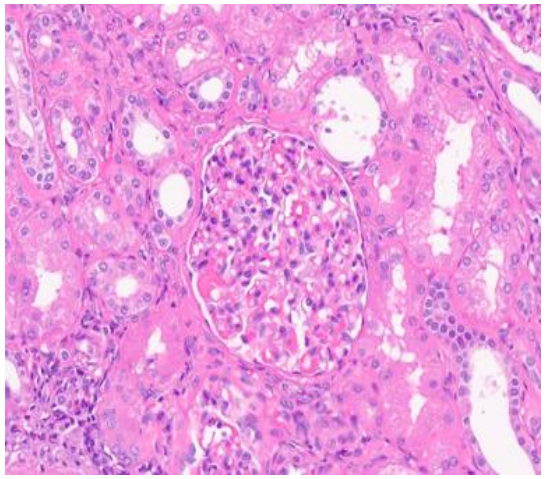
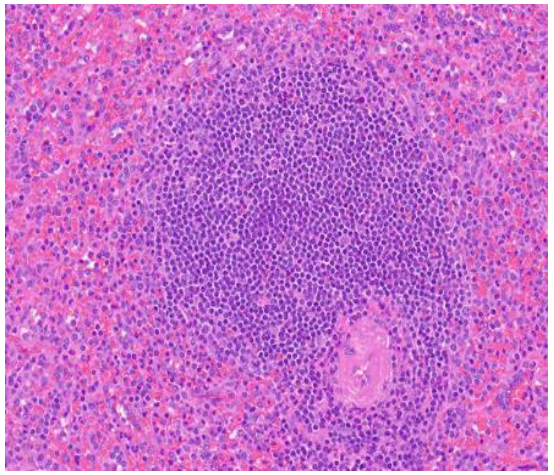
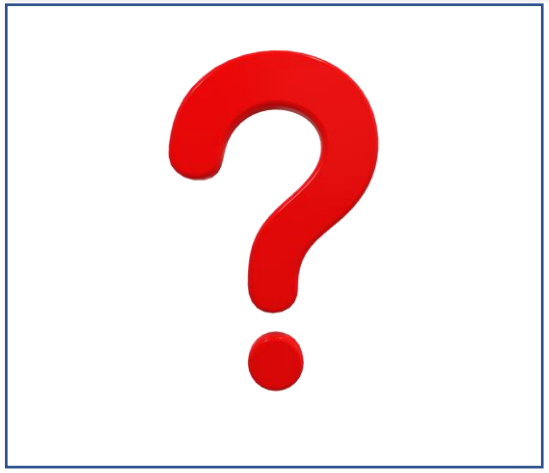


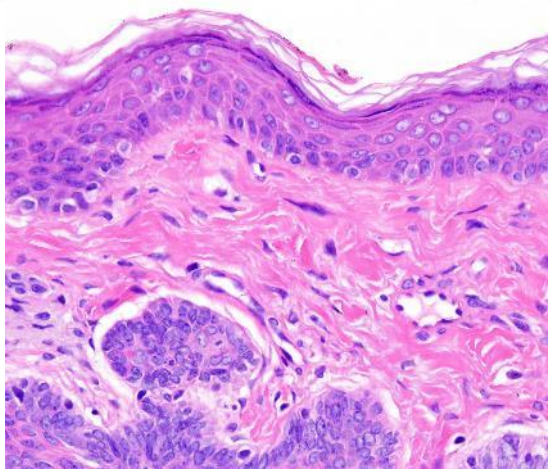


Tissue Processing

Frequently Asked Questions

Andrew R. Lisowski
Sr. Technical Content Global Marketing Manager





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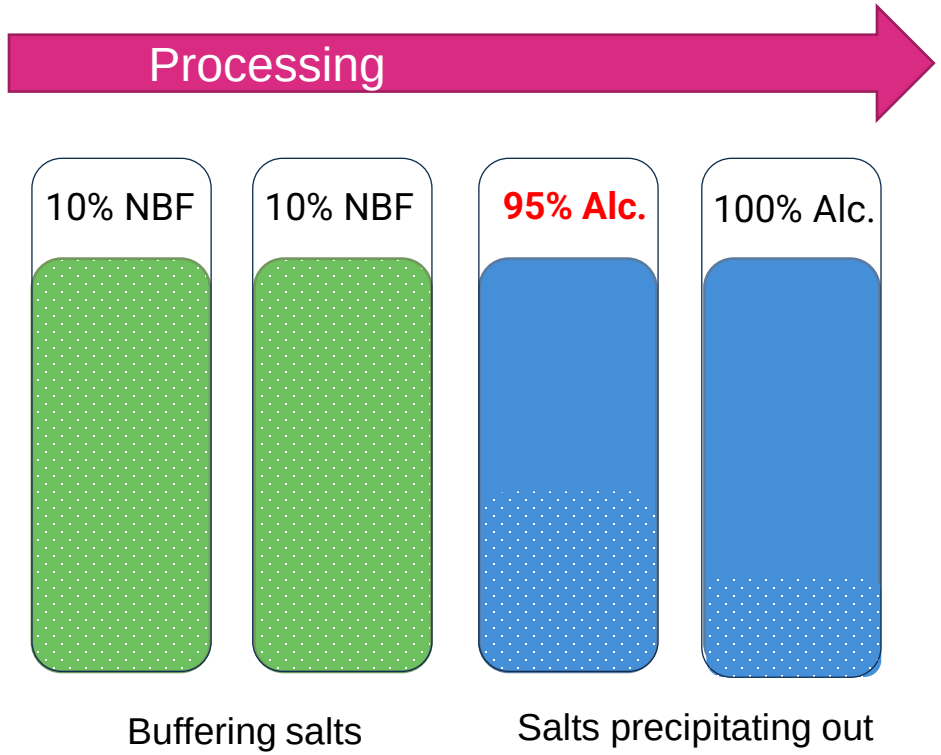
Enhancing precision cancer diagnostics

Top 7 Frequently Asked Questions

1. What is the precipitate in the processor chamber and what's causing it?
2. What is DMSO and why is it in paraffins?
3. How should I process fatty tissues?
4. How does heat affect processing?
5. How can I recover an under-processed specimens?
6. How can I recover over-processed specimens?
7. Paraffin in my paraffin tank is cloudy. What's causing it?
8. I am melting my paraffin off-line and accidently heated paraffin to close to 90˚C. Did I ruin the batch?
9. What are the carry-effect of biopsy bags/pads?

1. What is the precipitate in the processor chamber and what's causing it?

- Laboratory personnel have noted that the chamber and reagent bottles sometimes contain a **white precipitate**. If they are using buffered formalin, the precipitation is most likely due to the **formation of formalin salts** (sodium phosphate salts).



