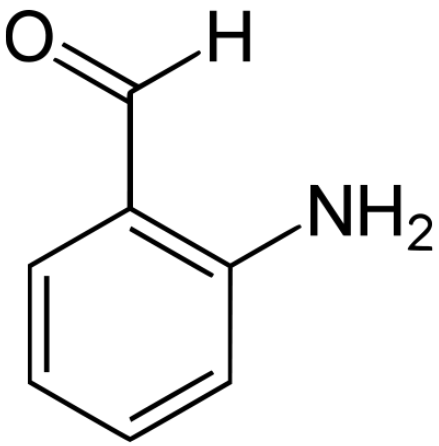
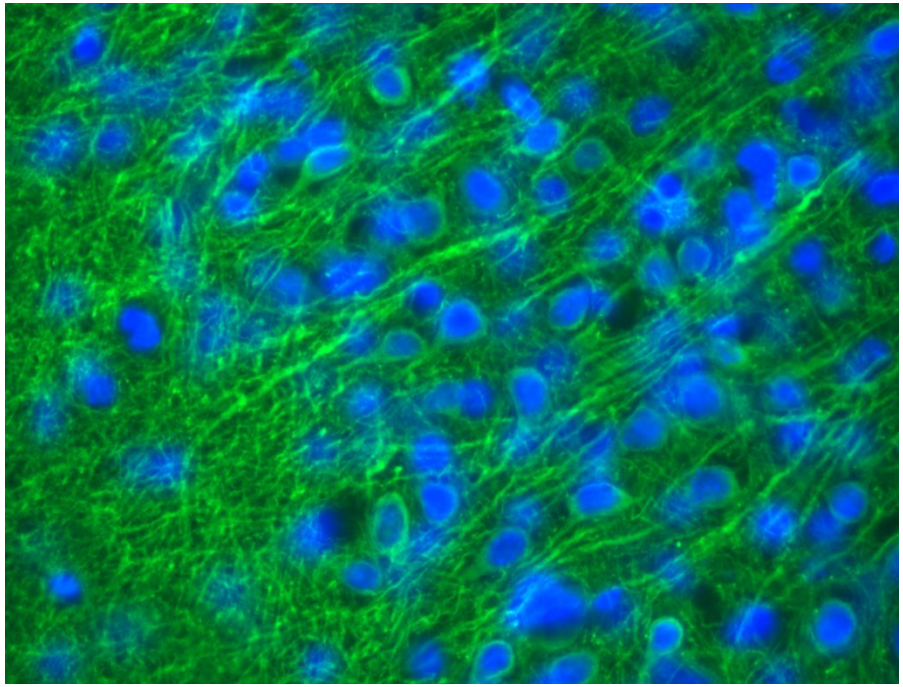
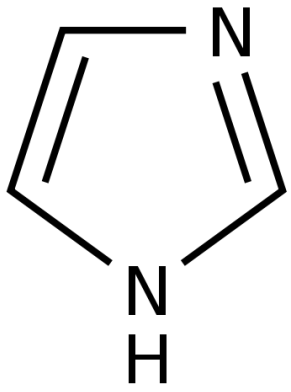


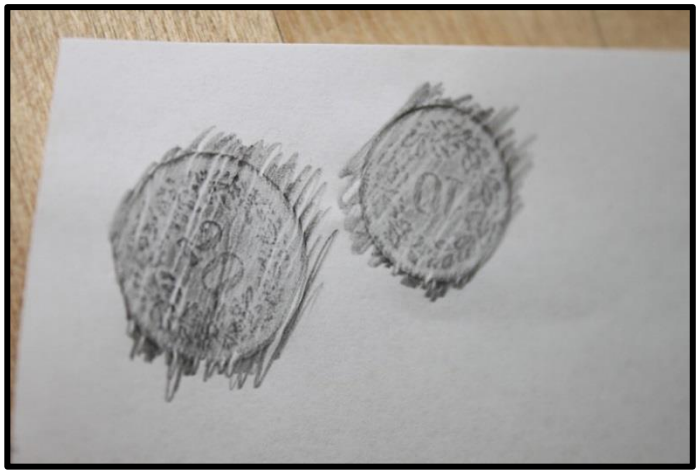


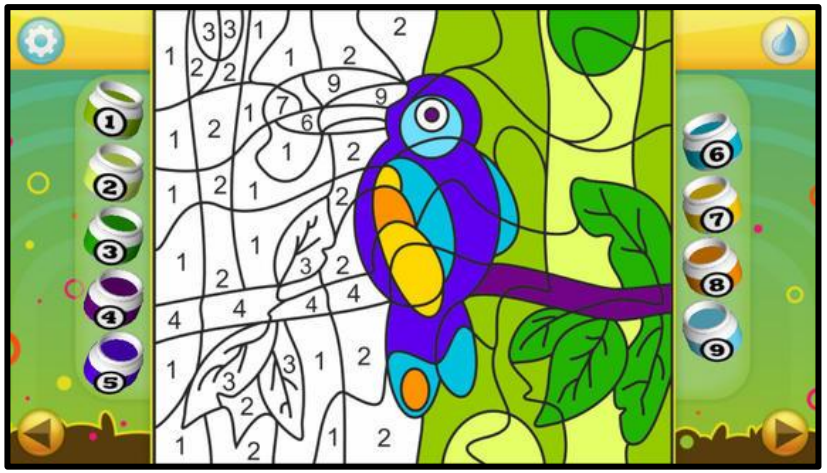


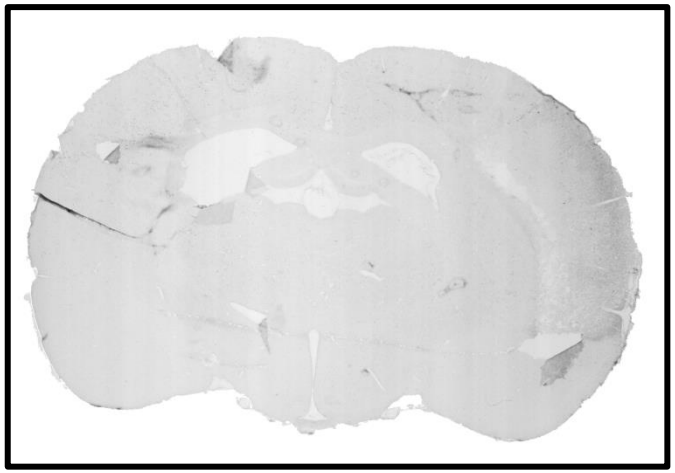
Teil 1 – **Präparationsmethoden**

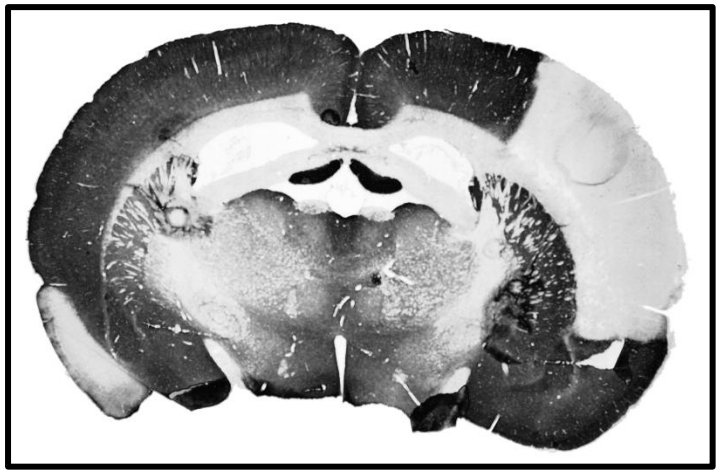
Teil 2 – **Färbemethoden**

Färben









Färbungen - Übersicht

i. **Konventionelle Färbungen**

ii. Enzymhistochemie

iii. **Immunhistochemie, Immunmarkierung**

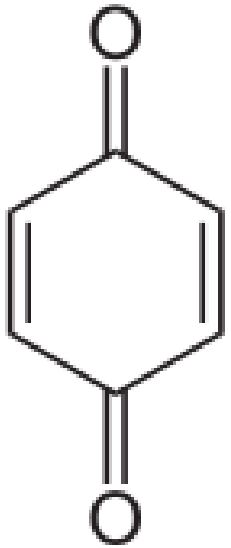
iv. In situ Hypridisierung

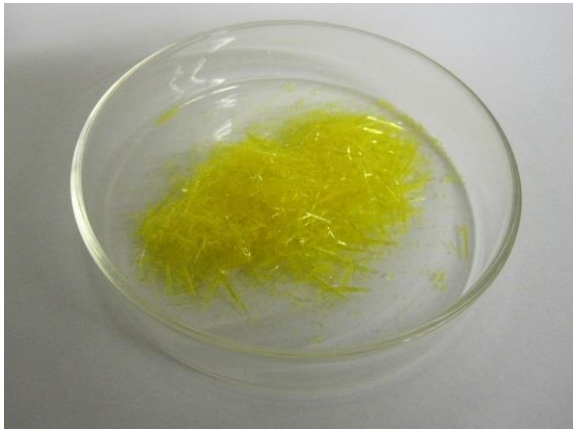
v. Reporter

i. Konventionelle Färbungen

Chromophor (Farbträger)

