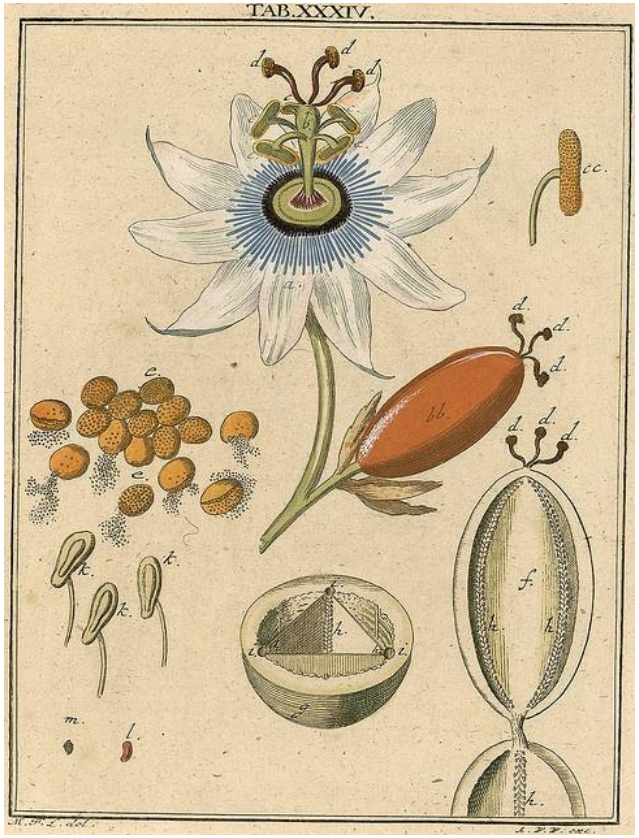
Vorlesung Mikroskopische Bildgebung

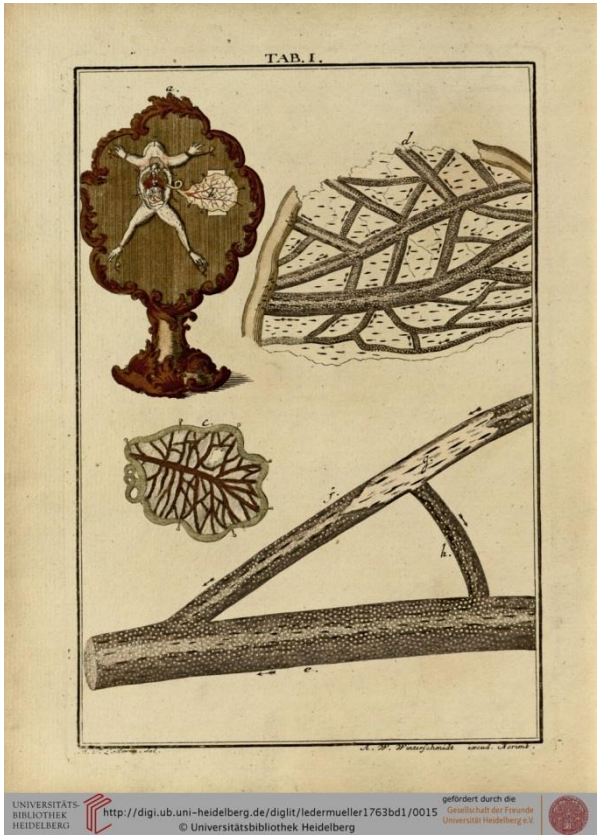
Teil 1 – **Präparationsmethoden**

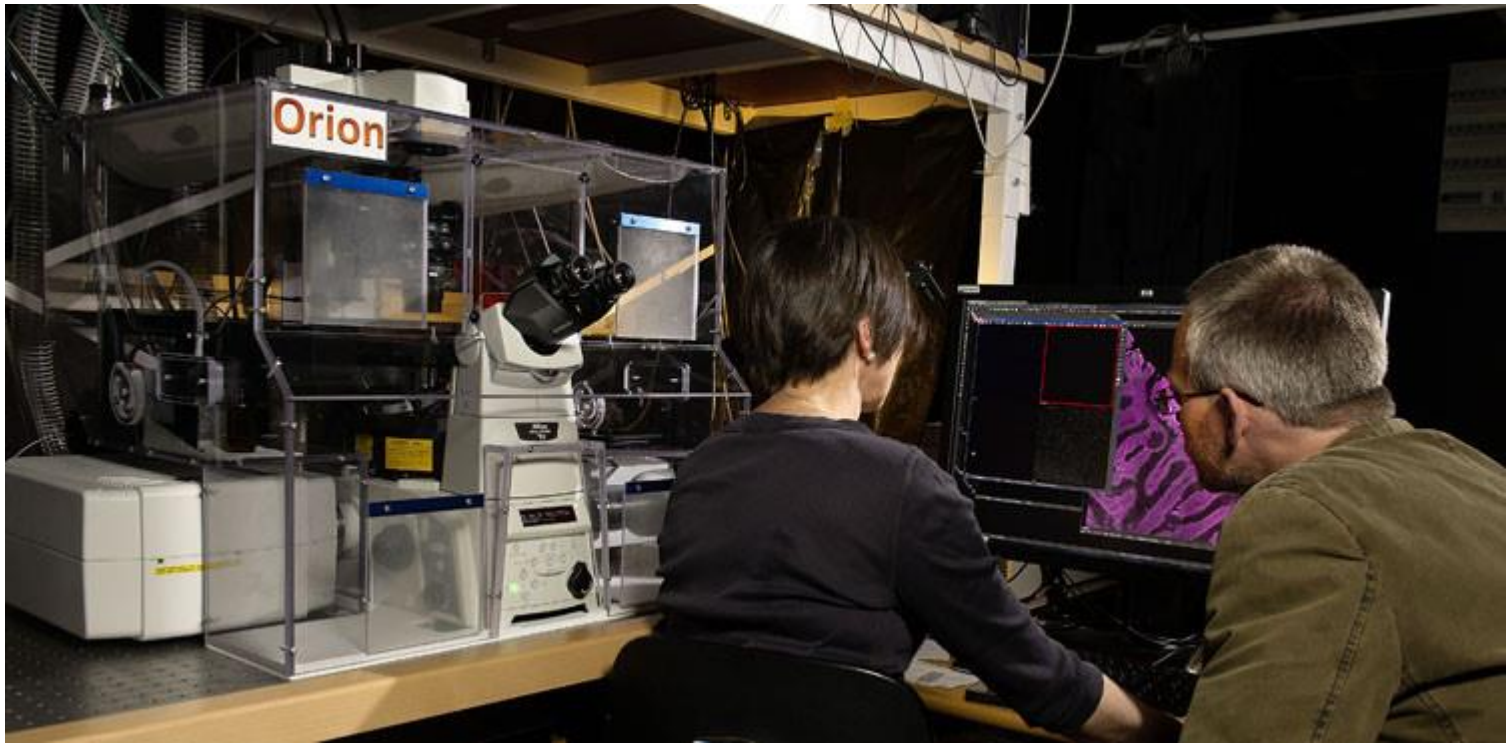
Teil 2 – **Färbemethoden**

Mikroskopische Gemüths- und Augen-Ergötzung

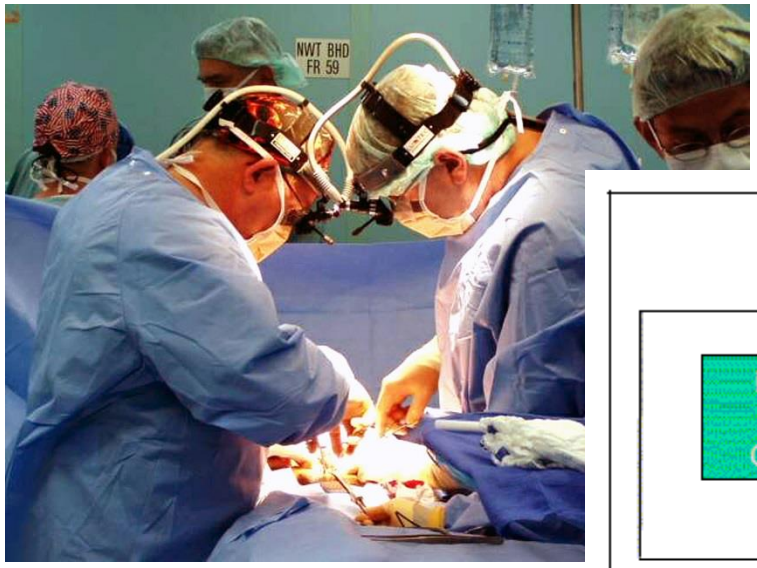
Frobenius 1719-1769

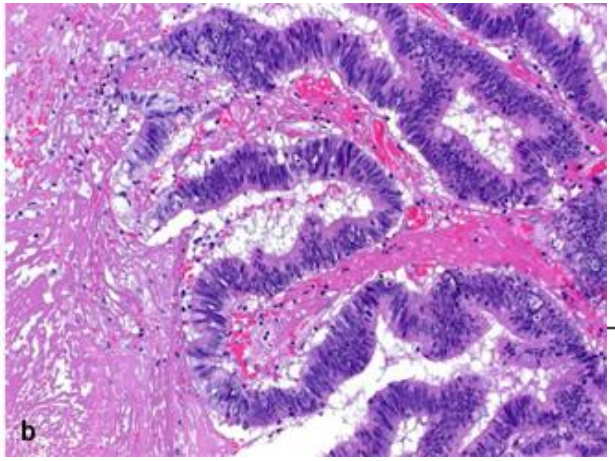


