

# Seminars in Histology

From basic principles to advanced histological techniques

*Sample collection, preparation, fixation, embedding and cutting*

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Histology Core Facility  
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24.08.2018

# Introduction

Marcello Malpighi (1628-1694), an Italian anatomist, is in fact considered the true “Father of Histology”. Malpighi described a series of microscopic structures never seen until then; for instance, was the first scientist to observe the capillaries.

In the 19th century, histology was an academic discipline in its own right.

In 1819, A. Mayer created the term Histology. He made use of two classical Greek root words (histos = tissue and logos = study).

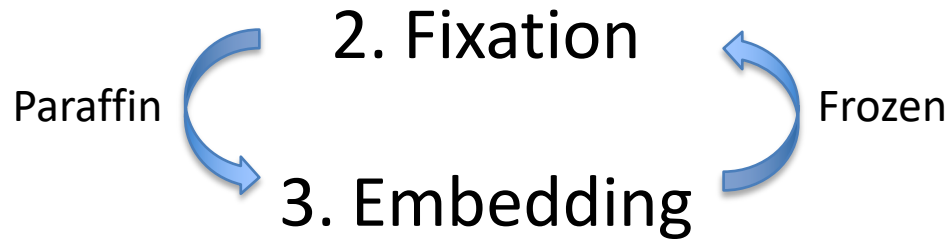
Histology is a branch of biology concerned with the composition and structure of plant and animal tissues in relation to their specialized functions

The fundamental aim of histology is to determine how tissues are organized at all structural levels, from cells and intercellular substances to organs

The Department of Biomedicine Histology Core Facility enables researchers to perform histological assays aimed at answering questions relative to the composition of tissues and the in-situ localization of organic molecules

# Common tissue processing steps

1. Sample collection



4. Sectioning

5. Staining

# Sample collection

## Animal models

### Pros

More abundant tissue

Easier to collect

No risk for the operator\*

Standard experimental conditions

### Cons

Histology may not fully resemble human

Interpretation of results

Species specific issues

Cross-reactivity of ABs

## Human

### Pros

Human!

More abundant AB availability

### Cons

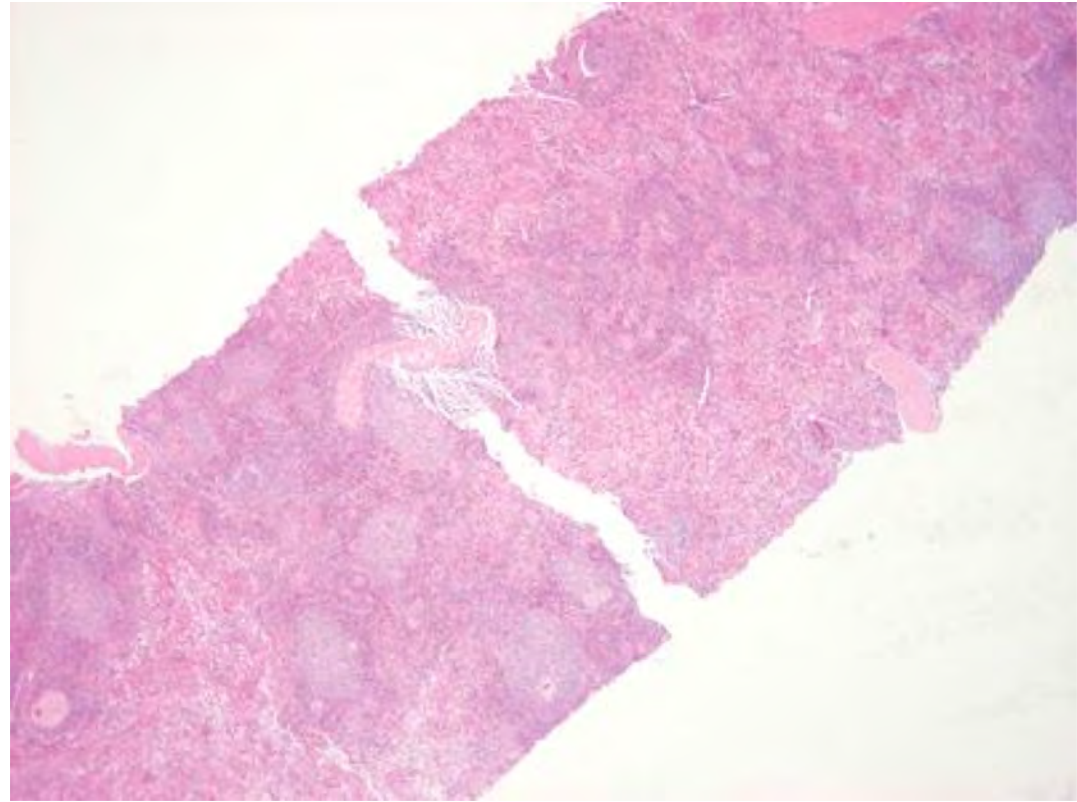
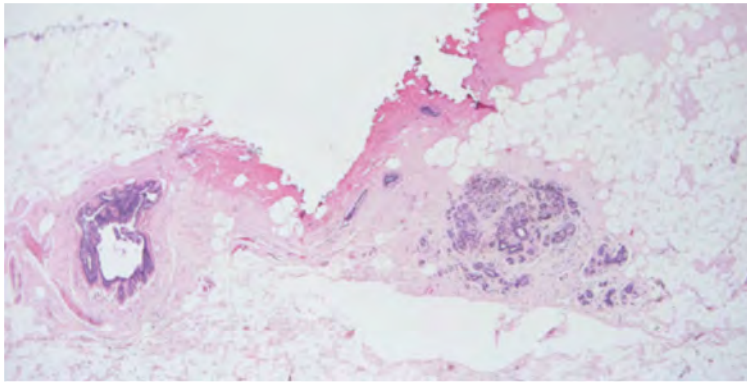
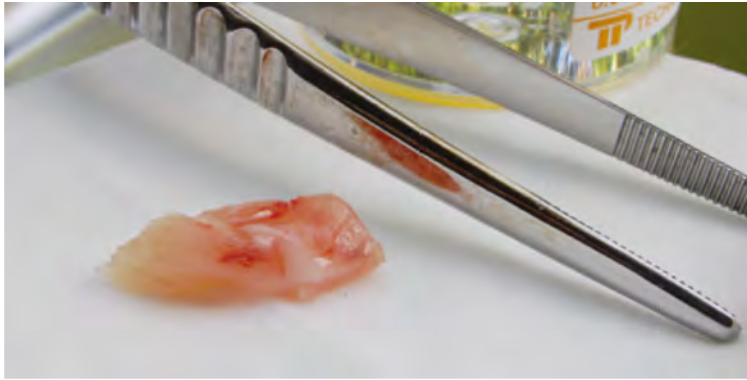
Biosafety concerns

Little amount of tissue

Tissue alteration/degradation

\* Not valid for models transduced or transgenic for human pathogens

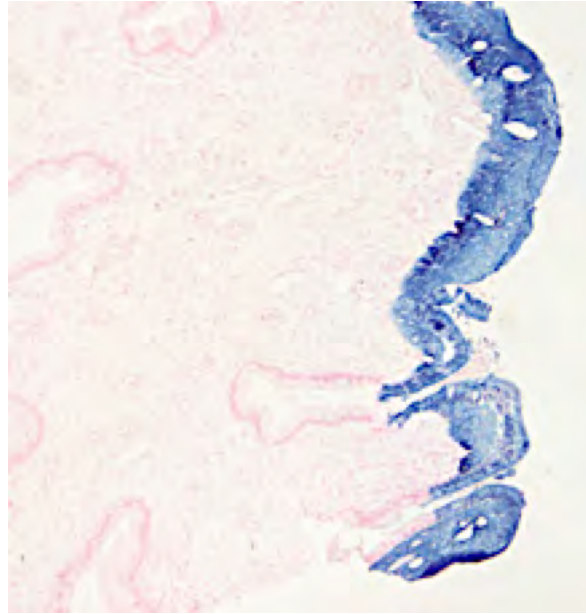
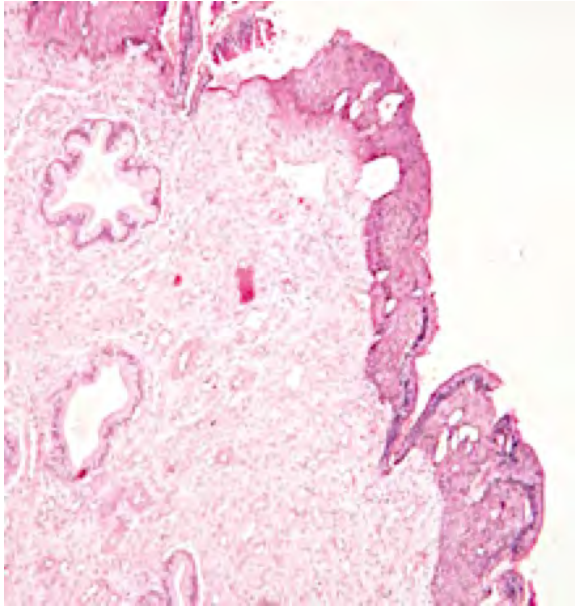
# Sample collection



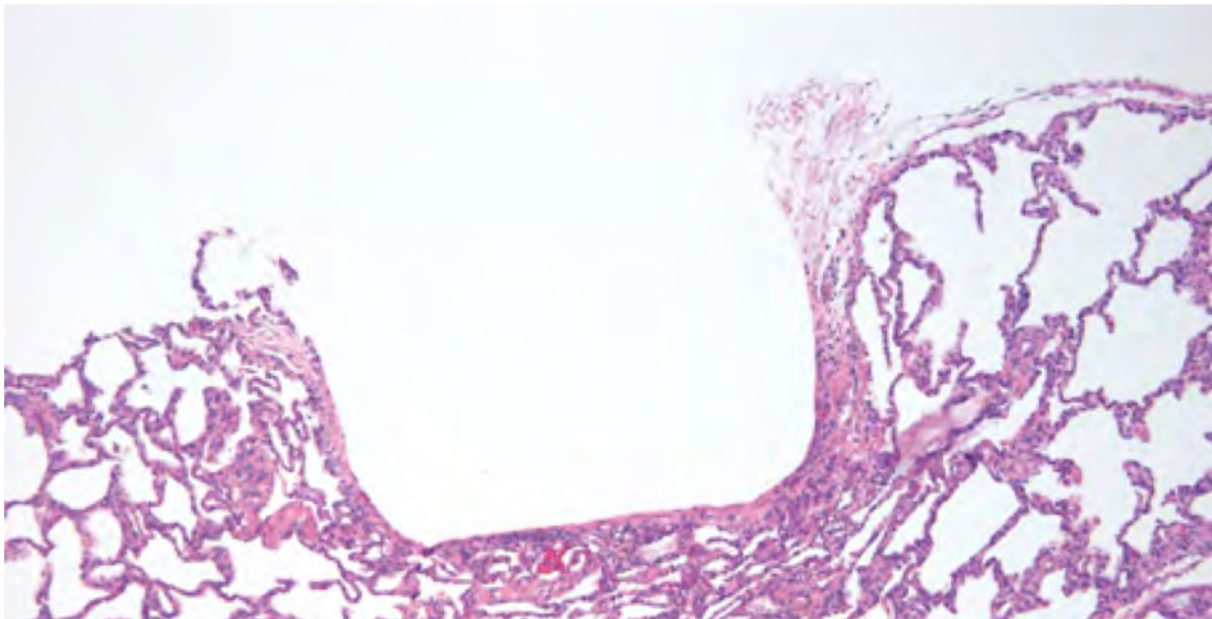
Tissue drying  
Heat damages  
Mechanical damages

Handle gently your tissues and process them as soon as possible after collection

# Sample collection



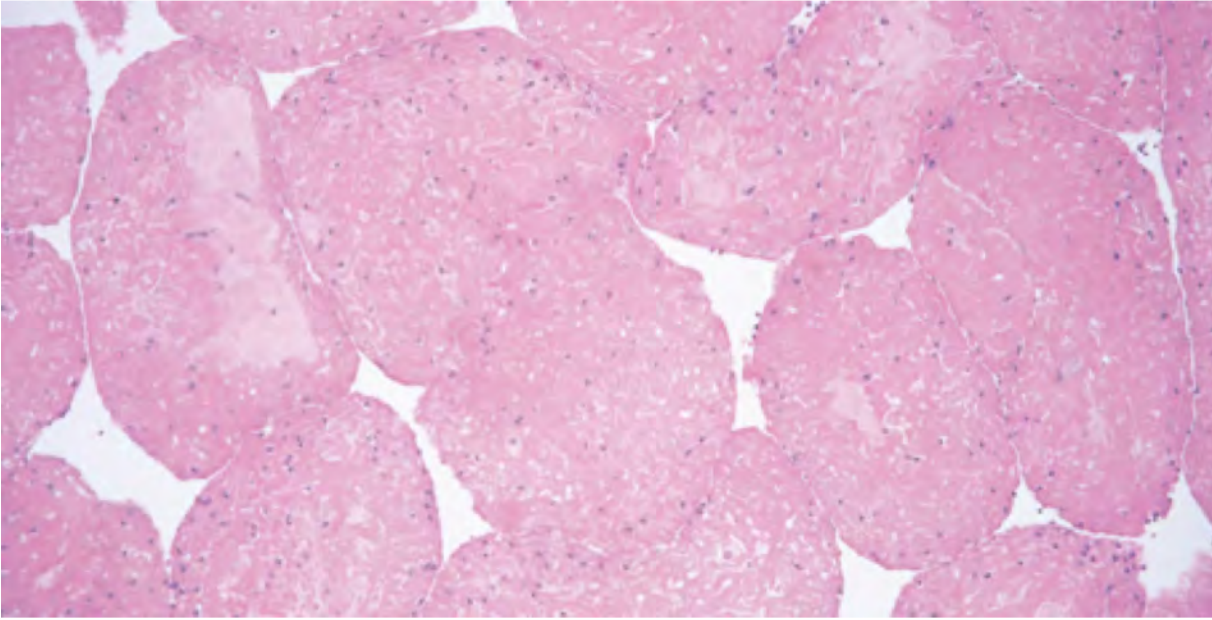
Monse's solution  
(ferric subsulphate solution)



