



# **Lessons from histology and immunohistochemistry**

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**Measurement techniques for Bioinformatics  
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## **Histology and immunohistology**

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

# Embryonic organs

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

# Embryonic organs

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

# 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

# Adult organs

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

# Fixatives

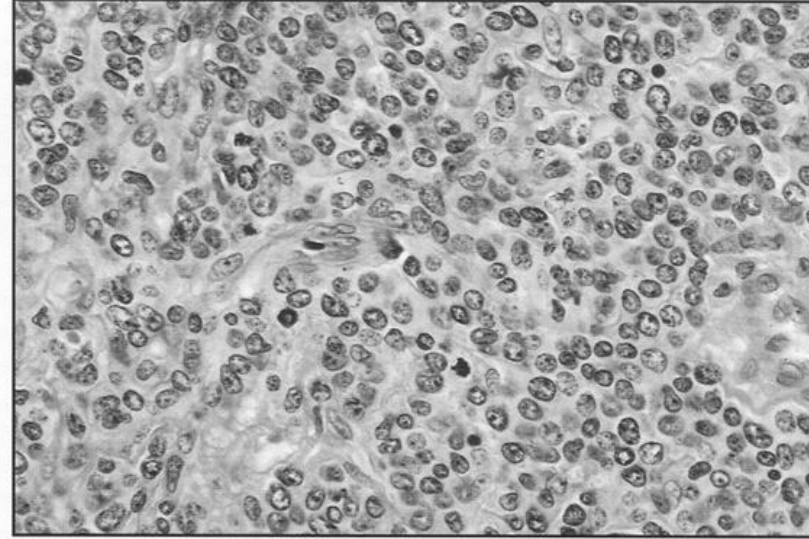
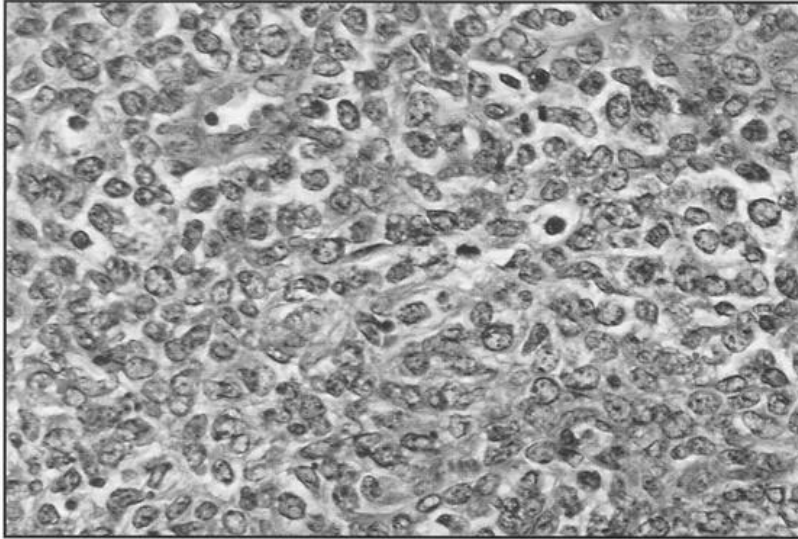
- fixative alters the tissue by stabilizing the proteins, changes the soluble contents of the cell into insoluble
- immersion fixatives are often so called additive fixatives that chemically react with protein
- with non-additive fixatives the fixative molecule itself does not combine with the protein

## 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



# Fixation affects the tissue morphology





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### **Immersion fixation:**

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

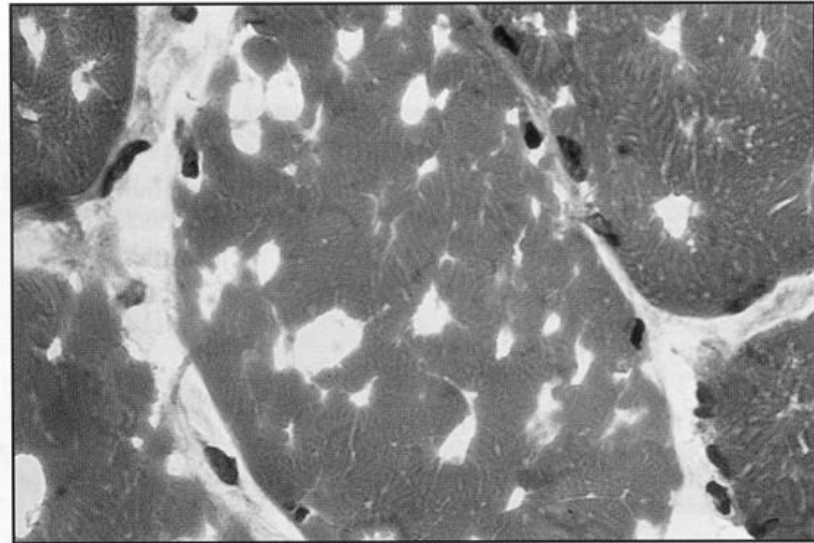
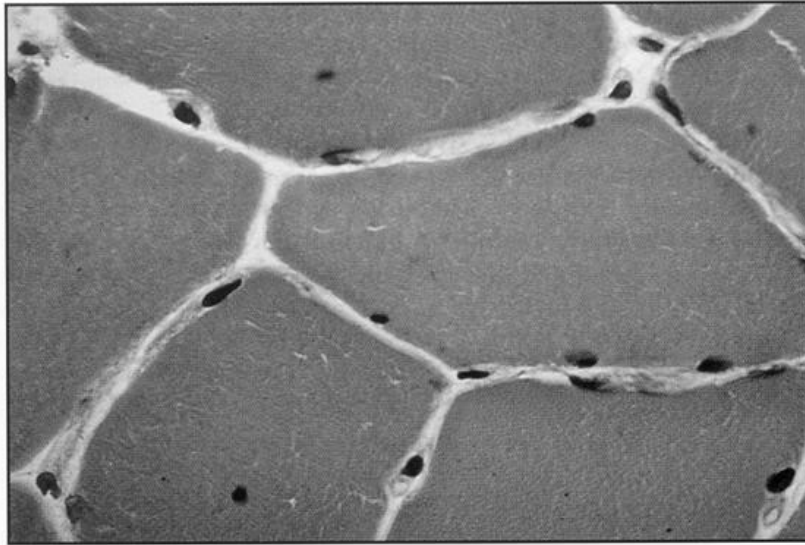
### **Perfusion fixation:**