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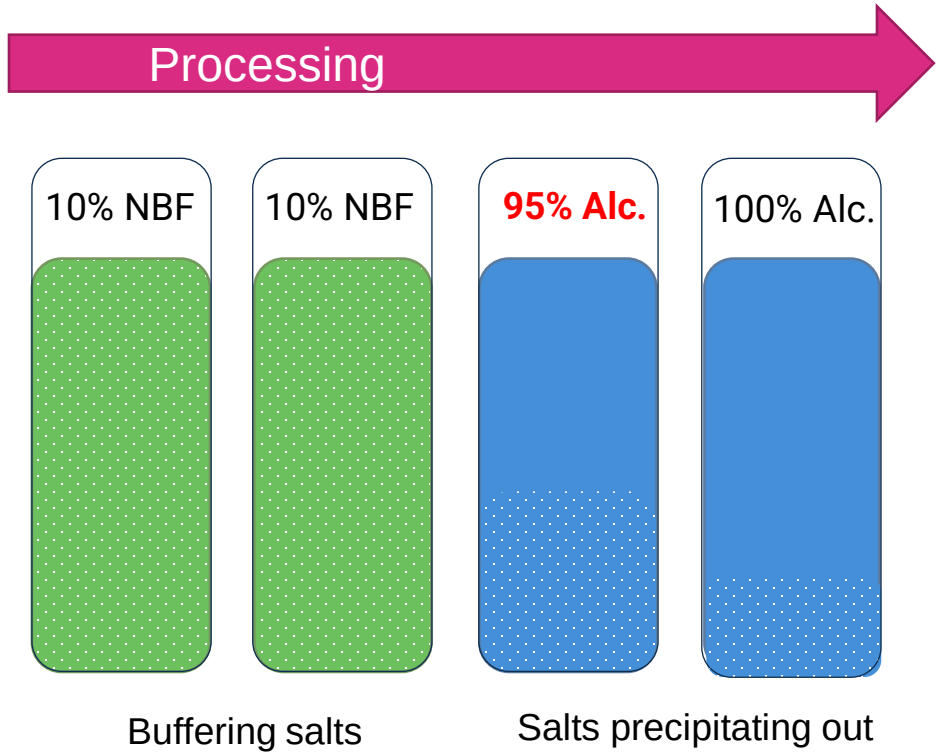
- Sodium salts are added to formalin, which is an aldehyde, to prevent the aldehyde from changing to acid over time. Acidic pH may damage tissue proteins and may create formalin pigments.
- Most NBFs use a combination of two salts, sodium phosphate monobasic and sodium phosphate dibasic.
- These **salts are soluble in water**, and the solubility of these salts decreases as the concentration of the alcohol increases.



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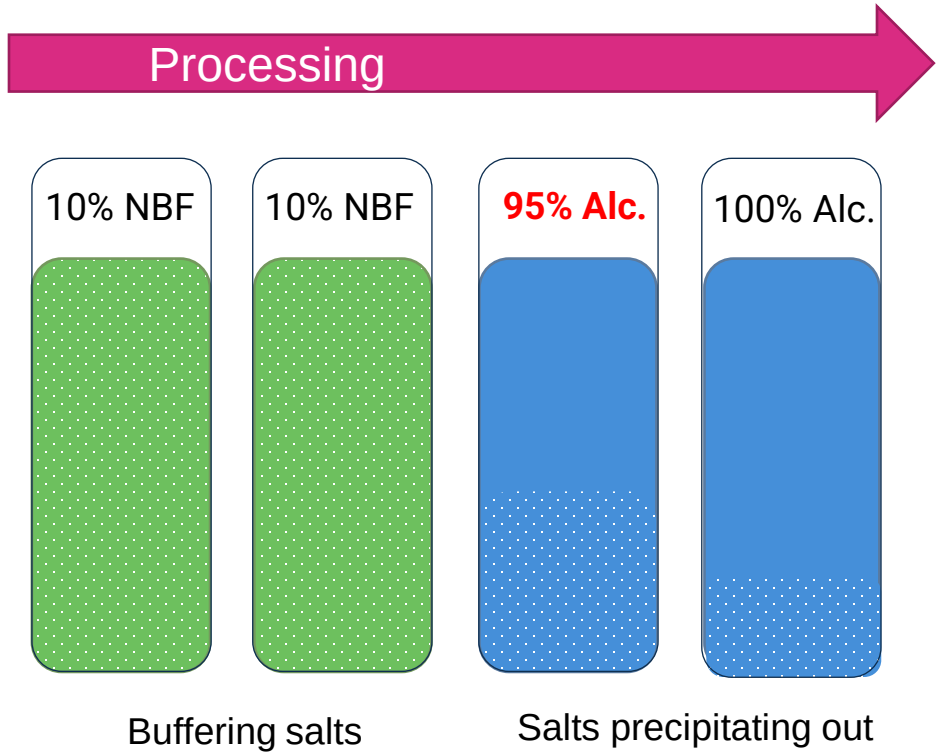
- Precipitation occurs when buffered formalin is used for fixation and then dehydration starts at an alcohol concentration **higher than 70%.**



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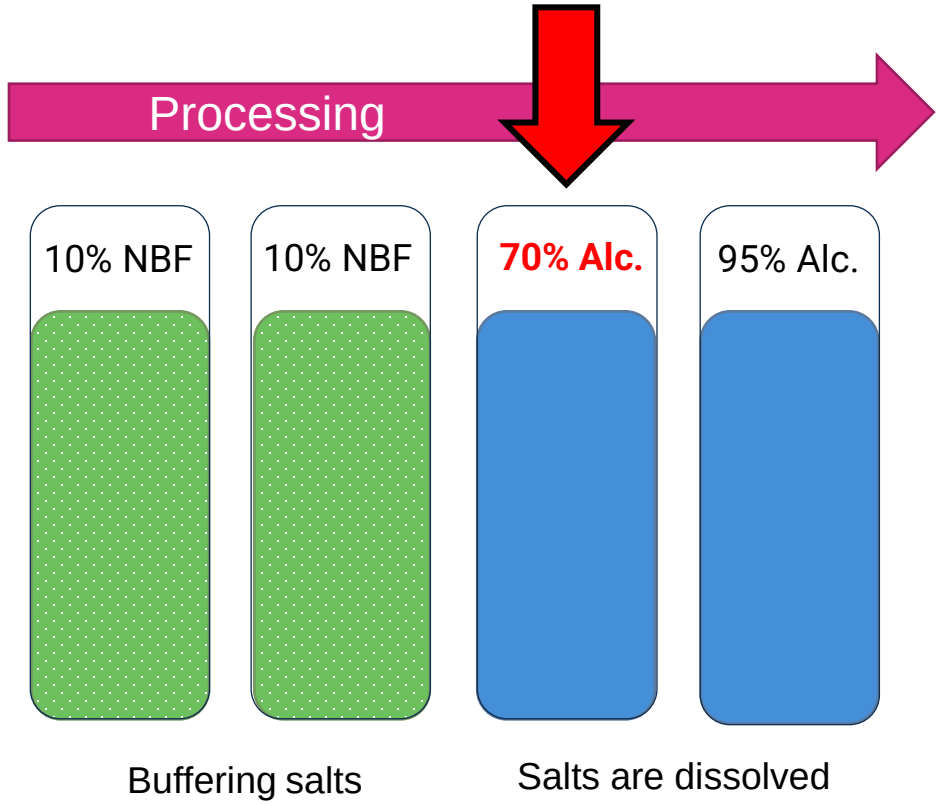
This can be prevented or corrected by beginning dehydration with 60-70% alcohol.