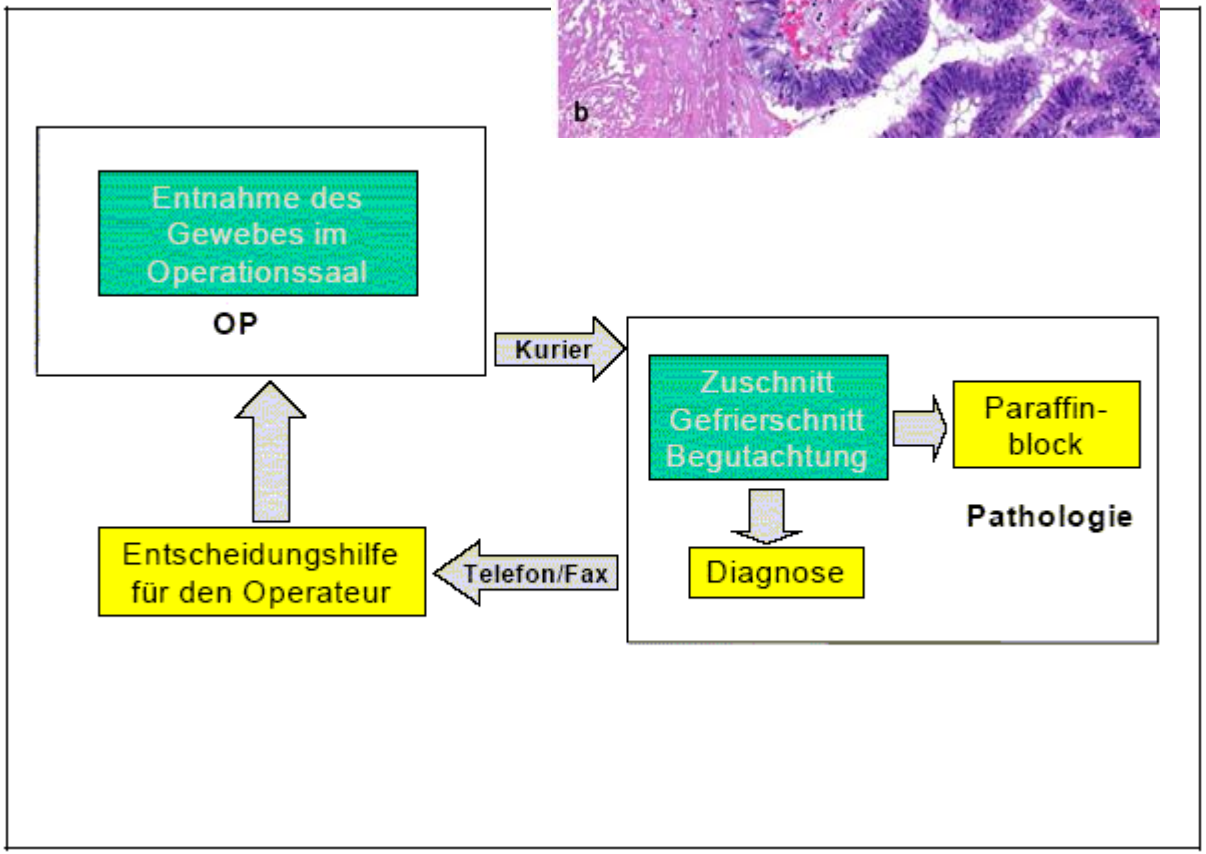




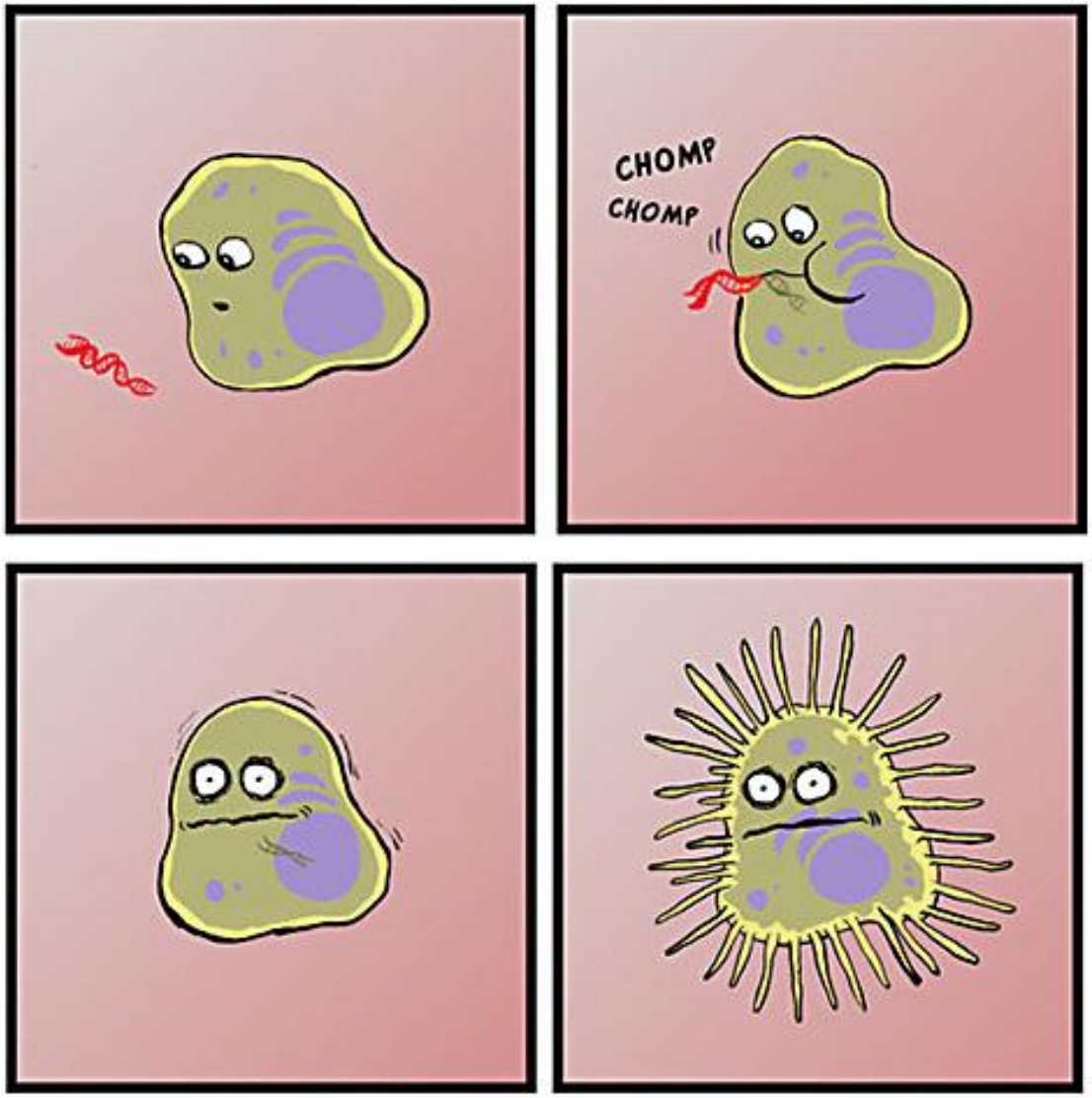
Schnellschnittuntersuchung



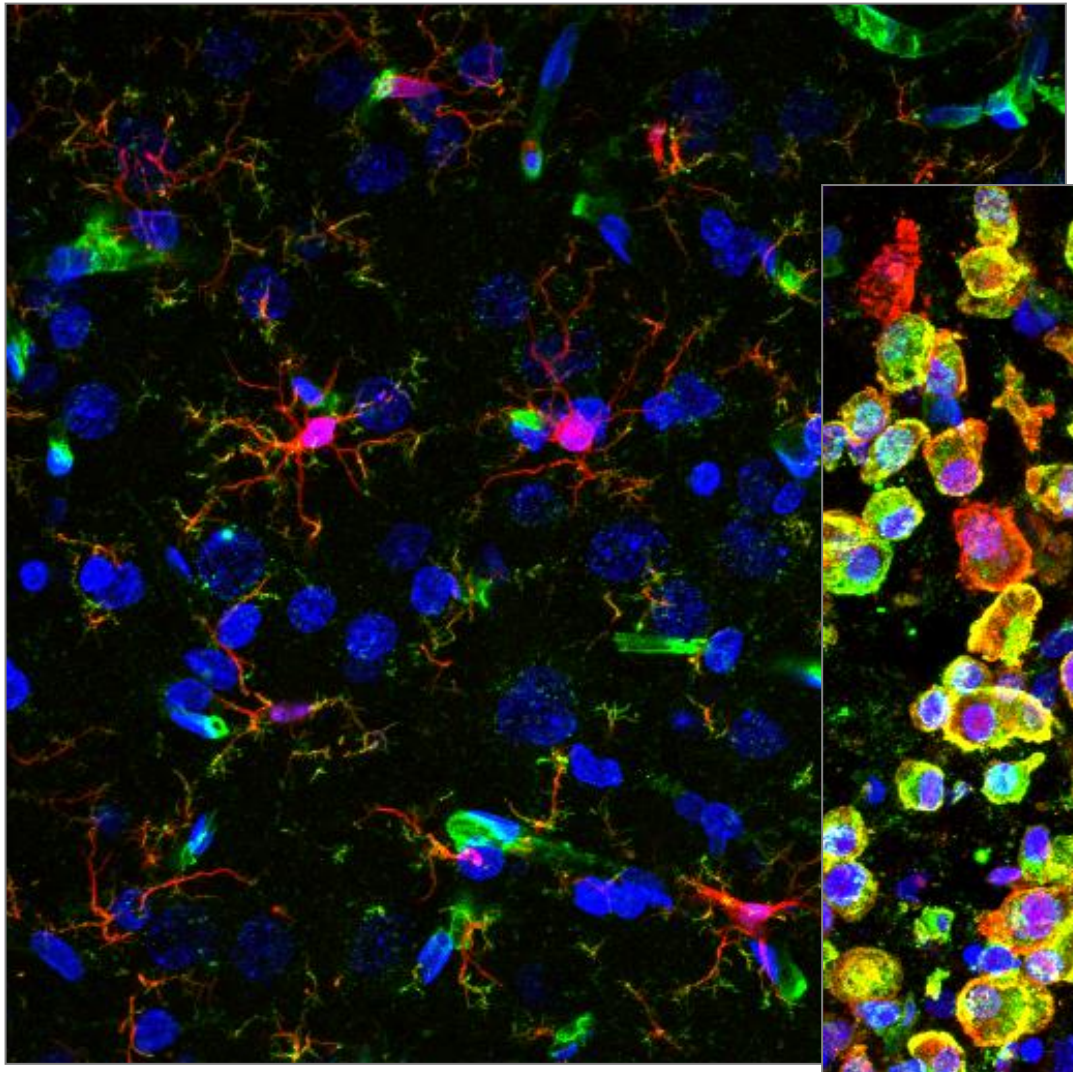




Fraunhofer IZI

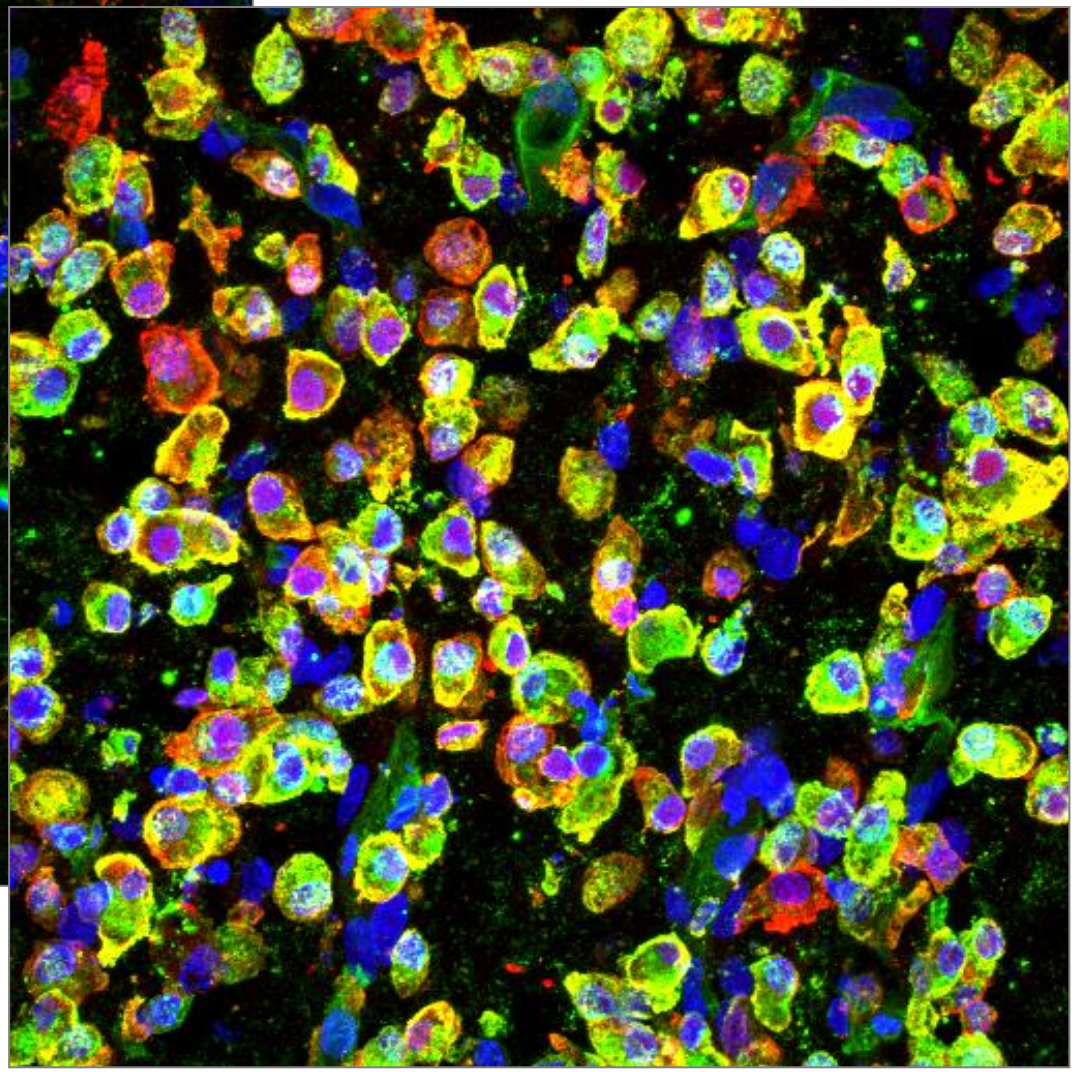


Pedro Veliça

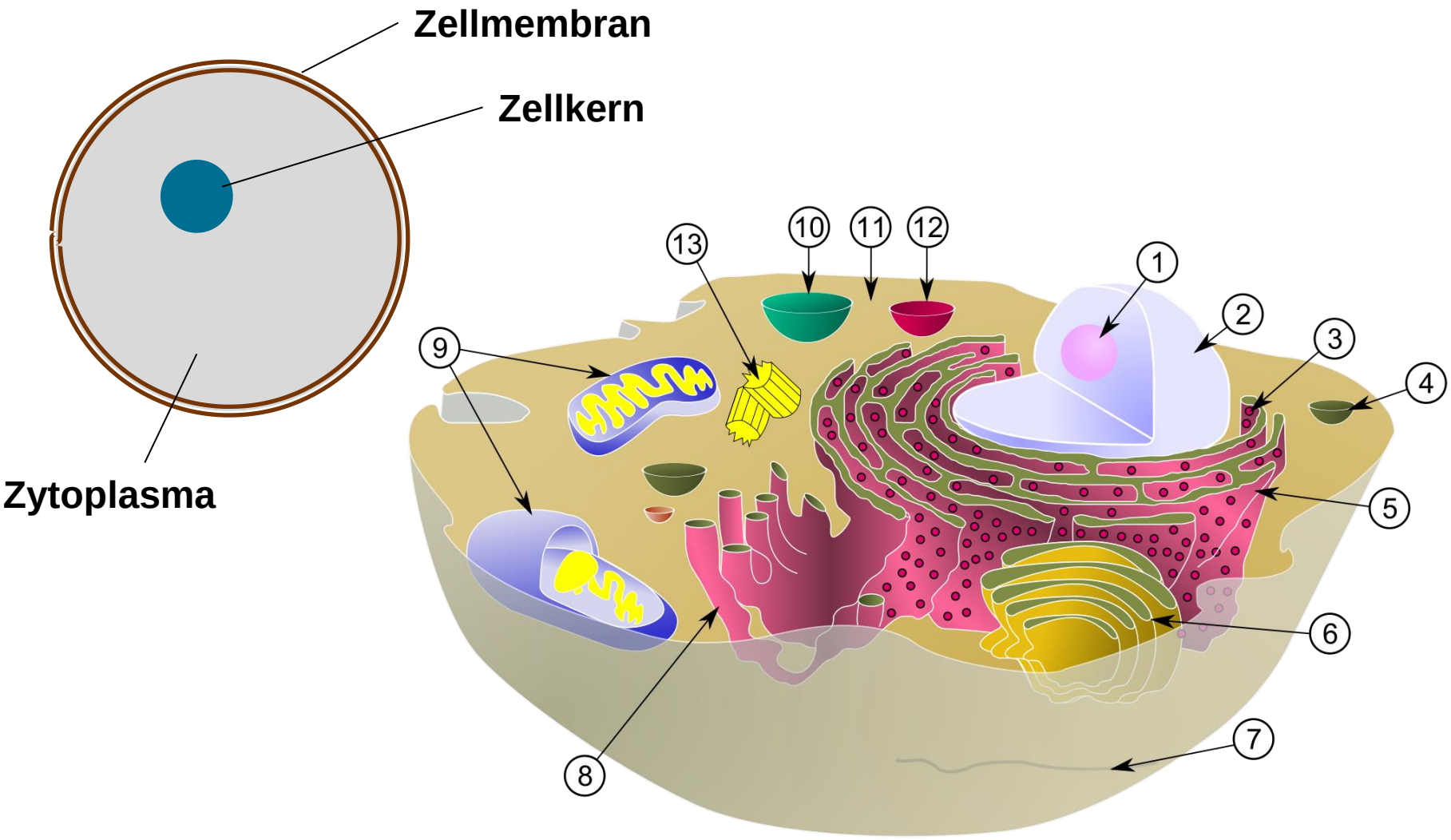


Gesundes Gehirn

Schlaganfall

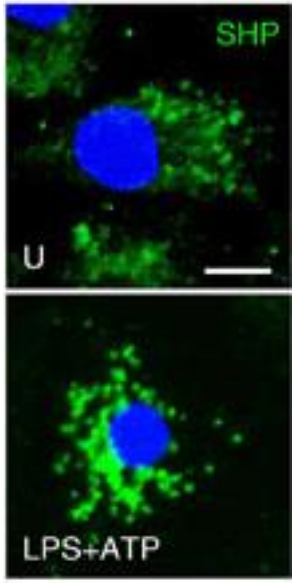


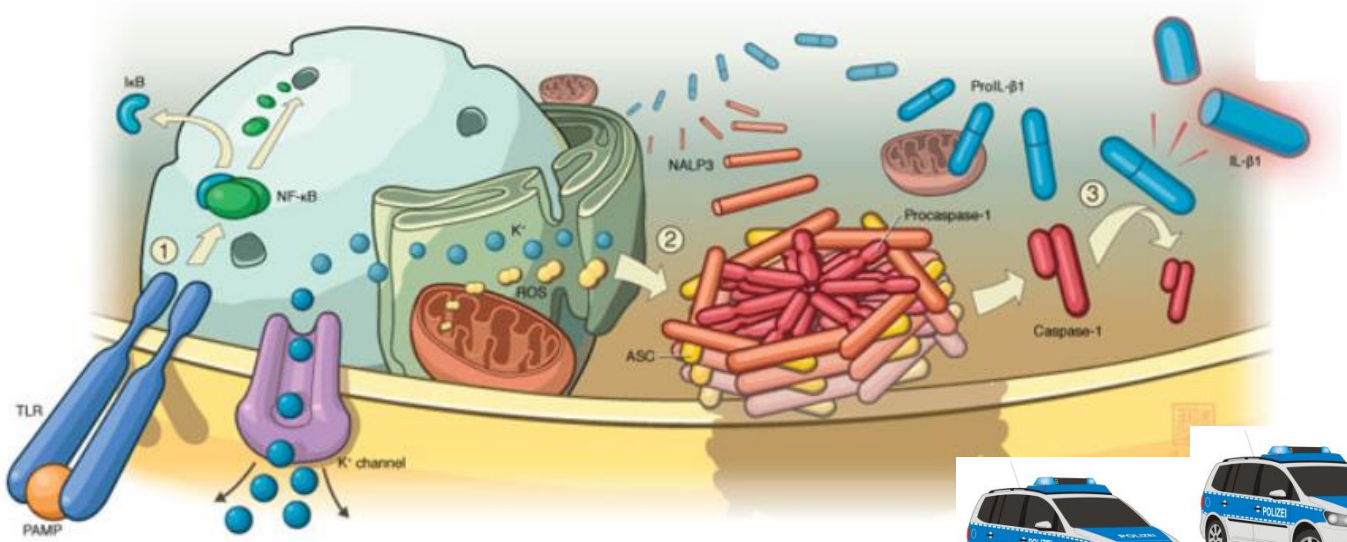
