# Lessons from histology and immunohistochemistry

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# Histology and immunohistology

- handling of the tissue depends on the further use
- methods of choice:
  - immersion fixation
  - perfusion fixation
  - freezing of tissue

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# Embryonic organs

- Loose
  - less extra cellular matrix
  - more water
- Small
  - easy to fix
  - difficult to process
  - easy to do whole-mounts

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# Embryonic organs

- Lack mostly immunological response elements
  - in immunohistochemistry background problems less severe than in many adult organs

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# Adult organs

- Dense
  - less undifferentiated areas, more extracellular matrix
  - less water
- Large
  - more difficult to fix, easier to process
  - almost impossible to do whole-mounts

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# Adult organs

- Immunological systems well developed
  - problems with background
- More blood cell
  - more background in immunofluoresence
- More enzymatic activity
  - more background in immunodetection

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# Fixatives

- fixative alters the tissue by stabilizing the proteins, changes the soluble contents of the cell into insoluble

- immersion fixatives are often so called additive fixatives that chemically react with protein

- with non-additive fixatives the fixative molecule itself does not combine with the protein

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# Factors influencing fixation

- temperature
- size of the tissue
- tissue to volume ratio
- osmolality
- time used for the fixation
- choice of fixative
- penetration
- tissue storage
- pH

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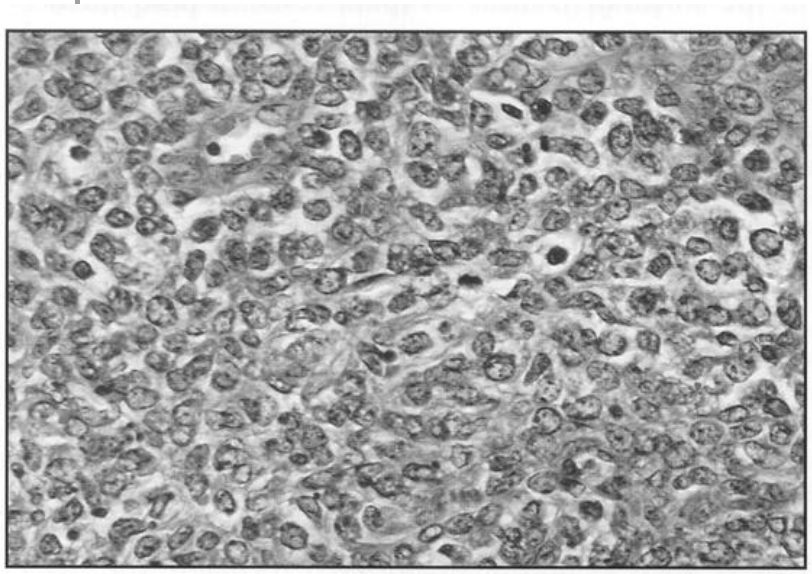
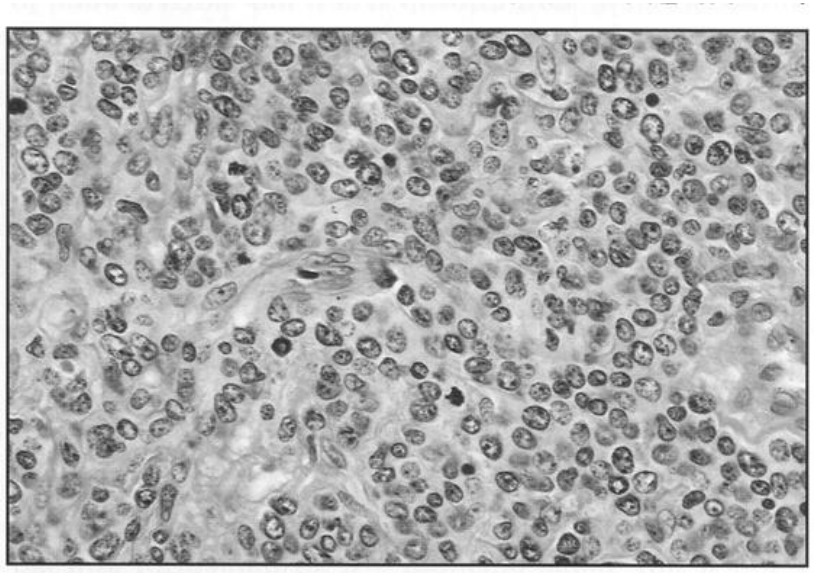
# Fixation affects the tissue morphology