Chemical contamination  
Post-collection mechanical  
damages



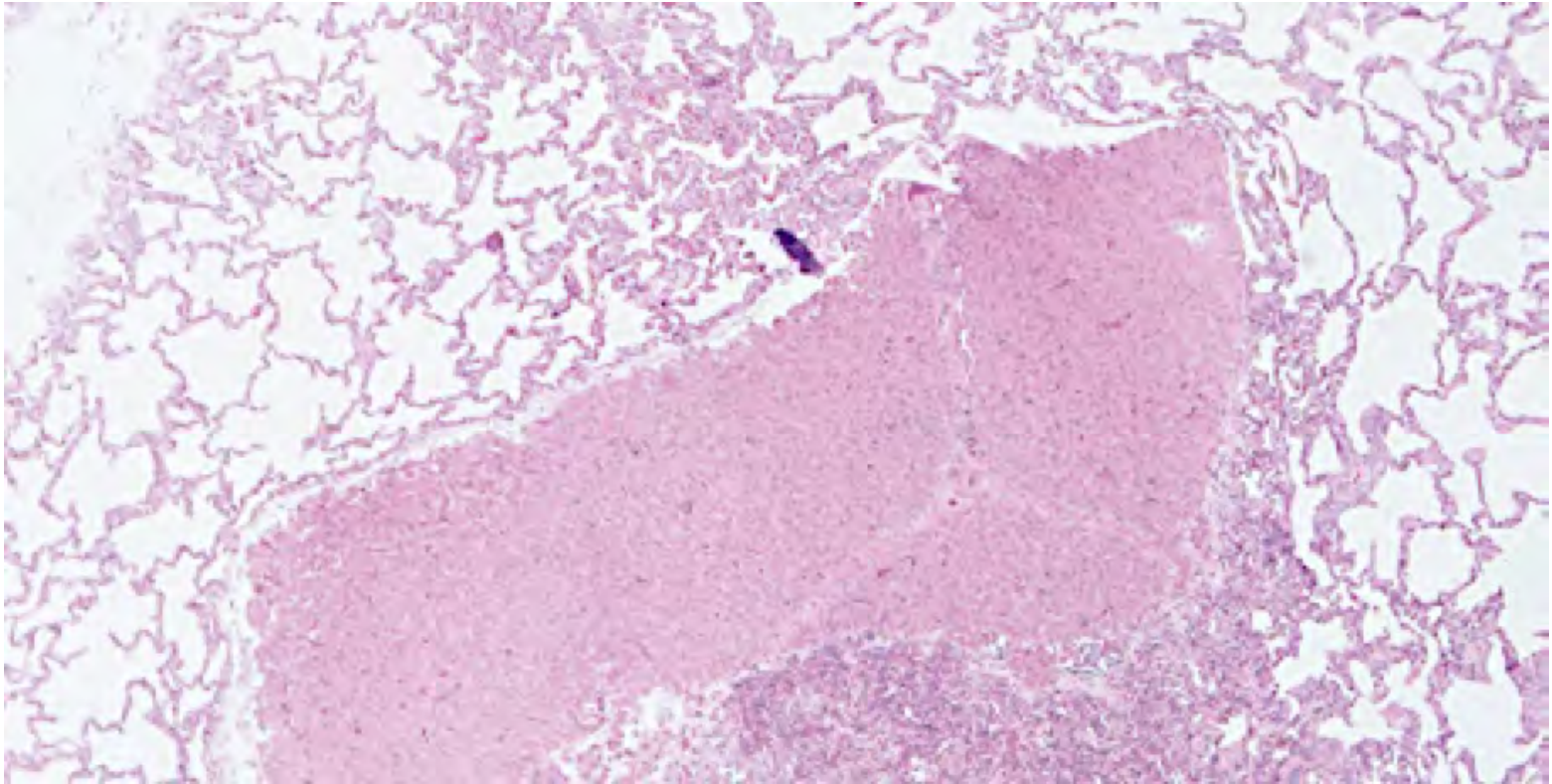
# Sample collection



Post-collection mechanical  
damages

Do not compress your samples in between  
biopsy sponges

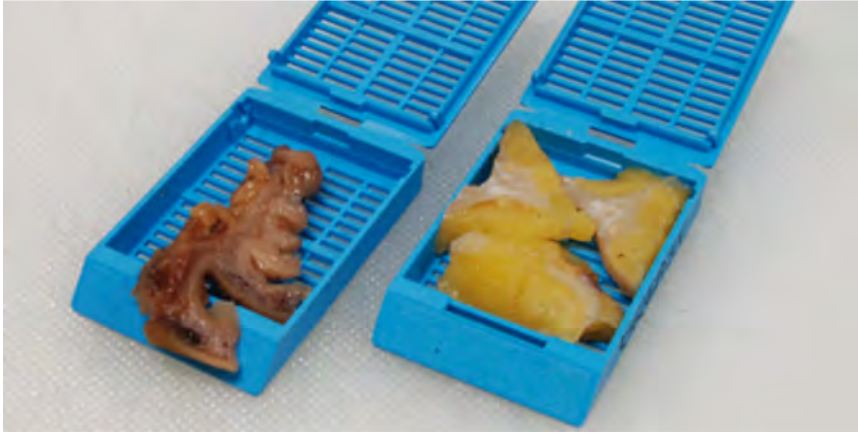
# Sample contamination



Keep clean the working area

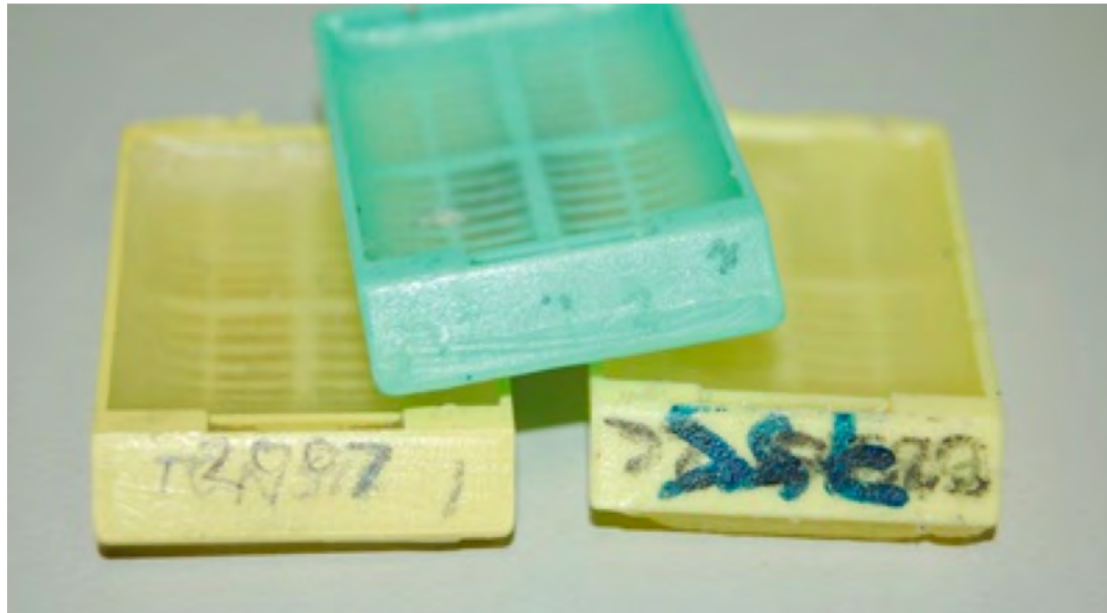
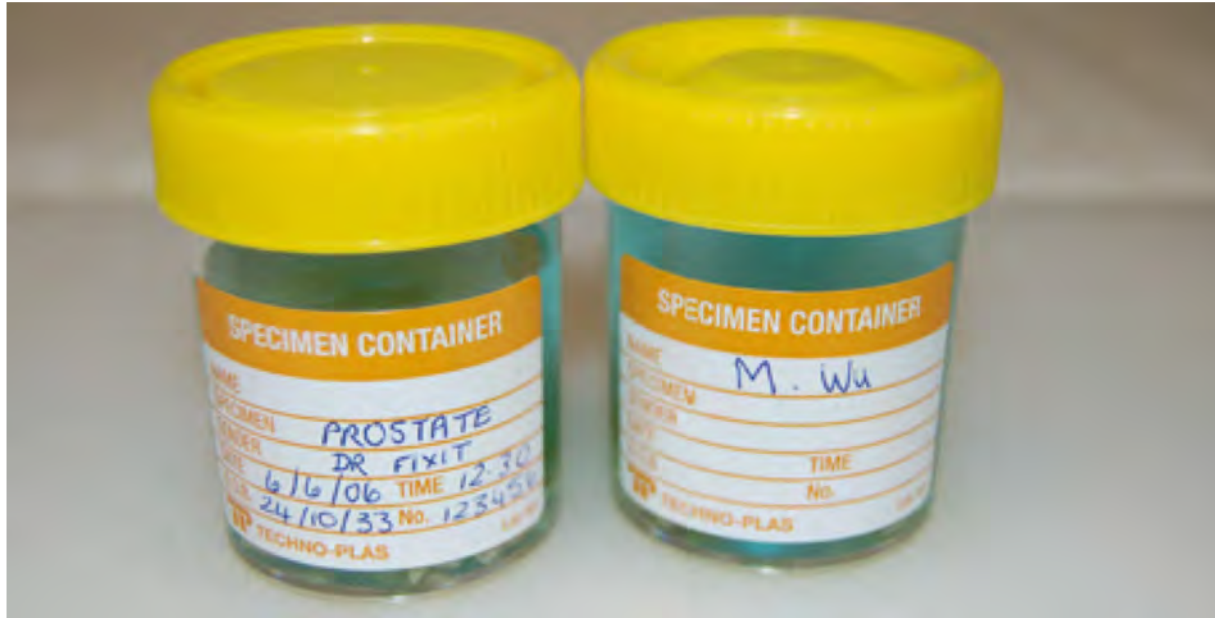


# Sample grossing



Use the correct cassette size and resize you sample for a fast and effective fixation

# Sample labeling



# Sample molding

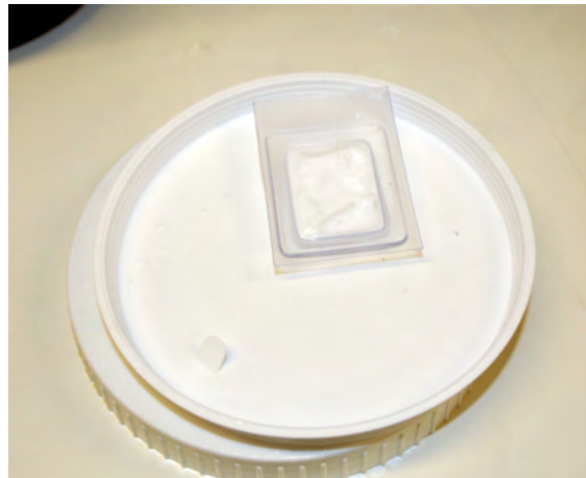
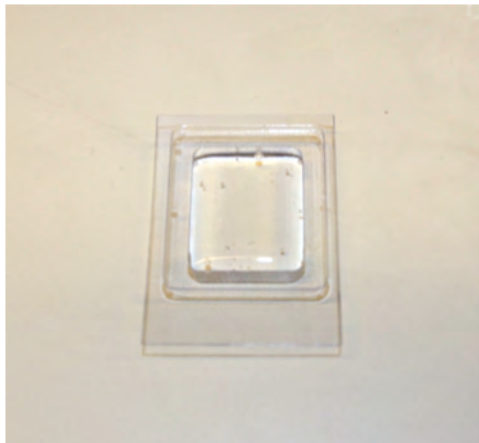


Use the correct amount of paraffin during the block molding





# Fresh tissue OCT embedding



Store at -80°C!!!

# Fixation

In the fields of histology, pathology, and cell biology, fixation is the preservation of biological tissues from decay due to autolysis.