Zelle: kleinste Organisationseinheit des Körpers



Fraunhofer IZI

Wheel of death: Beobachten von Immunfunktion





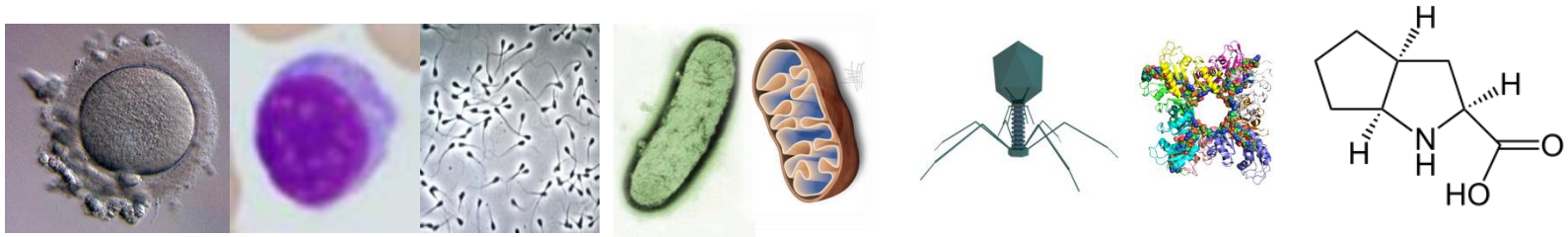
Fraunhofer IZI

Auge

Lichtmikroskop

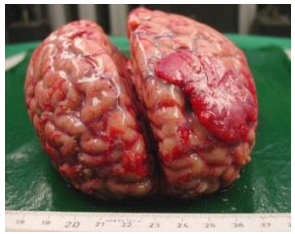
STED Mikroskopie

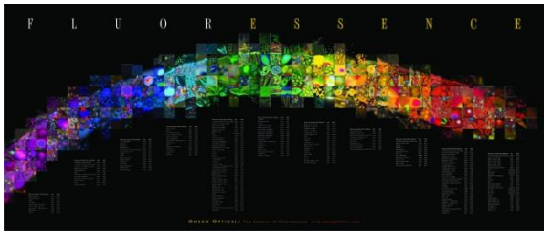
Elektronenmikroskop



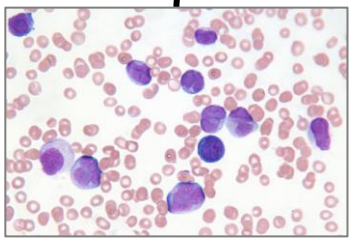
1 mm | 100µm | 10µm | 1µm | 100nm | 10nm | 1nm | 1A

