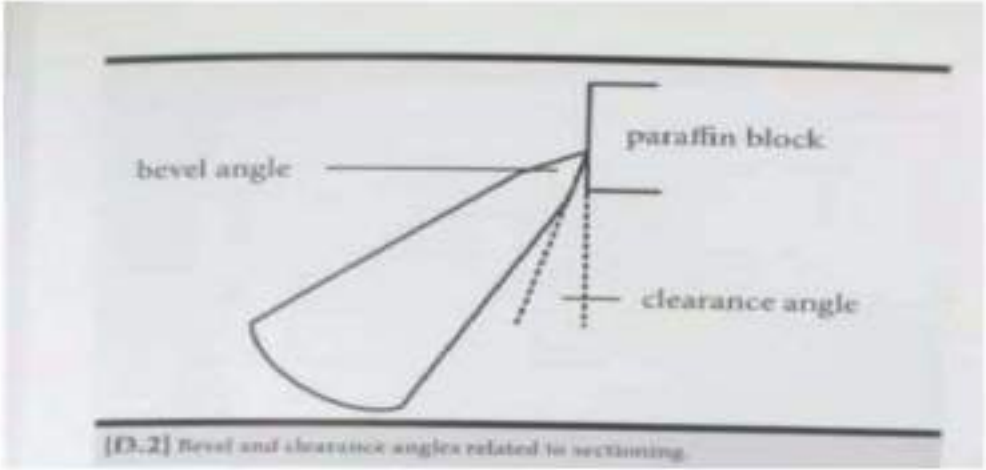
Troubleshooting in microtomy

Failure of ribbon to form

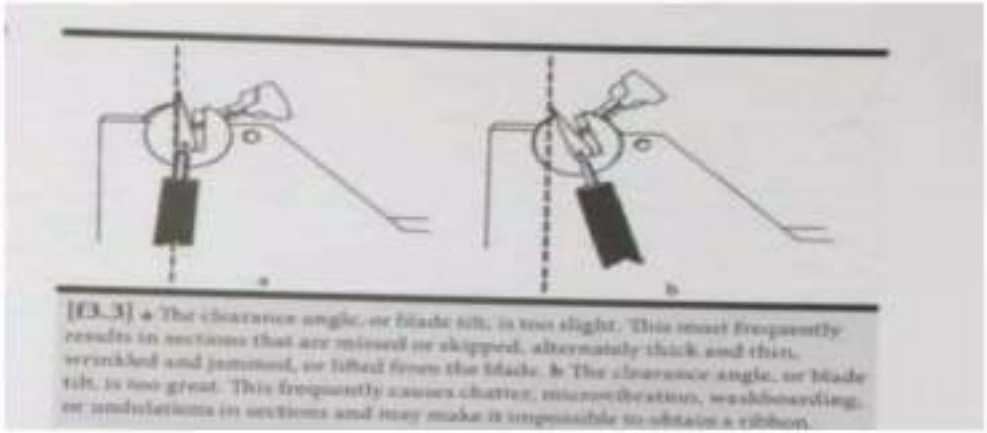
- Commonly caused by dull blade. Could result from too hard paraffin, too much blade tilt

Prevention:

- Paraffin with lower melting point
- Decrease blade tilt
- Change room temperature



[D.2] Bevel and clearance angles related to sectioning.



[D.3] a The clearance angle, or blade tilt, is too slight. This most frequently results in sections that are missed or skipped, alternately thick and thin, wrinkled and jammed, or lifted from the blade. b The clearance angle, or blade tilt, is too great. This frequently causes chatter, microcleavage, washboarding, or undulations in sections and may make it impossible to obtain a ribbon.