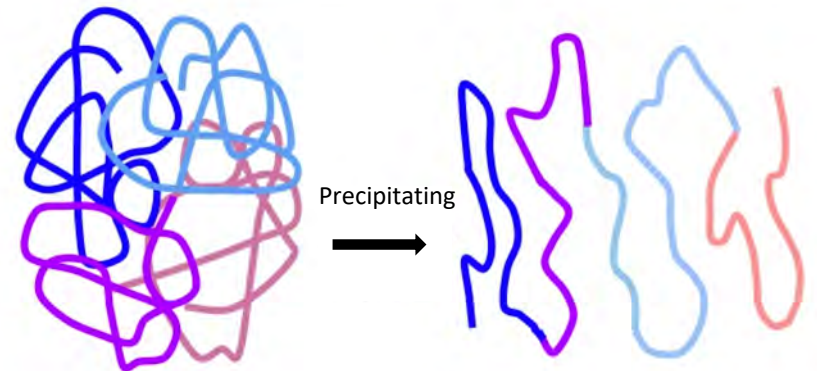
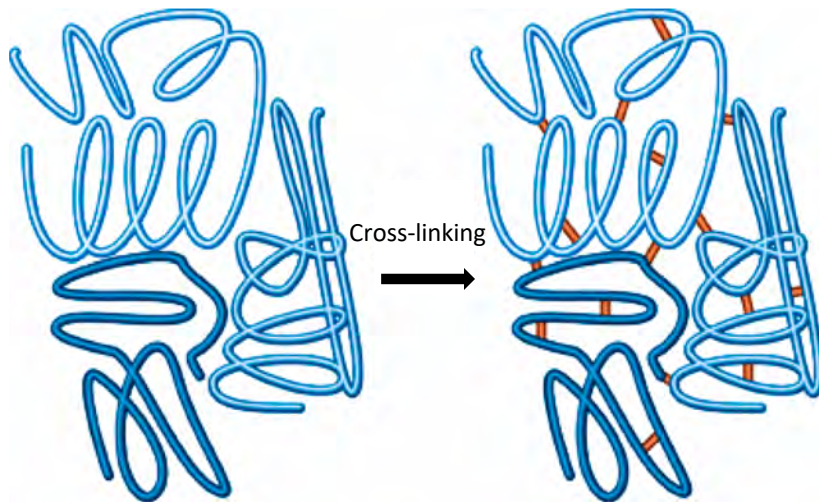
- Kill the tissue so that postmortem decay (autolysis and putrefaction) is prevented.
- Protects a sample from extrinsic damage.
- Alter the cells or tissues on a molecular level to increase their mechanical strength or stability

**In most of the case a fixation step is required to preserve the tissue during the following processing and to preserve a good tissue morphology.**



# Fixation

In performing their protective role, fixatives denature proteins by coagulation, by forming additive compounds, or by a combination of coagulation and additive processes.



# Fixatives

1. Phosphate buffered formalin
  2. Formal calcium
  3. Formal saline
  4. Zinc formalin (unbuffered)
  5. Zenker's fixative
  6. Helly's fixative
  7. B-5 fixative
  8. Bouin's solution
  9. Hollande's
  10. Gendre's solution
  11. Clarke's solution
  12. Carnoy's solution
  13. Methacarn
  14. Alcoholic formalin
  15. Formol acetic alcohol
- Aldehydes based fixatives
- Alcohol fixatives
- Other fixatives

<https://www.leicabiosystems.com/pathologyleaders/fixation-and-fixatives-4-popular-fixative-solutions/>

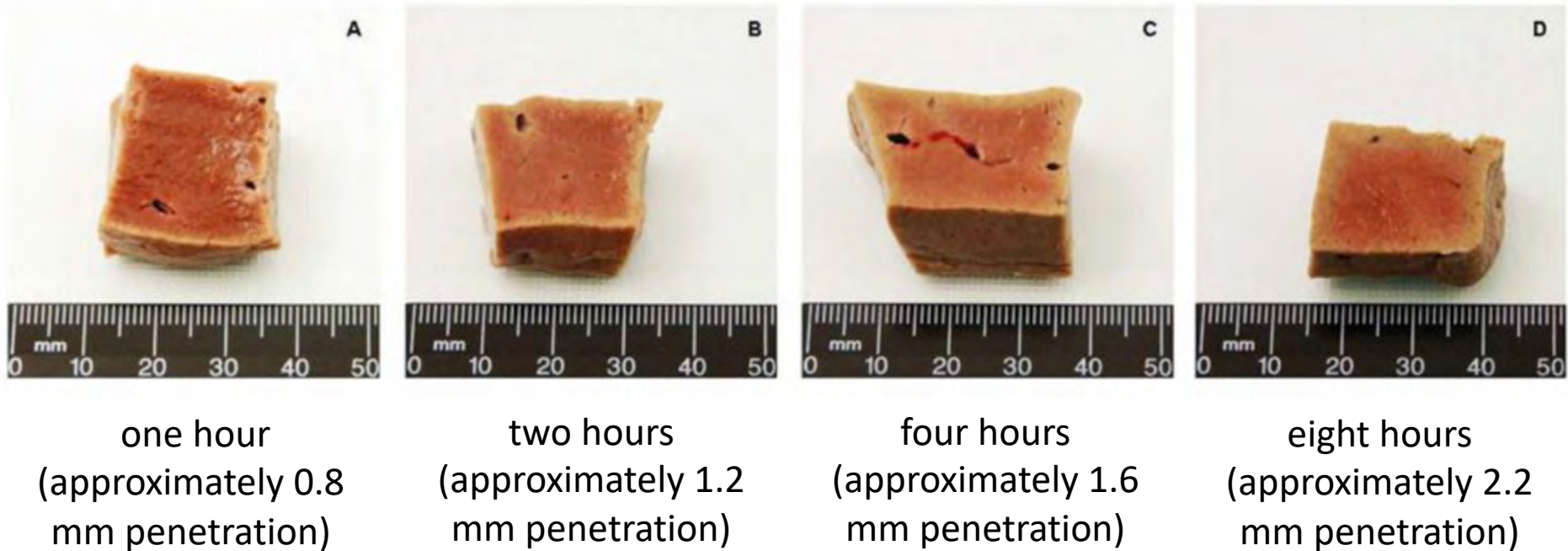
Target	Fixative/tissue of choice	Fixative to avoid
Proteins	Neutral buffered formalin, paraformaldehyde	Osmium tetroxide
Enzymes	Frozen sections	Chemical fixatives
Lipids	Frozen sections, glutaraldehyde/osmium tetroxide	Alcoholic fixatives
Nucleic acids	Alcoholic fixatives, HOPE	Aldehyde fixatives
Mucopolysaccharides	Frozen sections	Chemical fixatives
Glycogen	Alcoholic based fixatives	Osmium tetroxide

# Sample fixation



Use enough fixative for a fast and effective fixation  
The volume of fixative should be at least 3 times the volume of your sample.  
Adapt the container size accordingly.

# Formaldehyde penetration rate

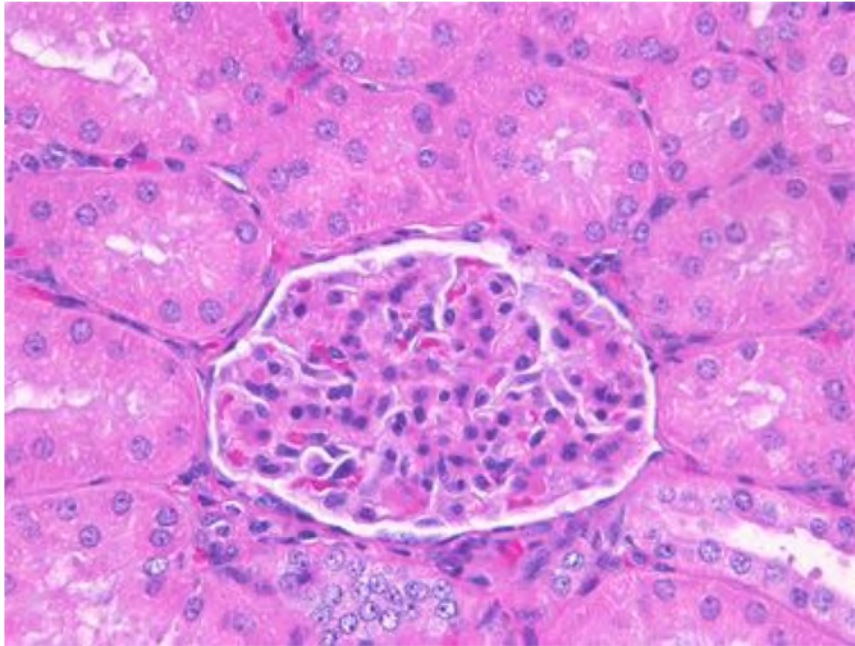


Aldehydes have a tissue penetration rate of 0.5-1mm per hour.  
Adapt the fixation time according with this rate and your sample size.  
Be consistent in your fixation procedure

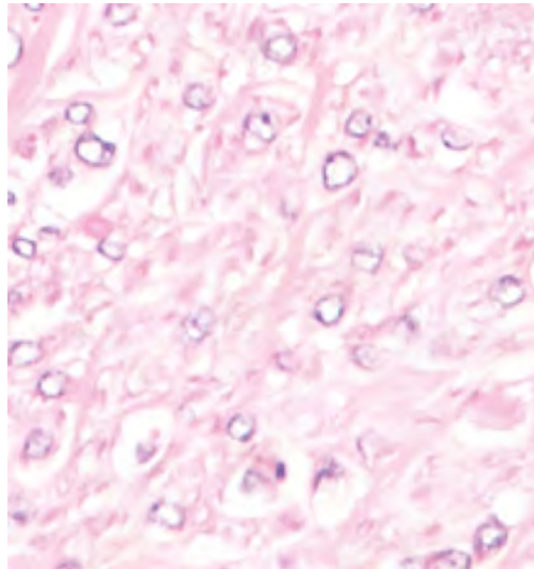
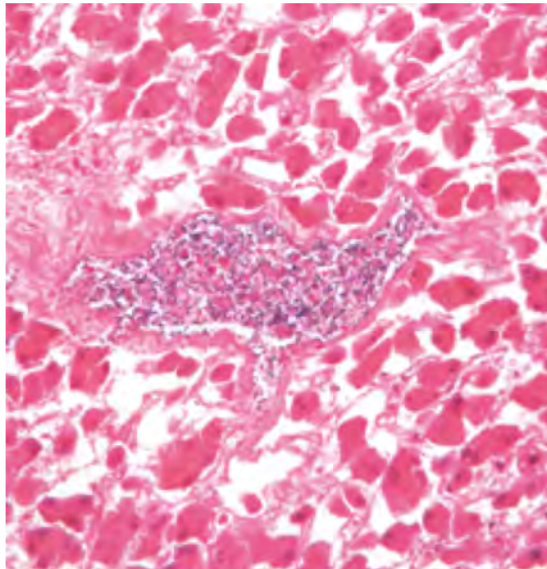
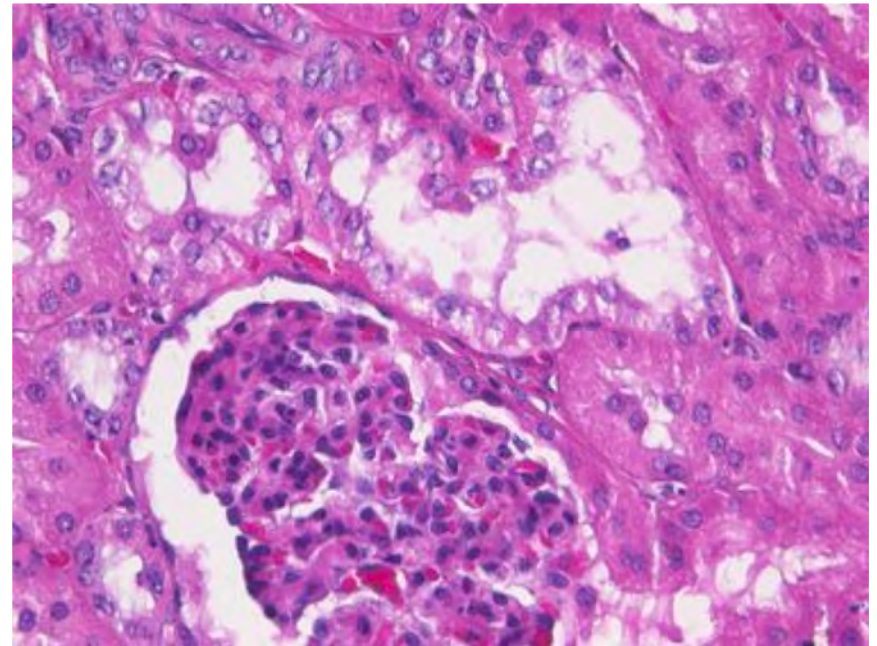