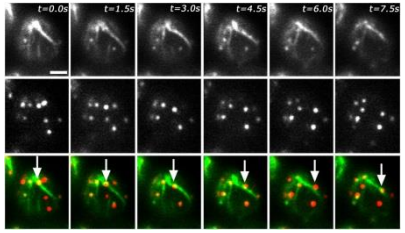
Fraunhofer IZI





P

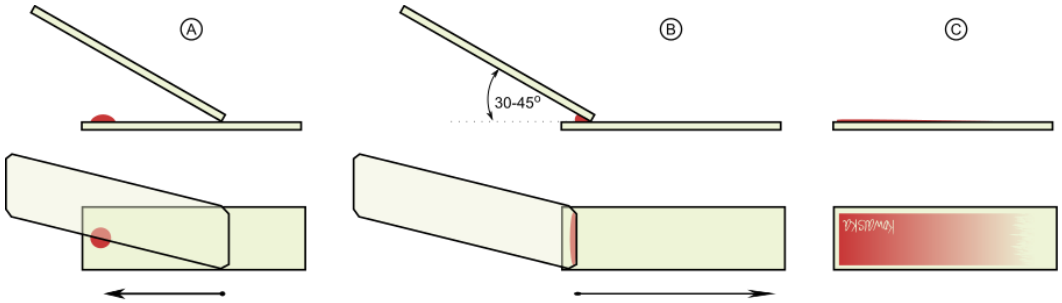




Fraunhofer IZI

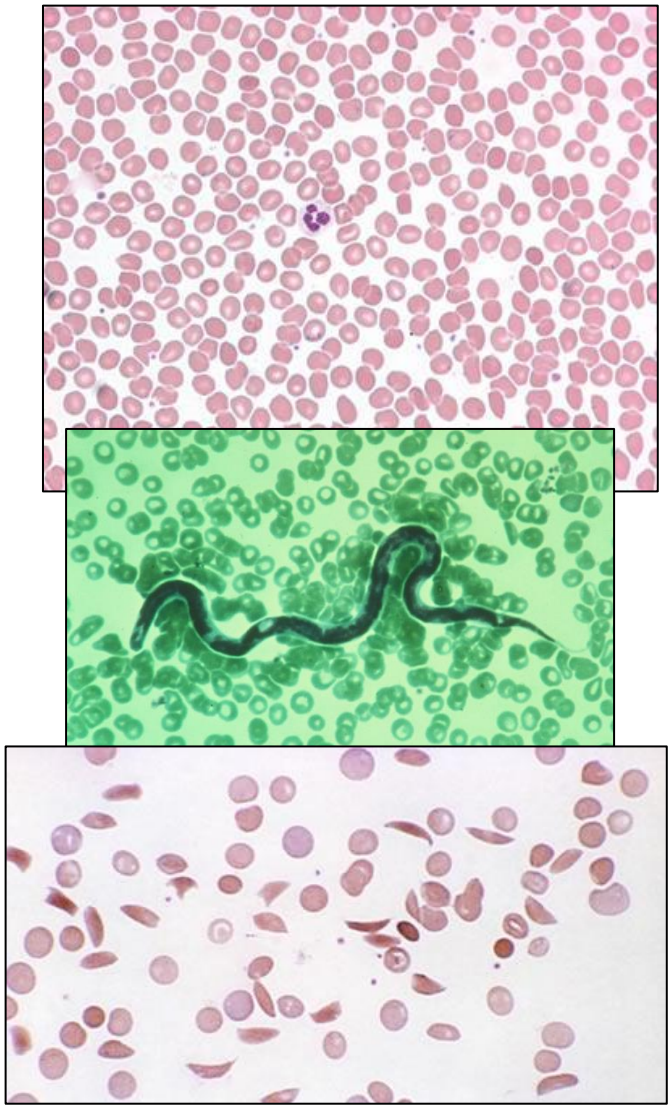
Flüssigkeiten und Zellen

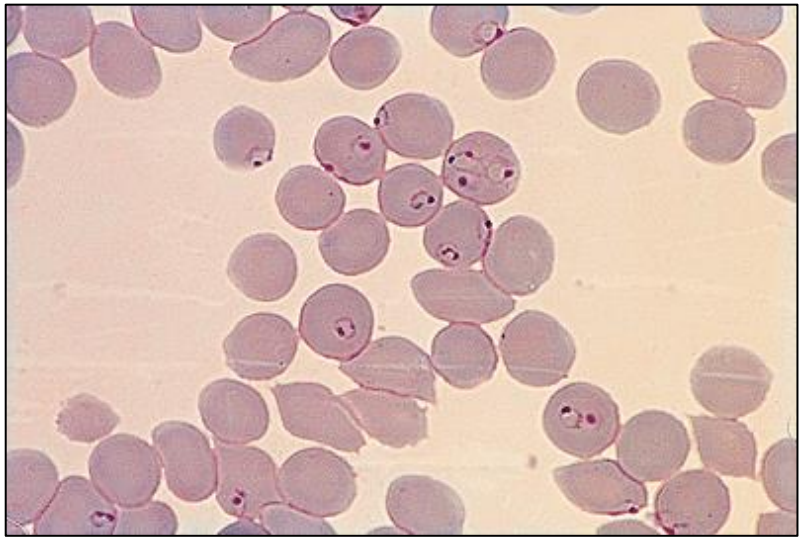
Ausstrich (Blut)

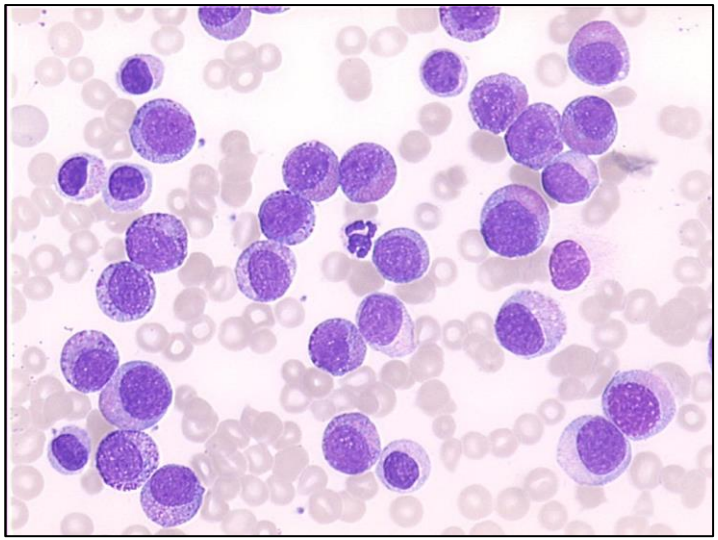


Giemsa-Färbung

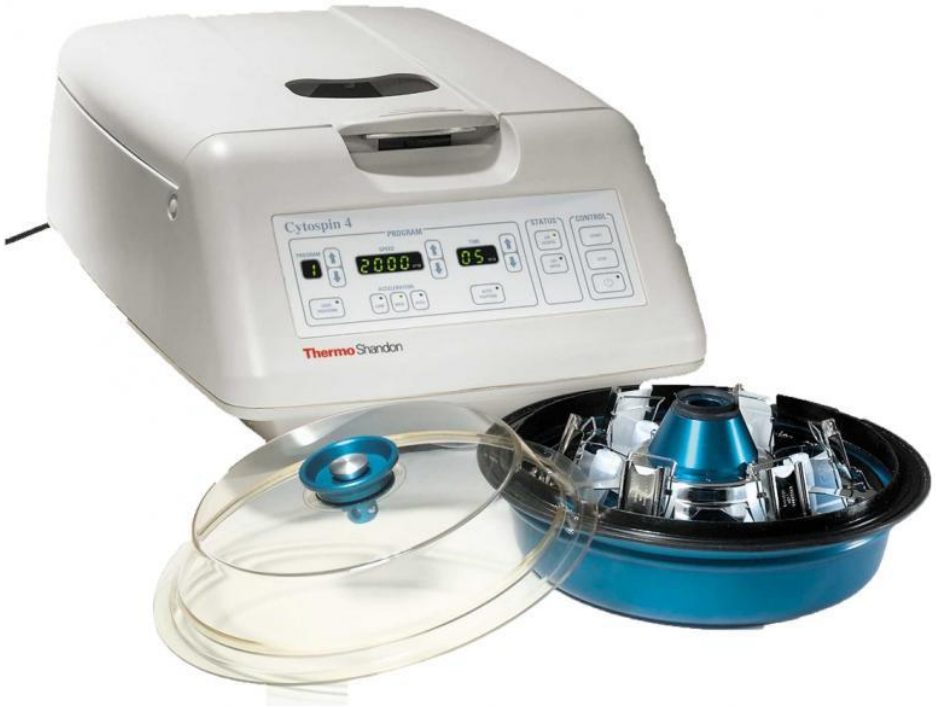
Ausstrich (Blut)