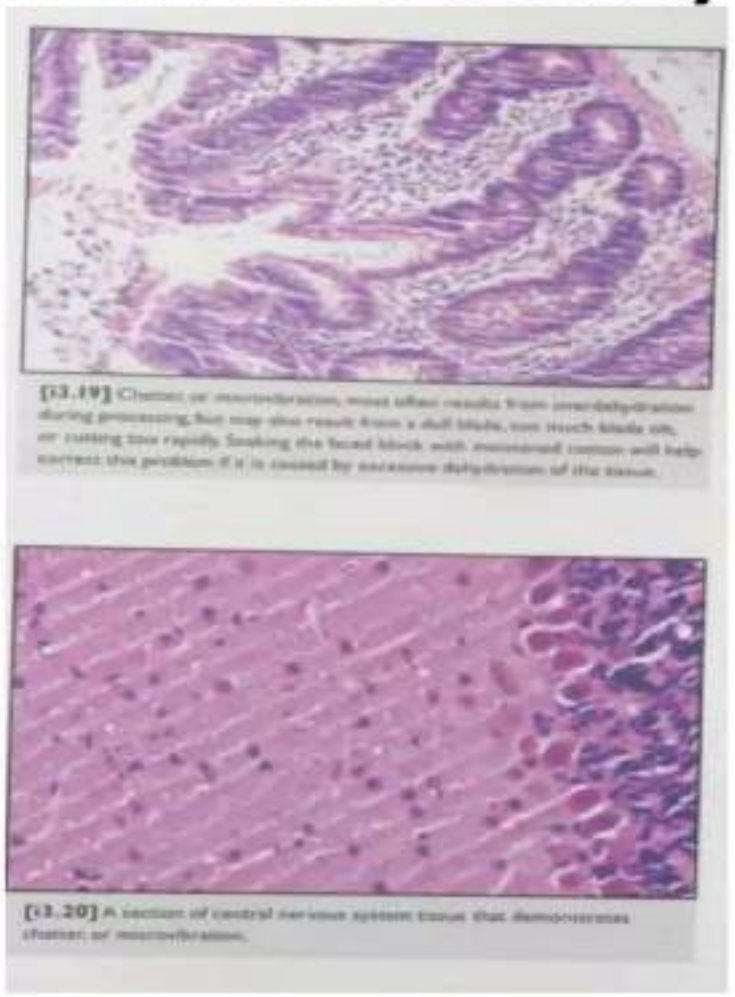
Troubleshooting in microtomy

Chatter or microscopic Vibration

Commonly caused by over dehydration or lack of moisture in the tissue. It could also result from dull blade or too much blade tilt which cause the section to be scrabed rather than cut. Or cutting too rapidly.

Prevention:

- Proper processing
- Restore moisture by facing the block down on an ice tray
- Decrease the blade tilt
- Decrease cutting speed: one wheel per second is considered reasonable speed.



Troubleshooting in H& E staining

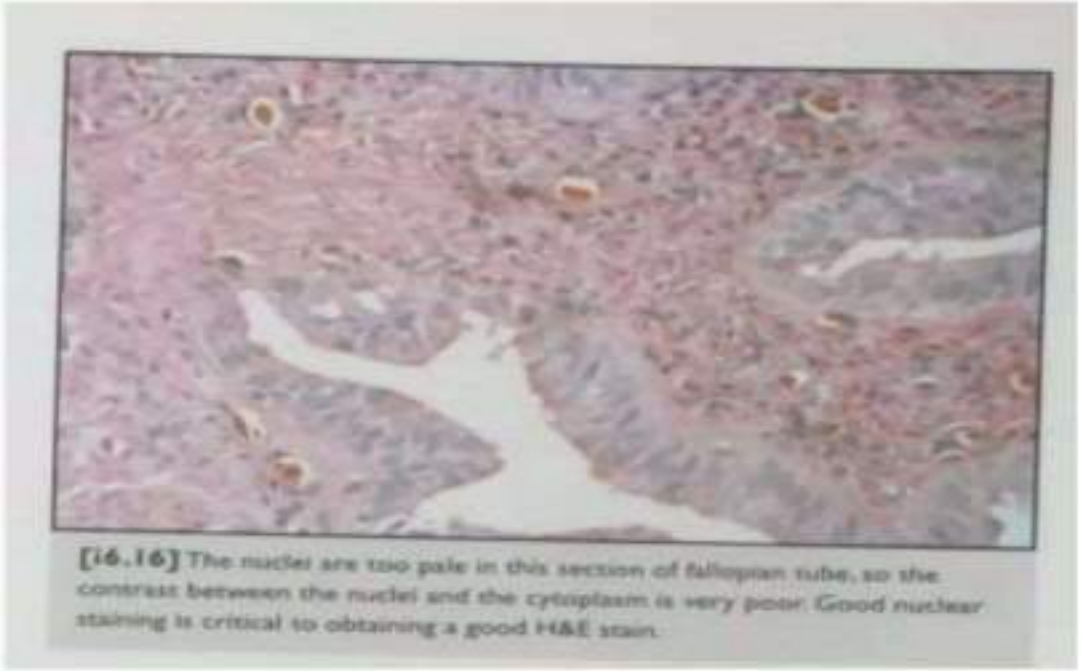
Pale nuclear staining

The Nuclei is too light due to :

- Slide not exposed to the hematoxylin long enough
- Exhausted (over oxidized or depleted) hematoxylin
- Over differentiation

Prevention:

- Leave the slide longer
- Use fresh hematoxylin
- Time the differentiation



[16.16] The nuclei are too pale in this section of fallopian tube, so the contrast between the nuclei and the cytoplasm is very poor. Good nuclear staining is critical to obtaining a good H&E stain.

Troubleshooting in H& E staining

Red or red-brown Nuclei

If the nuclei is stained red or reddish brown instead of blue, either the hematoxylin is breaking down or the blueing step was not properly done

- Prevention:
- New hematoxylin
- Prper blueing

[16.18] The nuclei are reddish-brown in this H&E-stained section of fallopian tube, indicating that the hematoxylin was not blued sufficiently or that the hematoxylin is breaking down (overoxidized).