# Effect of a poor fixation

Good

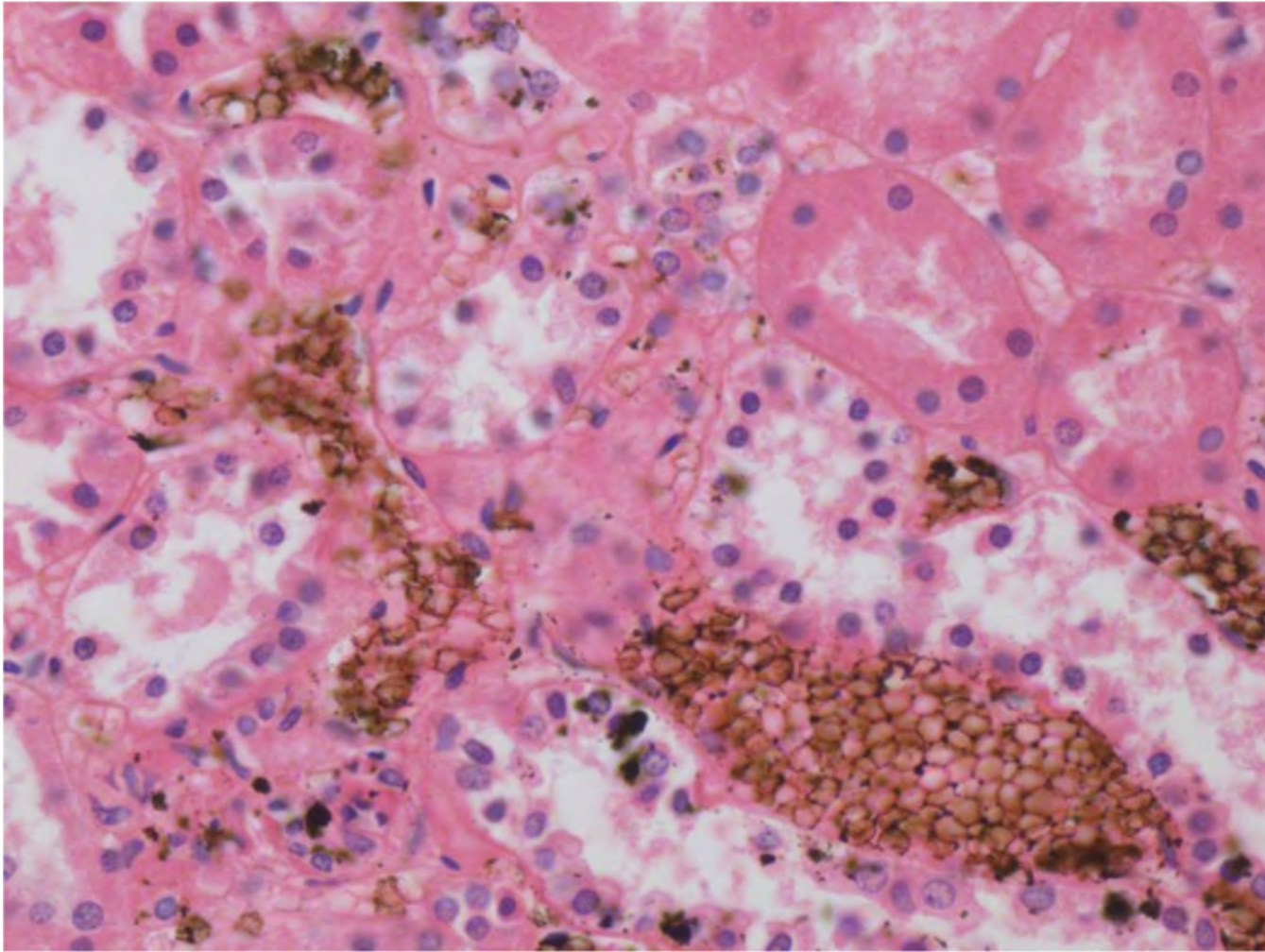


Poor



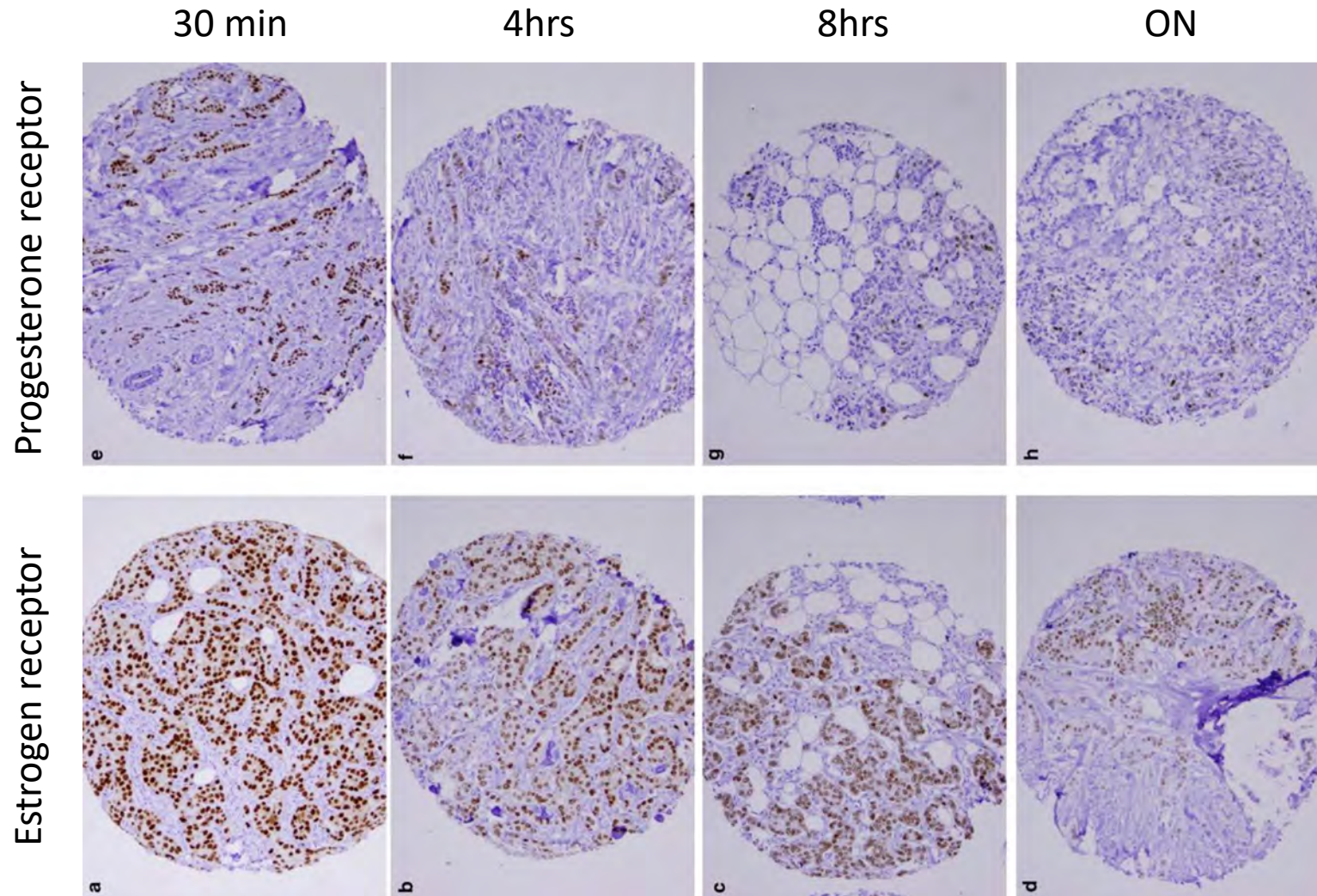


# Over-fixation



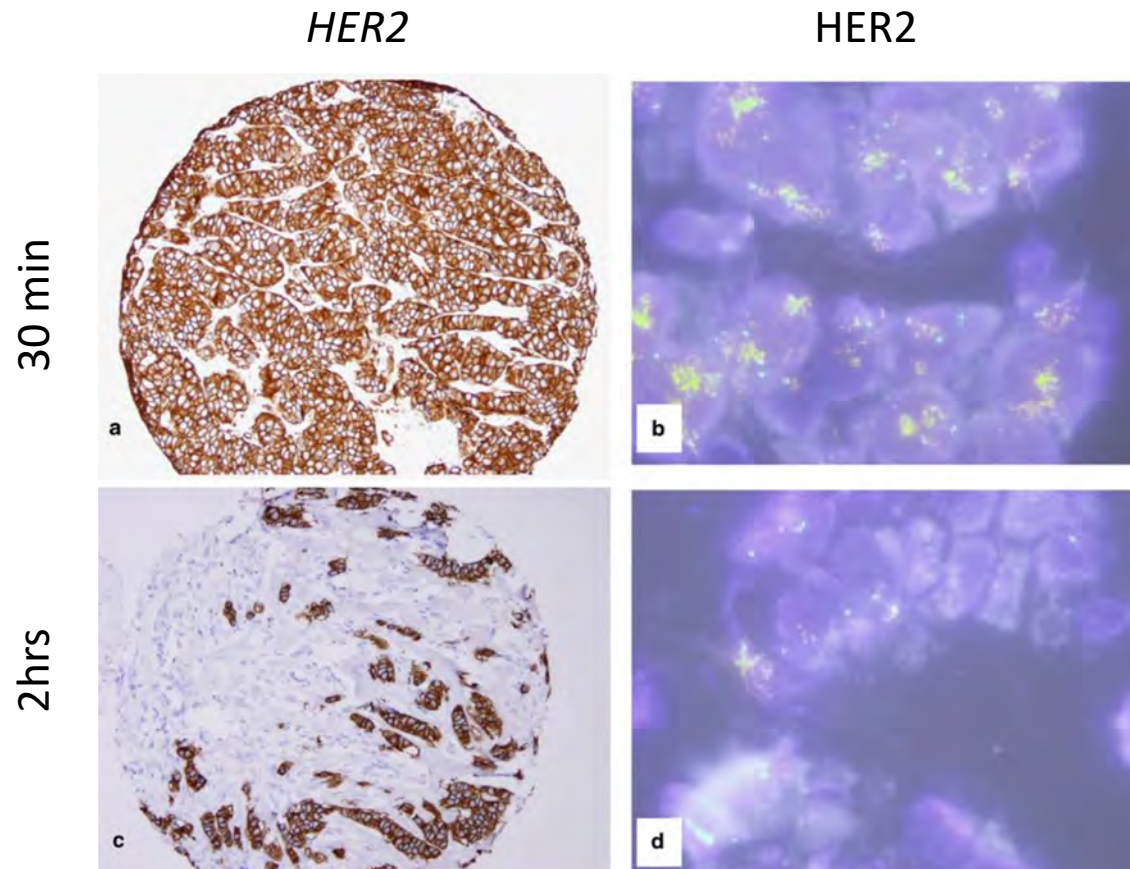
Deposition of acid formaldehyde hematin (formalin pigment)