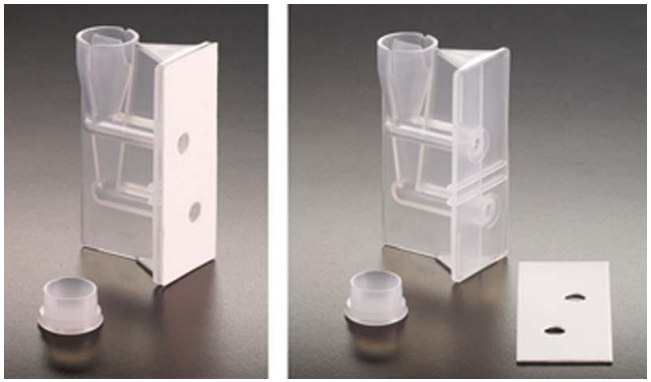


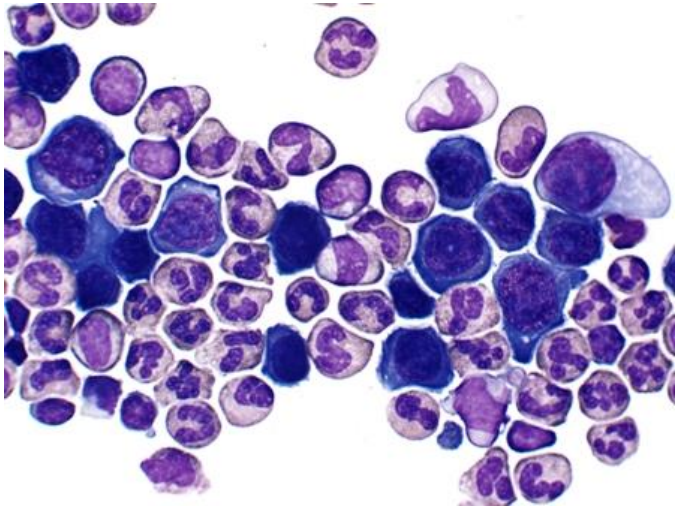




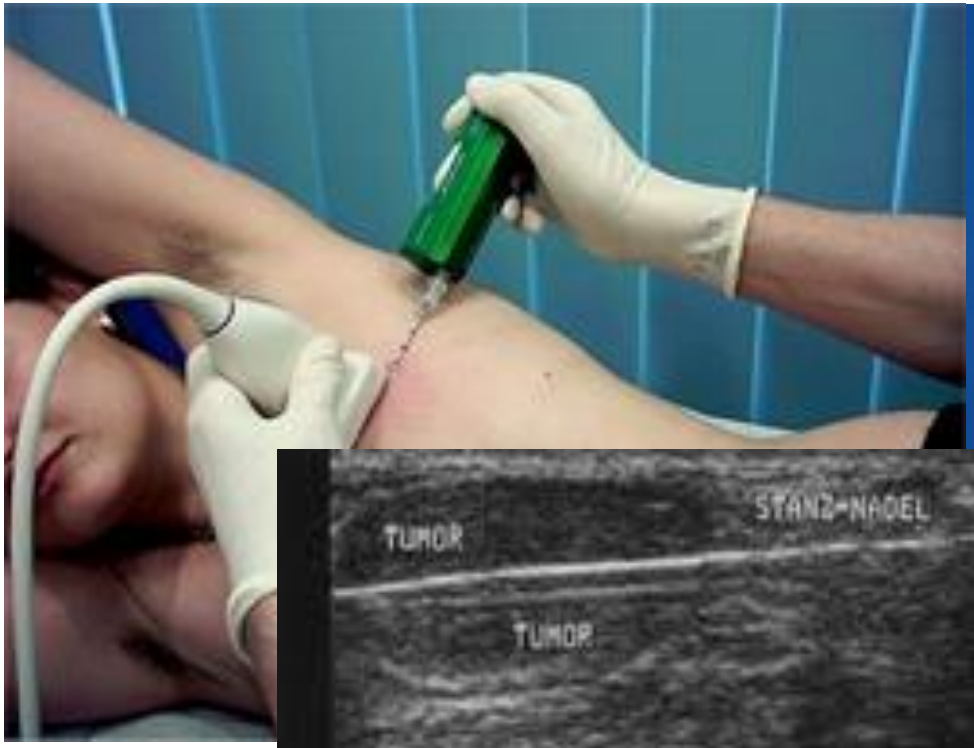
Cytospin

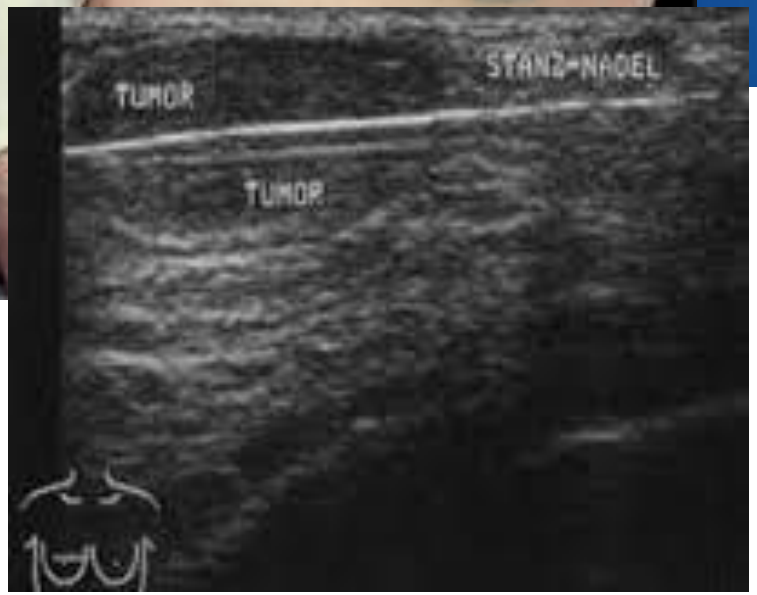


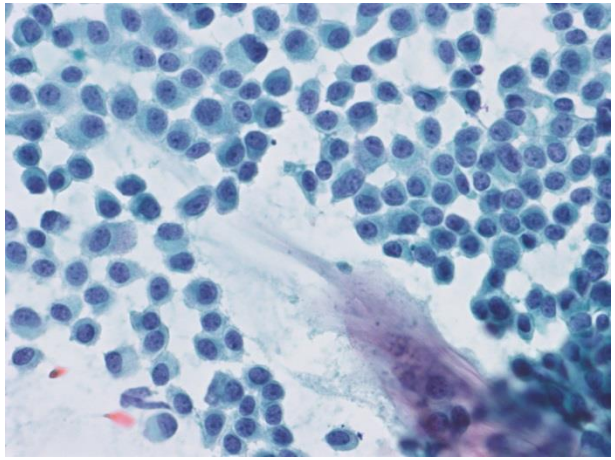




Biopsie





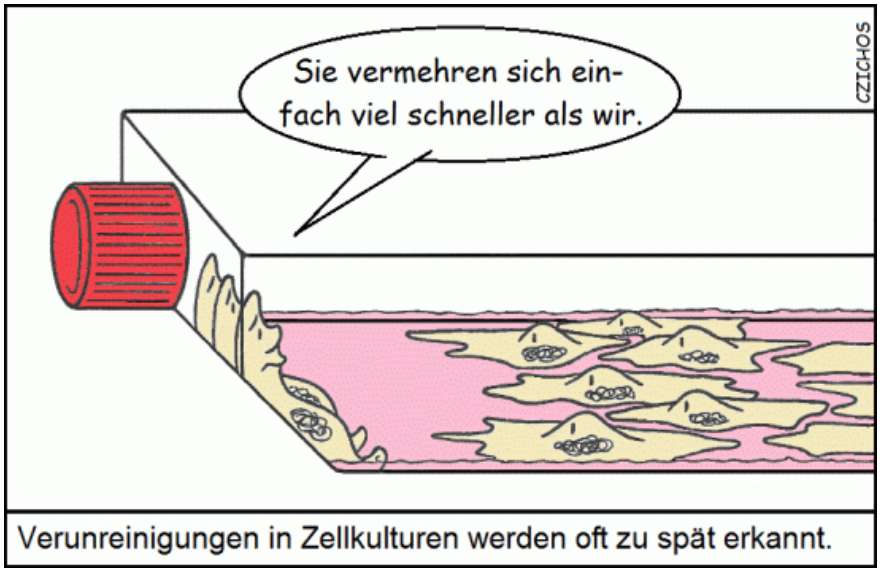


Zellkultur

Natives Material – lebendes Material

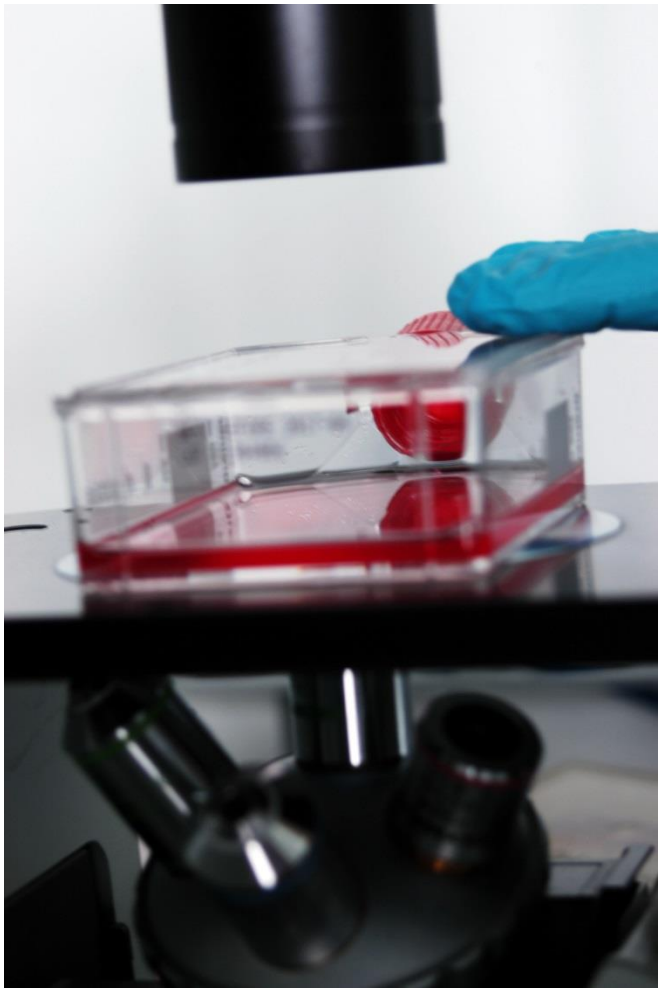
Limitationen

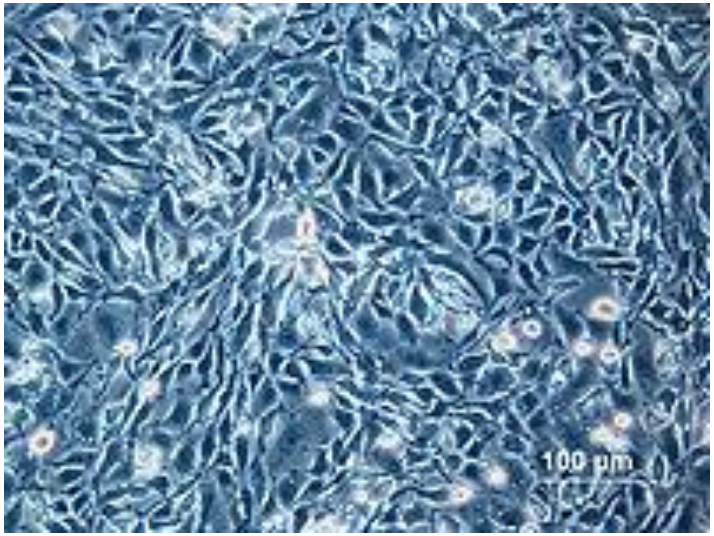
- Austrockung
- Nährstoffmangel
- Osmolarität
- pH
- Temperatur
- Fototoxität
- UV Strahlung
- pO2, pCO2
- Keime
- Oberflächen