.

- If there are two formalin bottles on the processor, you can optionally change the second one to **alcoholic formalin**:
  - There is enough water to dissolve the buffering salts
  - There is enough formalin to continue with fixation
  - There is a high enough percentage of alcohol to begin the dehydration step
- Remove precipitate by rinsing the chamber with a diluted solution of acetic acid (5% - 20%).



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POLL QUESTION #1

What is the difference between processing paraffins and embedding paraffins?

A. **Melting point**

B. **Only embedding paraffins can contain color dye**

C. **Presence of DMSO**

D. **Embedding paraffins infiltrate tissues slower but more thorough**

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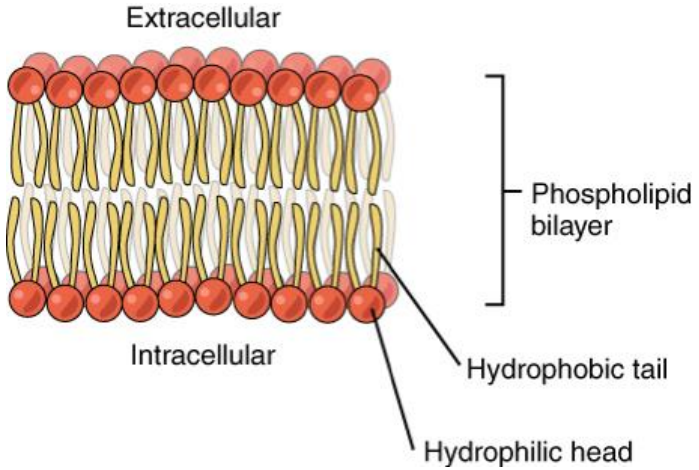
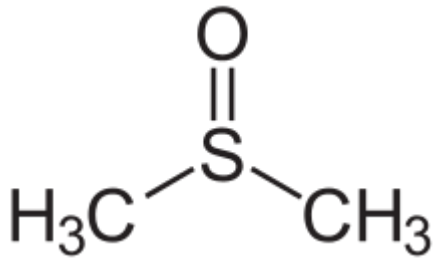
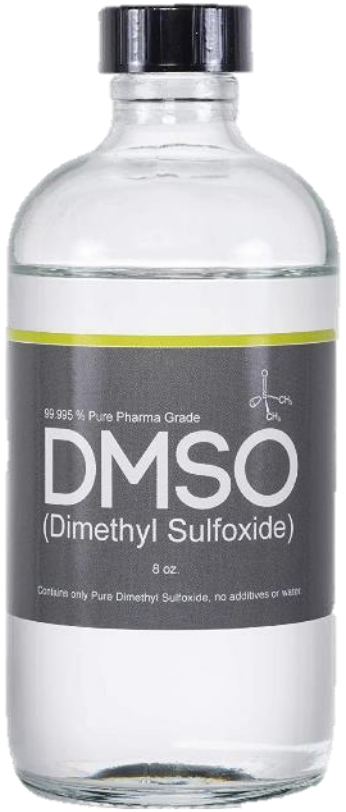
2. What is DMSO and why is it in paraffins?

- The story of dimethyl sulfoxide (DMSO) is an unusual one. This by-product of the paper making process was discovered in Germany in the late 19th century. It's a colorless liquid that gained notoriety for its **ability to penetrate** the skin and other biological membranes.







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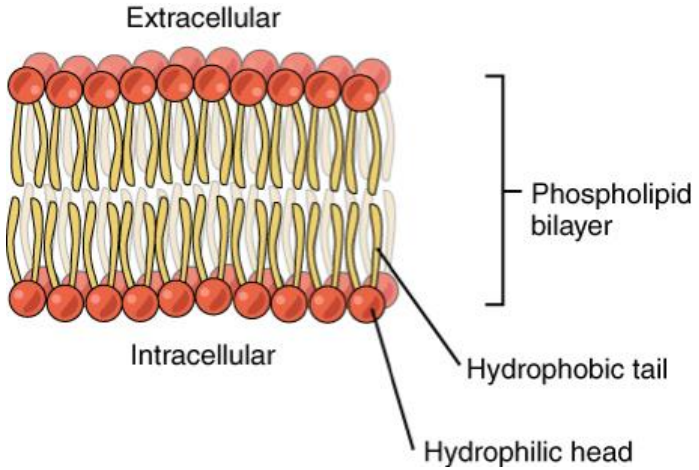
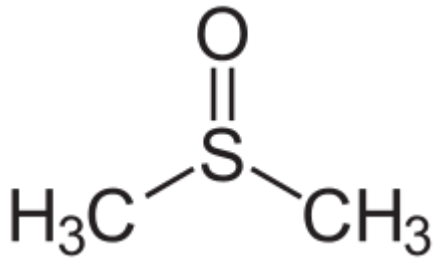
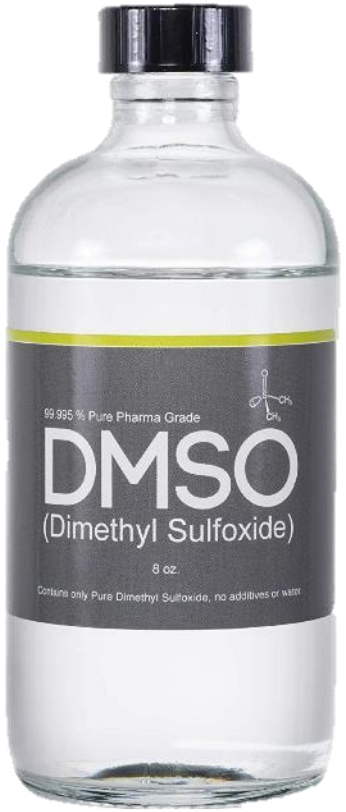
- Scientists discovered that they could use DMSO as a transportation device to pass small molecules through skin in the 1960s. Since then, scientists have researched the potential benefits and risks of using DMSO to treat a variety of conditions. This research is ongoing.