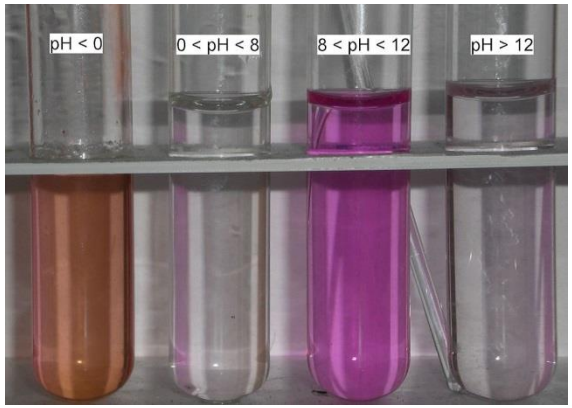
Chromophore Gruppen





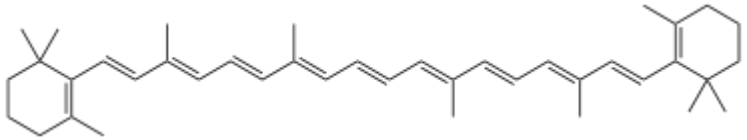
1,4-Benzochinon

C=C	Ethylen
C=O	Carbonyl
C=S	Sulfin
C=N	Carbimin
N=N	Azo
N=O	Nitroso



Phenolphthalein

Zahl & Anordnung der DB & pH bestimmen Farbe

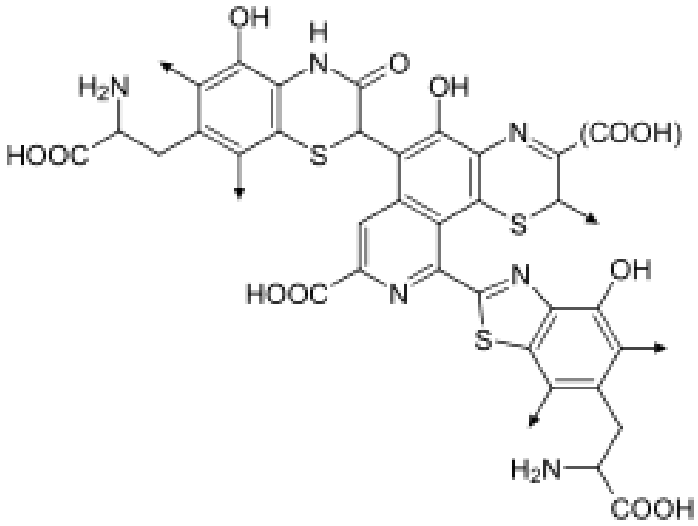


B-Carotin

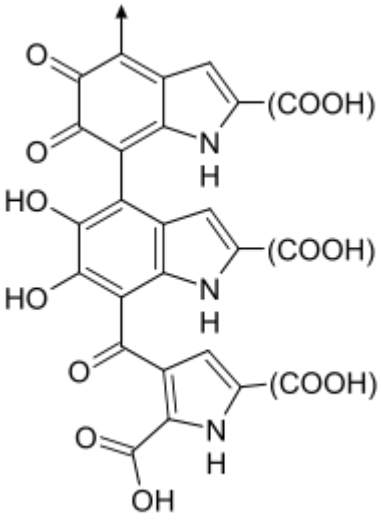




Rot-gold



Phäomelanin



Eumelanin

Schwarz-braun

Ladung von Farbstoffen

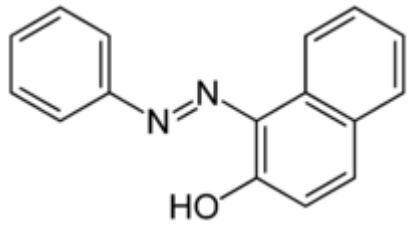
Auxochrome Gruppen

- **Basenbildend (+)**
  - NH_2 Amino
  - $NH(CH_3)$ Dimethylamino

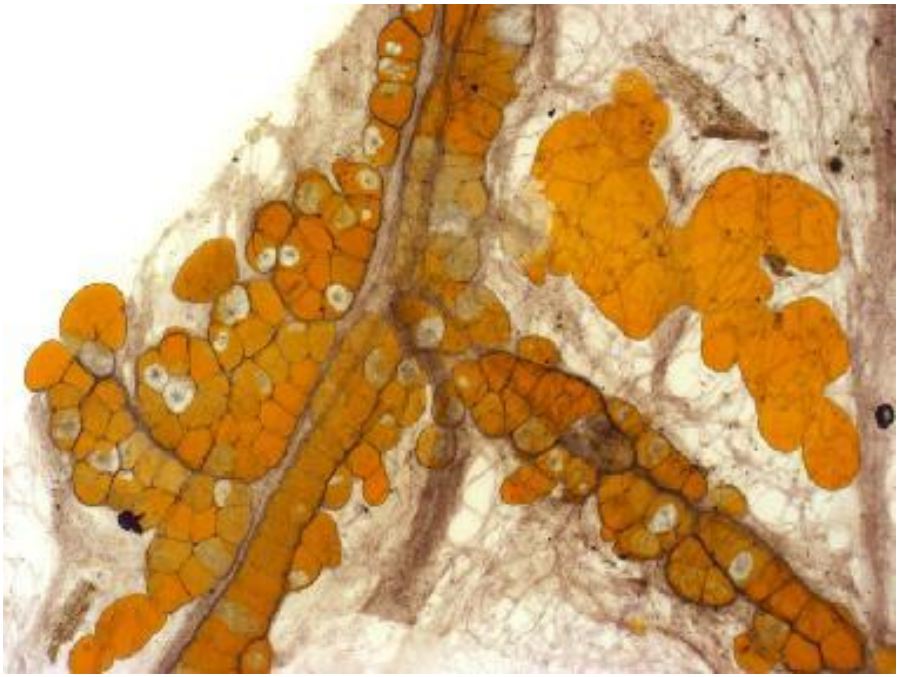
Fixierung am Substrat

- **Säurebildend (-)**
  - $COOH$ Carboxyl
  - NO_2 Nitro

Oder keine Ladung…

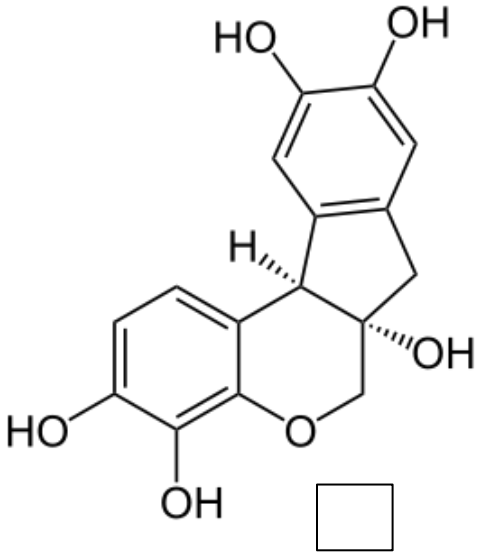


Sudan I



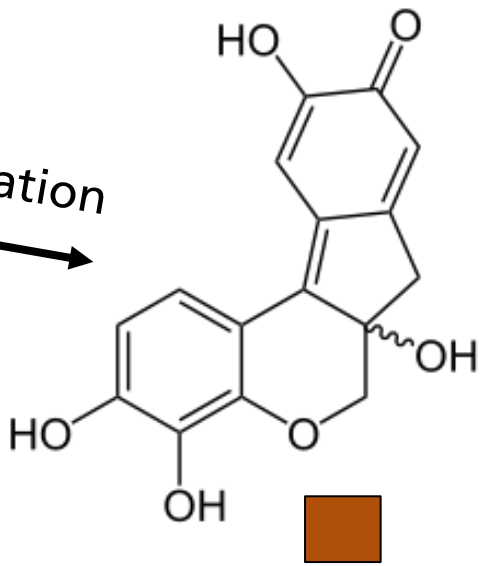
Beispiel Hämatoxylin

Campechebaum

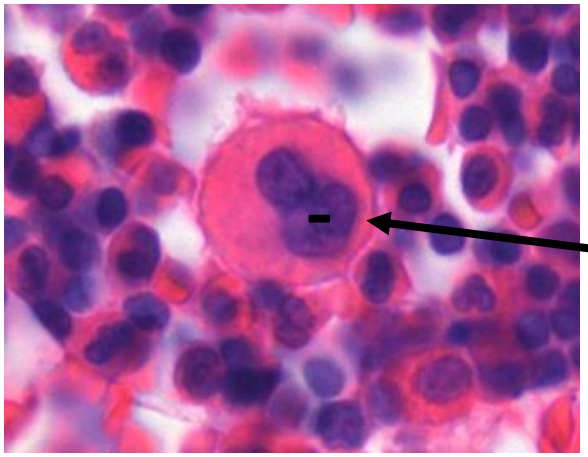


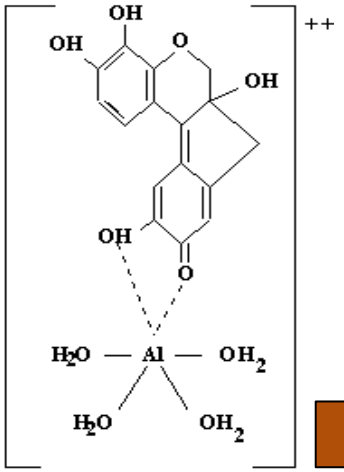
Hämatoxylin

oxidation



Hämatein





Hämalaun

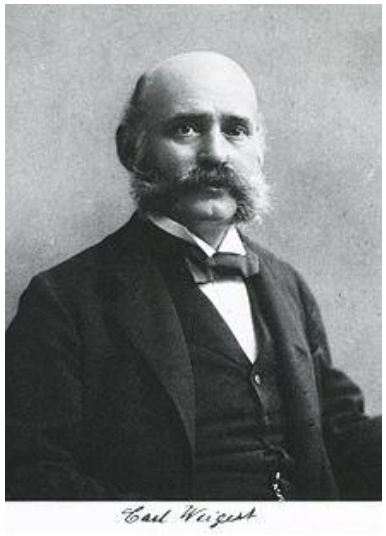
bläuen

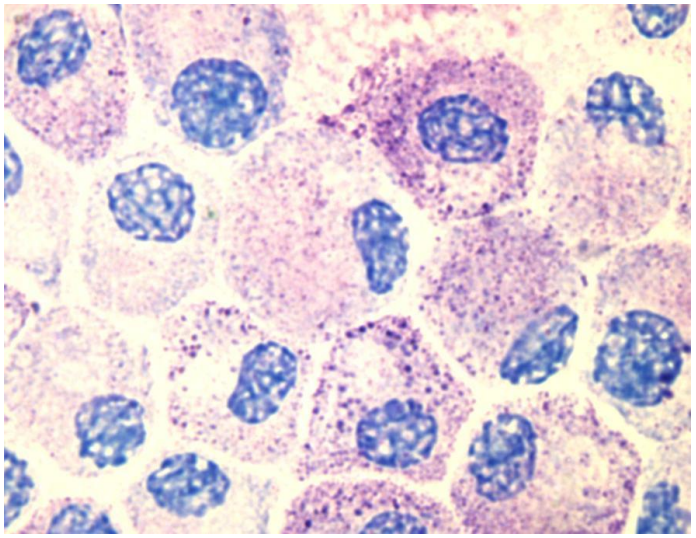
sauer

Saure

Basische Farbstoffe → azidophile / basophile / neutrophile Strukturen