# Over-fixation

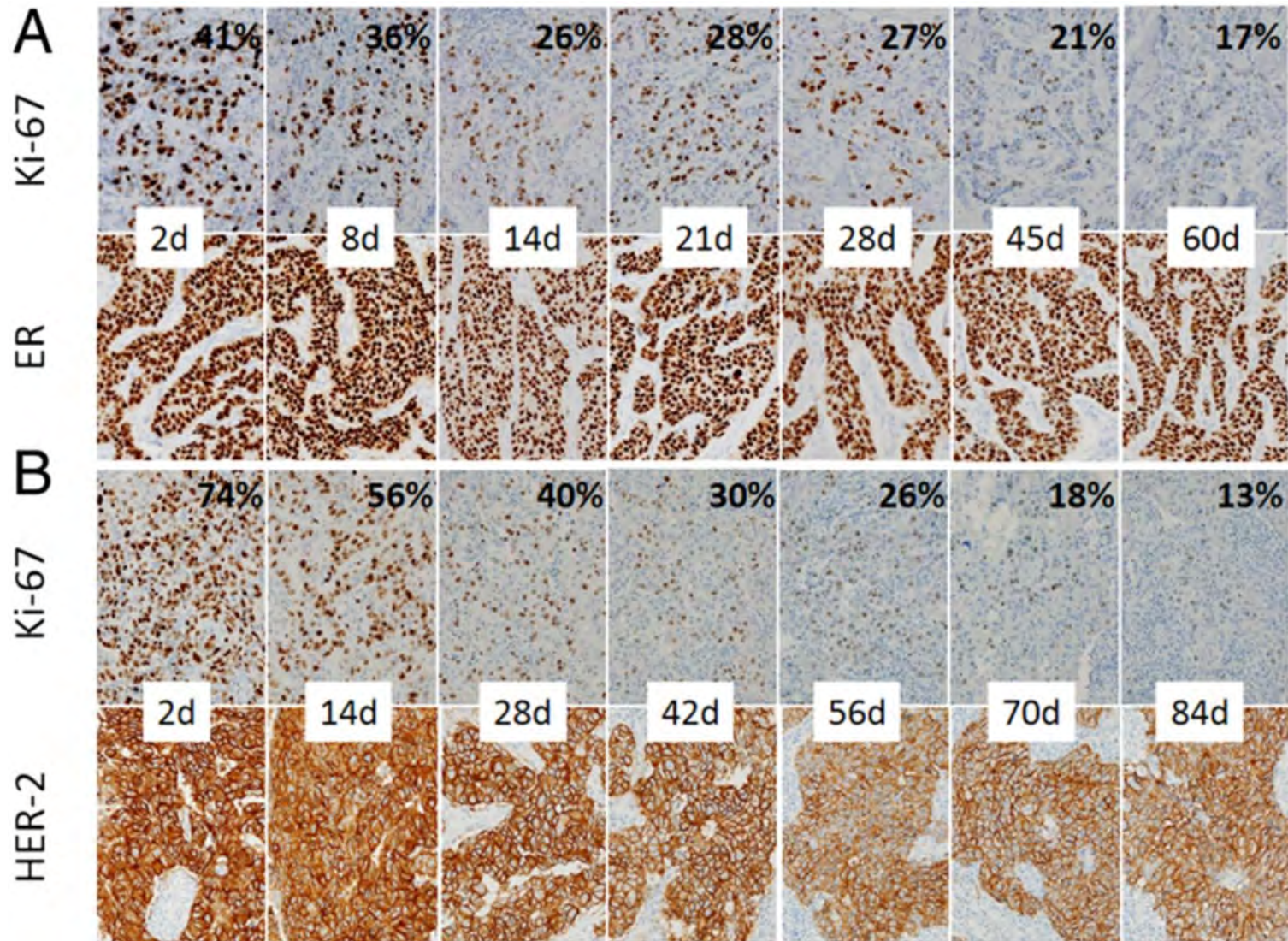




# Over-fixation

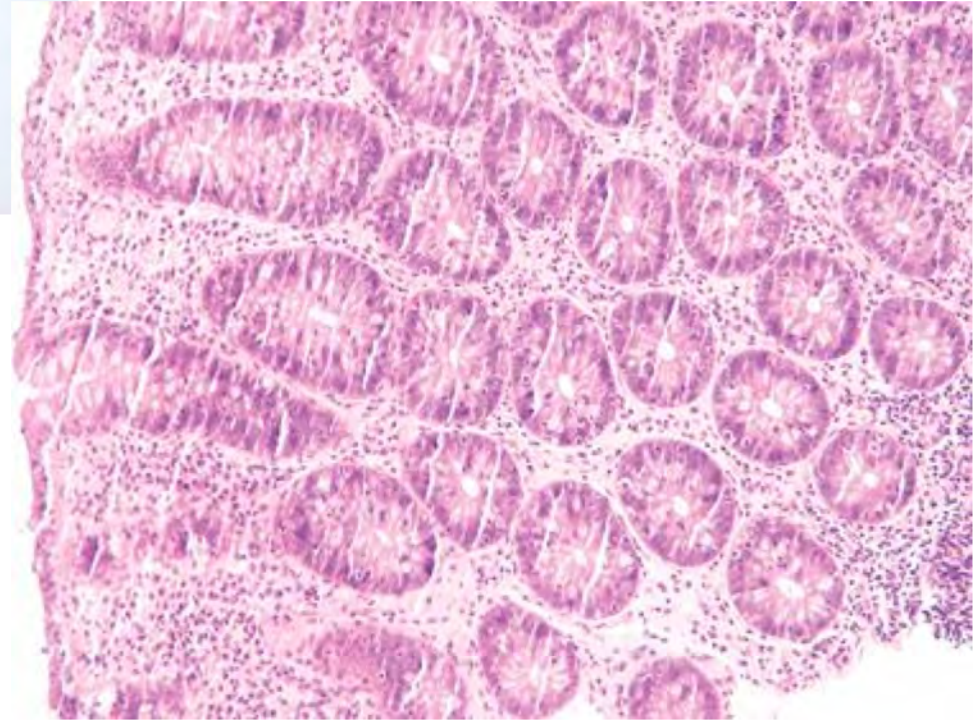
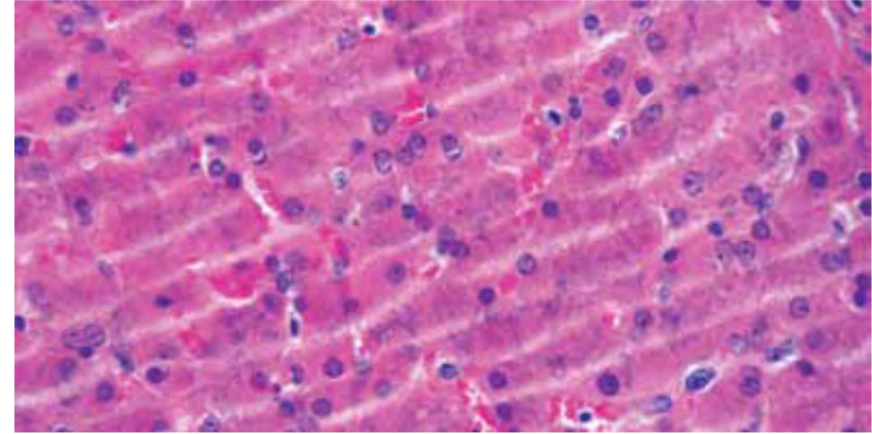
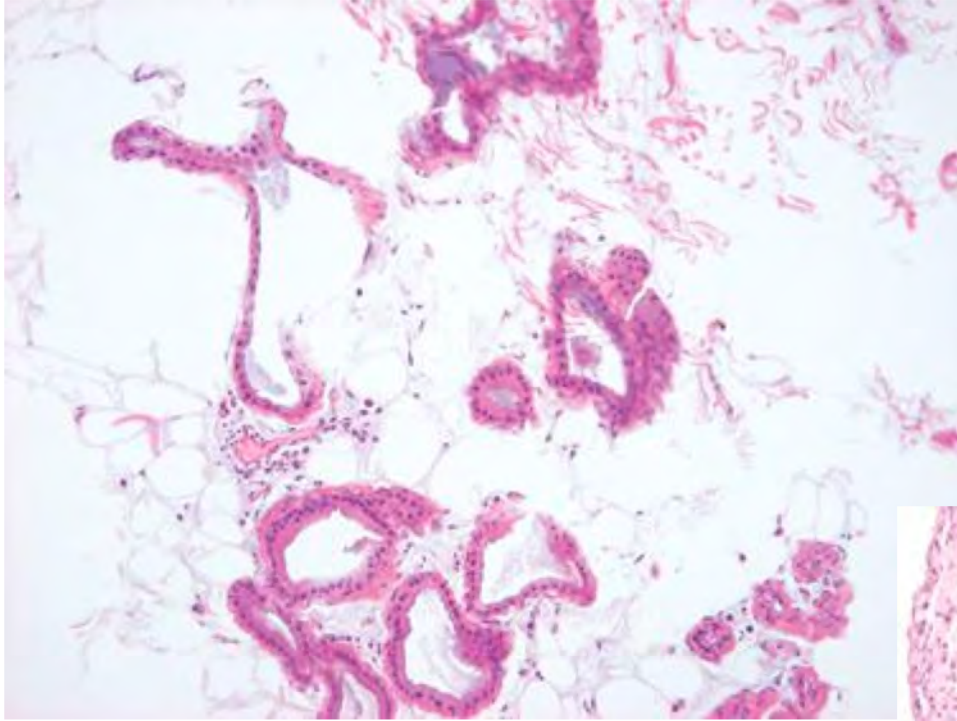