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**Immersion fixation:**

- whole tissues, pieces of tissue or whole embryos are fixed *in toto*

**Perfusion fixation:**

- animals are fixed *in toto* via blood circulation

**Freezing of tissues:**

- tissues/embryos are frozen *in toto*

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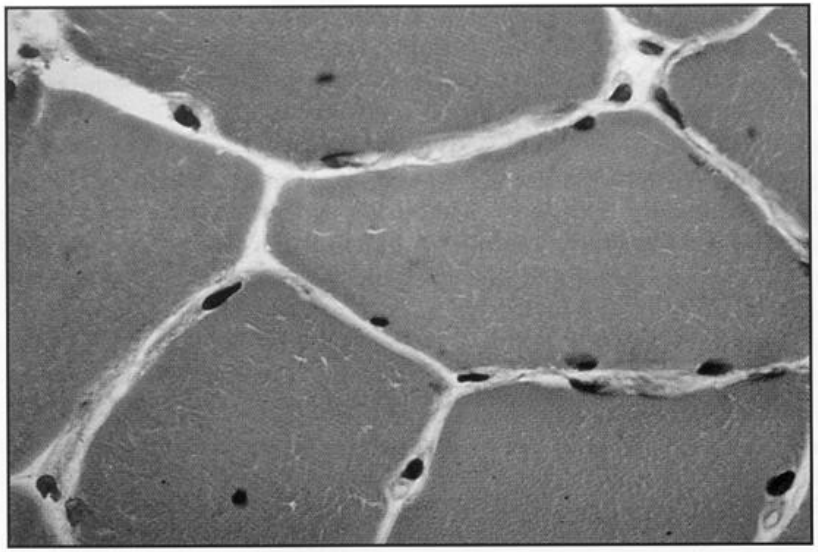
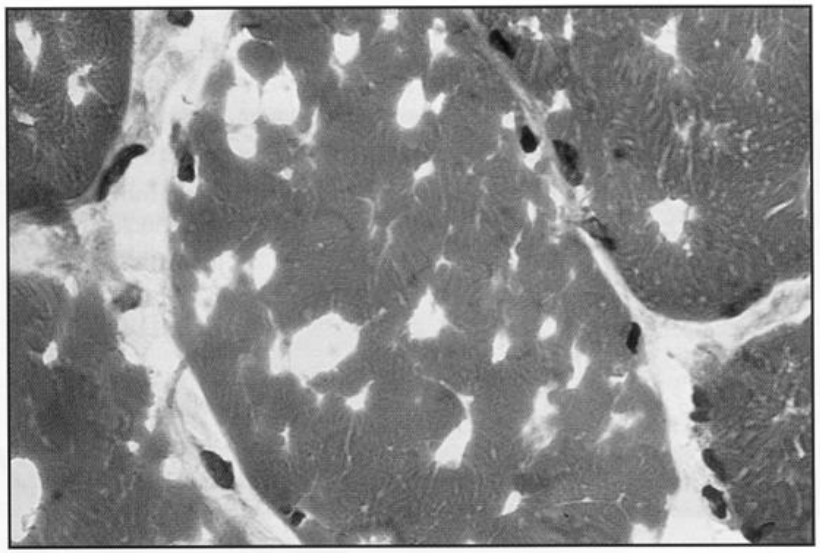
# Frozen sections





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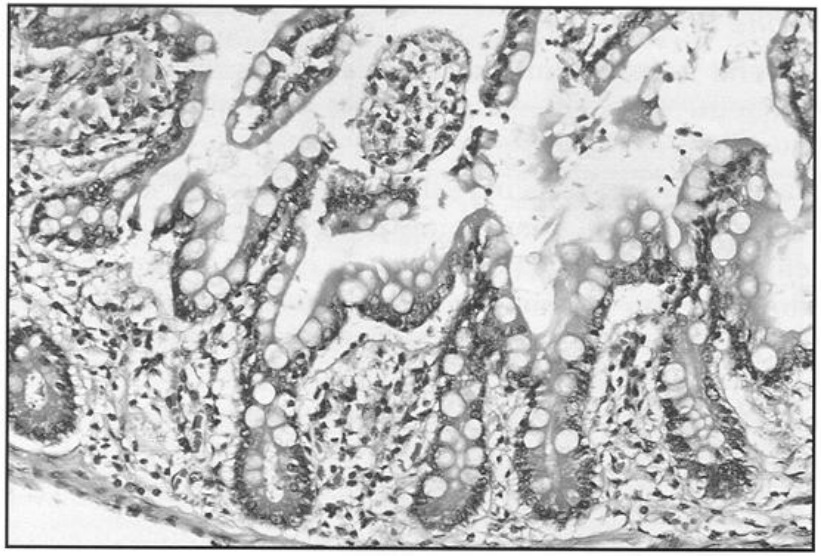
# Paraffin sections



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# Paraffin

- paraffin is the common name the alkane hydrocarbons with the general formula C*n*H2*n*+2
- Paraffin wax refers to the solids with n=20–40
- Wax melts in app. 55-60ºC, and tissues can be embedded into this wax, let the wax to

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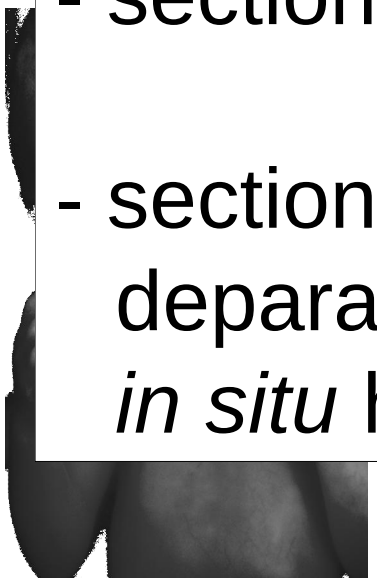
# Paraffin sections

- additive fixatives often used

- after fixations tissues are dehydrated, cleared and embedded in paraffin

- sections are cut at 2 - 10 μm with microtome

- sections are collected to objective slides, dried, deparaffinized and processed for histology, *in situ* hybridization or immunohistochemisty



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