Neutrale





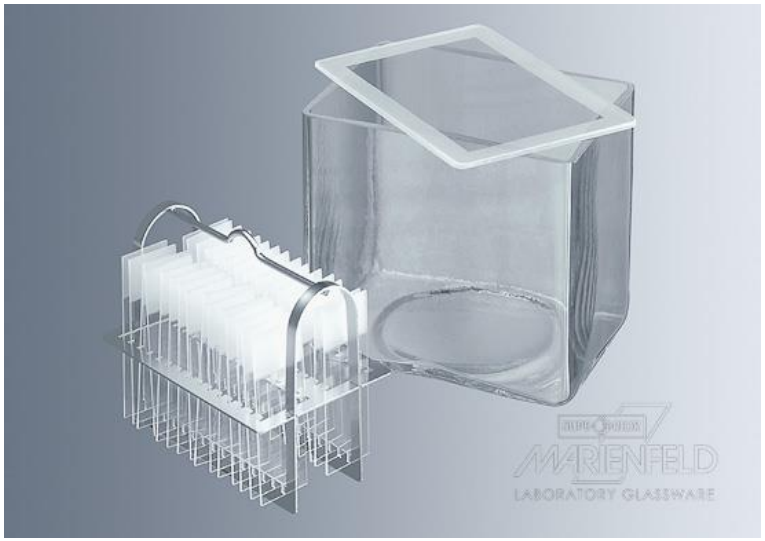
Färbetheorie

Färbungen wurden empirisch ermittelt

i. Chemische Färbungen

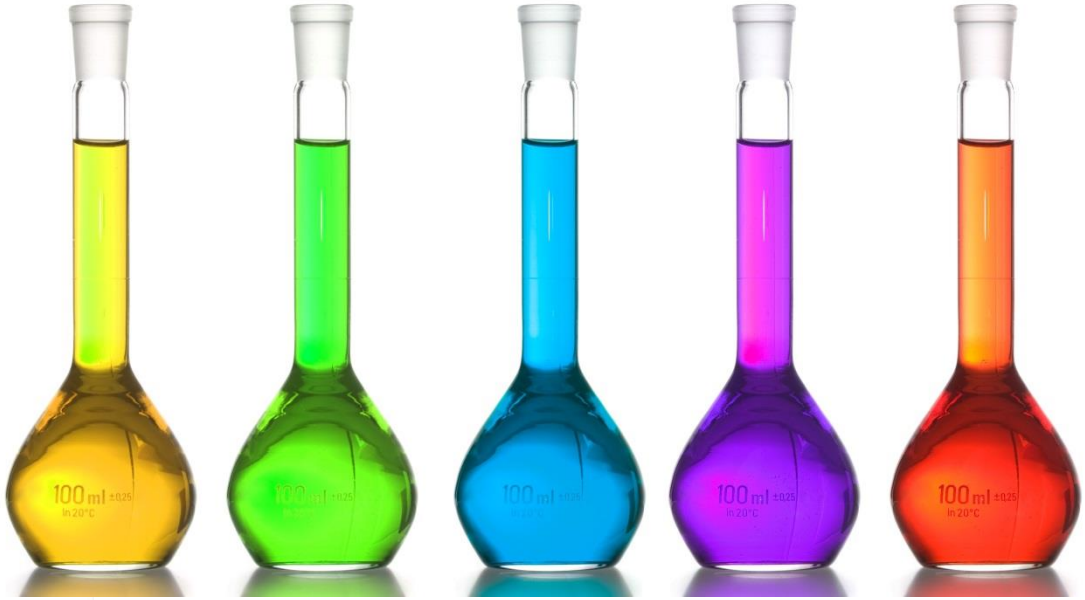
ii. Physikalische Färbungen
   - Löslichkeit
   - Gewebedichte
   - Dispersität (Zeit)

Färbezubehör



Fraunhofer IZI

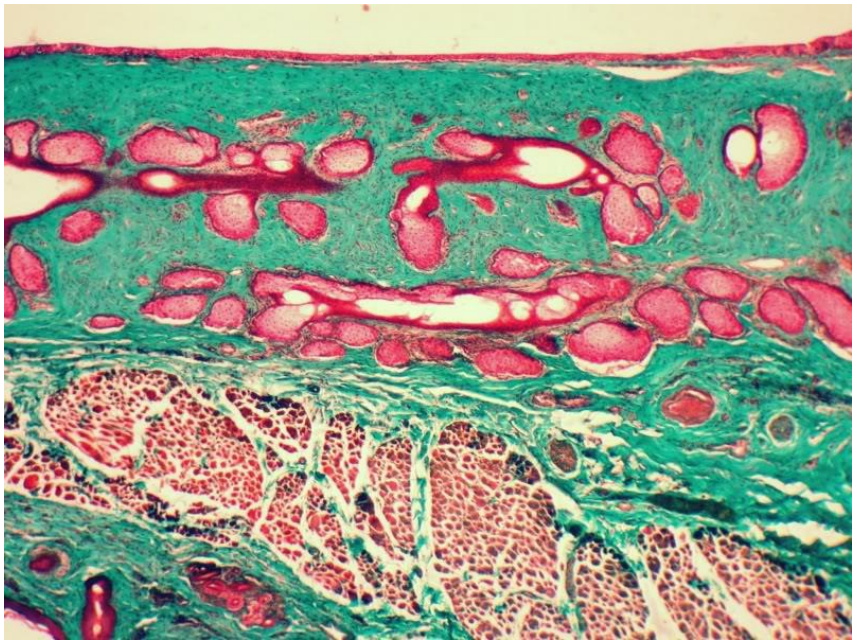
Färbezubehör



Färbezubehör

Beispiele…

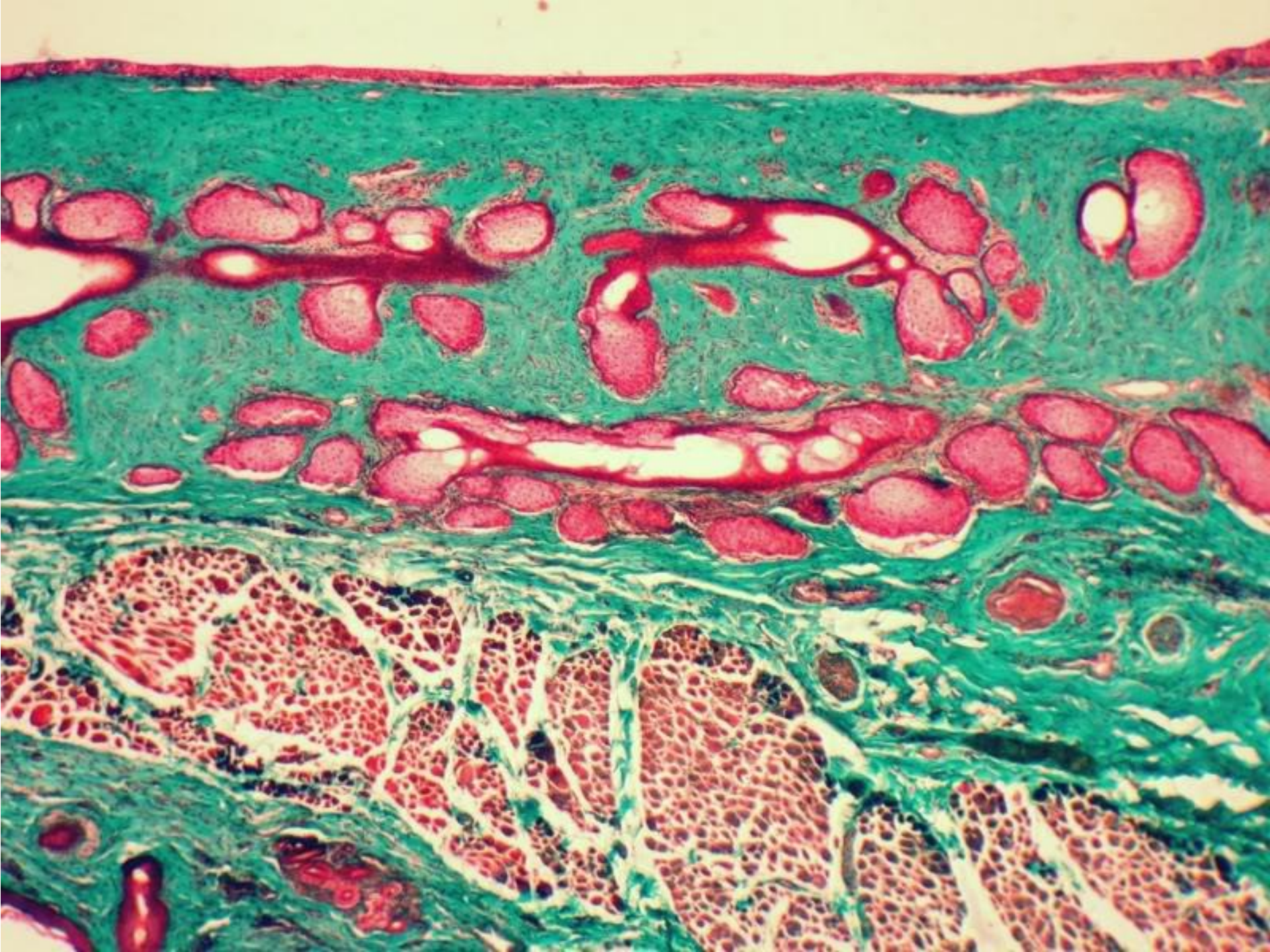
Beispiel 1: Masson-Goldner-Färbung (Trichrom)



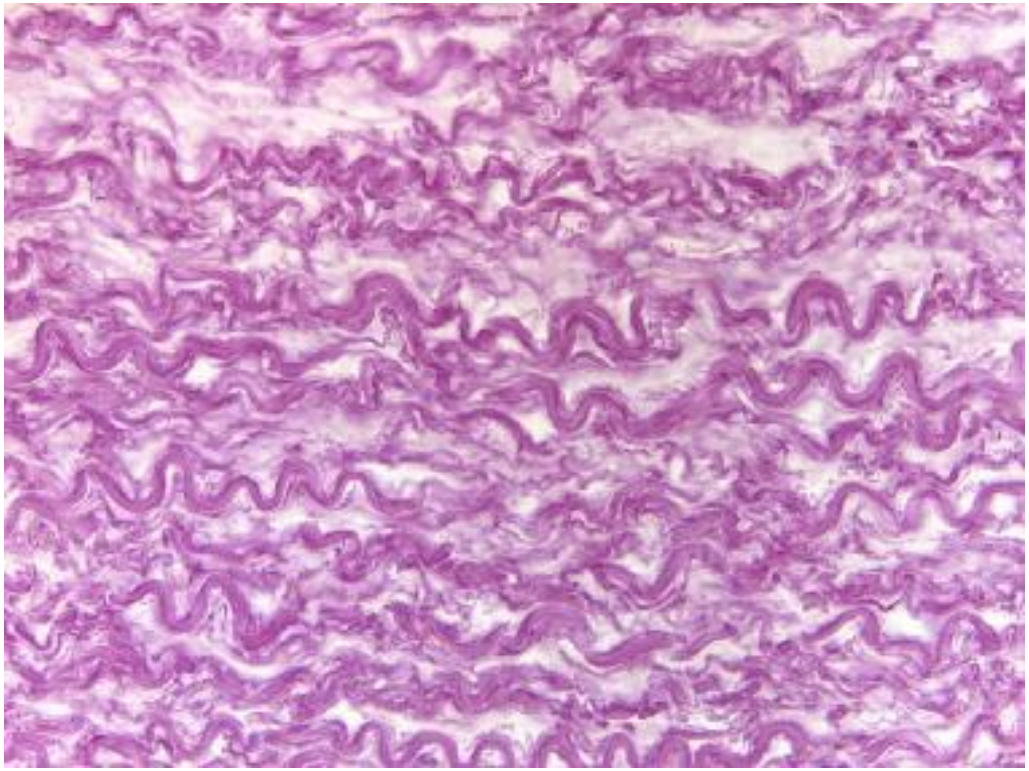
Augenlied

1. Eisenhhämatoxylin 2min
2. Leitungswasser 10min
3. Azophloxin 5min
4. 1% Essigsäure spülen
5. Phosphorwolframsäure-Orange 15s-30s Diff
6. 1% Essigsäure: Unterbrechnung Diff
7. Lichtgrün1-3min
8. 1% Essigsäure

Dispersität

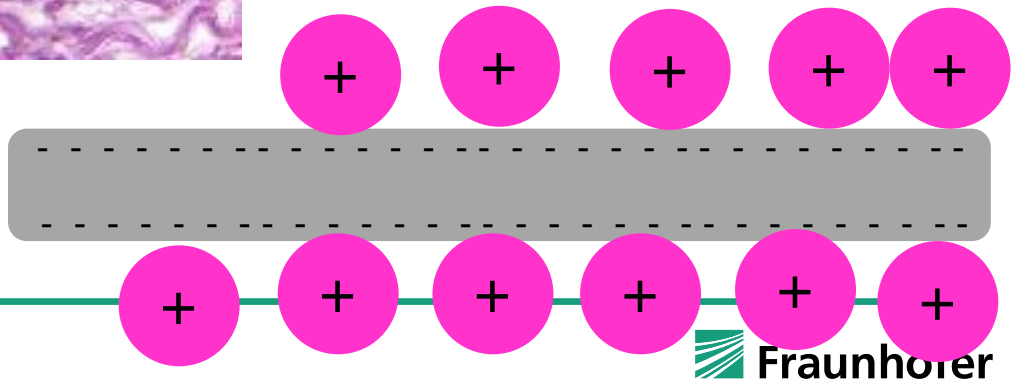


Beispiel 2: Resorcinufchsin-Färbung nach Weigert Elastikafärbung



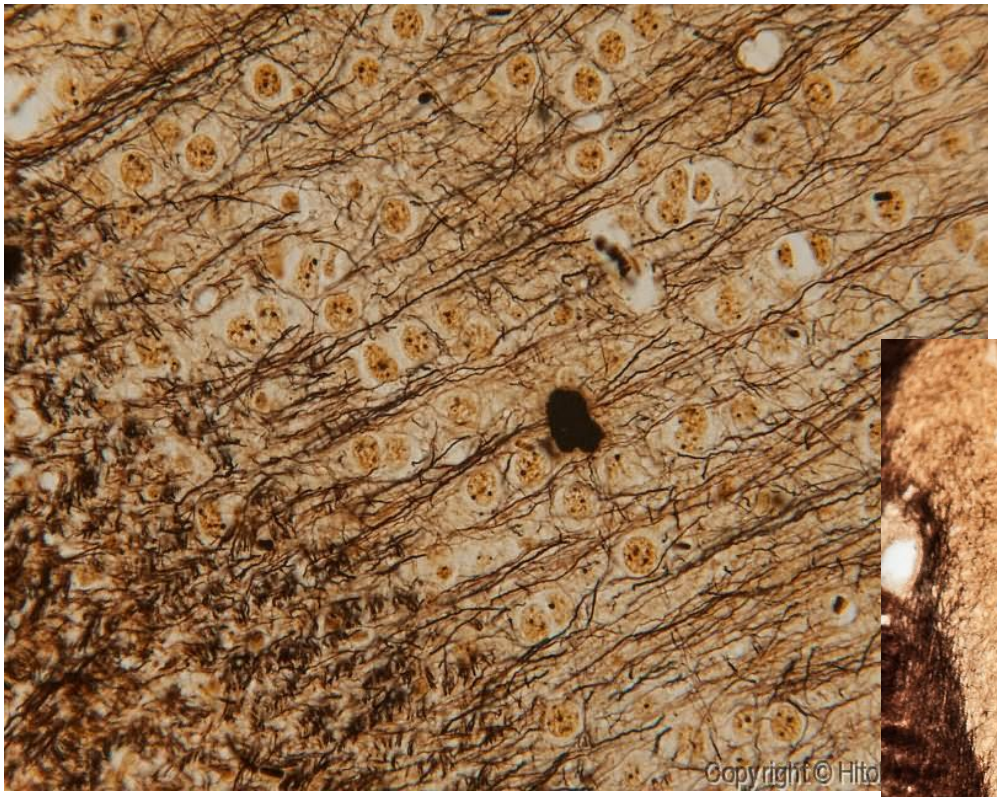
A. carotis communis

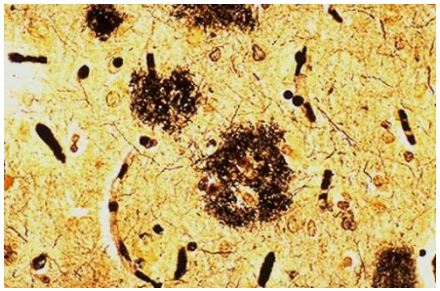
1. Basisches Fuchsin + Resorcin + Eisen-II-chlorid = Resorcinfuchsinlösung (+) 30min
2. Leitungswasser
3. Differenzierung in 96% Ethanol