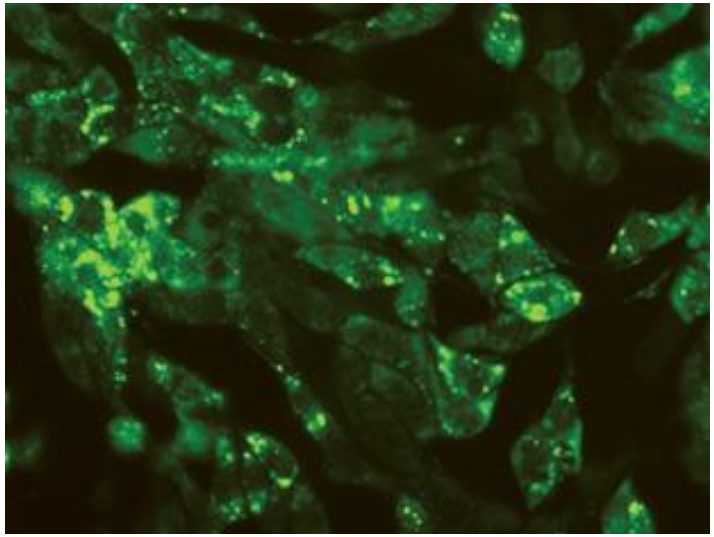


Verunreinigungen in Zellkulturen werden oft zu spät erkannt.