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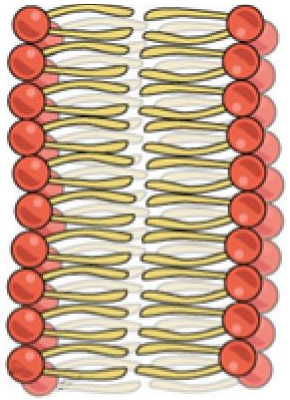
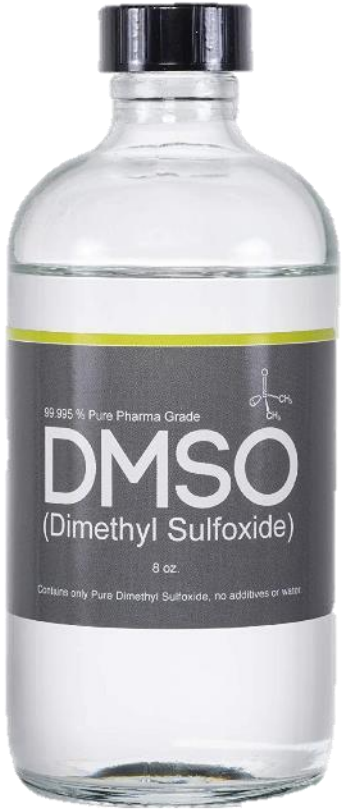
- Dimethyl sulfoxide (DMSO) has been broadly used in biology as an enhancer of membrane permeability.
- Although the effects of DMSO on the membrane structure have been extensively studied, the precise mechanism by which DMSO invokes its effect on lipid membranes (cell walls) is unsolved.



Cell wall



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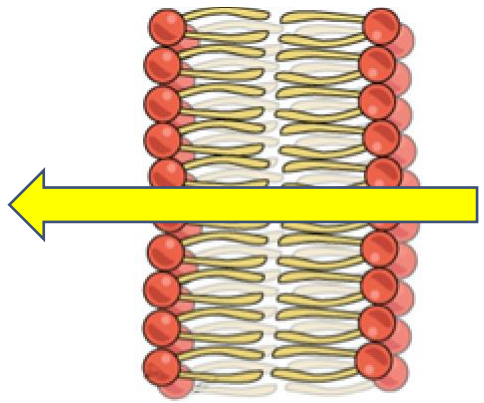

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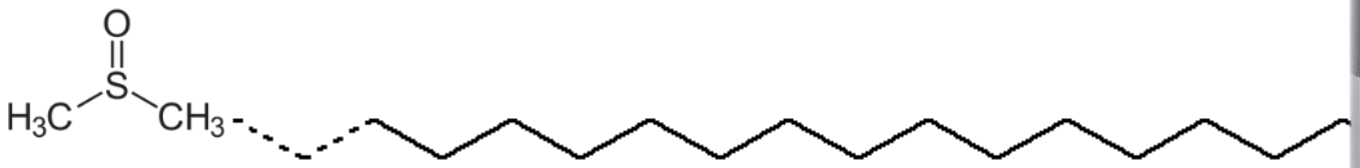
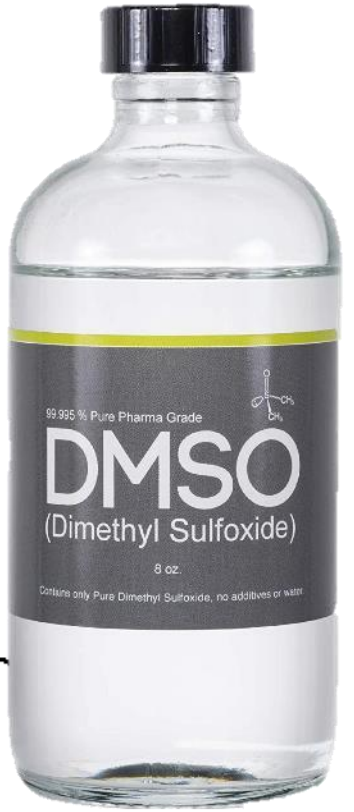


Wax carbohydrate attached to DMSO



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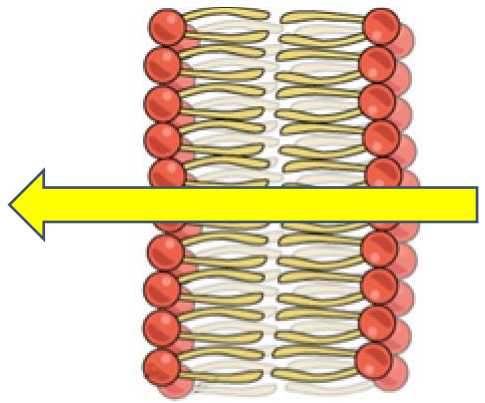
- Thanks to unique penetrating ability **DMSO increases the rate of penetration of the paraffin**.
- DMSO carries paraffin across the cell membrane in less time for more complete penetration.



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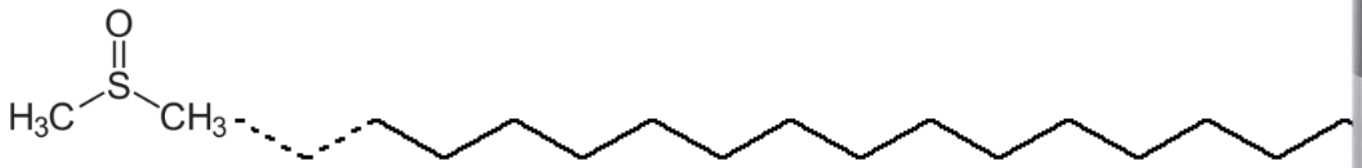

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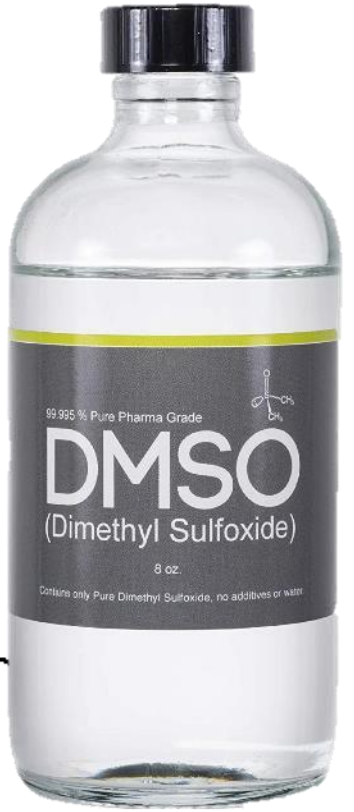