# Over-fixation





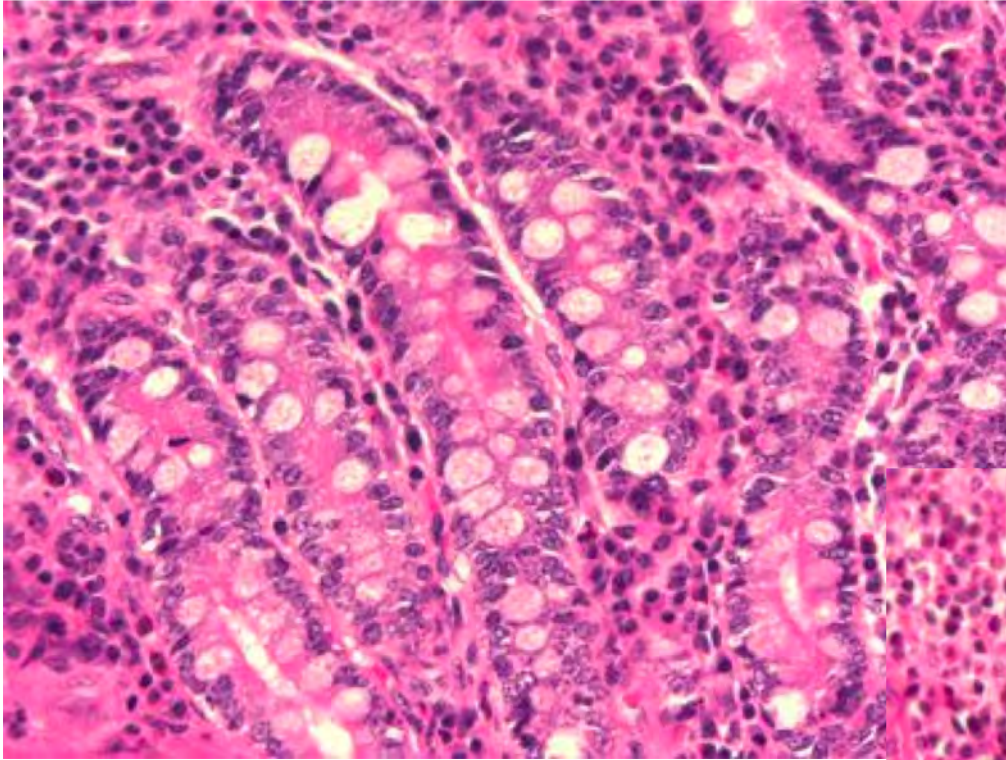
# Under- Vs. over-processing



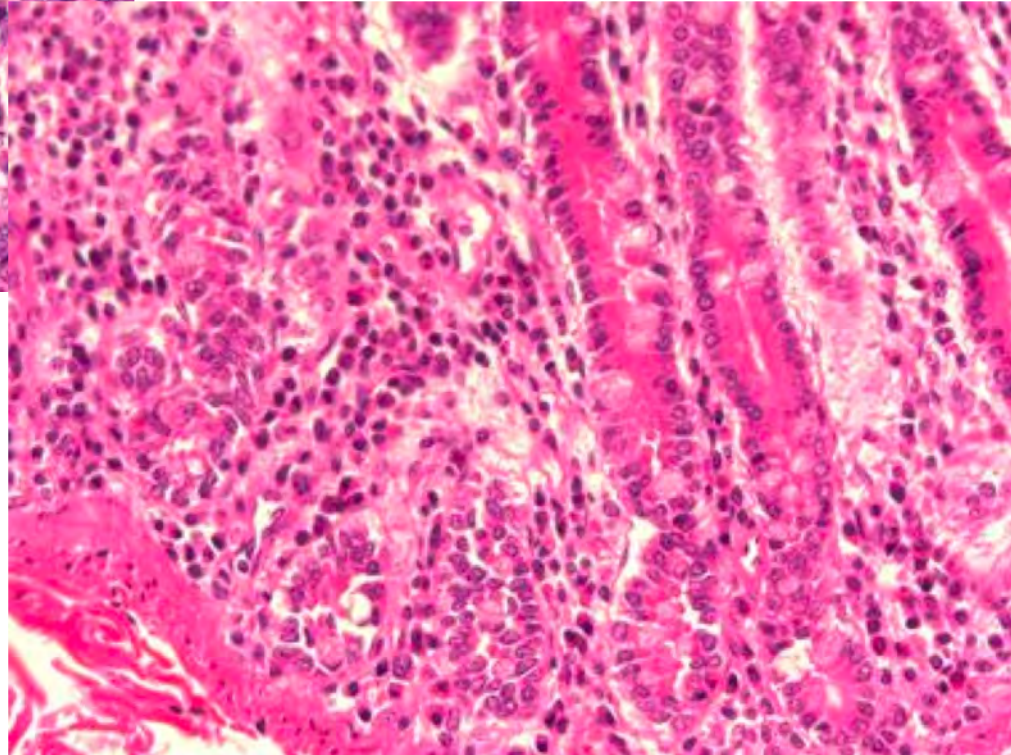


# Differences between fixatives

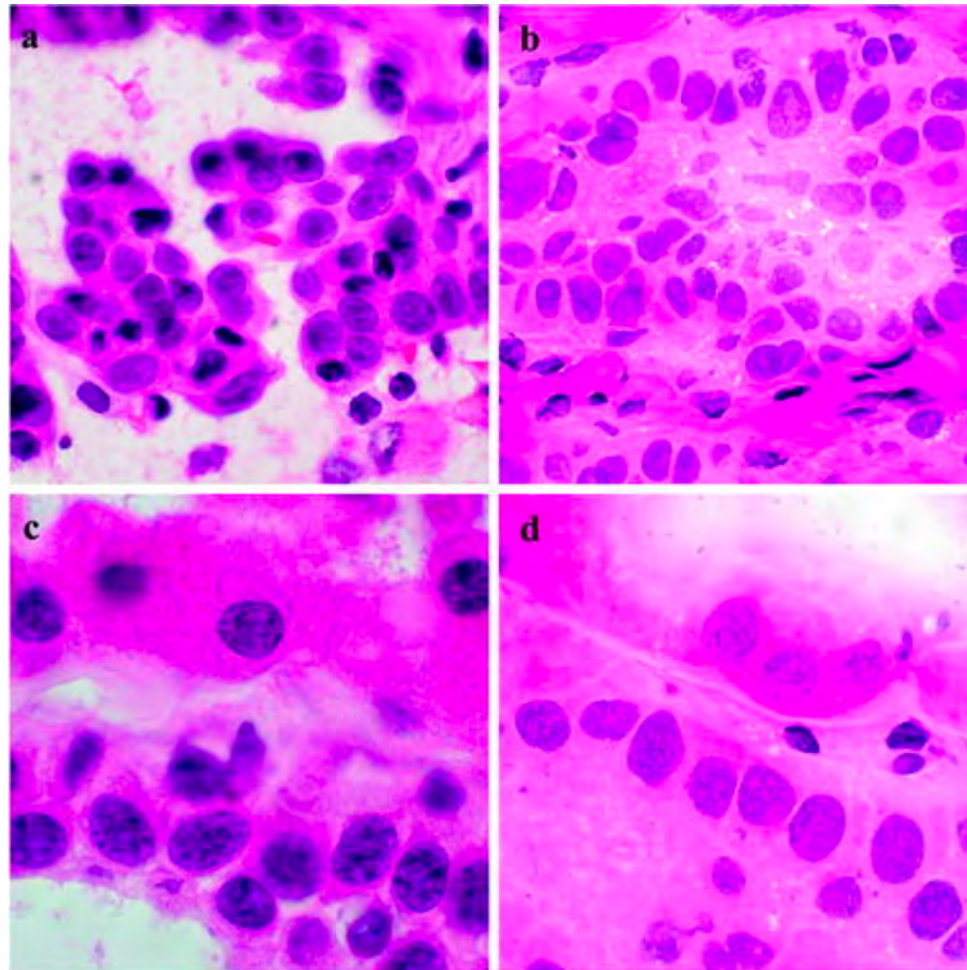
Formalin



Etoh 95%



# Immediate Vs. delayed fixation



Immediate fixation  
95% ETOH

15 second delayed fixation  
95% ETOH

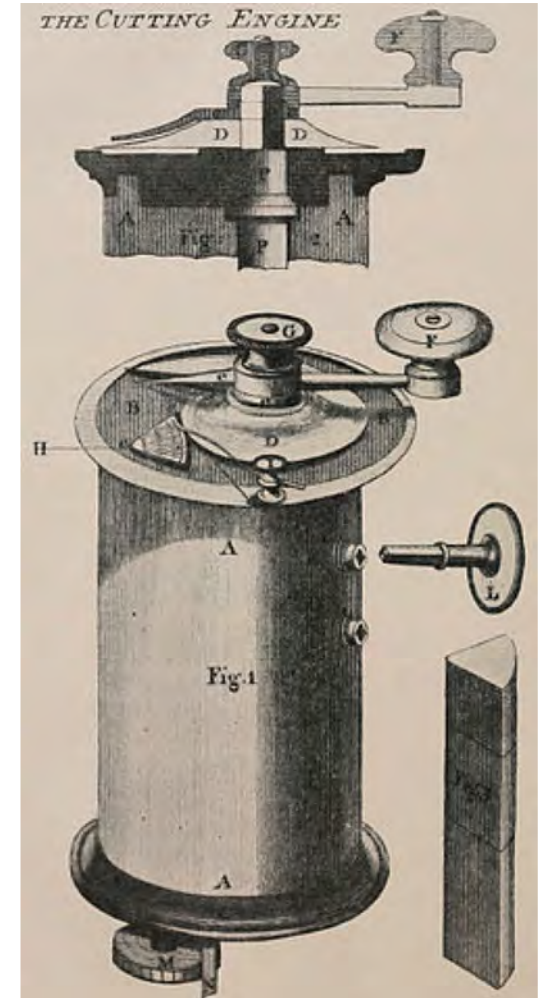


# Sample sectioning

First microtome has been designed in 1770

First vibratome in 1835

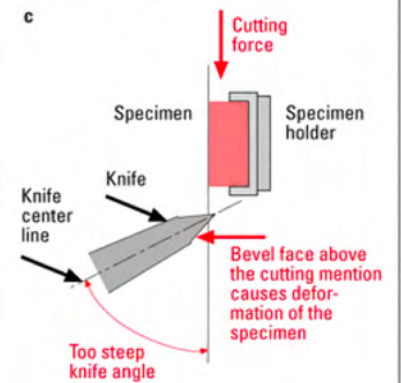
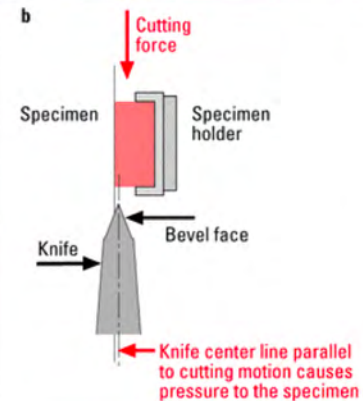
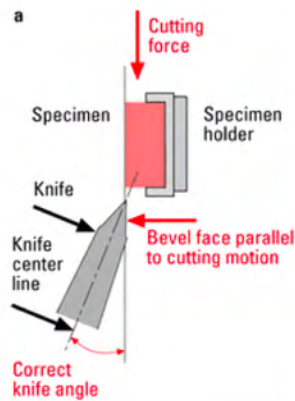
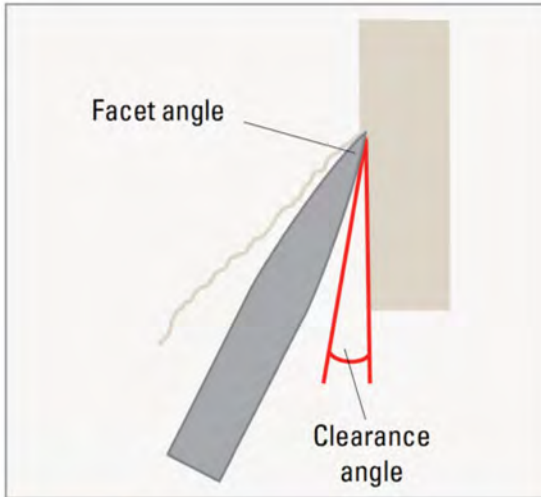
At the end of the 1800s, the development of very thin and consistently thin samples by microtomy, together with the selective staining of important cell components or molecules allowed for the visualization of microscope details.

























































Microtome drawn 1770



# Sample sectioning



# Blades

	Information	Material	Dimension (LxWxH)	Edge Finish		Soft tissue	Hard tissue	Large block	Cryostat	Thin sectioning	Ribbon sectioning	Technical information	Trimming	Type of Microtome	Package
	<b>S-35 "Fine"</b> For thin sectioning of paraffin embedded tissue blocks. It has earned a high reputation for superior cutting performance and durability.	Stainless Steel	0.254 x 8 x 80 Edge Angle 35°	Resin Coating Platinum Sputtering								Standard blade for routine sectioning. Both for rotary / sledge microtomes. Good sectioning quality in initial sectioning.	Short trimming time.	Sledge / rotary	Plastic Dispenser Box of 50 blades
	<b>R-35 "Routine"</b> For thin sectioning of paraffin embedded tissue blocks. Especially suited for routine microtomy operations. Double sputtering increases edge strength and durability even when sectioning right after trimming with the same blade position.	Stainless Steel	0.254 x 8 x 80 Edge Angle 35°	Resin Coating Platinum Sputtering								Special hardened blade edge. Easy to get ribbon sectionings. For hard tissue.	Short trimming time.	Sledge / rotary	Plastic Dispenser Box of 50 blades
	<b>A-22</b> For extra thin sectioning of paraffin embedded tissue blocks. Especially suited for fibrous tissues / biopsy.	Stainless Steel	0.254 x 8 x 80 Edge Angle 22°	Resin Coating Platinum Sputtering								Standard blade for routine sectioning easy to get ribbon section.	Short trimming time.	Sledge / rotary	Plastic Dispenser Box of 50 blades
	<b>A-35 "Superior"</b> For thin sectioning of paraffin embedded tissue blocks. Especially suited for hard tissue block sectioning and ribbon sectioning. Durable and stable life after trimming.	Stainless Steel	0.254 x 8 x 80 Edge Angle 35°	Resin Coating Platinum Sputtering								Durable and stable sectioning quality also for ribbon section.	Short trimming time.	Sledge / rotary	Plastic Dispenser Box of 50 blades
	<b>N-35 "Long Duration"</b> For thin sectioning of paraffin embedded tissue blocks. Long blade life, no need to move the blade in real sectioning after trimming.	Stainless Steel	0.254 x 8 x 80 Edge Angle 35°	Resin Coating Platinum Sputtering								Long and stable sectioning quality. Very good for hard tissue. Also suitable for ribbon sectioning.	After short trimming, excellent durability.	Sledge / rotary	Plastic Dispenser Box of 50 blades
	<b>N-35HR</b> For thin sectioning of paraffin embedded tissue blocks. Especially recommended for hard tissue and routine sectioning.	Stainless Steel	0.254 x 8 x 80 Edge Angle 35°	Resin Coating Platinum Sputtering								Especially for hard tissue. Also suitable for ribbon sectioning. Very good service life.	Short trimming time.	Sledge / rotary	Plastic Dispenser Box of 50 blades
	<b>S-22 "Super Fine"</b> For extra thin sectioning of paraffin embedded tissue blocks. Especially recommended for laboratory use. Under some circumstances, blade life may be limited because of the ultra-thin edge.	Stainless Steel	0.254 x 8 x 80 Edge Angle 22°	Resin Coating Platinum Sputtering								For soft tissue and extremely thin sectioning. (Research)	Short trimming time.	Sledge / rotary	Plastic Dispenser Box of 50 blades
	<b>HIGH-PROFILE</b> For thin sectioning of paraffin embedded tissue blocks. Especially suited for ribbon sectioning with rotary microtome.	Stainless Steel	0.31 x 14 x 75.7 Edge Angle 35°	Resin Coating Platinum Sputtering								Standard blade for routine sectioning easy to get ribbon section.	Short trimming time.	Sledge / rotary	Plastic Dispenser Box of 50 blades
	<b>C-35</b> For thin cryo-sectioning. Specially ground for cryostat use to eliminate curling or wrinkling problems.	Carbon Steel	0.245 x 8 x 80 Edge Angle 35°									Carbon steel blade. For cryostat only.		Cryostat	Plastic Dispenser Box of 20 blades
	<b>S-35L</b> For thin sectioning of large tissue blocks. Same quality as the S-35. (120mm)	Stainless Steel	0.254 x 8 x 120 Edge Angle 35°	Resin Coating Platinum Sputtering								12cm long blade. For large tissue.	Short trimming time.	Sledge	Plastic Dispenser Box of 20 blades
	<b>S-35LL</b> For thin sectioning of large tissue blocks. Same quality as the S-35. (180mm)	Stainless Steel	0.254 x 8 x 180 Edge Angle 35°	Resin Coating Platinum Sputtering								18cm long blade. For large tissue.	Short trimming time.	Sledge	Plastic Dispenser Box of 20 blades

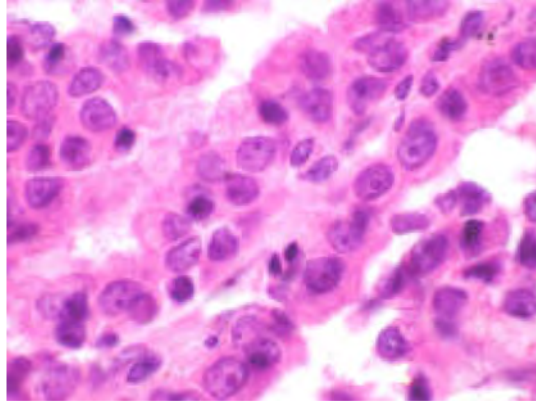
Actual size

 = BEST

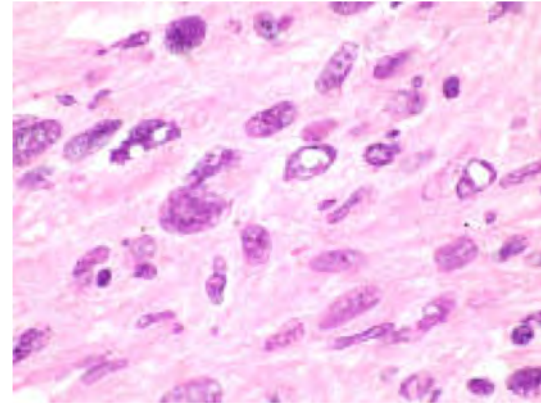
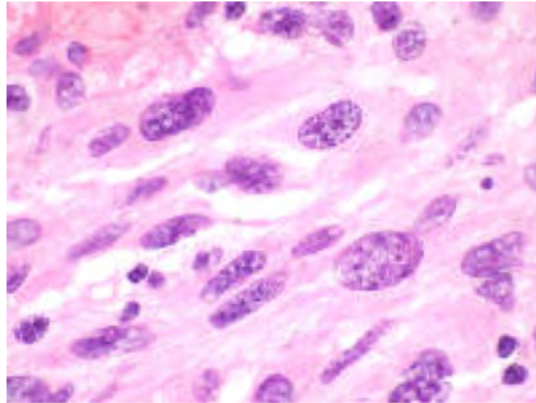
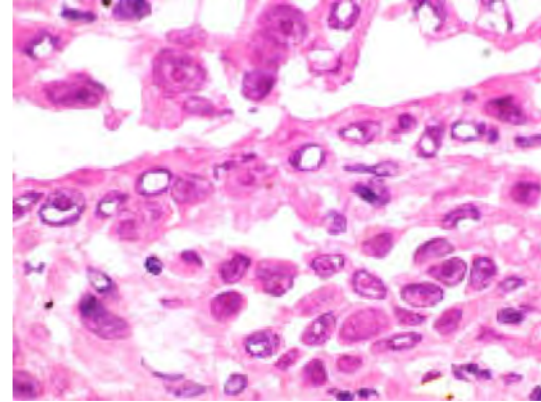
 = GOOD

# Section thickness

6 $\mu$ m



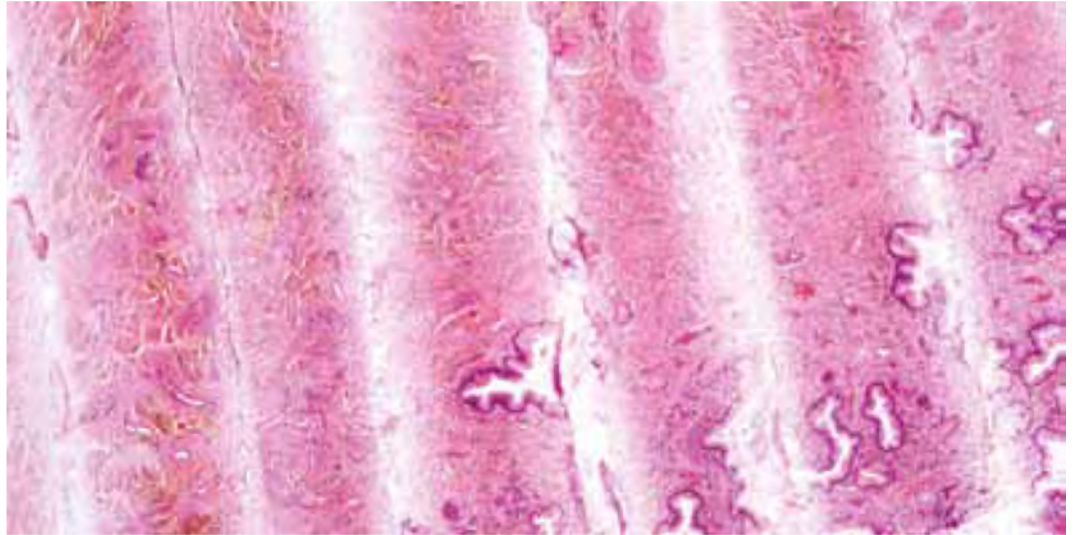
3 $\mu$ m



Section thickness should be adapted to the tissue cell density and the histological/molecular structure we aim to localize