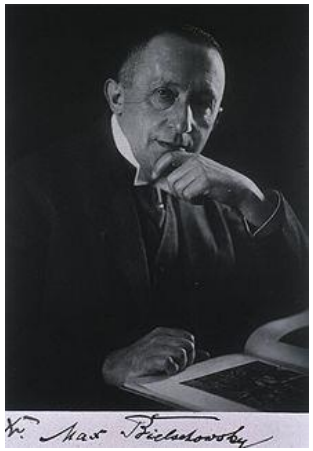


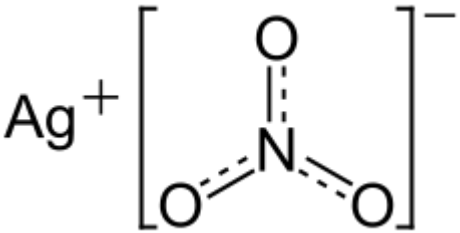
Fraunhofer IZI

Beispiel 3: Bielschowsky Versilberung



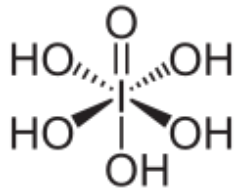




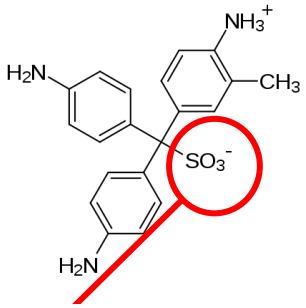


Fraunhofer IZI

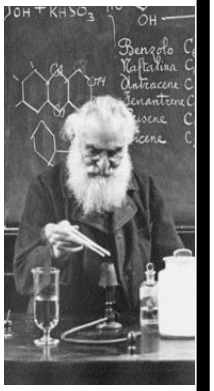
Beispiel 4: PAS Färbung – Nachweis von Glycoproteinen



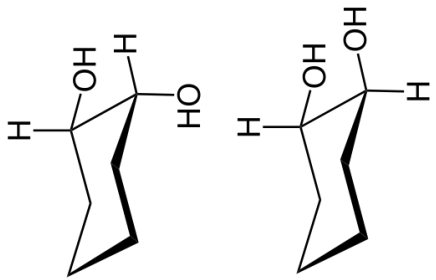
Periodsäure

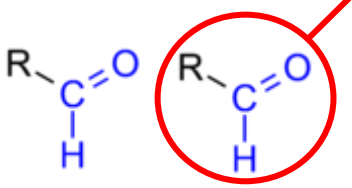


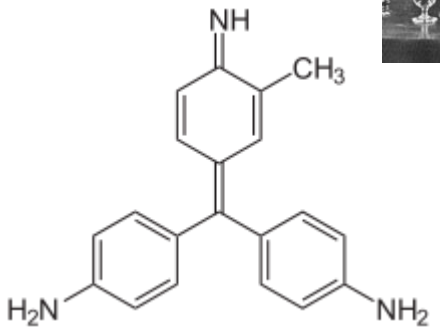
Fuchsinschweflige Säure



Schiff-Reaktion







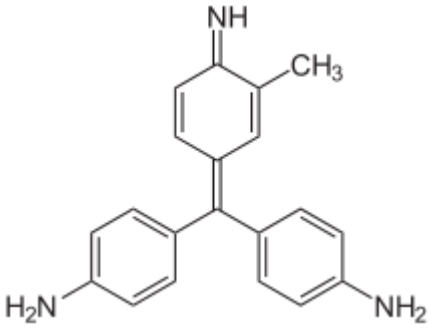
Fuchsin

Glycolgruppen Aldehydruppen

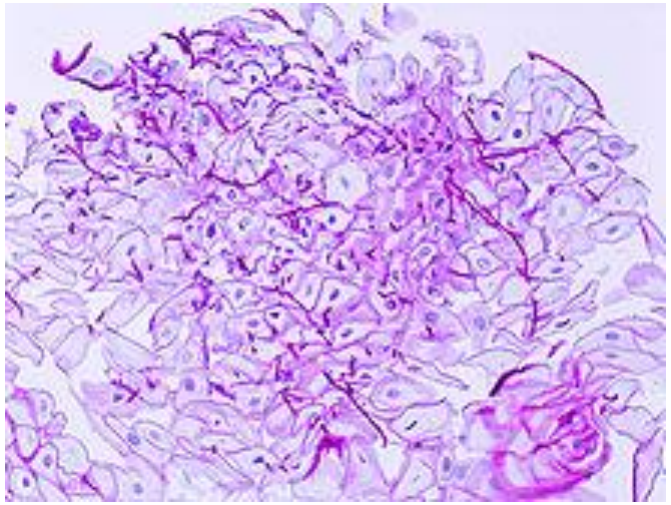
Glcyoprotein

Fraunhofer IZI

Beispiel 4: PAS Färbung – Nachweis von Glycoproteinen



Fuchsin

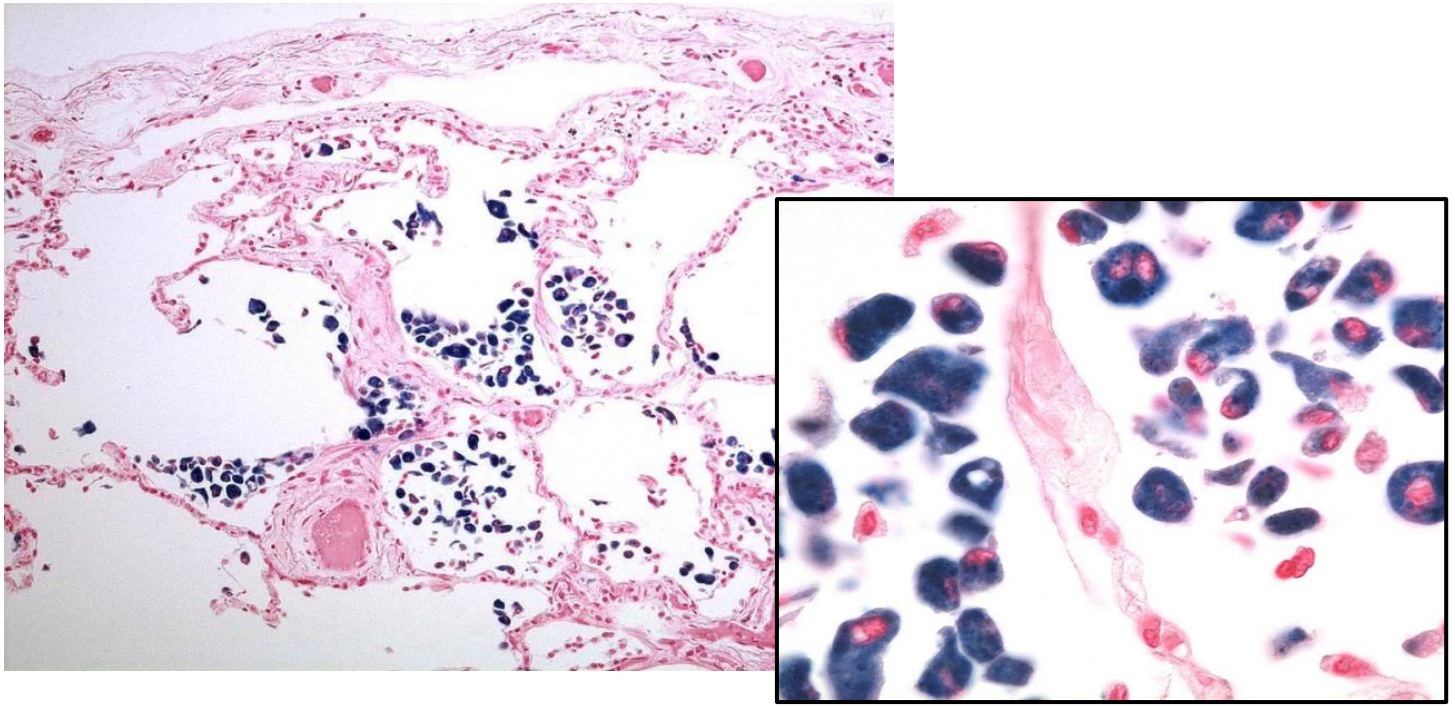


Beispiel 5: Berliner Blau Reaktion

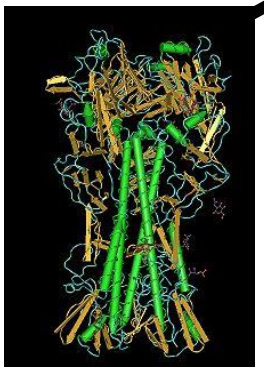
Fe^{3+}

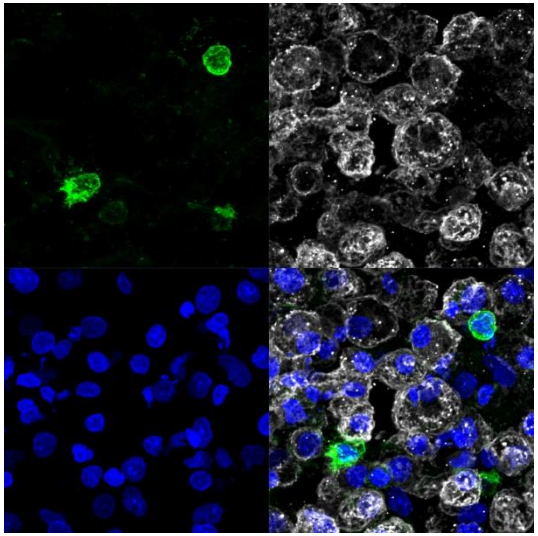
Kaliumhexacyanidoferrat(II) (*Gelbes Blutlaugensalz)*

Berliner Blau



Beispiel 6: Lektinfärbung



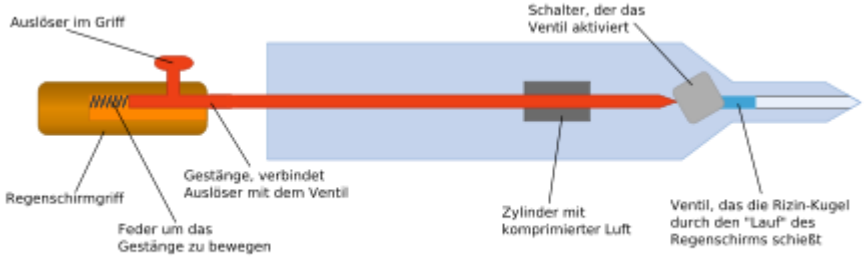


Lektine (legere - auswählen)