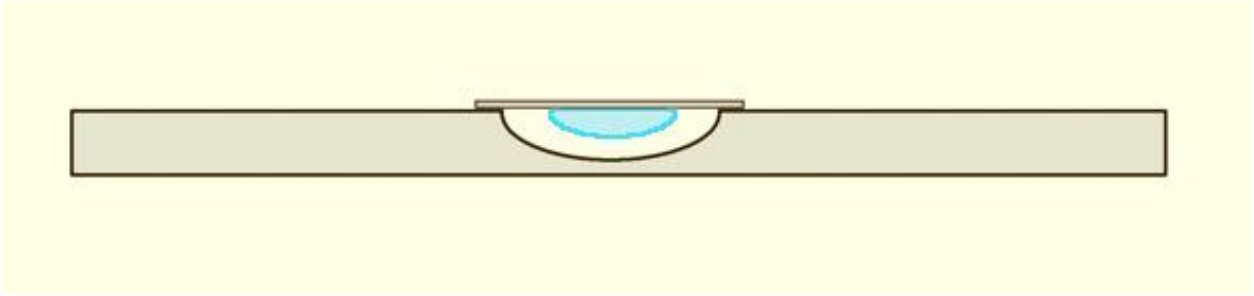
Zellkultur



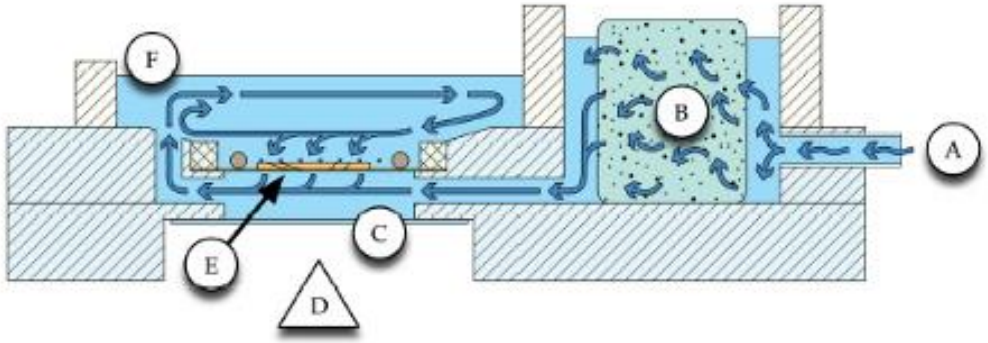




Hängender Tropfen



Gewebekulturen

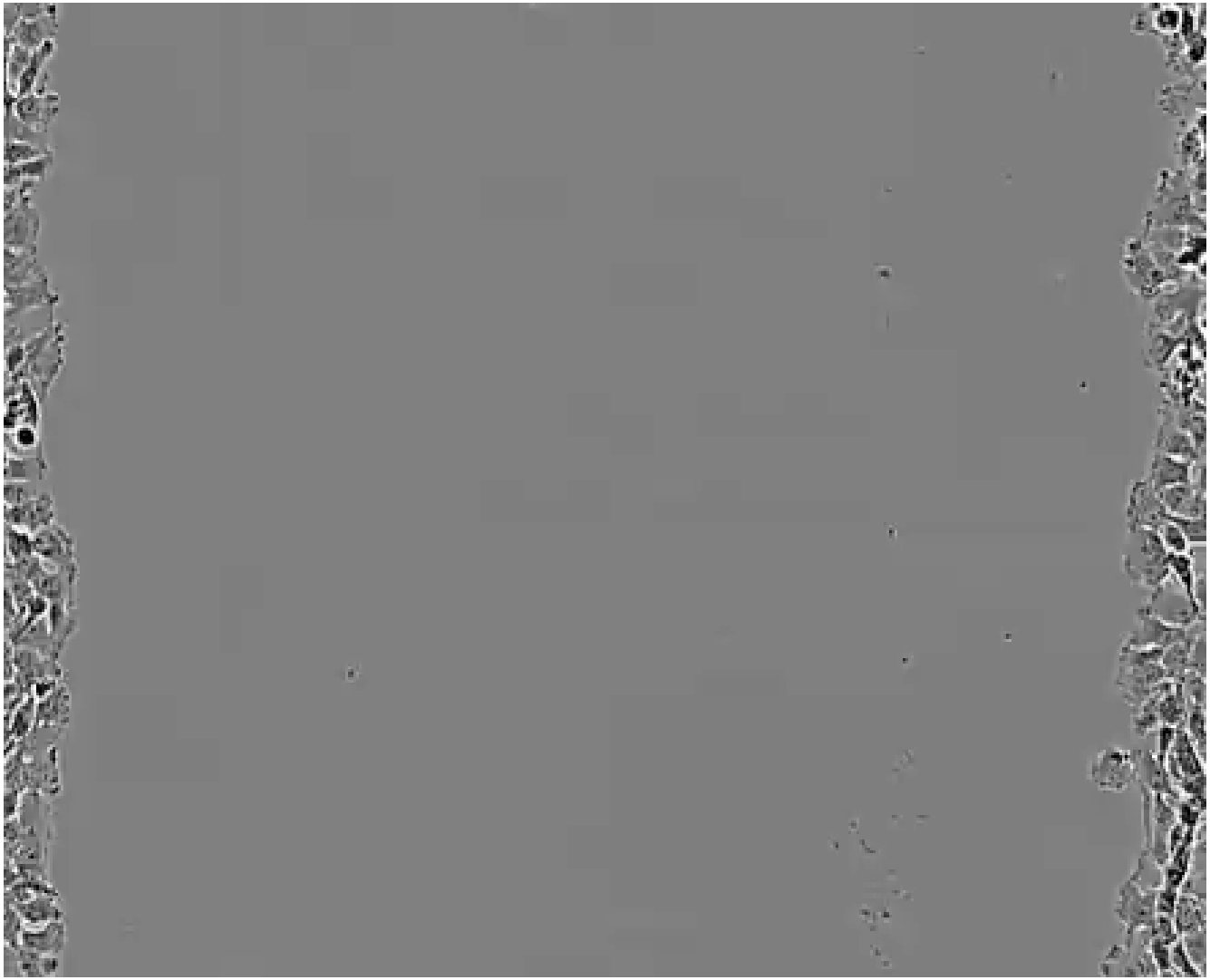


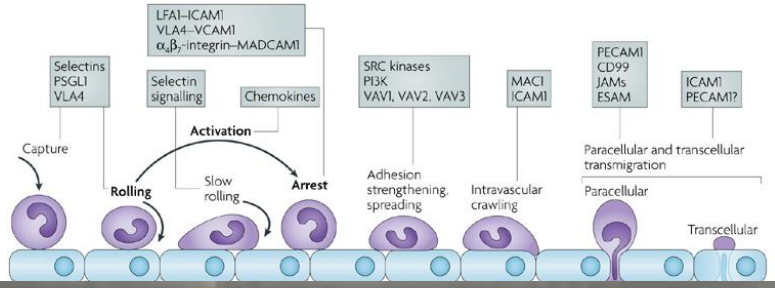
Fraunhofer IZI

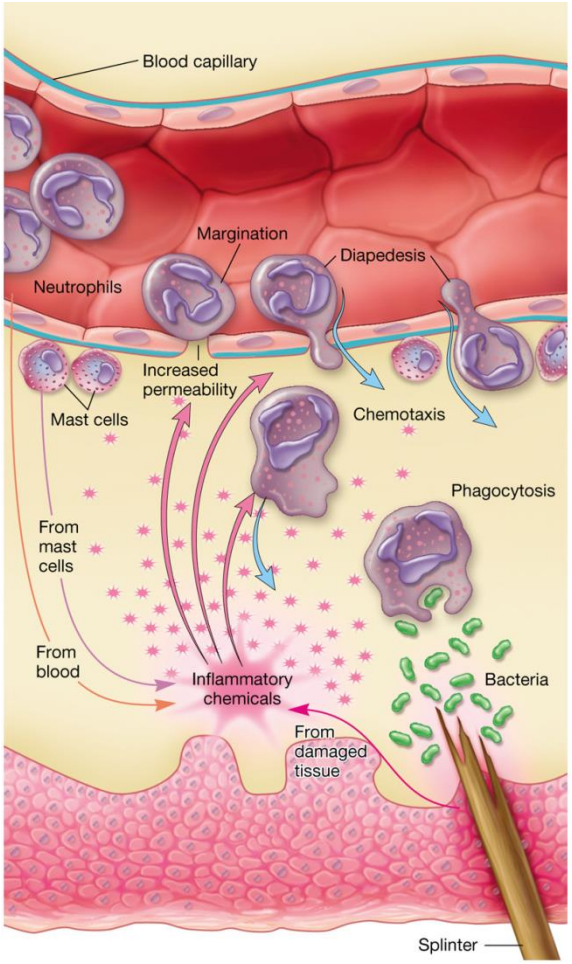
Live cell imaging

Fraunhofer IZI

Live cell imaging

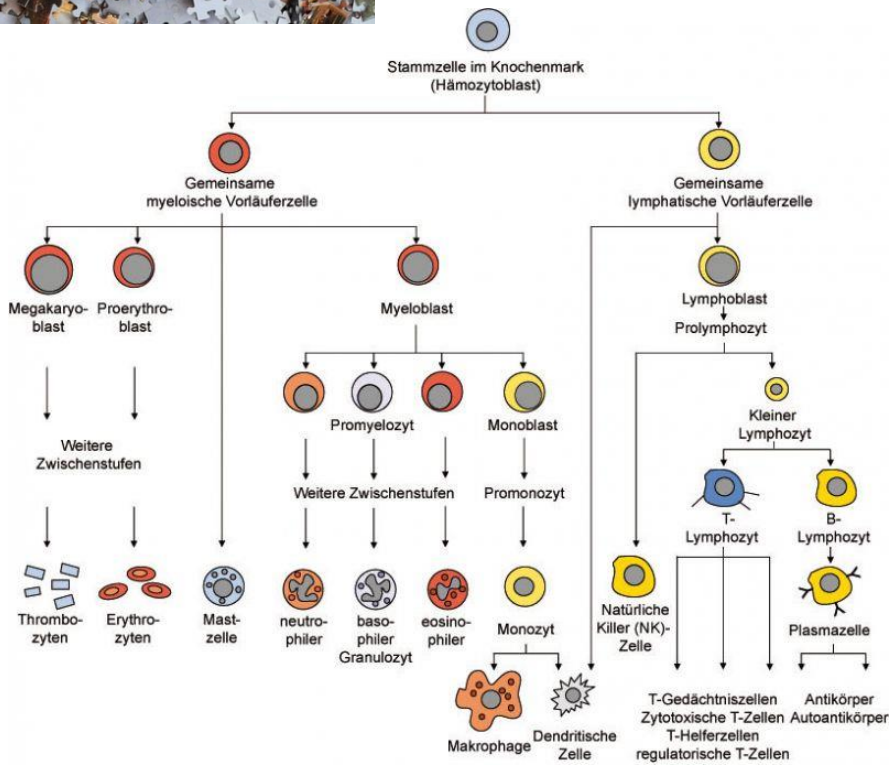


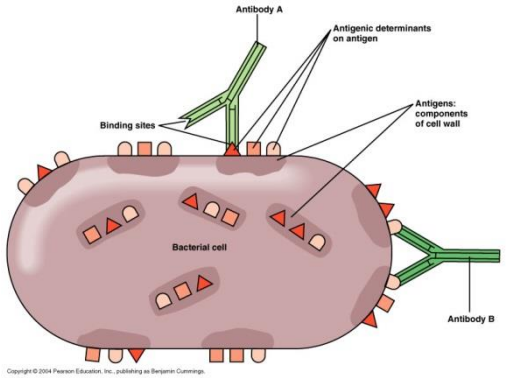