- animals are fixed *in toto* via blood circulation

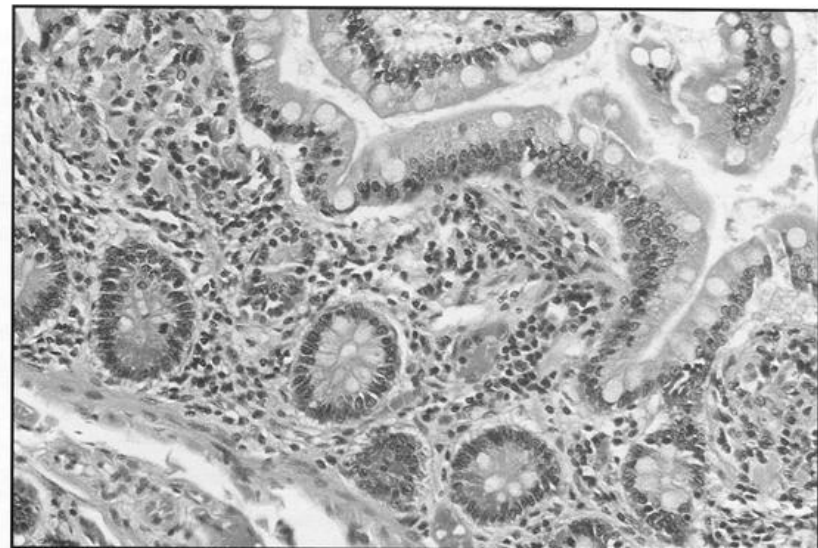
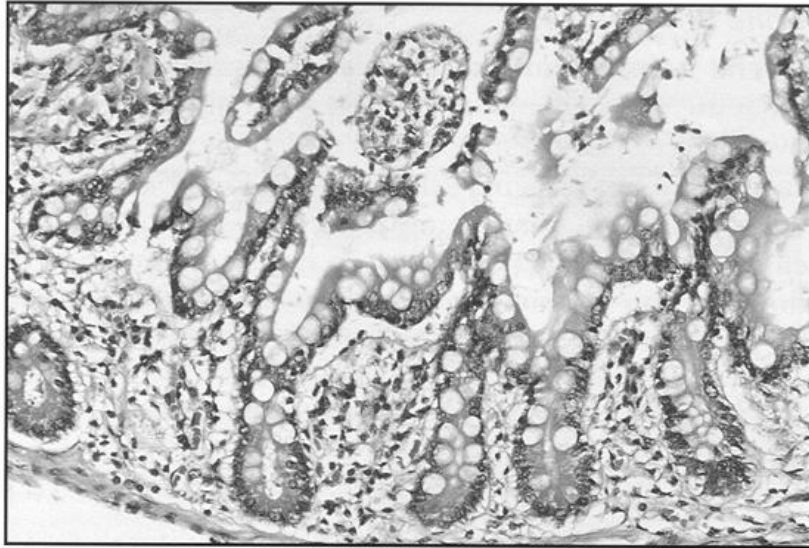
### **Freezing of tissues:**

- tissues/embryos are frozen *in toto*

# Frozen sections



# Paraffin sections



# Paraffin

- **paraffin** is the common name the alkane hydrocarbons with the general formula  $C_nH_{2n+2}$
- **Paraffin wax** refers to the solids with  $n=20-40$
- **Wax** melts in app.  $55-60^\circ\text{C}$ , and tissues can be embedded into this wax

# Paraffin sections

- additive fixatives often used
- after fixations tissues are dehydrated, cleared and embedded in paraffin
- sections are cut at 2 - 10  $\mu\text{m}$  with microtome
- sections are collected to objective slides, dried, deparaffinized and processed for histology, *in situ* hybridization or immunohistochemistry