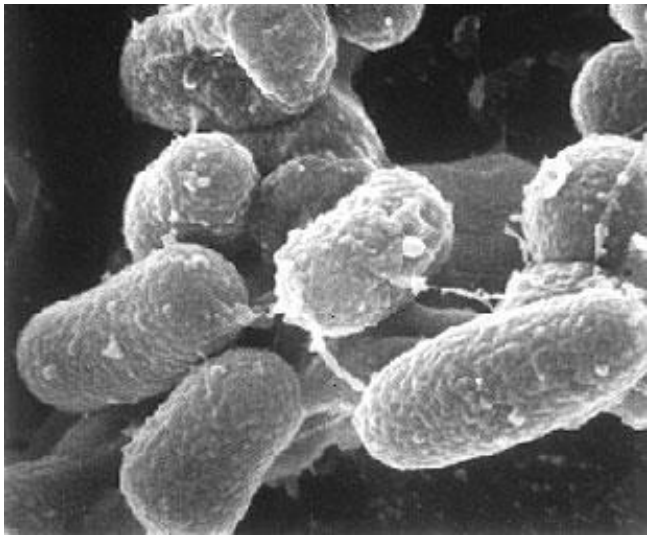
Proteine die spezifisch binden

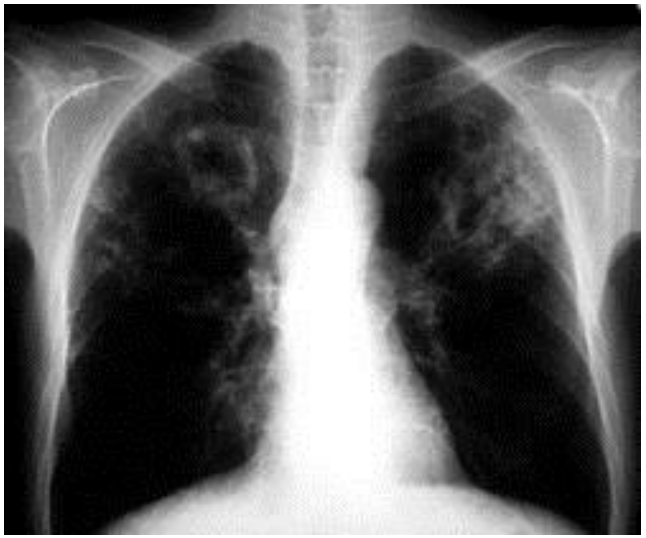
Ricinussamen

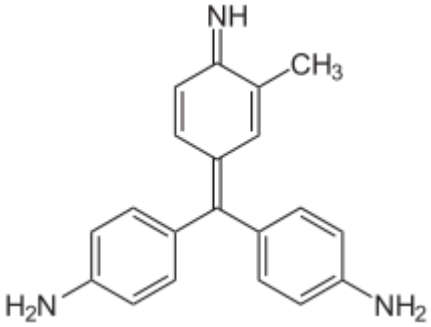
0,2mg



Beispiel 7: Ziehl-Neeslen Färbung

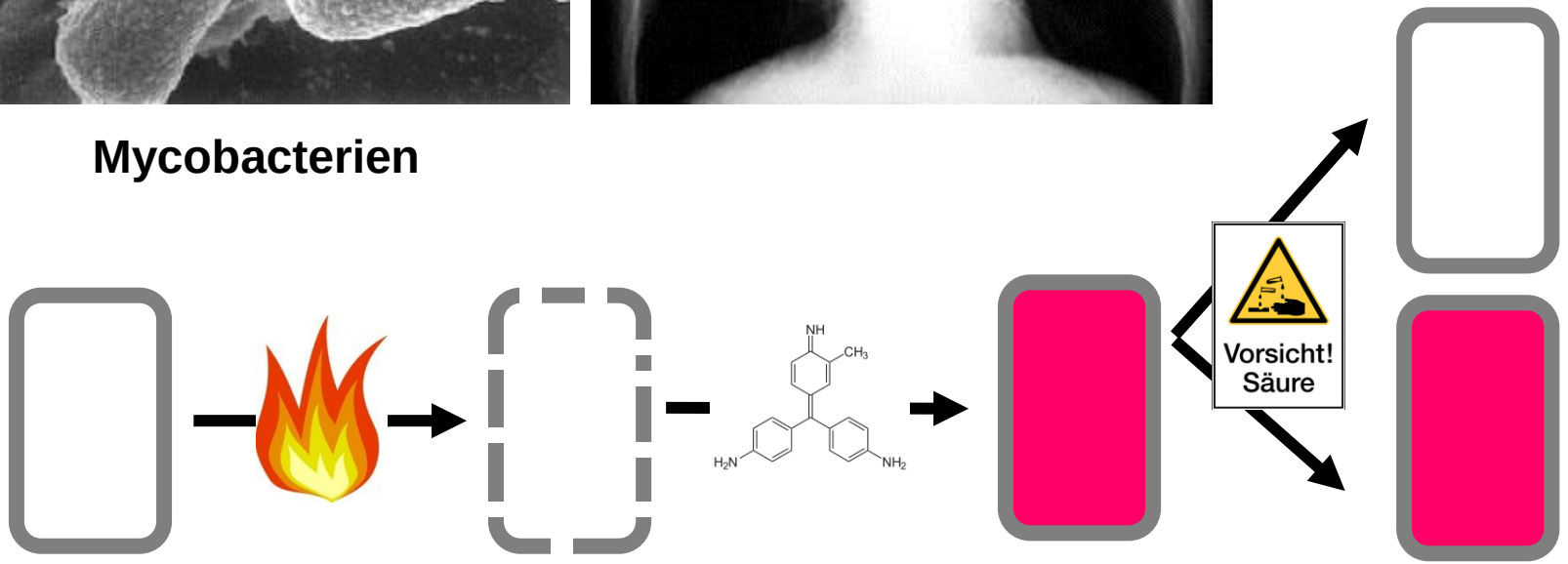






Fuchsin

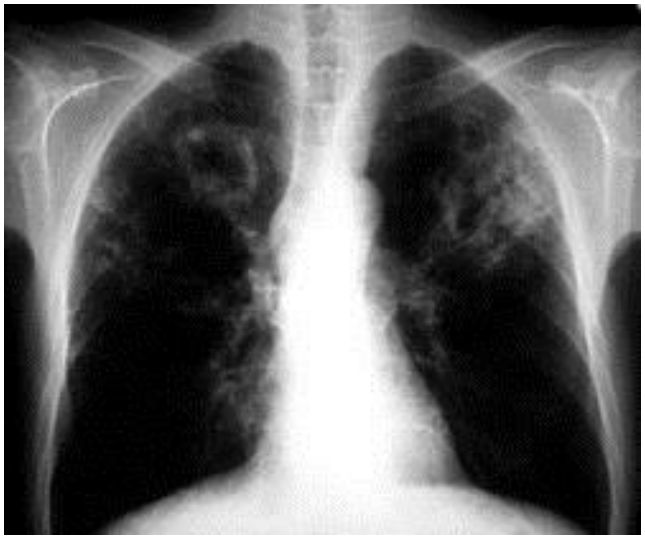
Mycobacterien

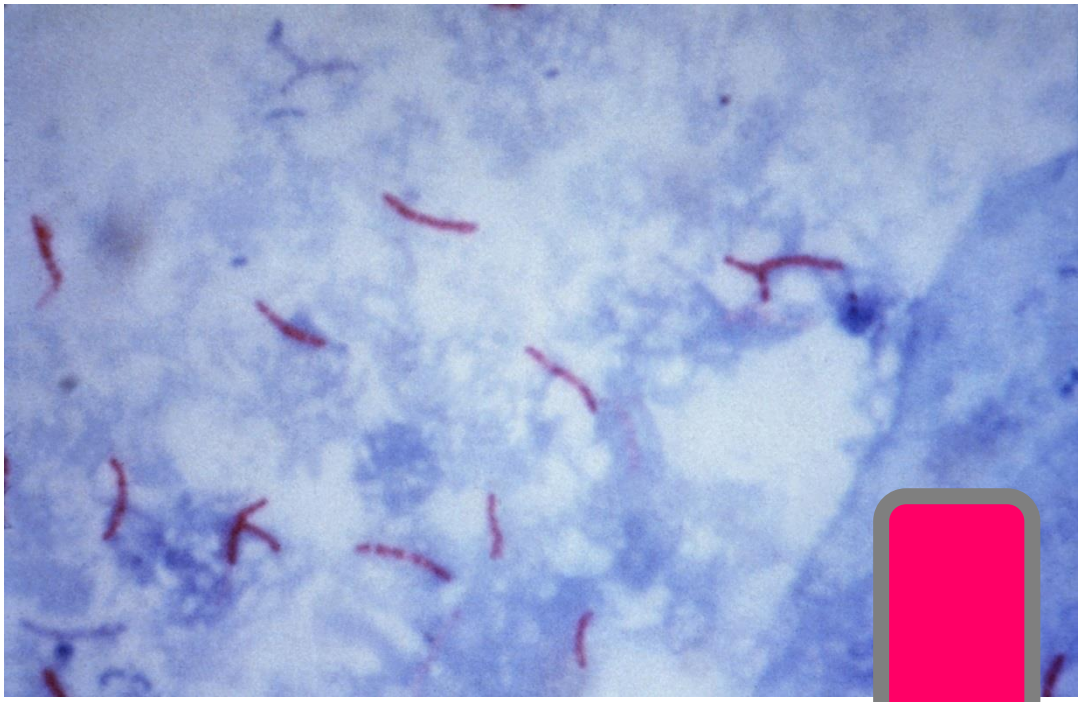


Säurefest

Fraunhofer IZI

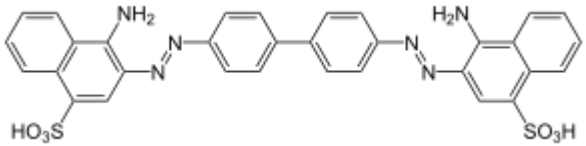
Beispiel 7: Ziehl-Neeslen Färbung





Säurefest

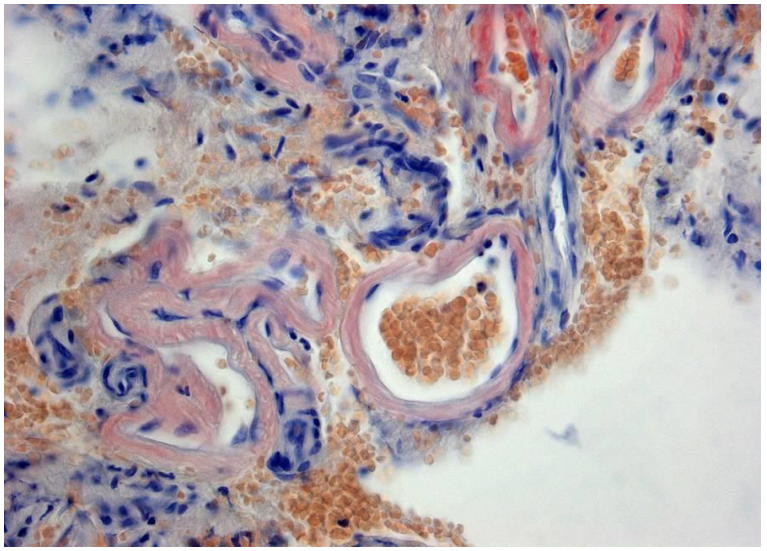
Polarisationsfärbung: Kongorot

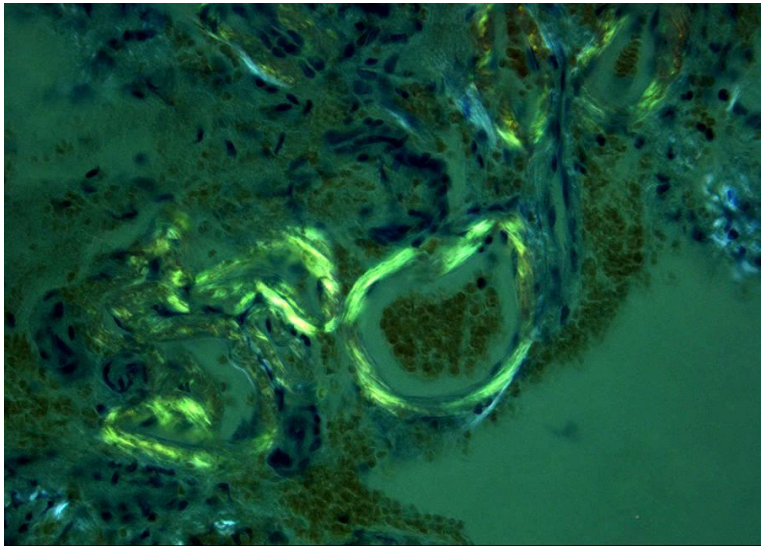


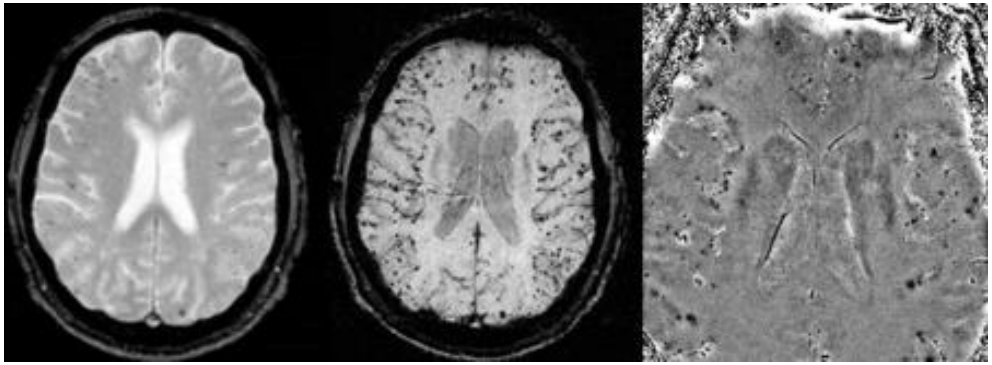
Topo-optische Reaktion =

Orientierte Anlagerung des Farbstoffes und

Erhöhung der Brechung



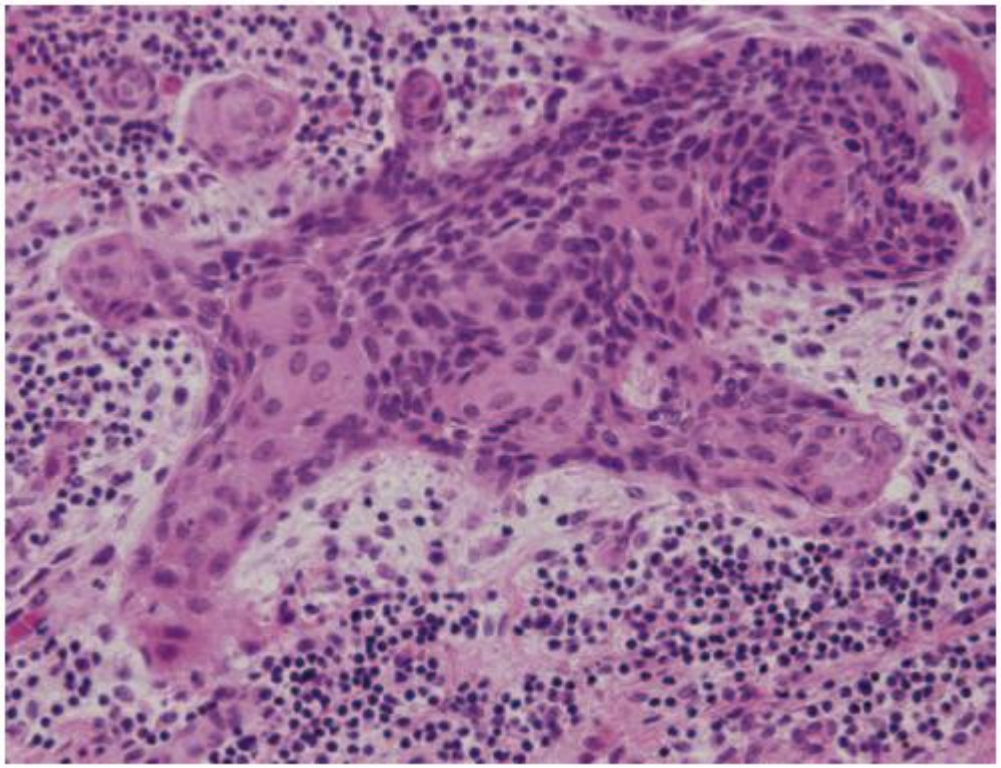


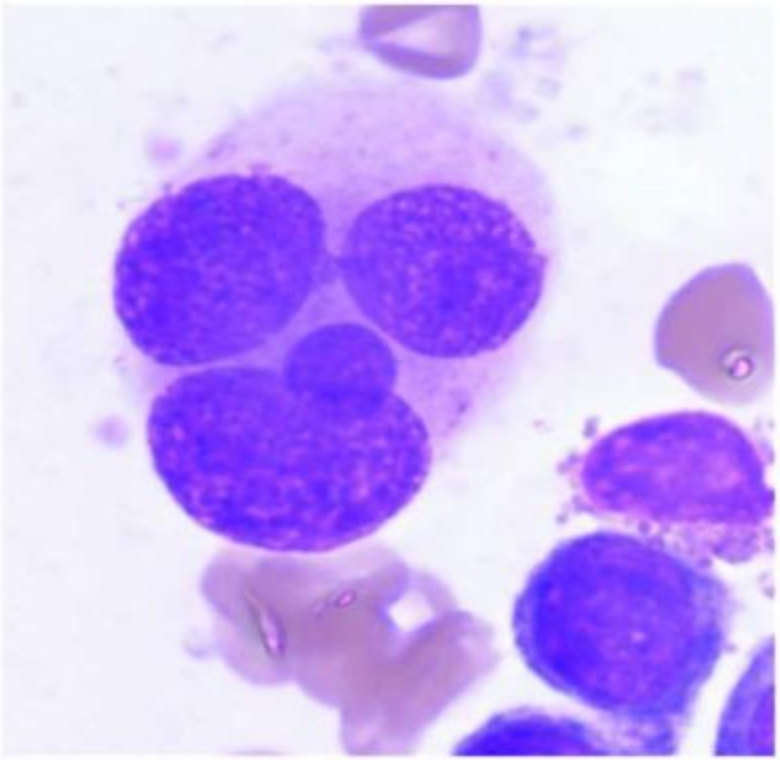


Fraunhofer IZI

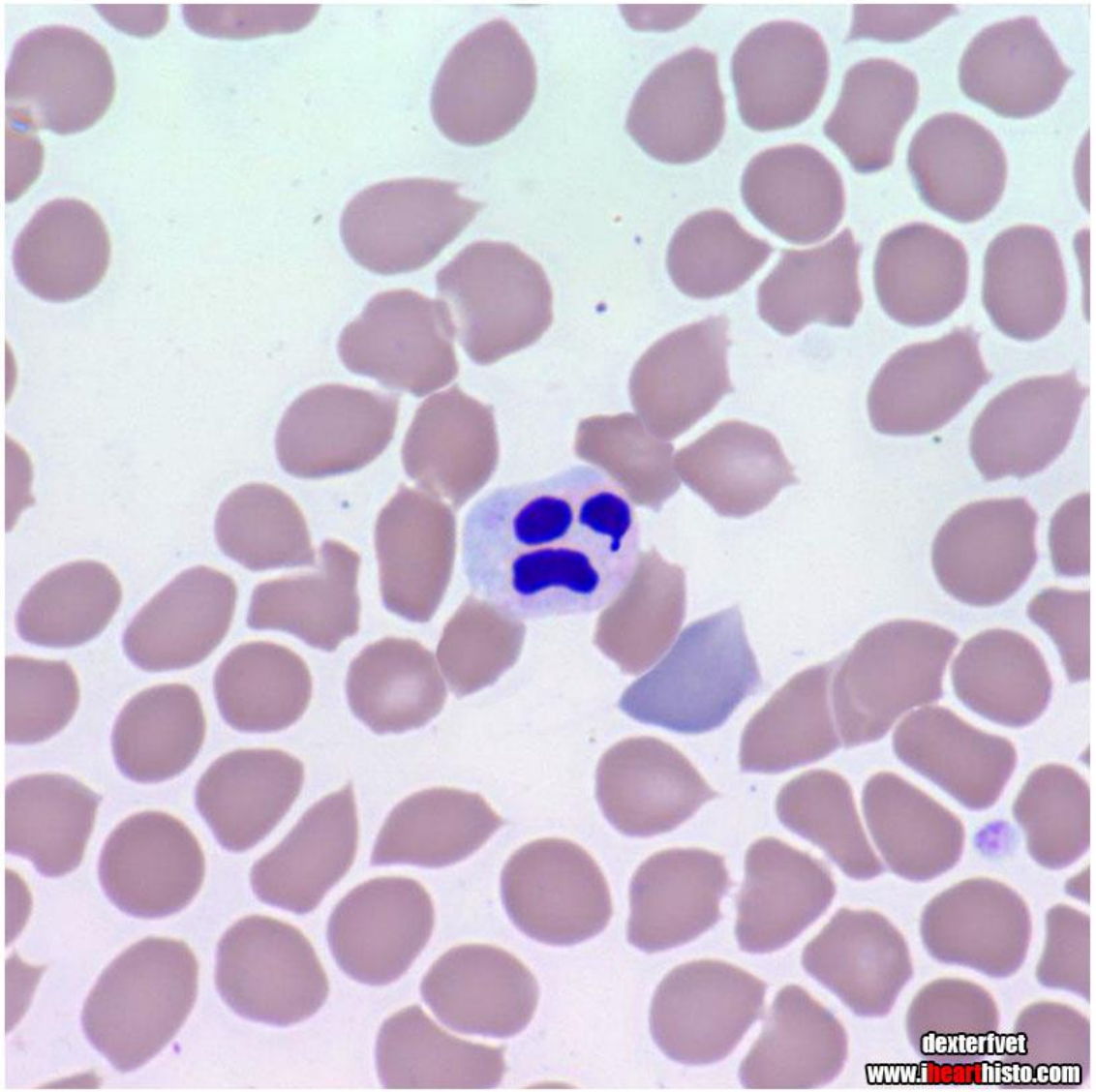
i ♥ histo

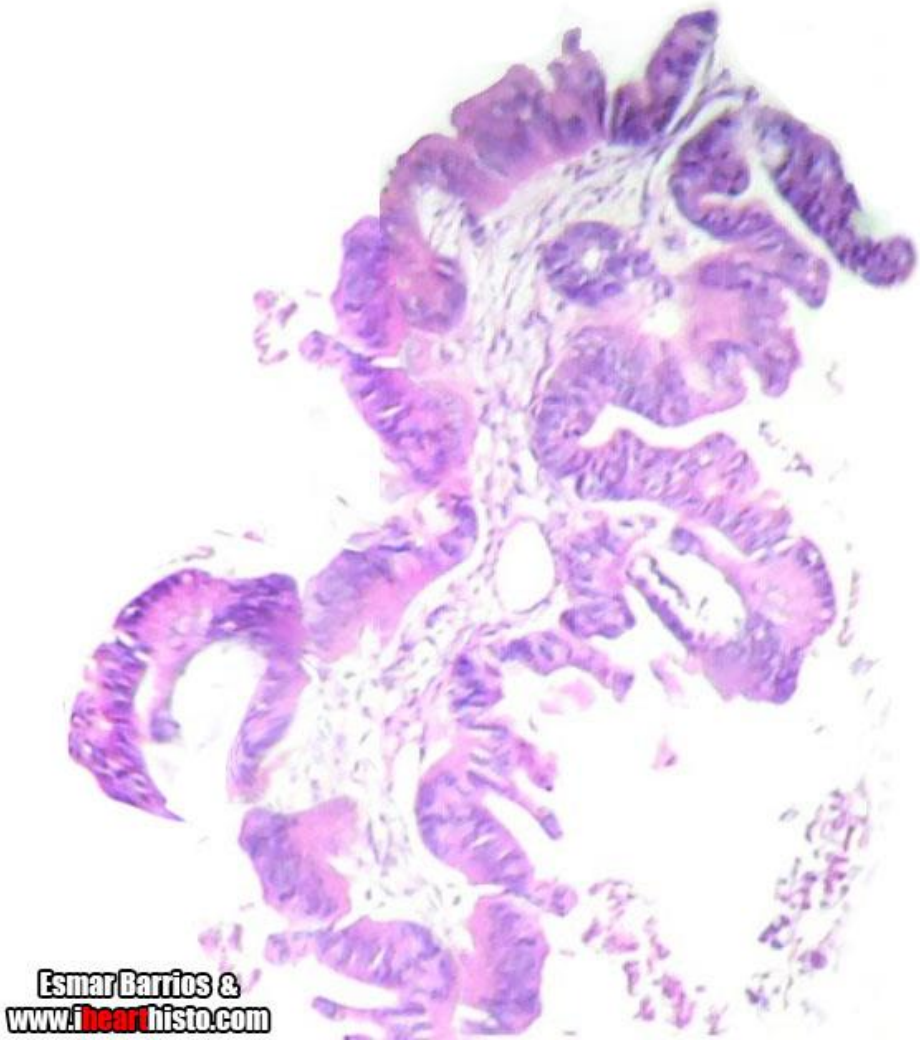
www.ihearthisto.com



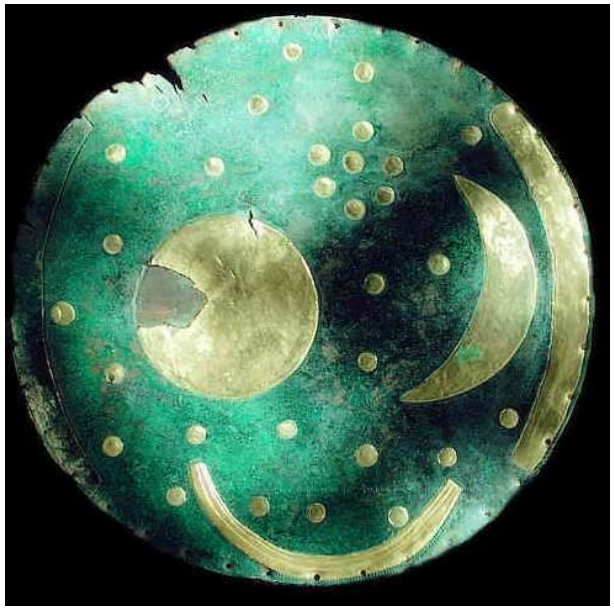


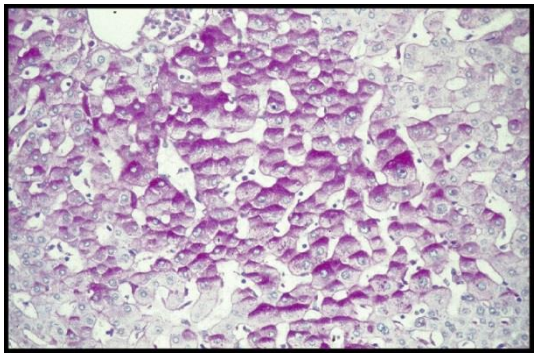
Histology's 'Placental villi'