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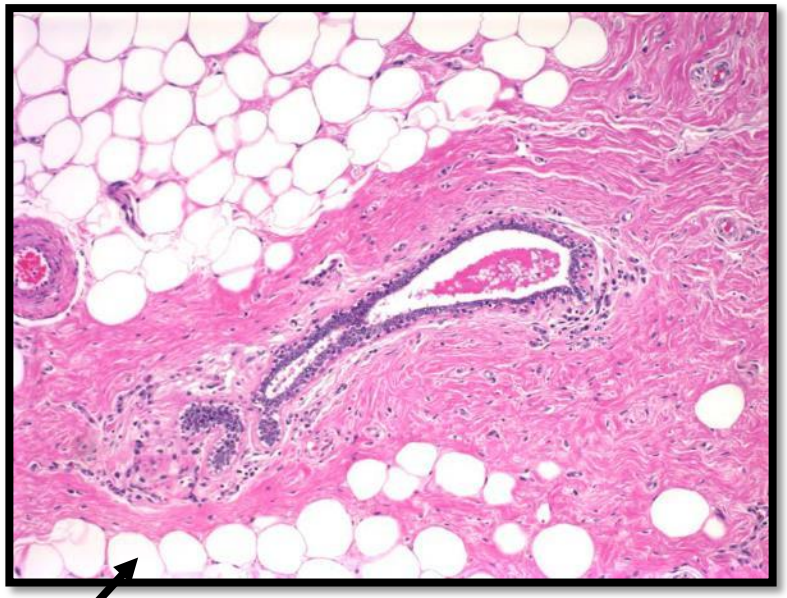
3. How to process fatty tissues?

Fat is fundamentally hard to penetrate. Whenever processing fails to remove lipids from the specimen, these areas will not be properly infiltrated with paraffin and, therefore, impossible to section.



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Breast specimen,
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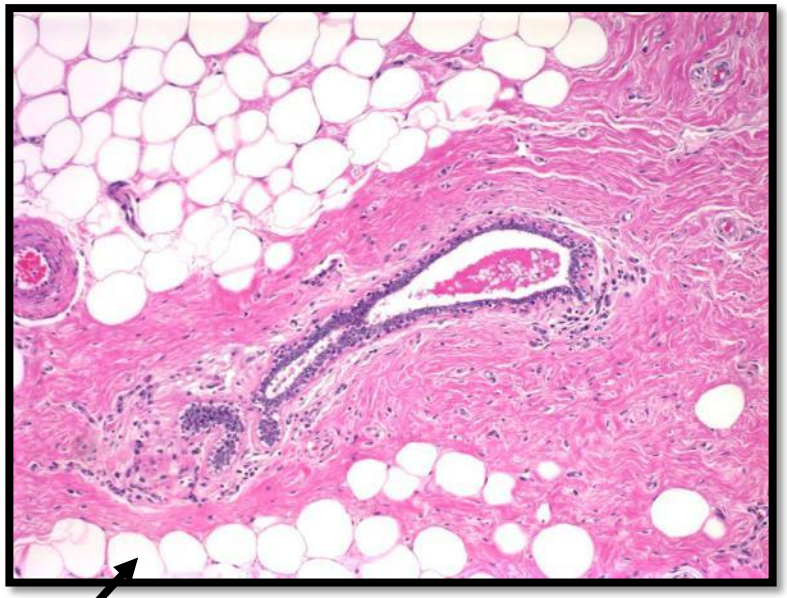

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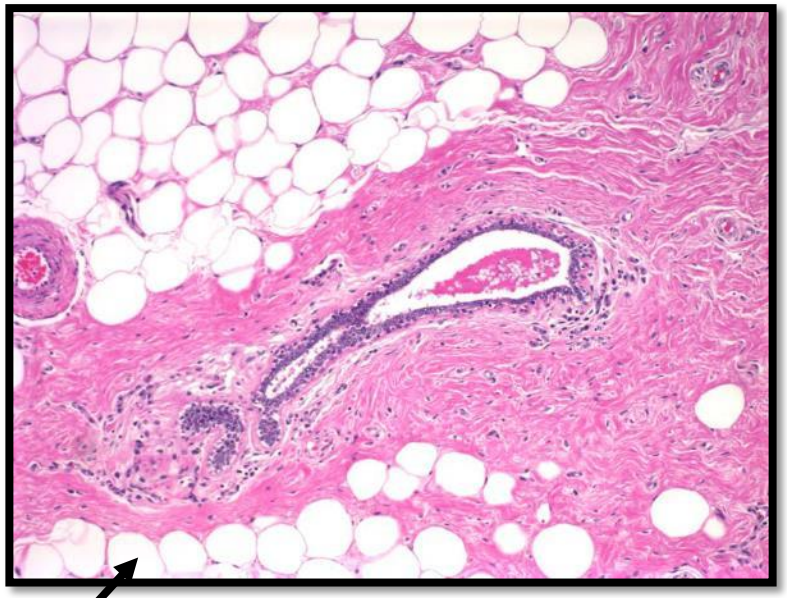

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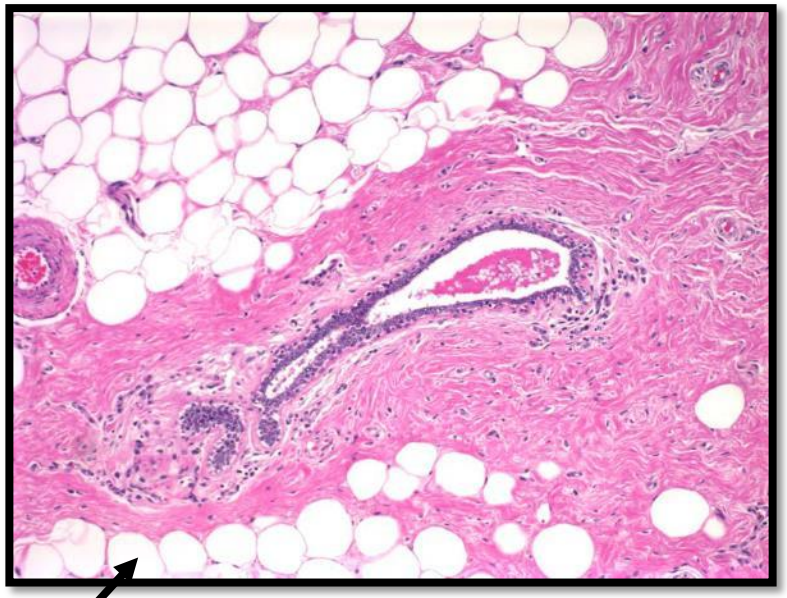