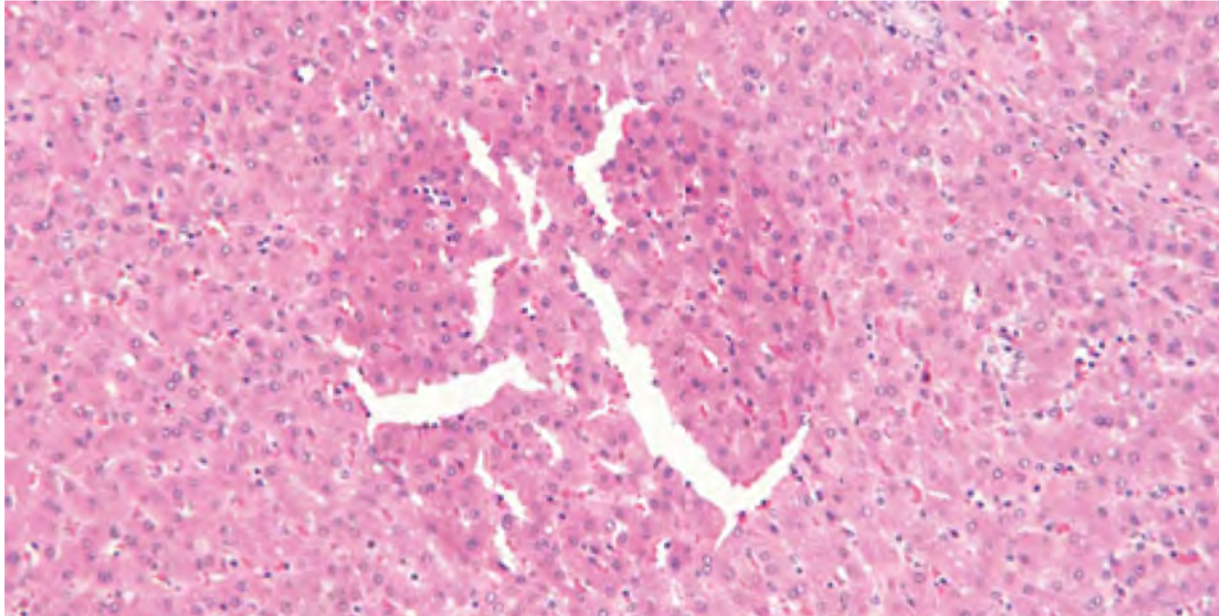


# Microtome/cryostat part moving



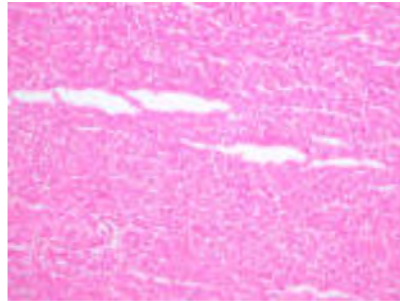
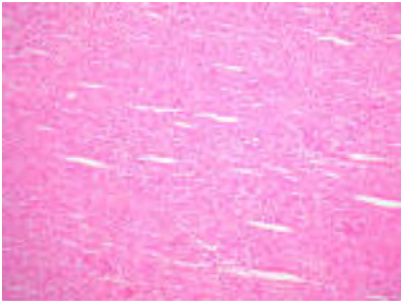
Lock all the moving mechanisms before starting the sectioning

# Water bath bubbles

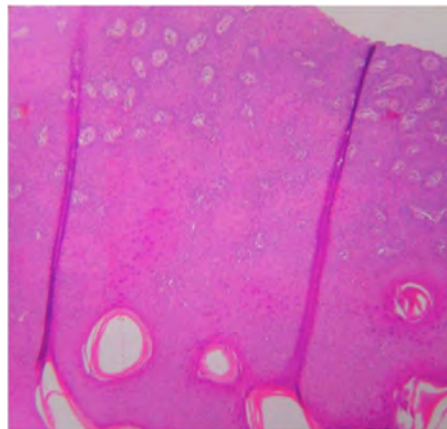
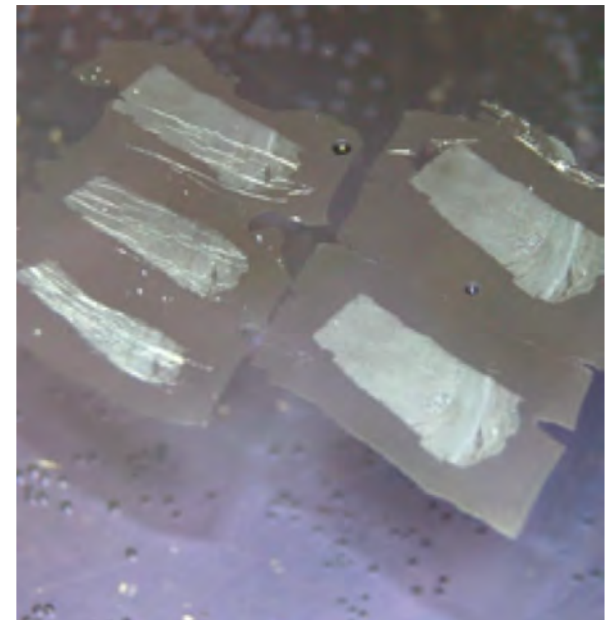


Debubble the water bath bottom before  
floating sections

# Cutting temperature



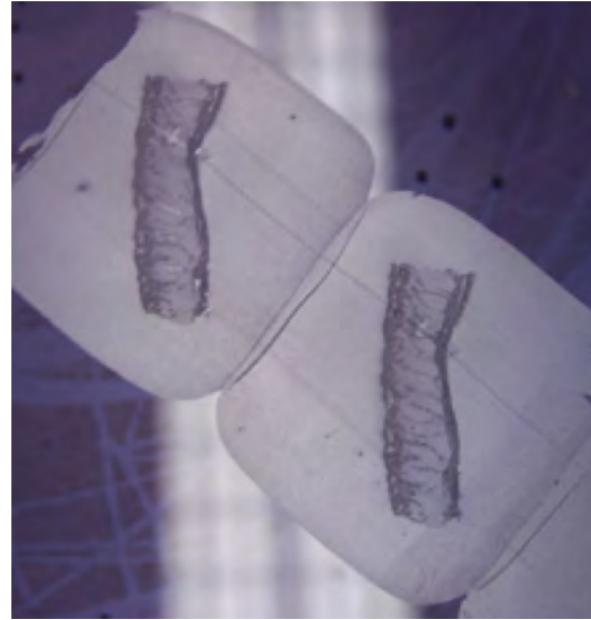
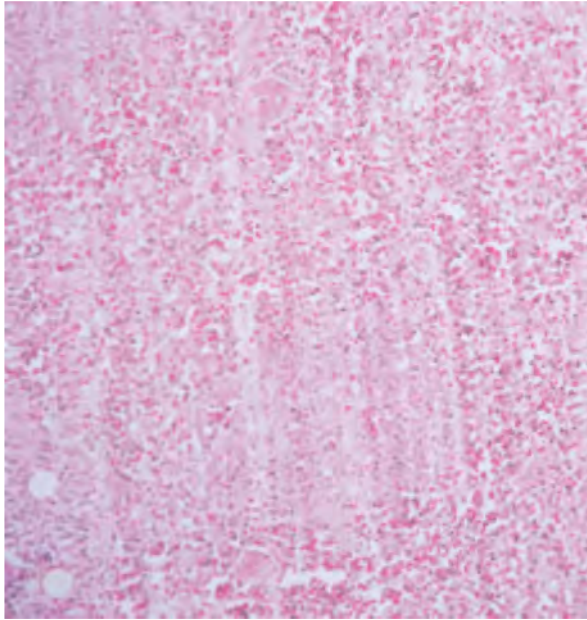
OCT -> cracking and shattering



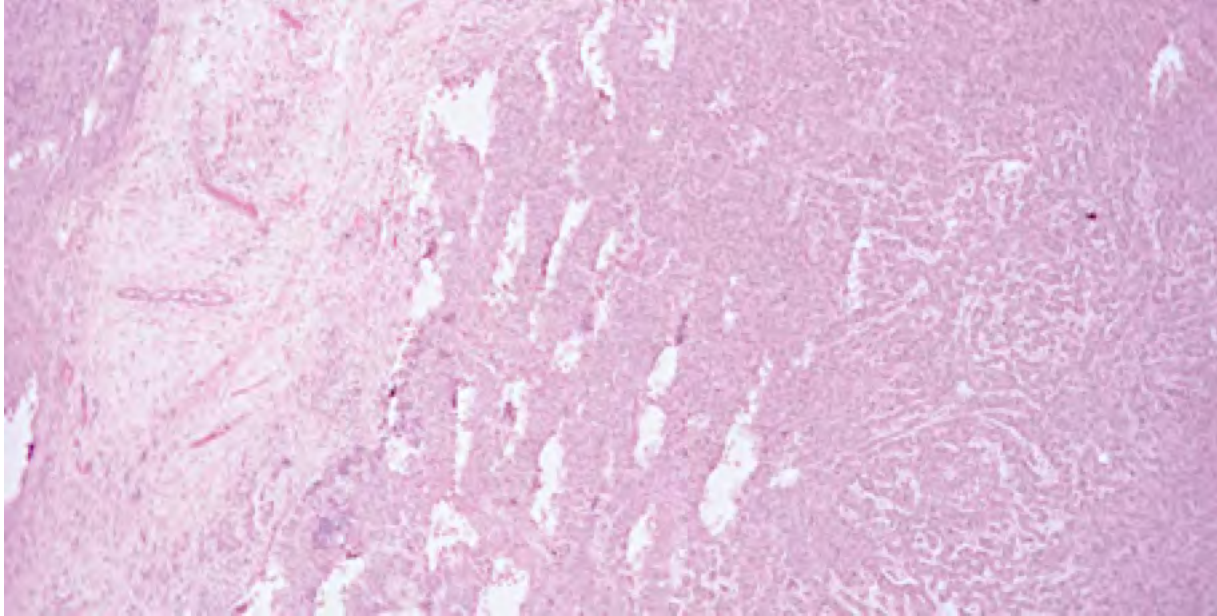
FFPE -> folding and compression



# Blade scratch, nick and dirt

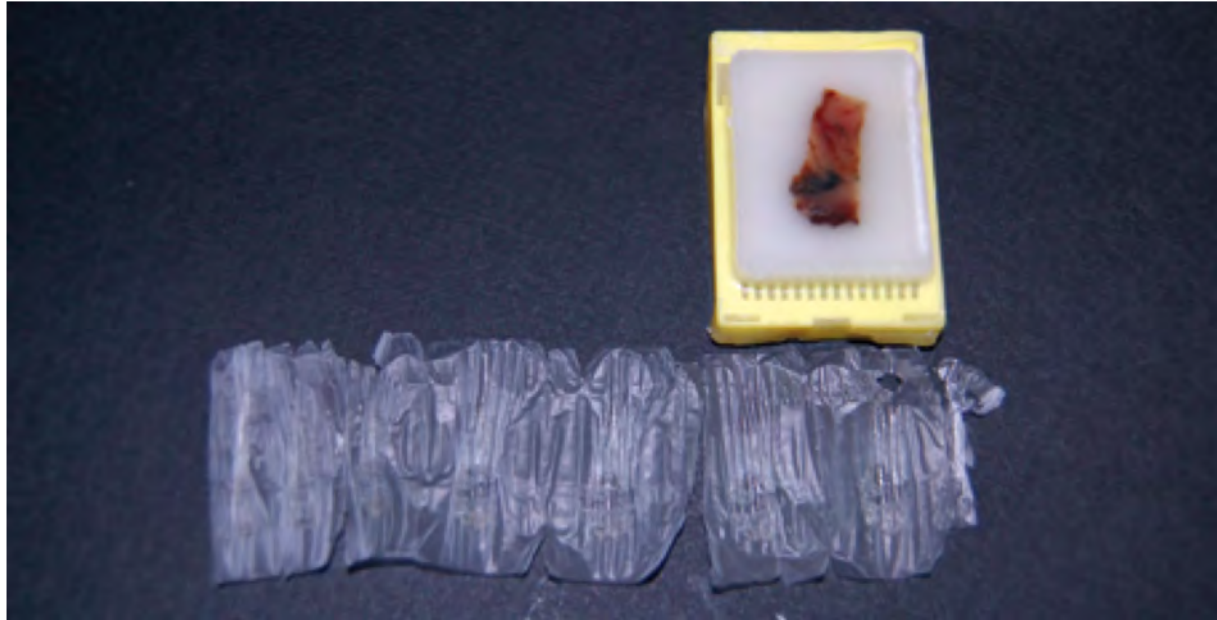


# Rough Trimming



Limit the trimming thickness setting and perform it slowly in order to preserve the blade