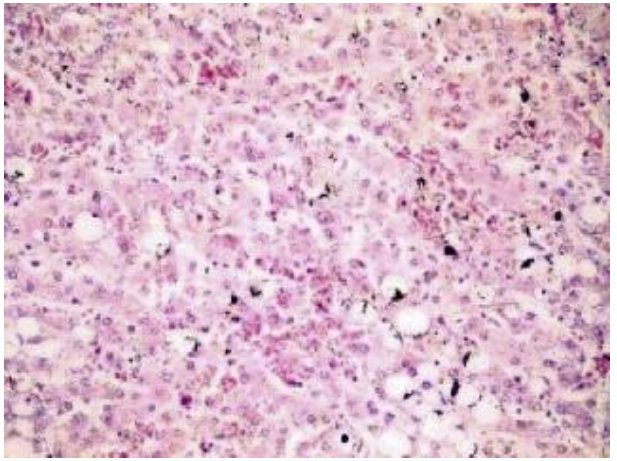


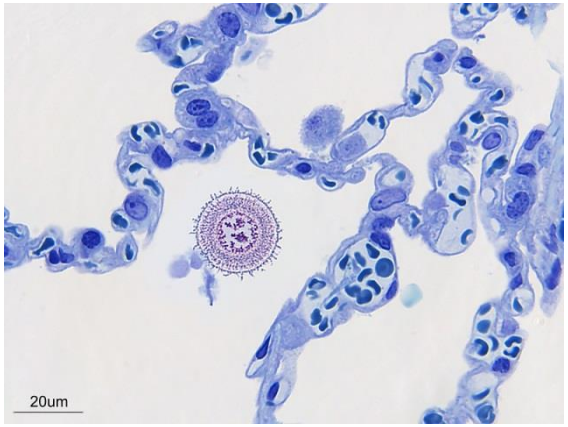


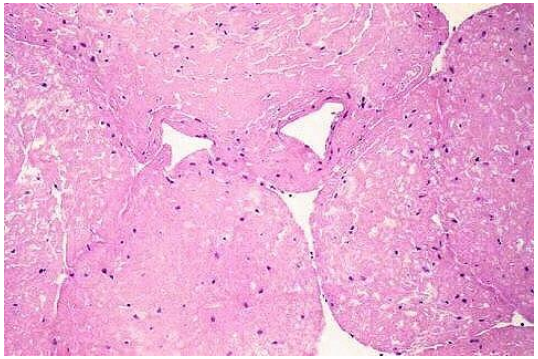
Artefakte

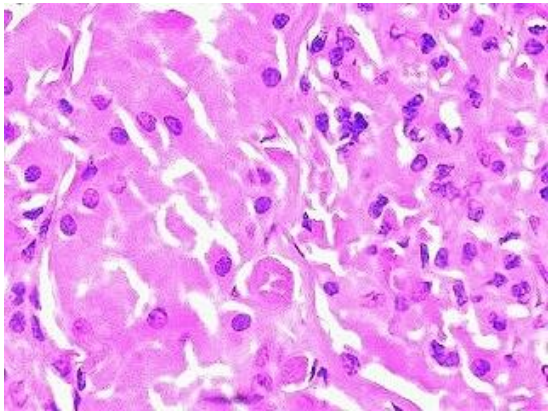






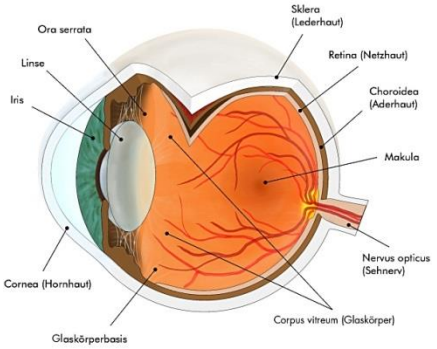


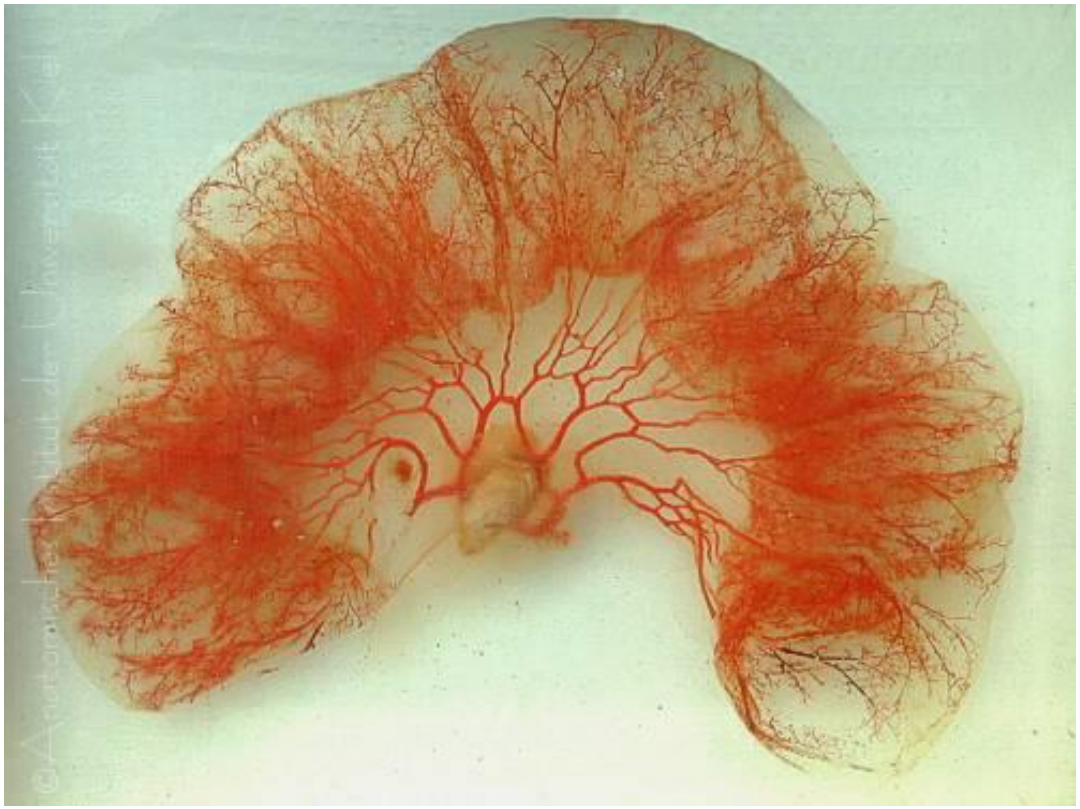


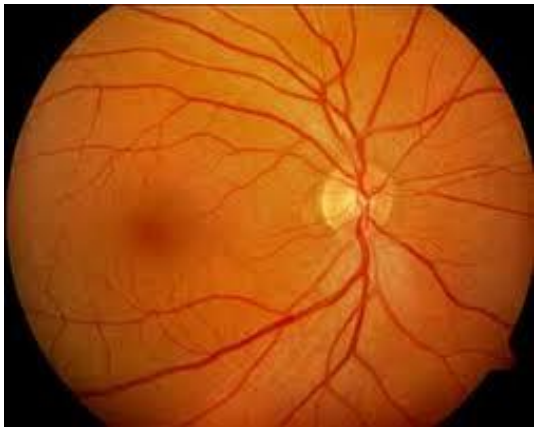


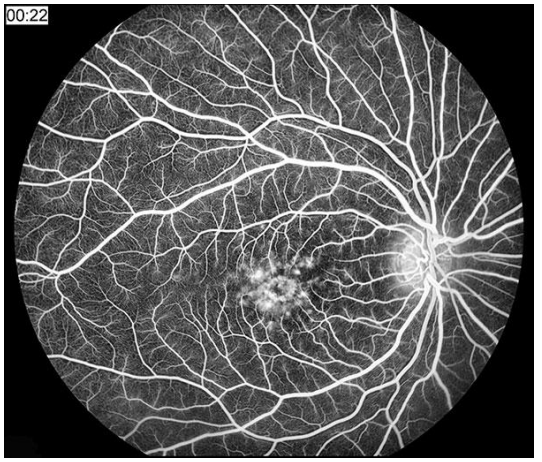
Fraunhofer IZI

Färbeverfahren ohne Bindung - Injektionen in hohlräume



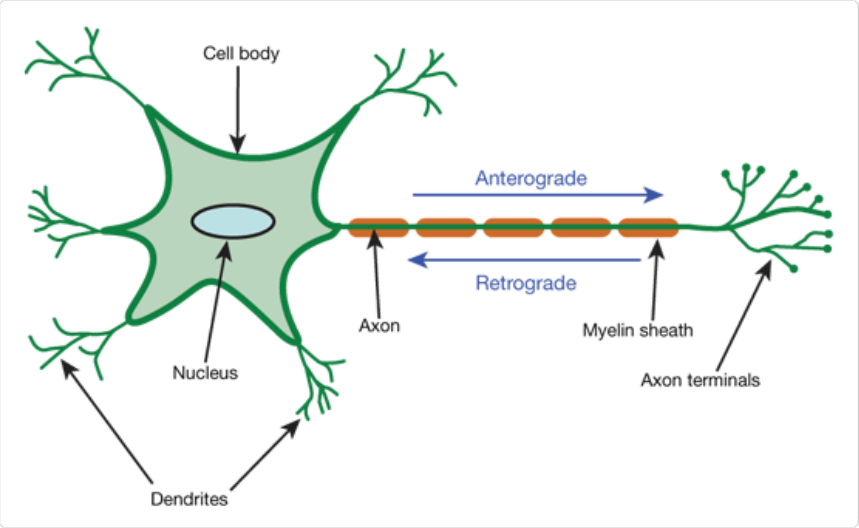


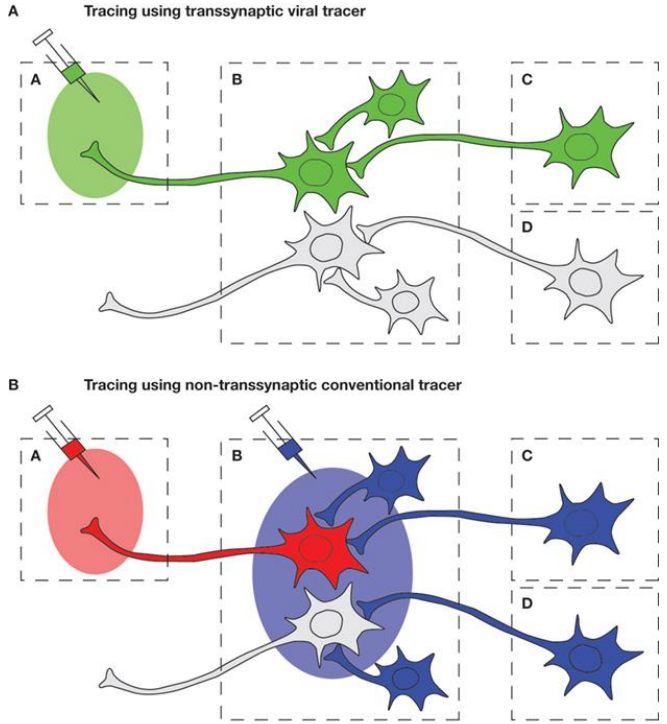


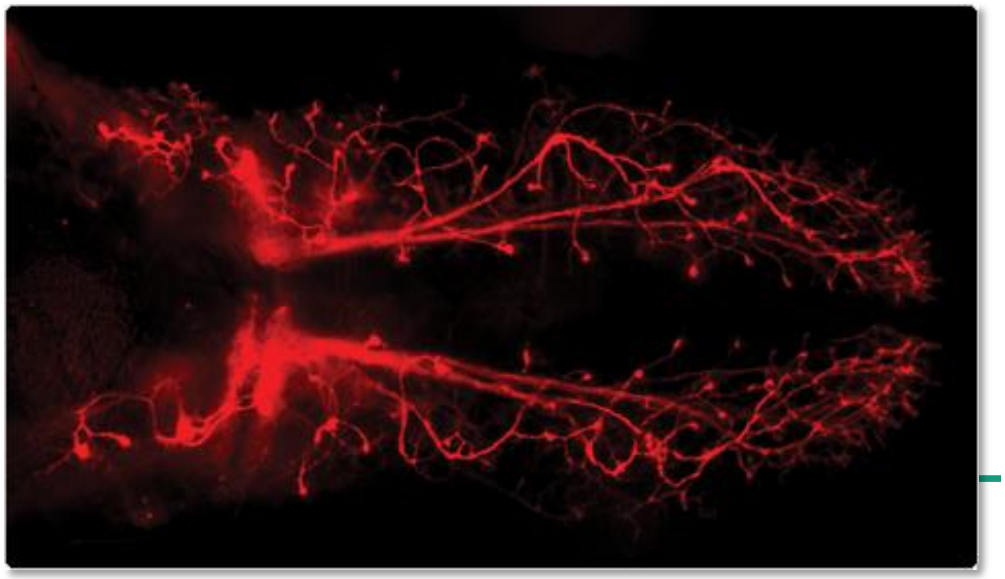


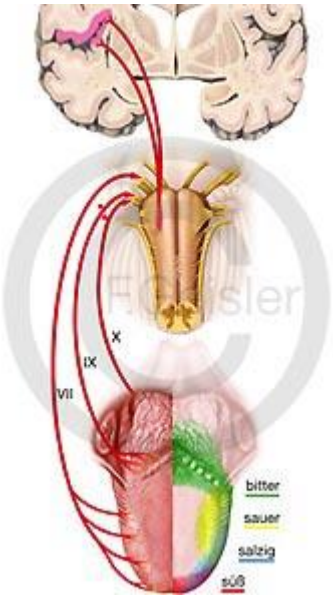
Fraunhofer IZI

Neuronales Tracing



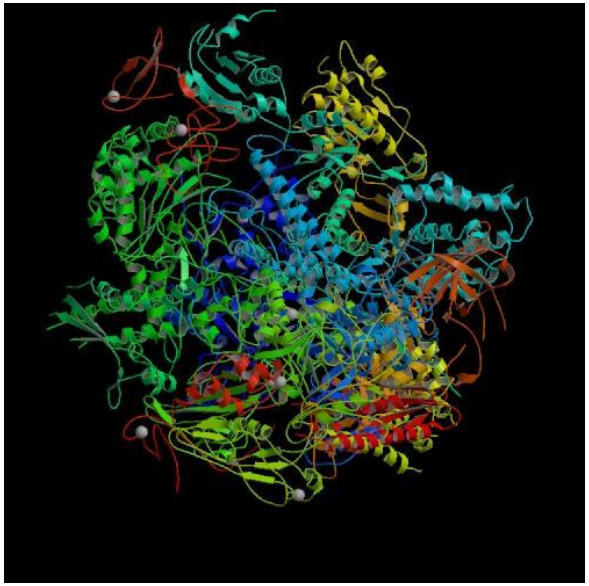






Fraunhofer IZI

Enzymhistochemie

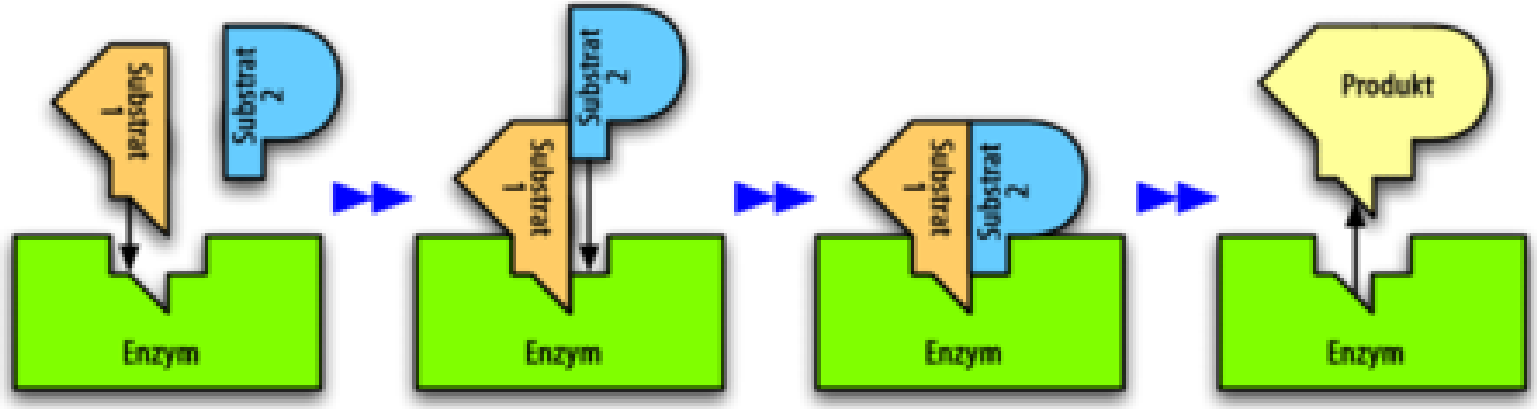


Enzyme = katalytische Proteine

Nachweis Enzymaktivität durch Umsatz

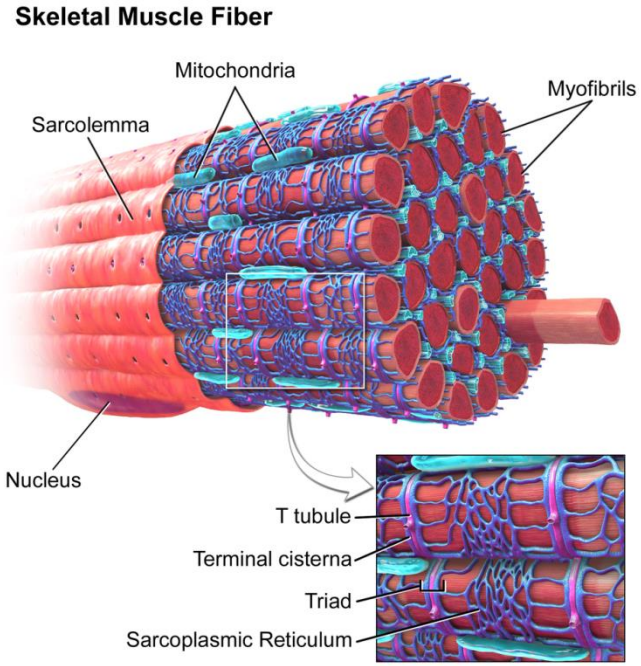
Löslich -> Unlöslich

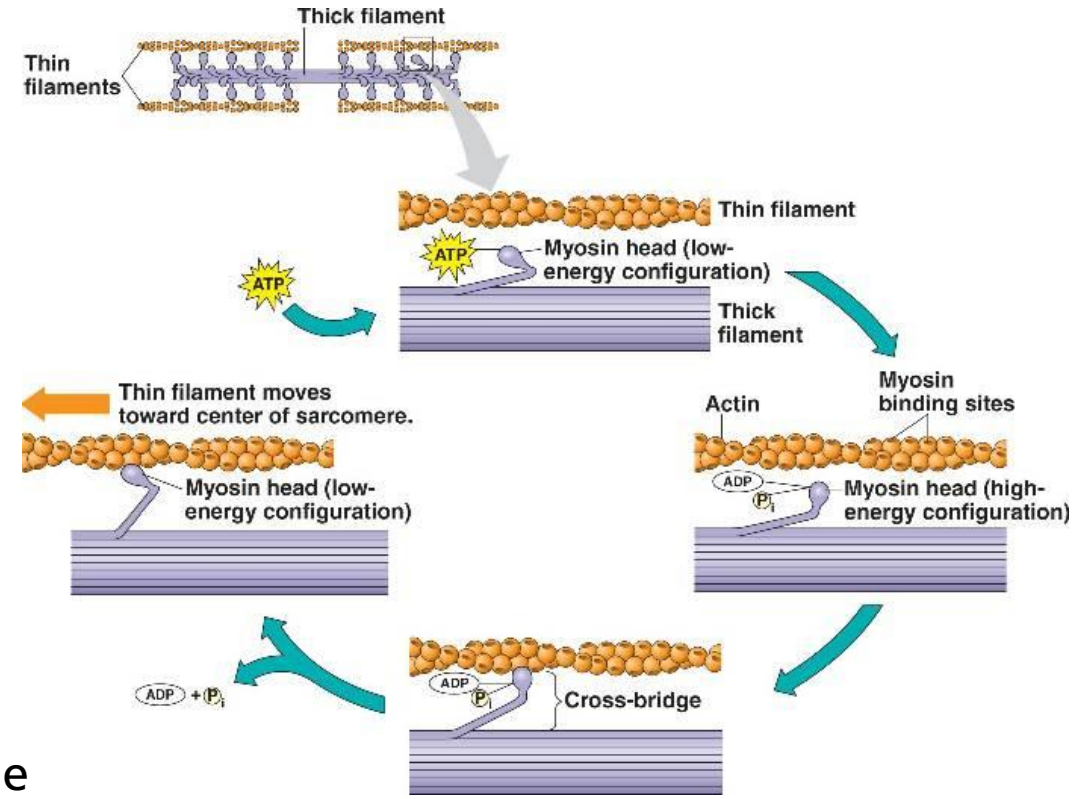
Vorsicht: Fixierung



Myosin ATPase

ATP + H2O ⟶ ADP + Phosphat





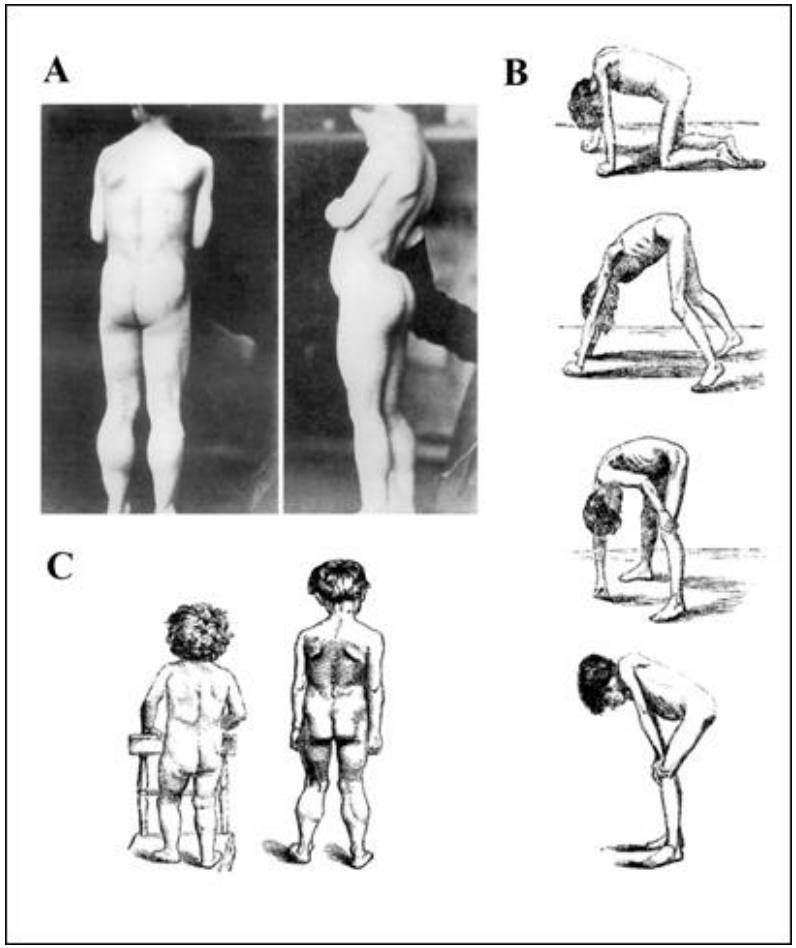
5-10 ATP-Moleküle pro Sekunde

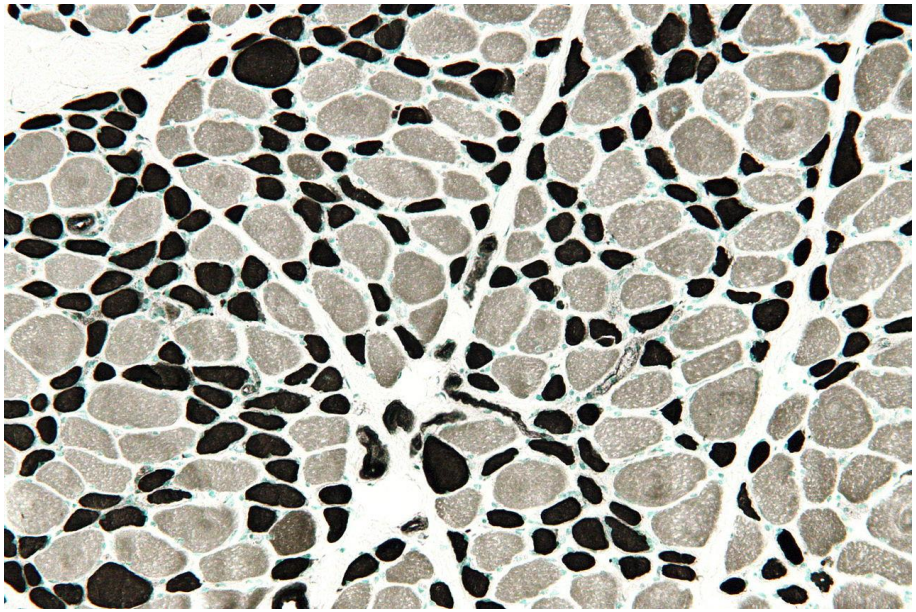
Fraunhofer IZI

ATP + H2O ⟶ ADP + Phosphat

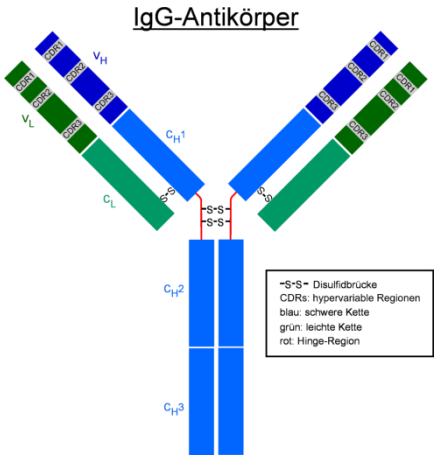
Bleisalz (Bleinitrat)