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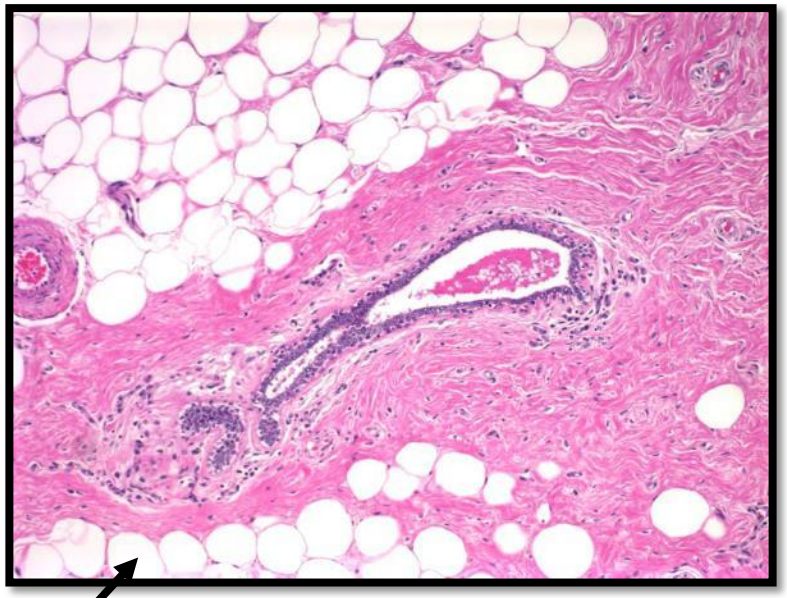

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4. Score the tissue to allow fixative to penetrate the tissue. Alternately, staple tissue to a cork during fixation to minimize folding and ensure fixative accessibility

3. How should I process fatty tissues?

Step	Reagent	Time (h:m)
1	10% Formalin	0:10
2	10% Formalin	0:10
3	Alcohol 75%	0:30
4	Alcohol 90%	0:30
5	Alcohol 95%	0:40
6	Alcohol 100%	0:30
7	Alcohol 100%	0:30
8	Alcohol 100%	0:40
9	Xylene	0:30
10	Xylene	0:30
11	Xylene	0:40
12	Paraffin	0:30
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14	Paraffin	0:40

Protocol Example

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Formaldehyde (37-40%) --------- 100 mL
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7. Use Infiltrating type paraffin (with little to no polymer).
8. Thick, fatty specimens may require a longer protocol.
   Extend the protocol or use the longest pre-installed.

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POLL QUESTION # 2

Why most of us use heat on a tissue processor?

A. To lower reagents viscosity

B. To increase reagents permeability

C. To decrease processing time

D. All of the above

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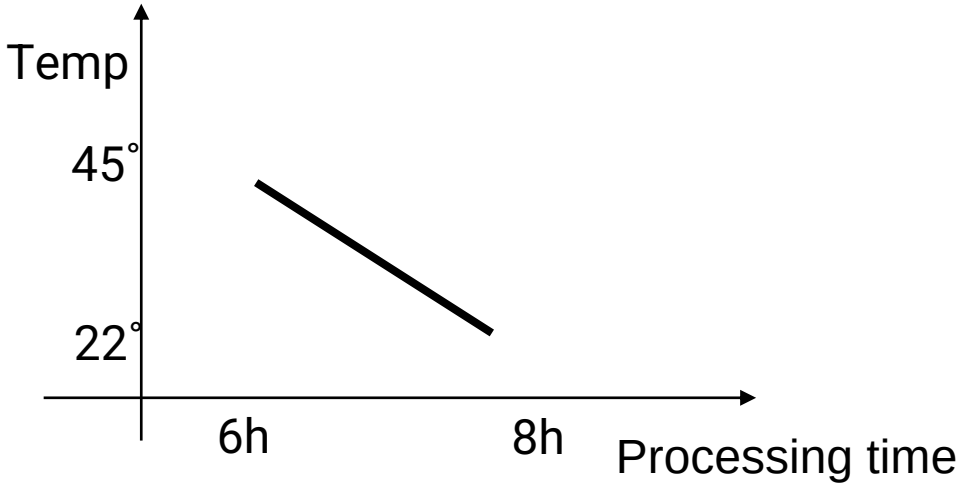

* Illustration, not based on real data

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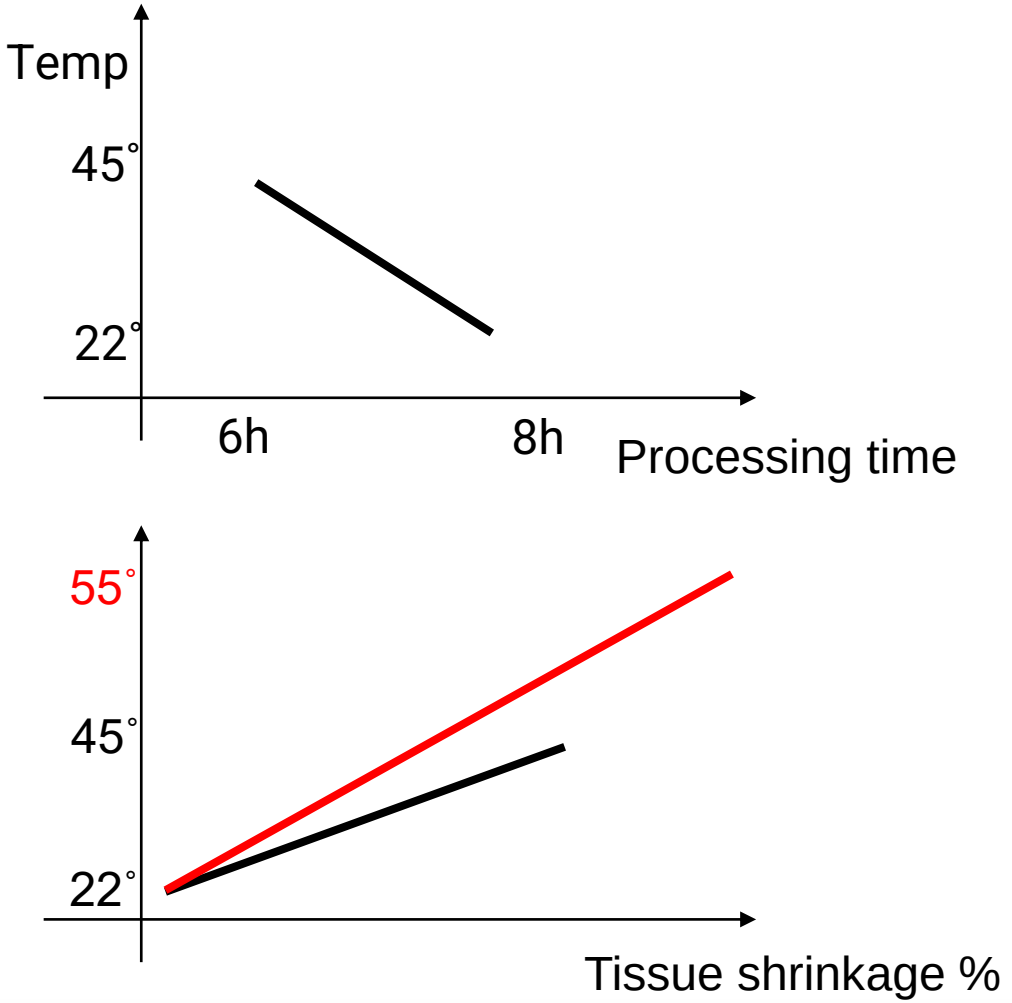