Troubleshooting in H& E staining

Dark nuclear staining

The nuclei is too dark due to :

- Slide exposed to the hematoxylin too long
- Section are too thick
- Differentiation step is too short

Prevention:

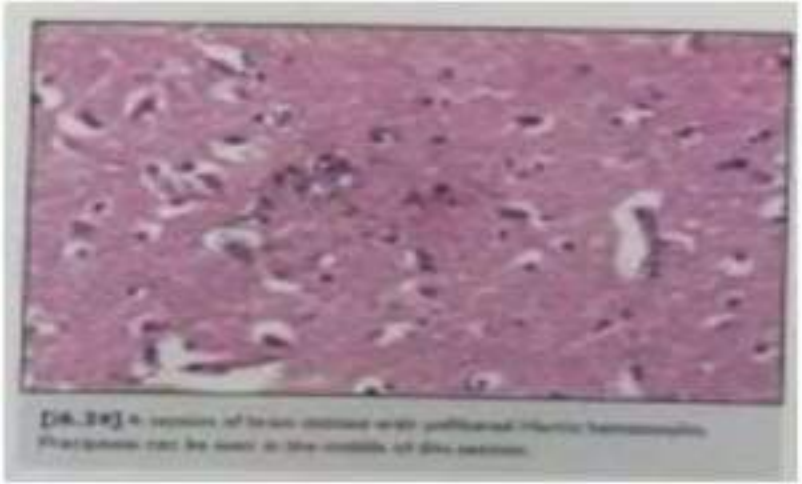
- Thin sections
- Decrease hematoxylin exposure
- Increase time for differentiation

[16.17] The nuclei are overstained in this section of skin, so that nuclear detail is lost. The cytoplasm, especially in the epithelium, contains some hematoxylin staining, and the contrast between the nuclei and cytoplasm is poor.

Troubleshooting in H& E staining

Blue black precipitate

- Hematoxylin precipetate
- Prevention:
- Filter the hematoxylin



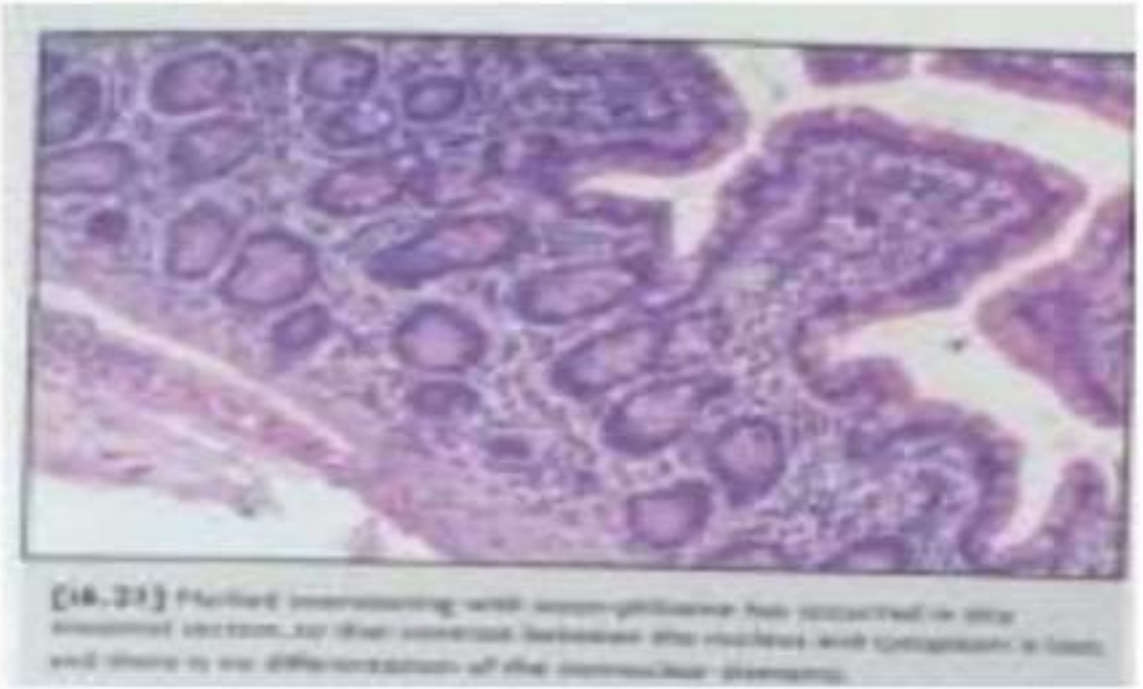
Troubleshooting in H& E staining

Dark Cytoplasmic staining

- If the cytoplasm is overstained, and the differentiation is poor, the contrast between the nucleus and the cytoplasm is lost

Prevention:

- Avoid overconcentration of eosin, dilute eosin solution
- Do not leave sections in eosin for long
- Allow sufficient time in dehydration solution, specially 70% alcohol, to allow good eosin differentiation
- Check section for proper thickness



Thank you