Fällung





Immunhistochemie - Immunlokalisierung

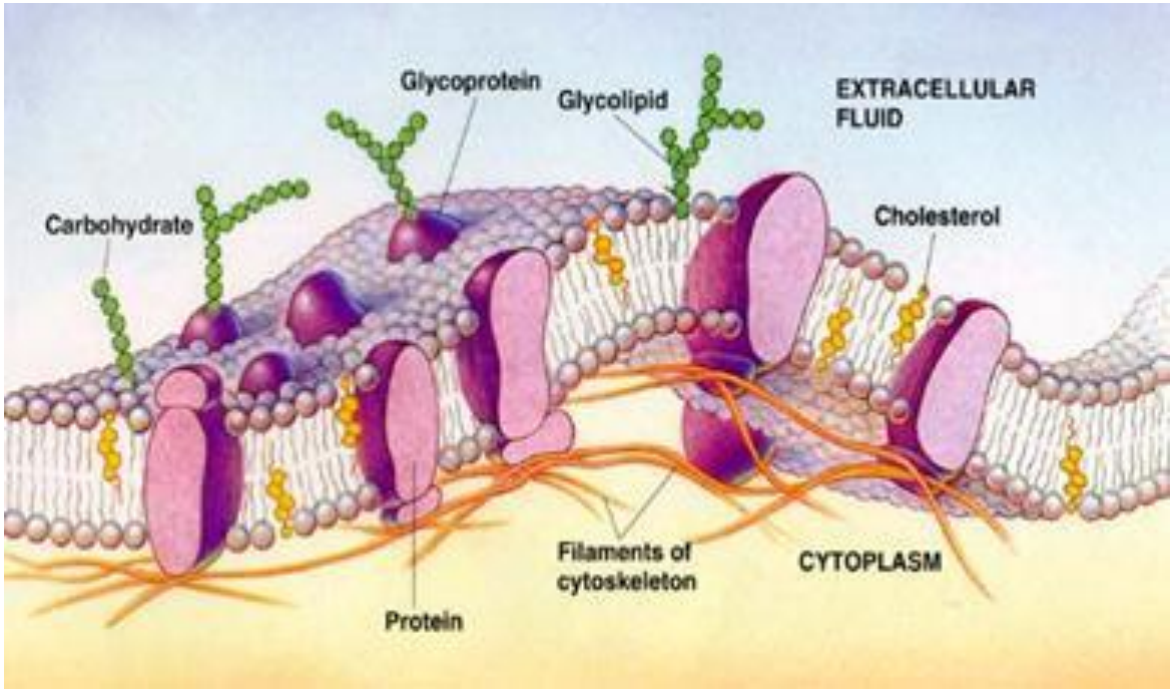


Konventionelle Färbungen

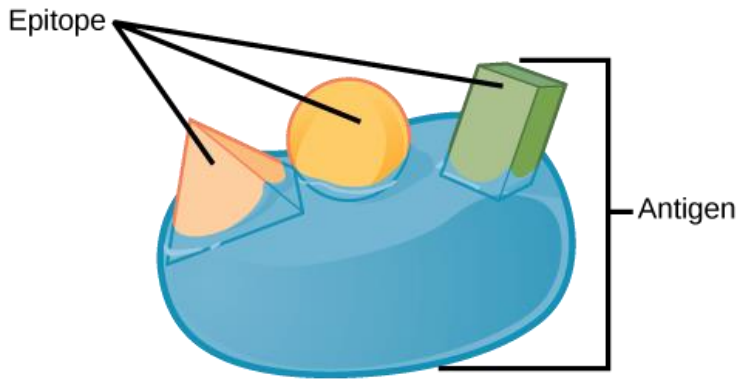
+ schnell
+ billig
- unspezifisch

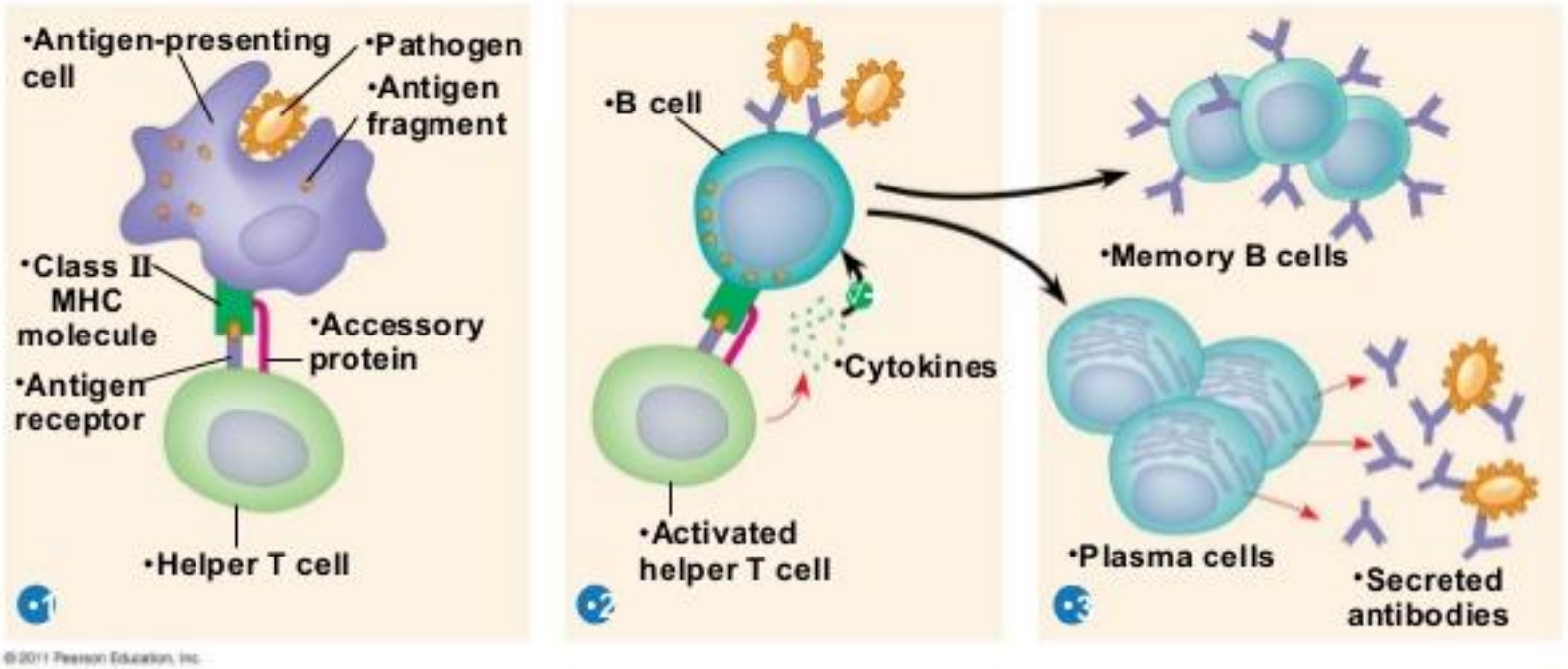
Immunhistochemie

- nicht schnell
- teuer
+ sehr spezifisch



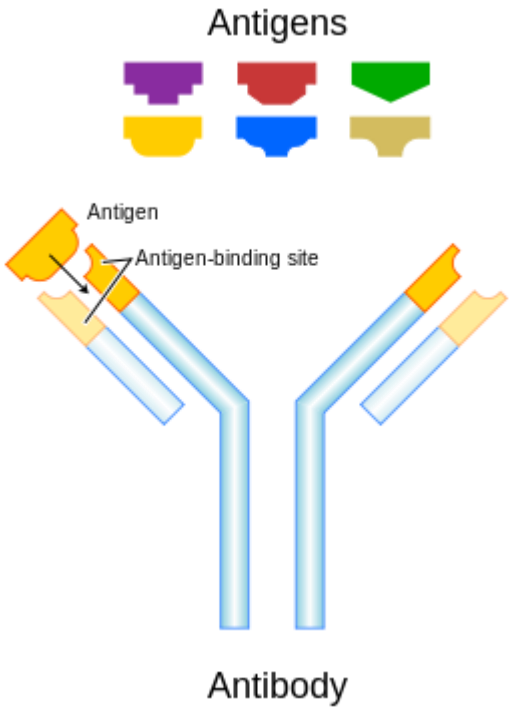
Fraunhofer IZI

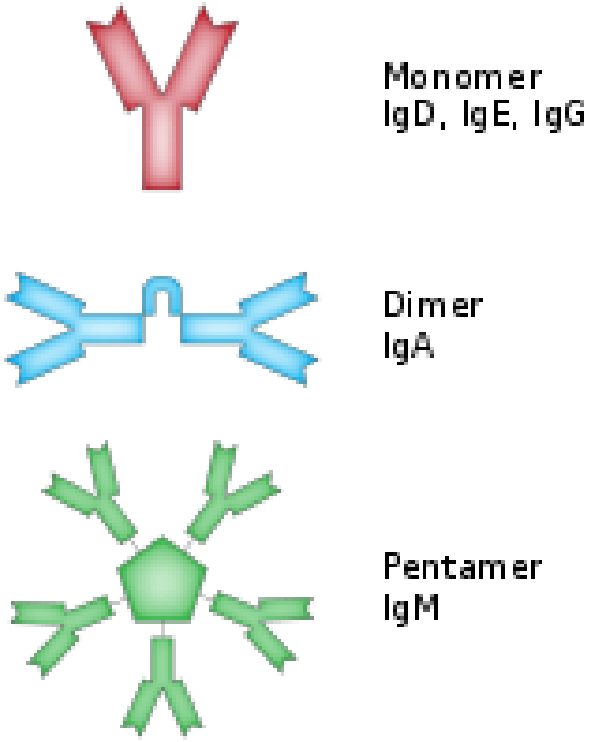




Fraunhofer IZI

Immunhistochemie - AK

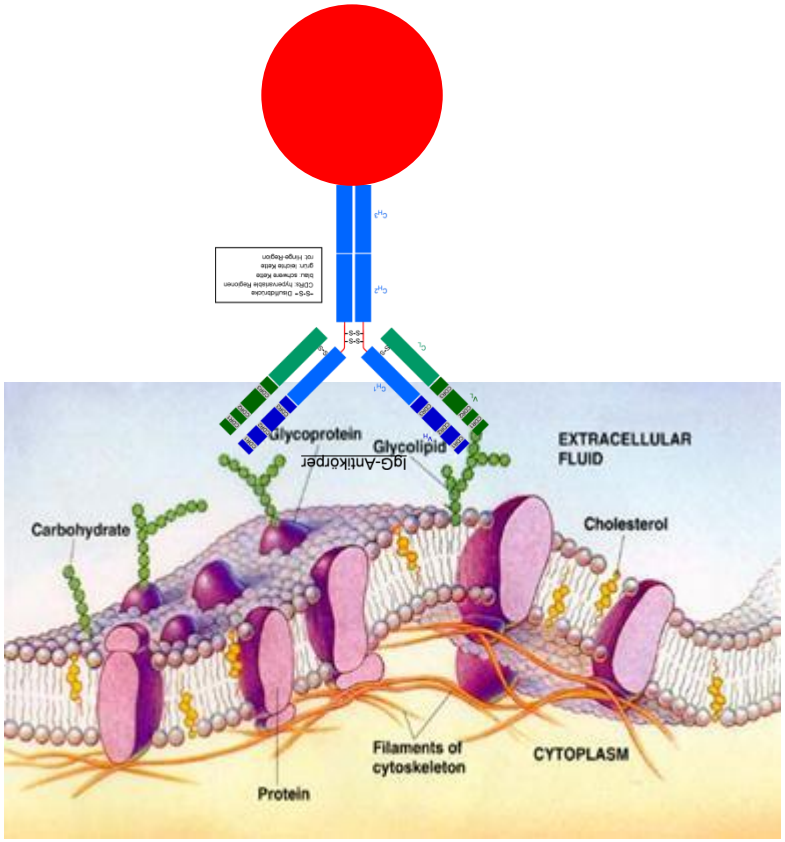




V(D)J-Rekombination

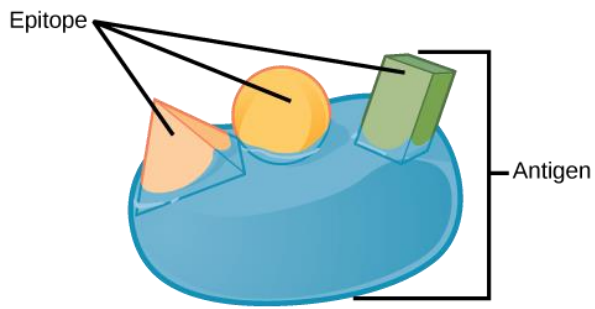
1,92 Millionen Kombinationsmöglichkeiten

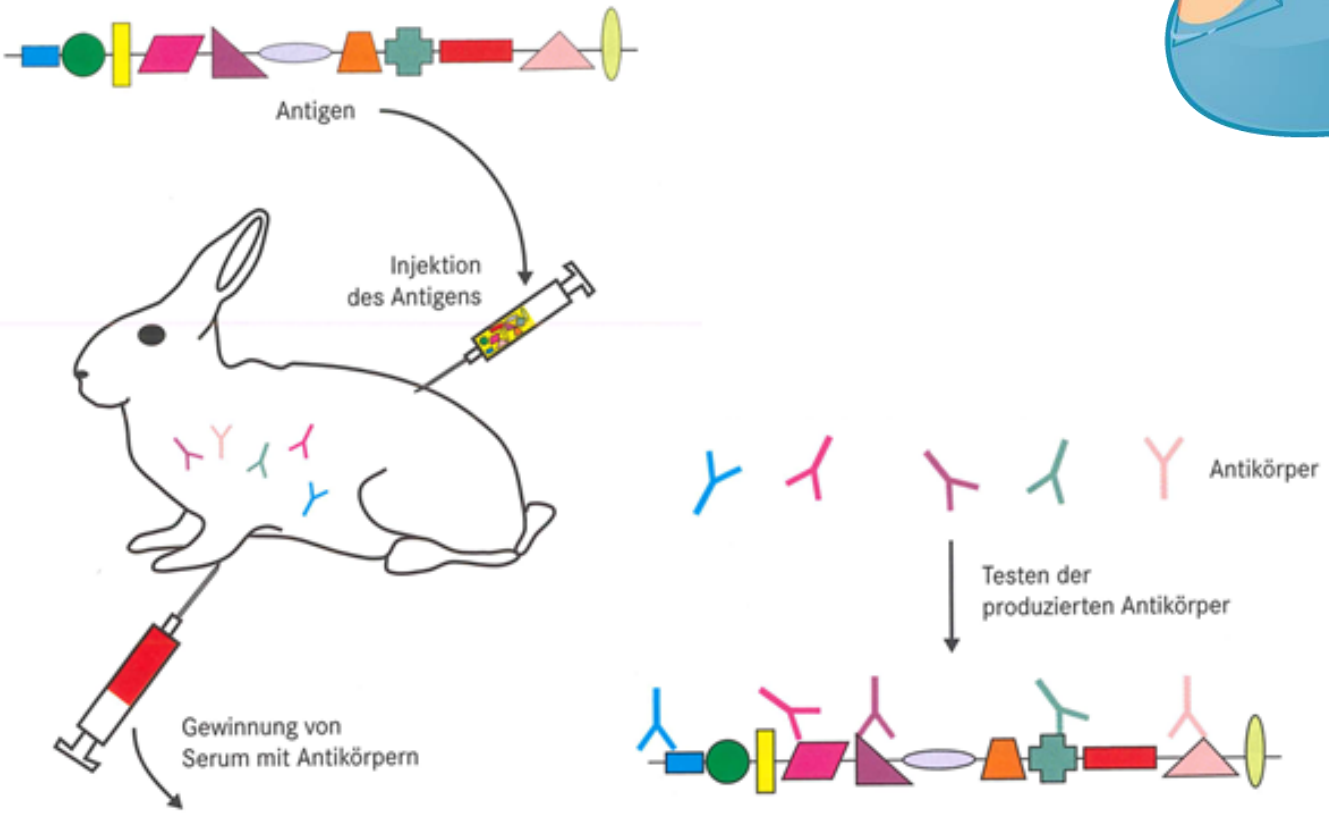
Immunhistochemie



Fraunhofer IZI

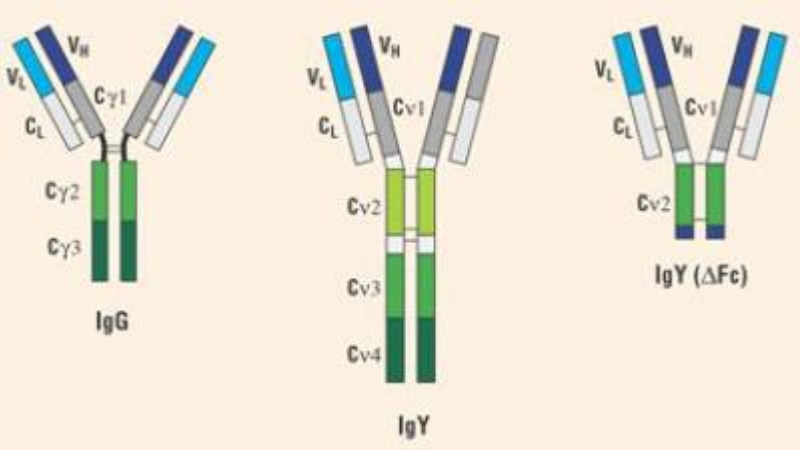
Herstellung von Antikörpern: polyclonal

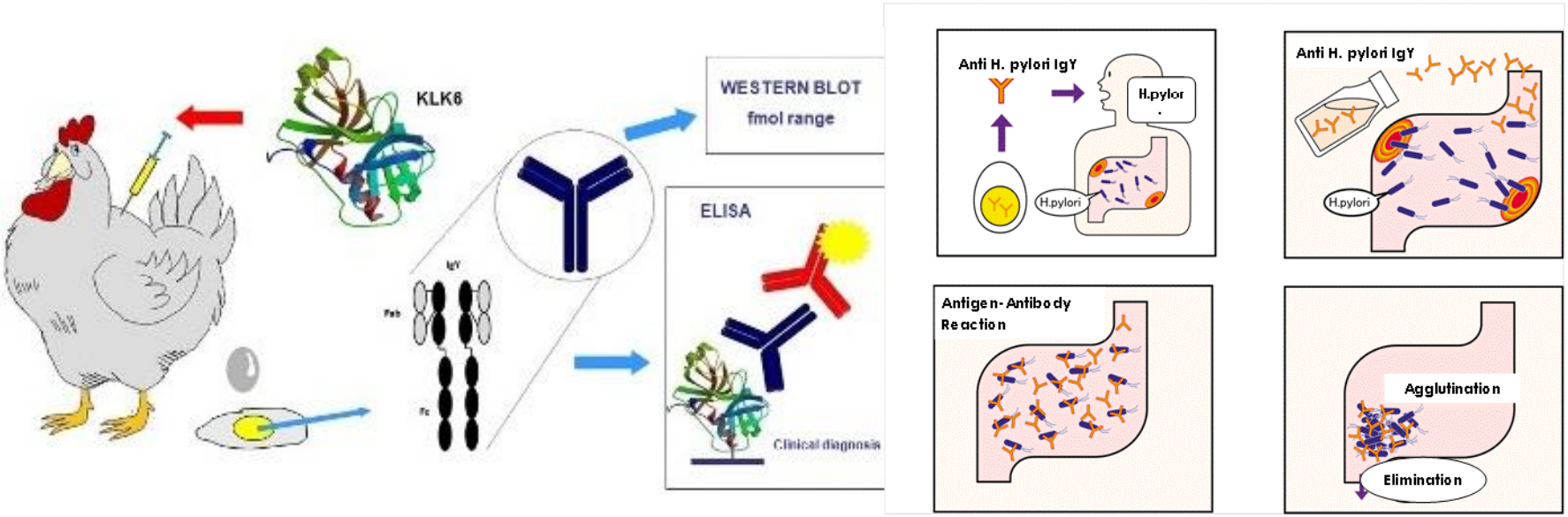




Fraunhofer IZI

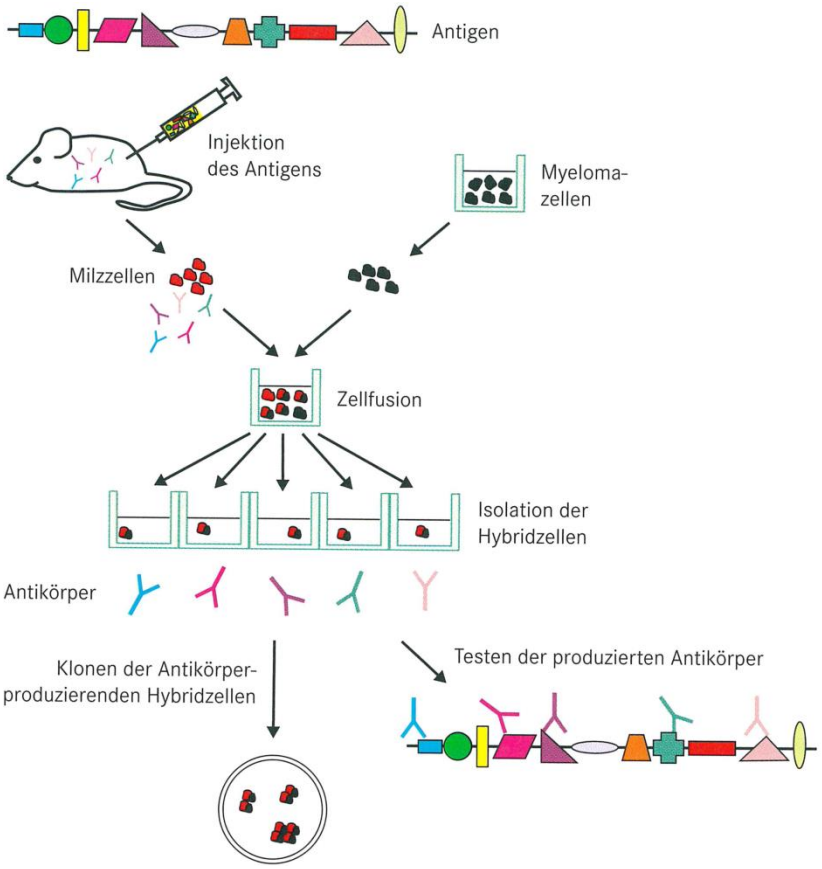
IgY

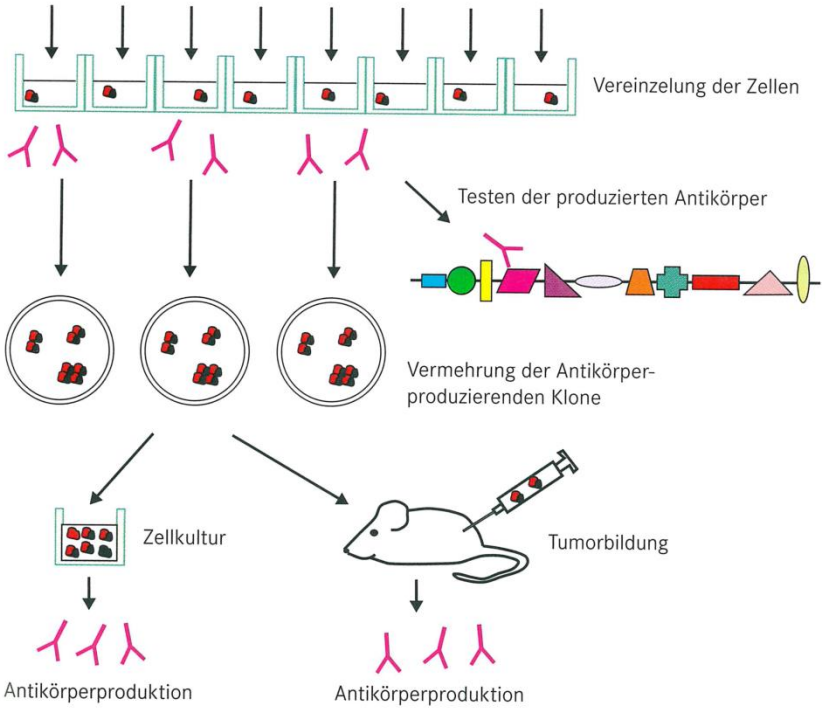




Fraunhofer IZI

Herstellung von Antikörpern: monoclonal





Fraunhofer IZI

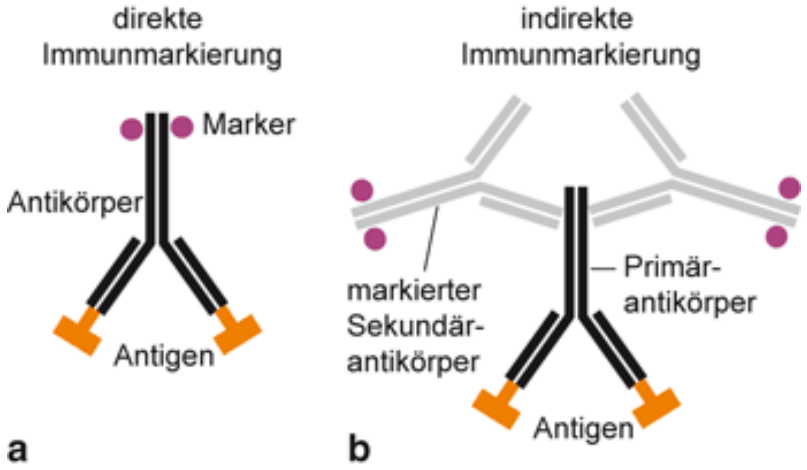
Monoclonal **versus** **polyclonal**

- weniger Bindung
- Weniger tolerant
+ hoch spezifisch
+ reproduzierbar

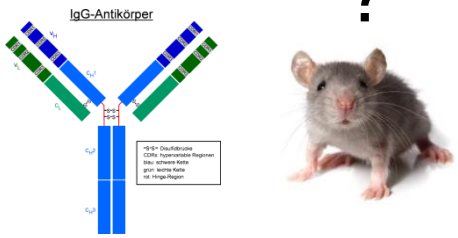
+ mehr Bindung
+ toleranter
- Kreuzreaktion

Nachweismethoden

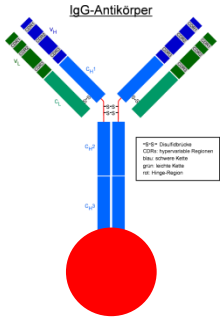
+ schneller
+ spezifischer
- schwächer
- teuer