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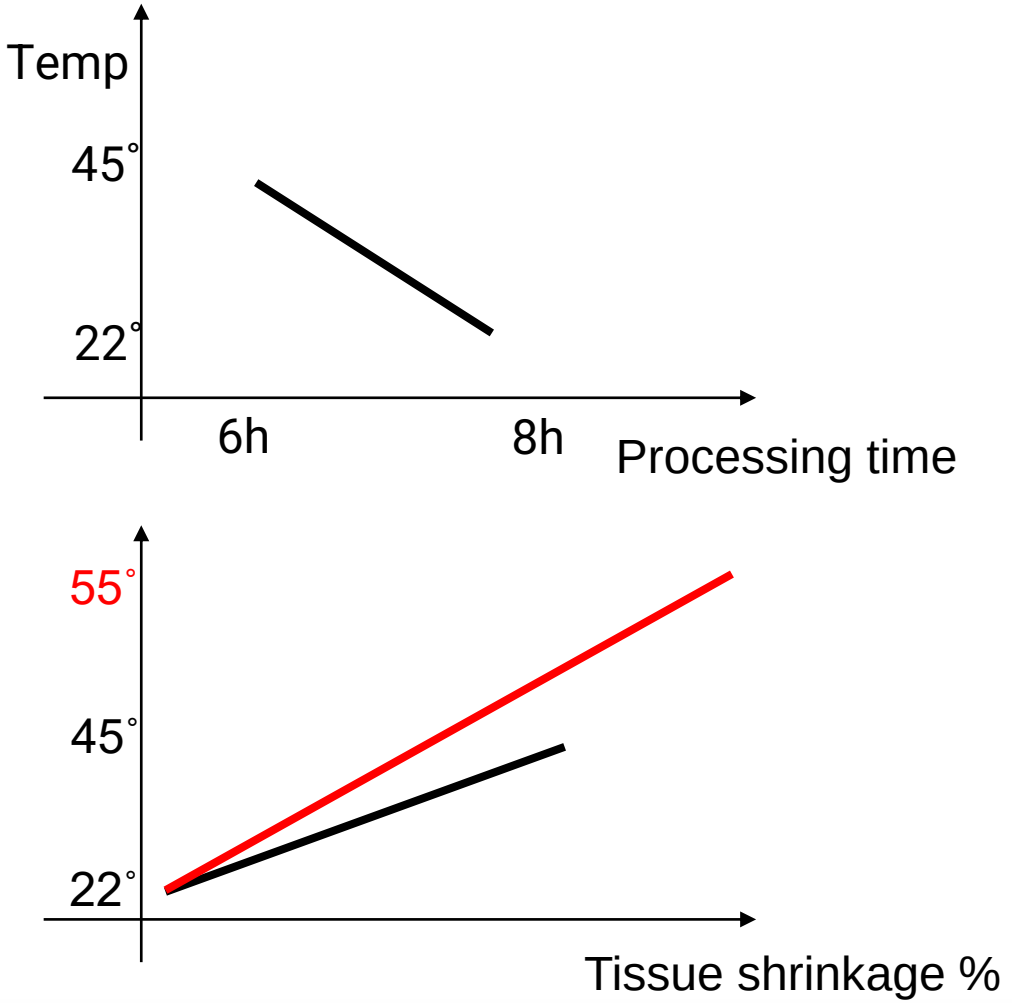
- When processing extremely small or delicate specimens, consider avoiding heat altogether.



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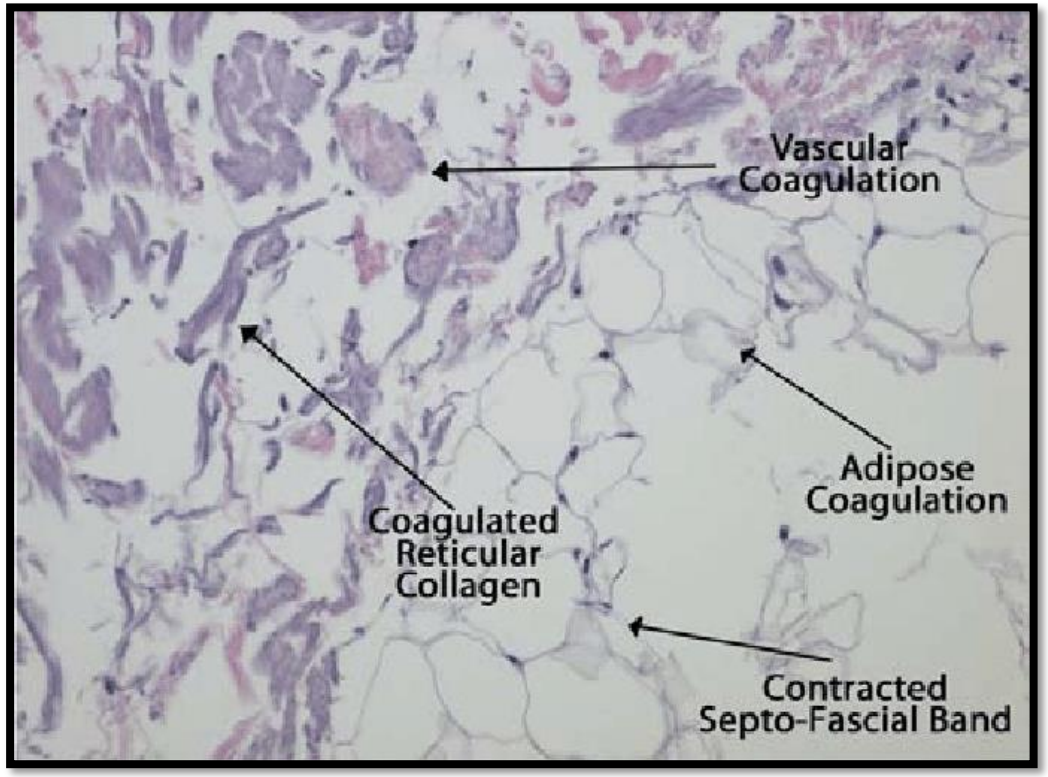