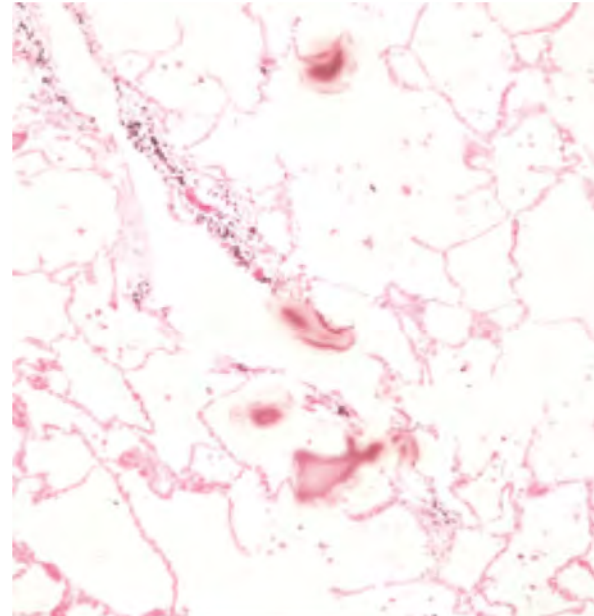
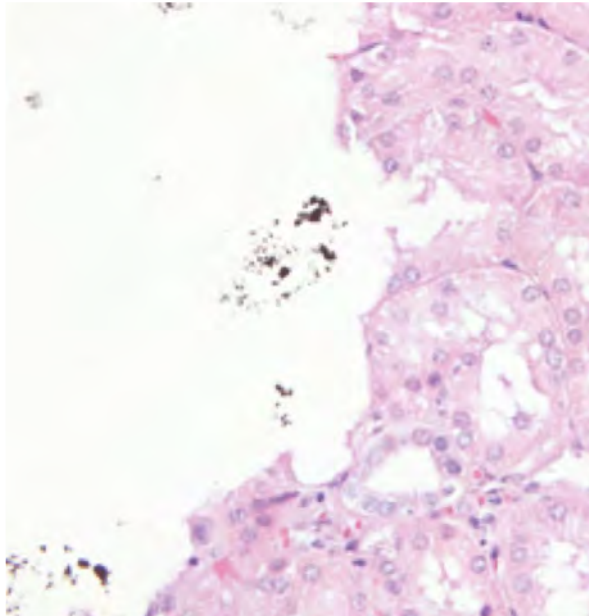
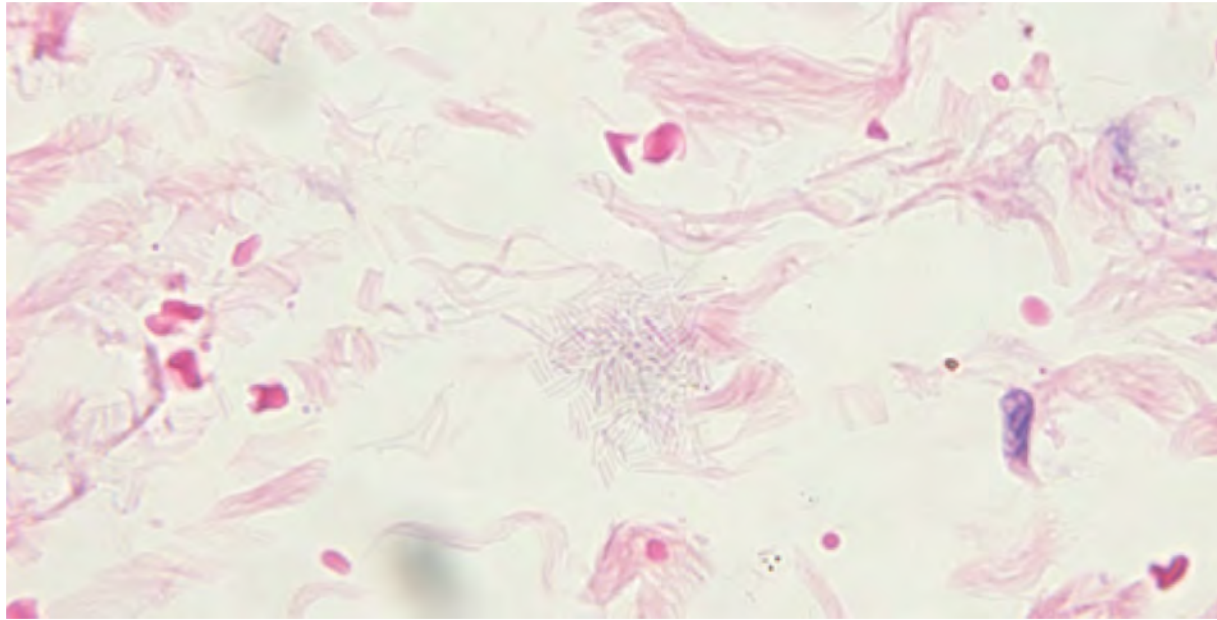
# Quality of the embedding media



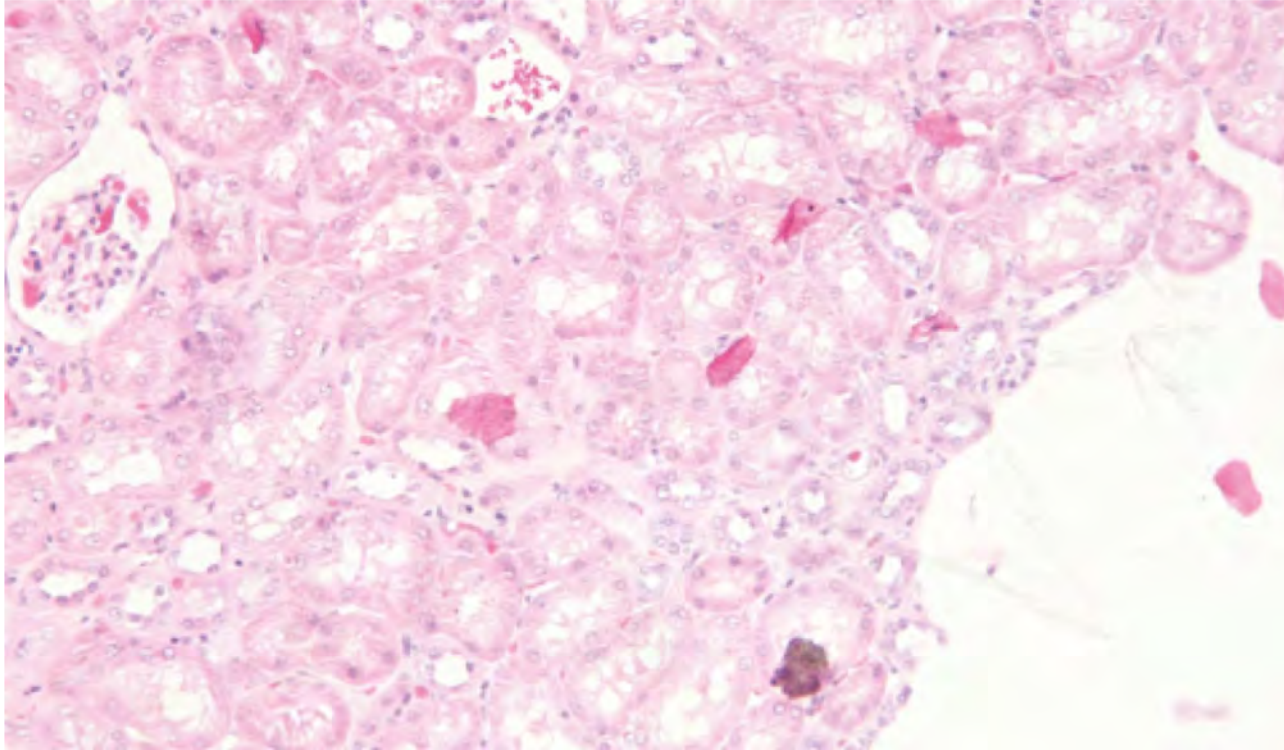
Bad quality paraffin leads to section folding although a correct chilling was applied



# Water bath/glass contamination

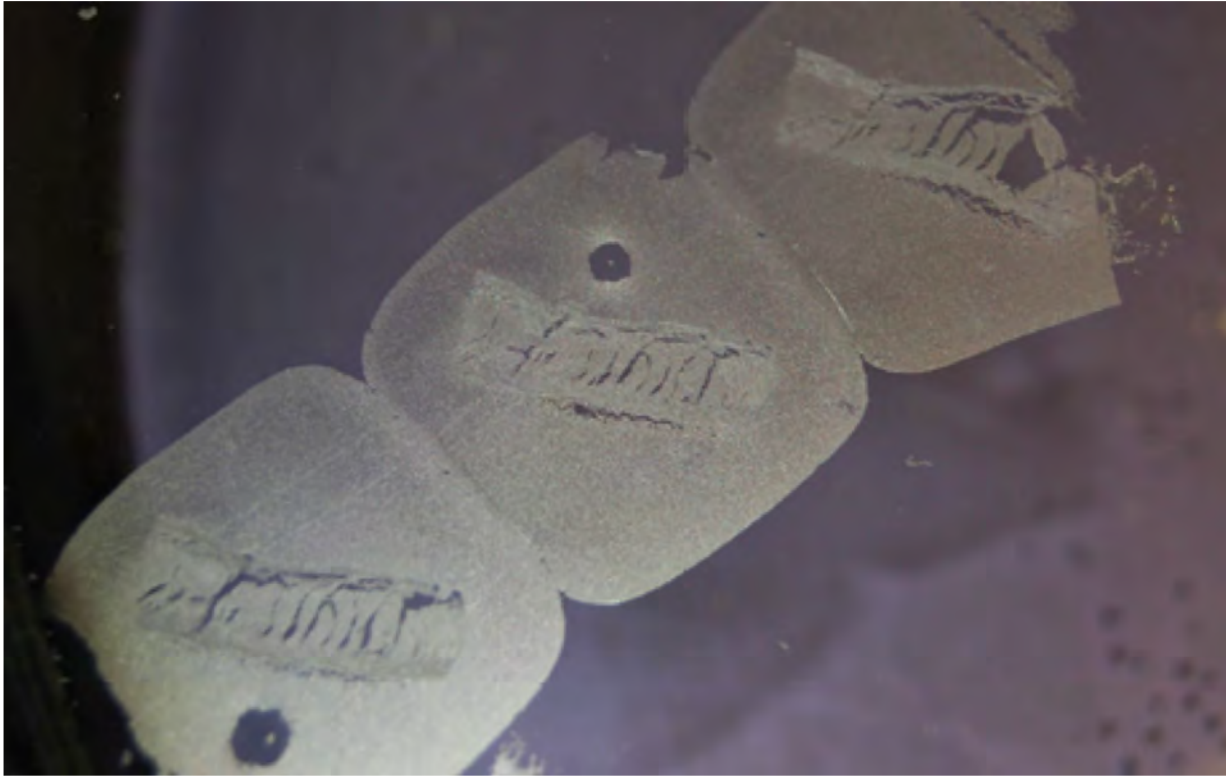


# Squamous contamination

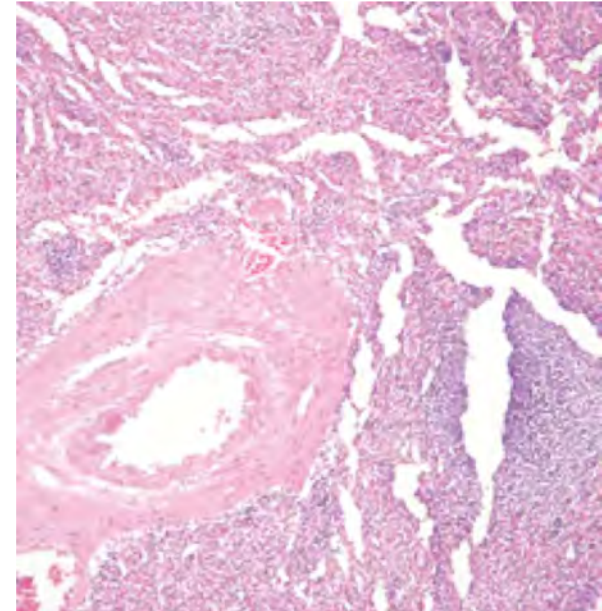


Do not recycle the floating water, clean the water bath after each use, clean the glass slides with acetone and let them dry before use, keep your tools clean.

# Over-floating/expansion

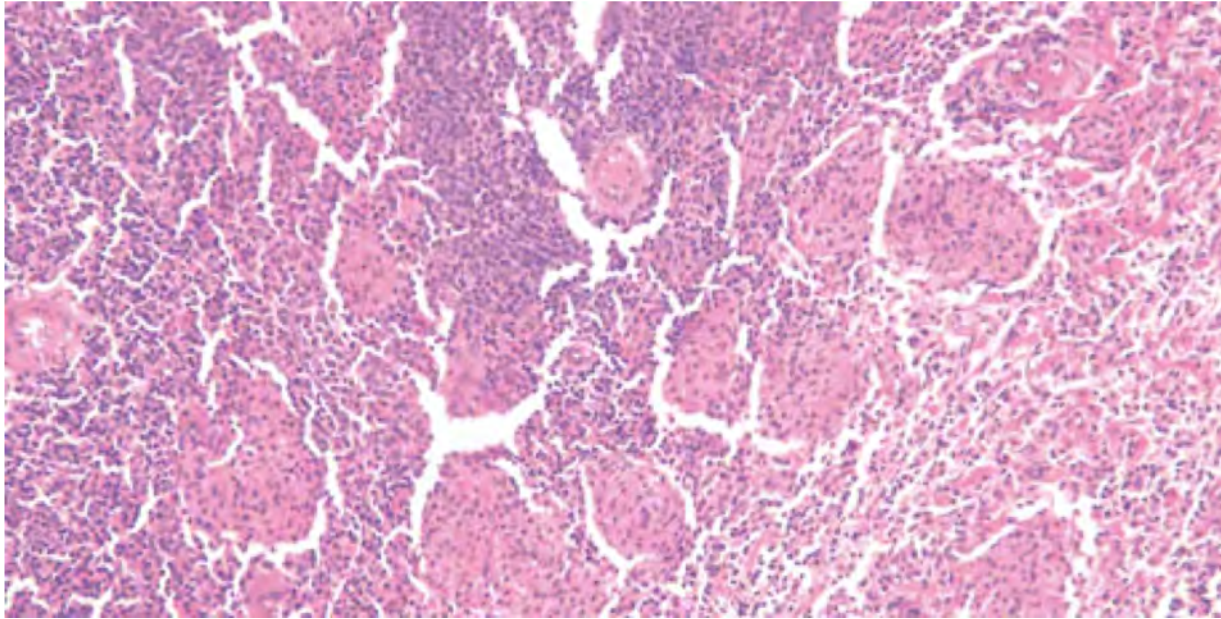


Do not over-float your sections





# Section drying



Do not forget your sections in the oven (nor anywhere else!)

# Conclusions

- The preliminary tissue processing steps may strongly influence the quality of your histological preparations, thus influencing the quality of your results
  - Be aware of the basic principle
  - Do not mess up reagents
  - Follow the protocols
  - Do not play around with the equipment settings

The best histology protocol rely on good tissue preparation and on good sectioning.

Q&A