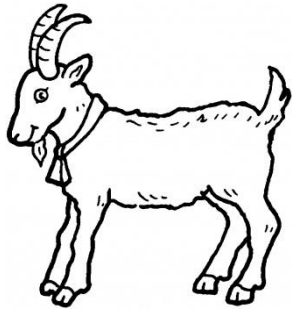


+ Signalverstärkung
+ Flexibilität
- Komplexer
- unspezifischer



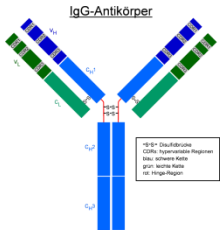
Primärantikörper

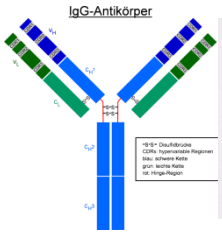




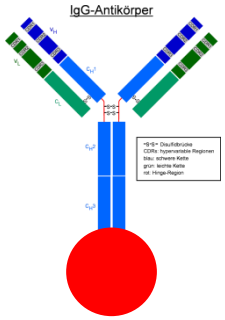
Sekundärantikörper

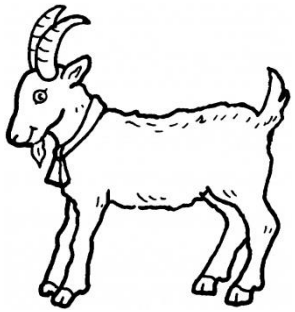
Immunhistochemie – Mehfachfärbungen

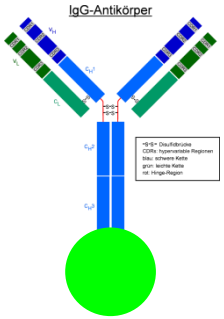


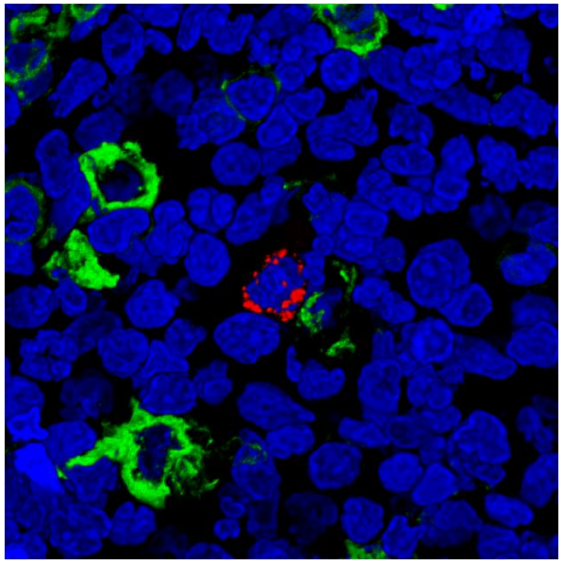


+



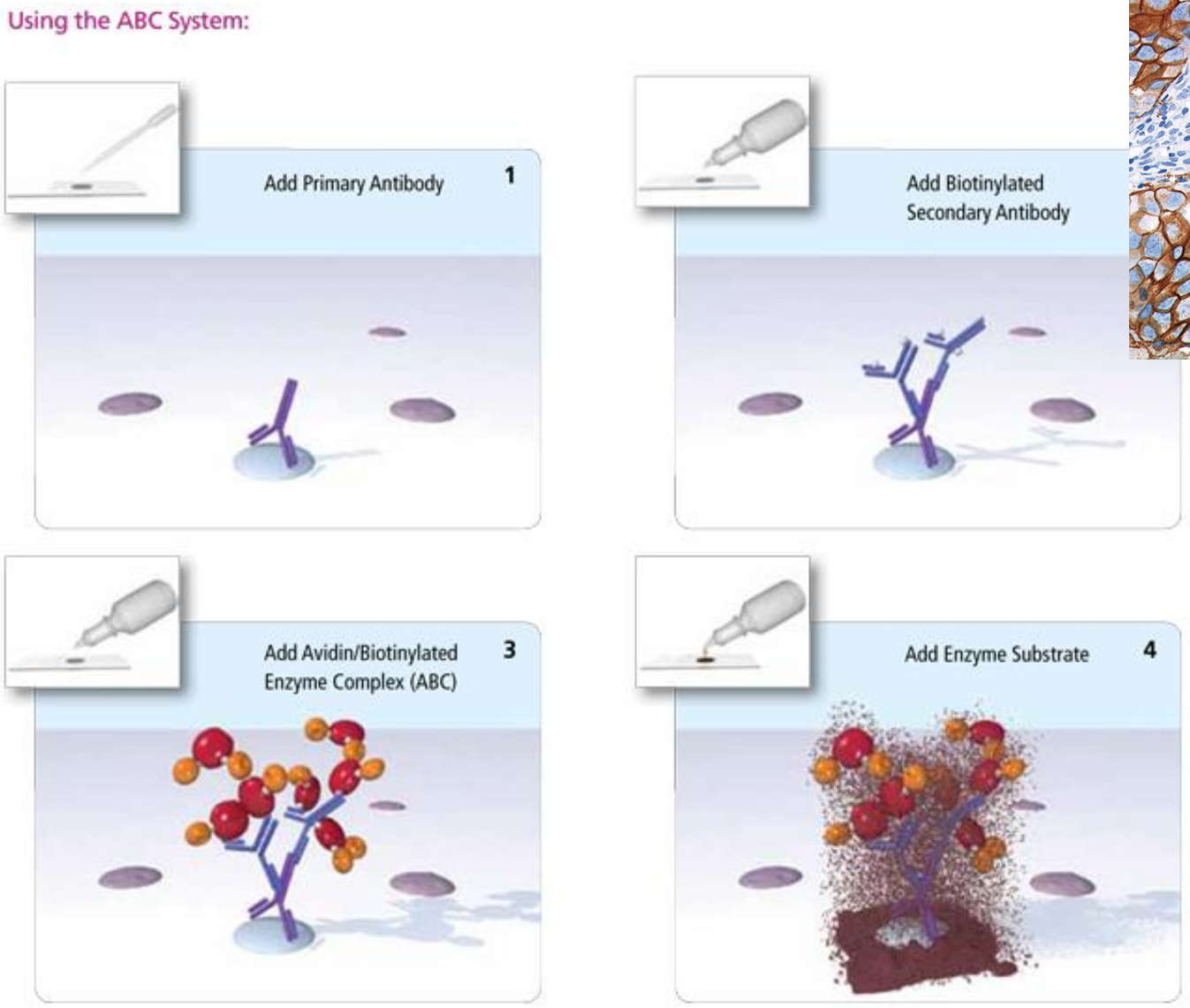


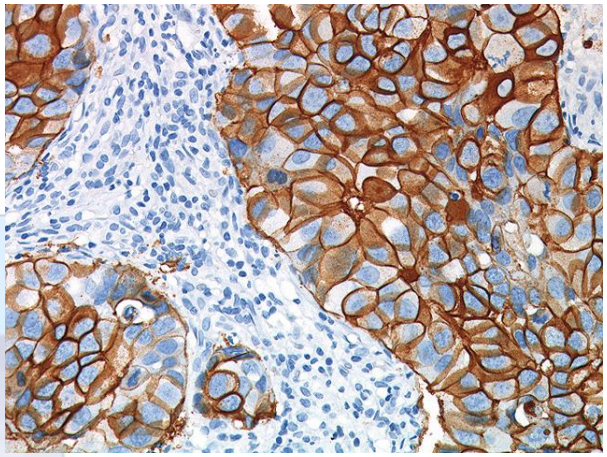




Fraunhofer IZI

Immunhistochemie – Signalverstärkung

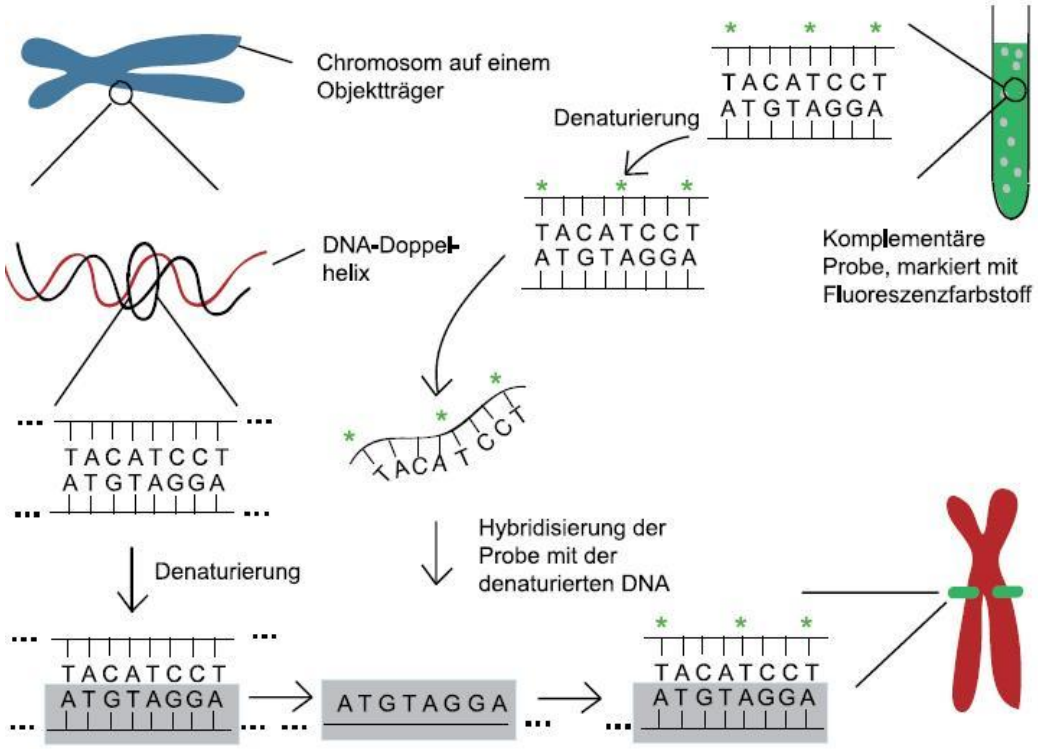


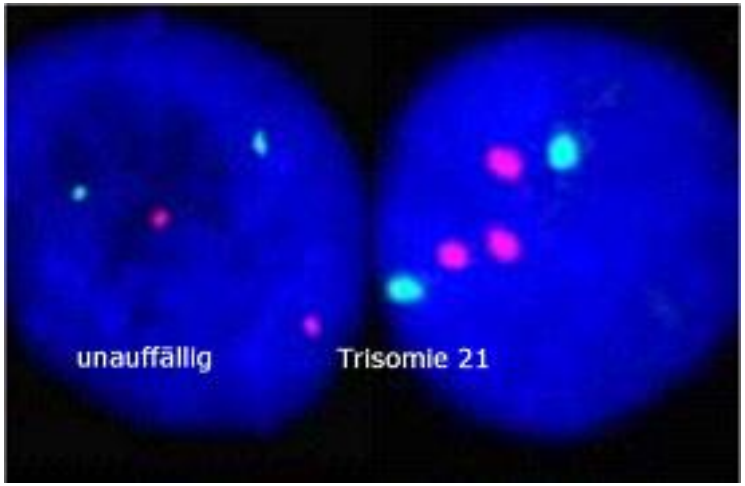


Fraunhofer
IZI

In-situ-Hybridisierung

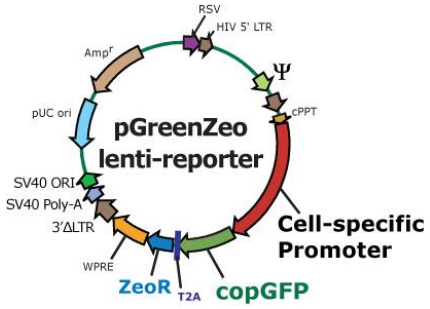
Nachweis von DNA oder RNA





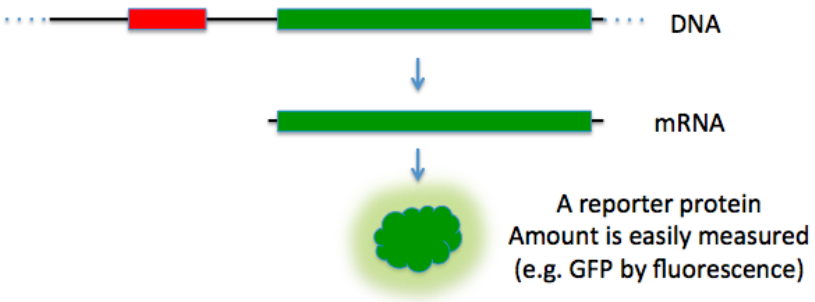
Fraunhofer IZI

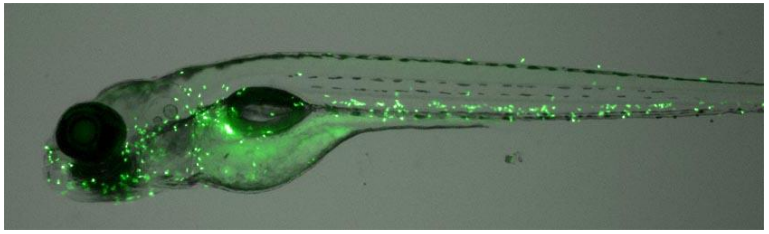
Reporterproteine

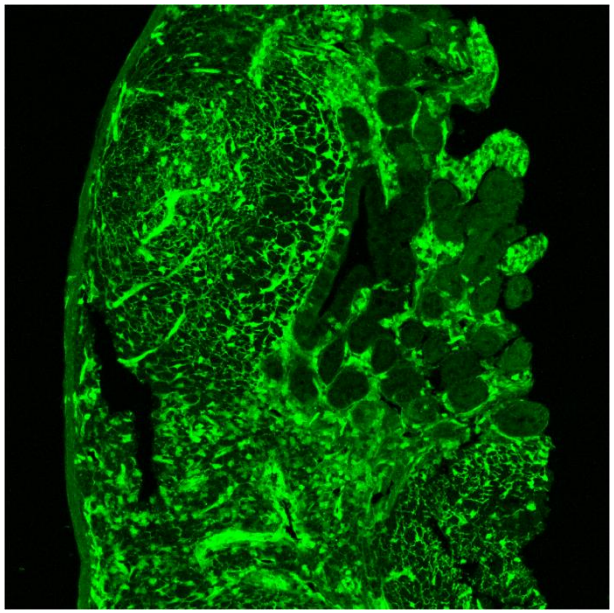


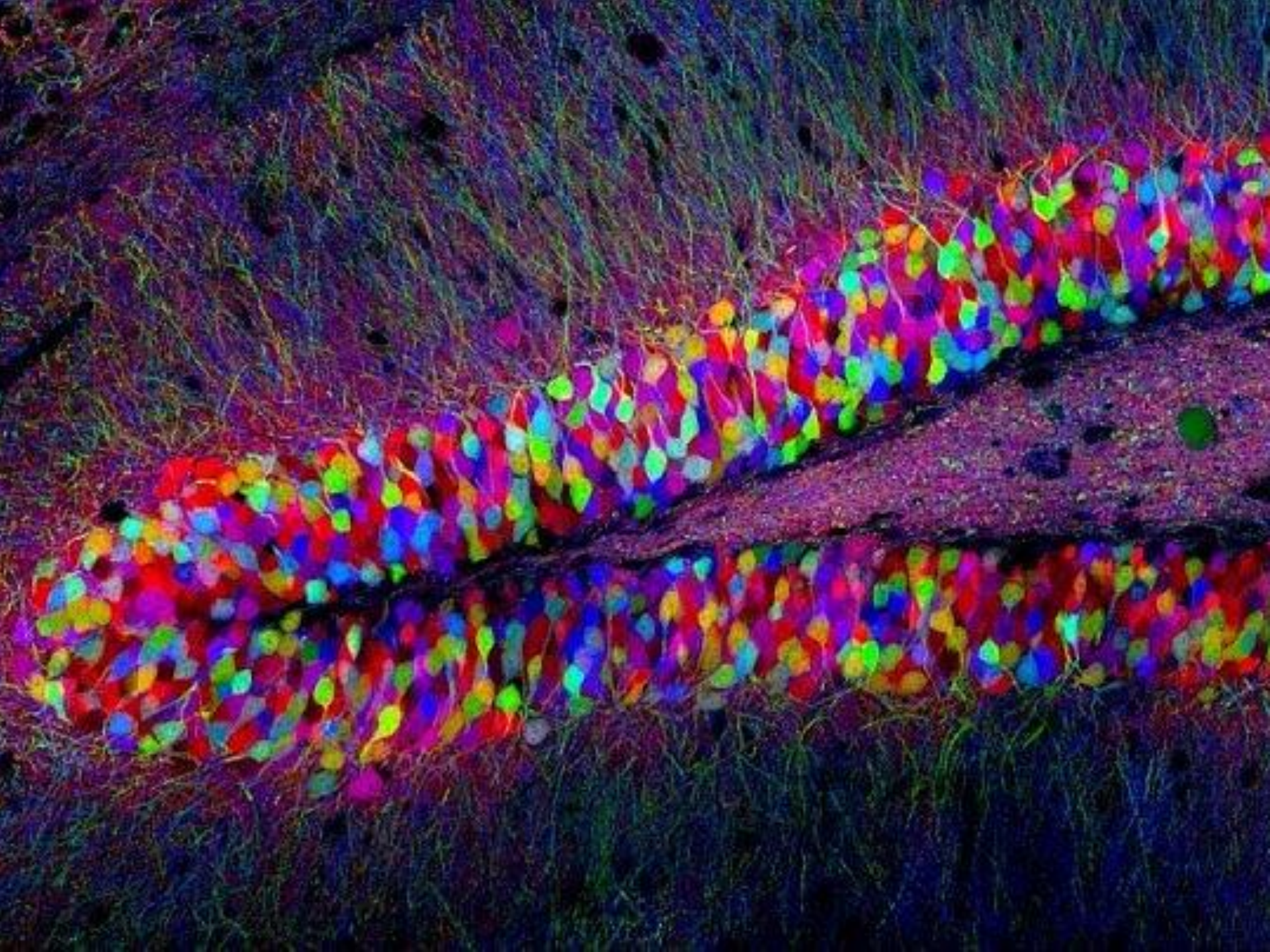
Regulatory sequence to be studied (e.g. a gene's promoter)

Reporter gene (e.g. encoding GFP or luciferase)









"Mr. Osborne, may I be excused?
My brain is full."