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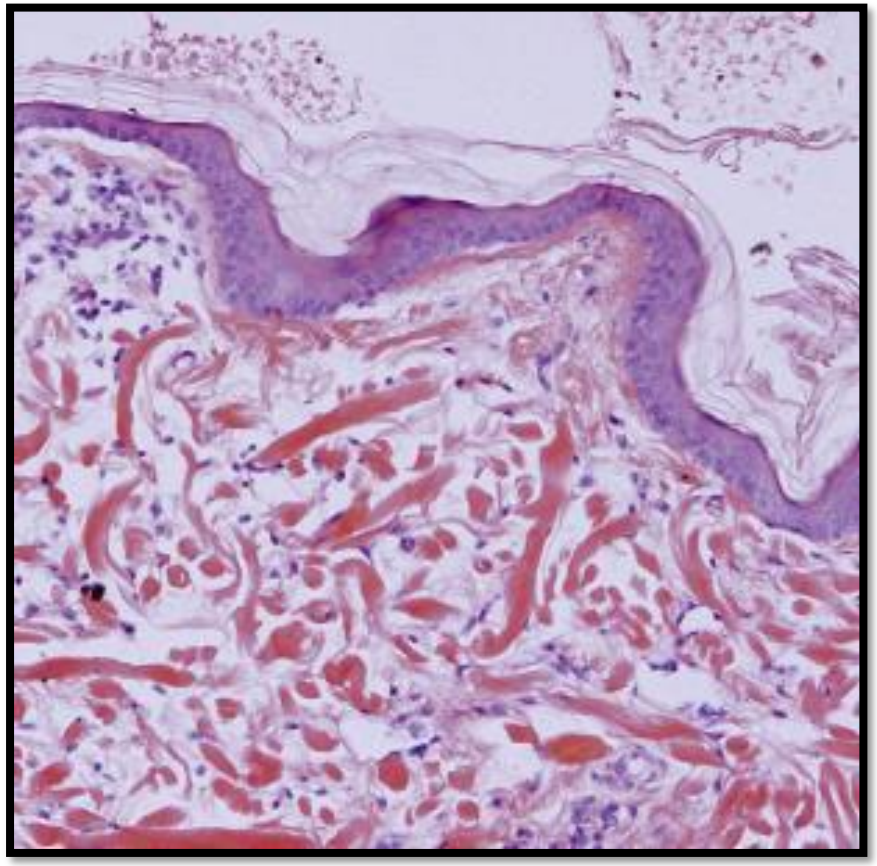

5. How can I recover under-processed specimens?

The most evident processing problems in histology laboratories are under-processed tissue samples.

Under-processing can be due to (1) **too short a protocol**, (2) **too large a specimen**, (3) **processor failure** or (4) **saturated, spent reagents**.

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Paraffin removal	Saline (0.9% NaCl) treatment	Processing

1. Melt paraffin
2. Remove excess paraffin
3. Place the specimen in a new cassette

1h @ 65˚C

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The signs of the over-processed samples	
Crisp, crunchy or brittle tissue	The tissue is likely to be over-processed. Reprocessing is unlikely to help. Soaking the block face in ice water or softening agent and careful re-cutting may produce an acceptable result: ▪ Chilling blocks: (freezer, melting ice, cold spray) ▪ Soaking and softening: ice water, fabric softener, potassium hydroxide
“Cooked” tissue	Tissue described in this way is usually over-processed or has been subjected to extreme conditions e.g., excessive heat. The damage may be irreversible. Sections can sometimes be obtained by soaking the block face in ice water or softening agent and carefully re-cutting
Dry or powdery tissue	Dry and powdery tissues can be the result of over-processing, particularly if the tissue contains a lot of blood. It is also frequently observed when rodent tissue is processed on excessively long schedules. Reprocessing will not help. Soaking the block in ice water prior to cutting is useful. Cutting the block very slowly after allowing it to warm a little may allow sections to be obtained. Cutting at a thinner thickness setting may help.
Outer rim is brittle, while central area is satisfactory	Indicates under-processing. Another possibility is that the specimen has been properly dehydrated and cleared but has had insufficient exposure to paraffin to produce complete infiltration. Melt down the block and apply additional infiltration. Place the cassettes back in the paraffin bath, melt them completely and process them with at least two additional paraffin steps using vacuum. Re-embed and section.

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In the petroleum industry, polymer "**cloud point"** refers to the temperature below which paraffin forms a cloudy appearance.

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Paraffin melting and dispenser tanks

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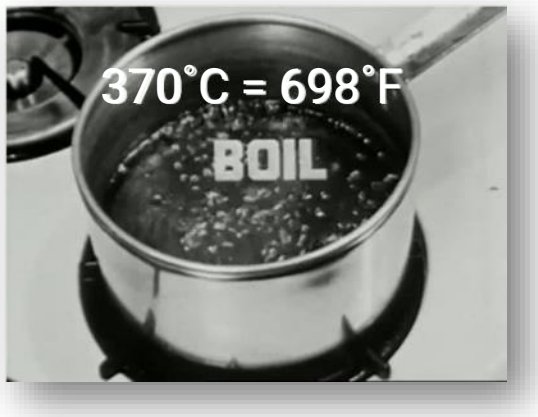

Paraffin melting and dispenser tanks

9. What are the carry-effect of biopsy bags/pads?

Biopsy sponge pads are made of cellular polyester urethane foam and are used to prevent tissues from being lost during processing. While they protect smaller samples from being lost, or immobilize samples from moving around, they can also pose a danger:

- Foam pads will carry-over large amounts of reagent from one station to another resulting in contamination.
- Biopsy foam pads may produce grid-like, triangular-shaped artifacts resembling vascular channels. Artefacts like that can impair biopsy interpretation.
- The foam pad(s) might compress your sample.
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What is the better way of protecting your samples?

- Biopsy bags are a better solution to reduce the risk of specimen loss during processing.
- Bags allow unrestricted fluid movement around tissue and excellent fluid exchange.
- Polyester bags are also acid resistant which mean that they can be used during decalcification.
- Alternatively, mesh cassettes can be used, they provide significantly less carry over than foam pads.


Thank you for attending!

The Epredia Tissue Processing Instrument Portfolio

Epredia Revos Rotational Tissue Processor

Epredia Excelsior AS Tissue Processor

Epredia STP 120 Spin Tissue Processor