Fraunhofer IZI

Cell sorting





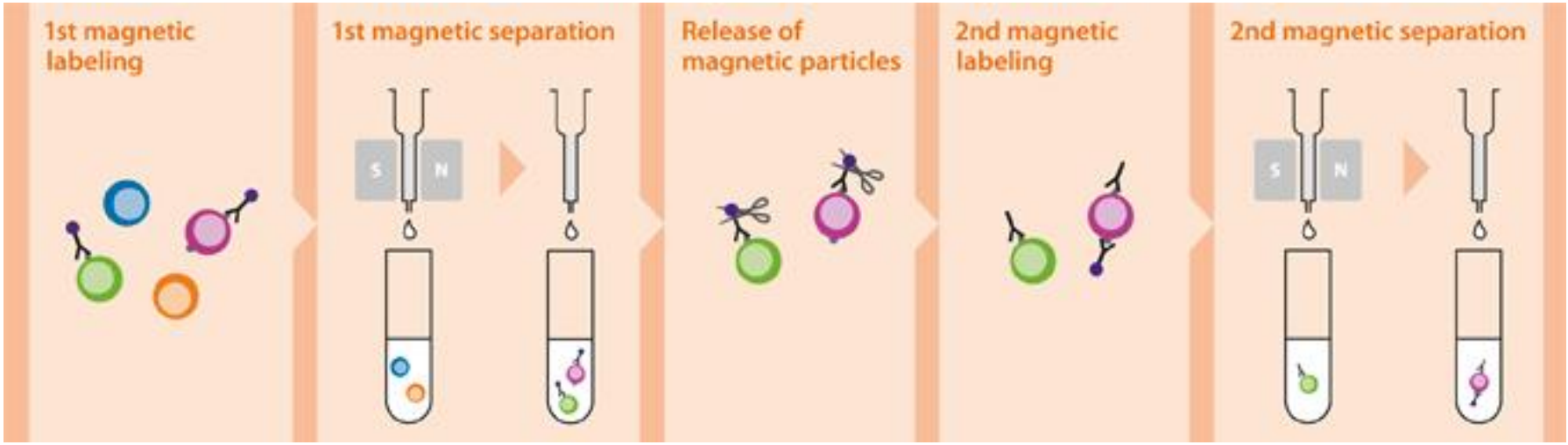
Fraunhofer
IZI

MACS sorting

MACS Columns used to contain the specimen of interest

Magnetic bar (MACS separator) to hold the columns and attracted to the metal plate (shown black in colour above)

http://textbookhaematology4medical-scientist.blogspot.sg/

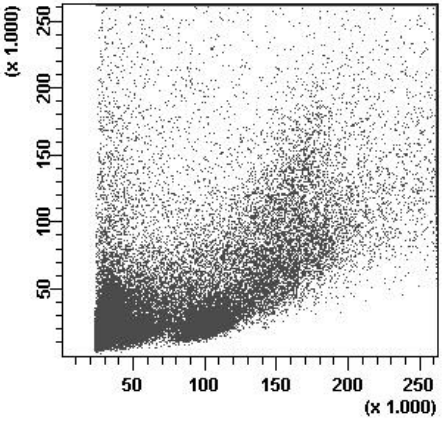


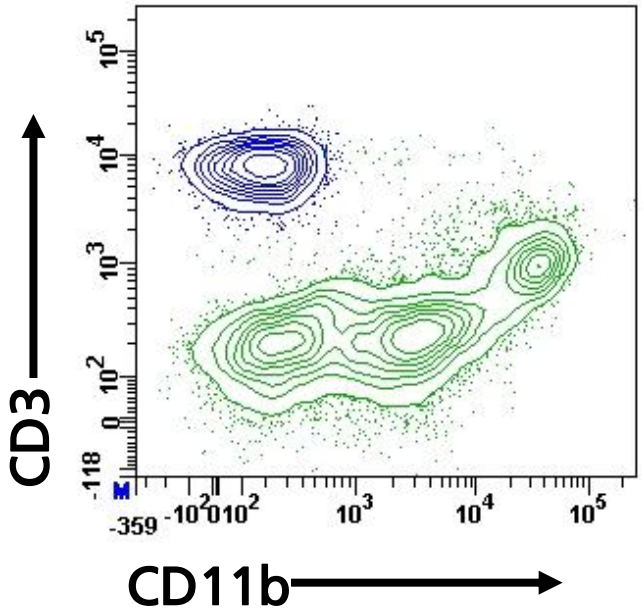
Fraunhofer IZI

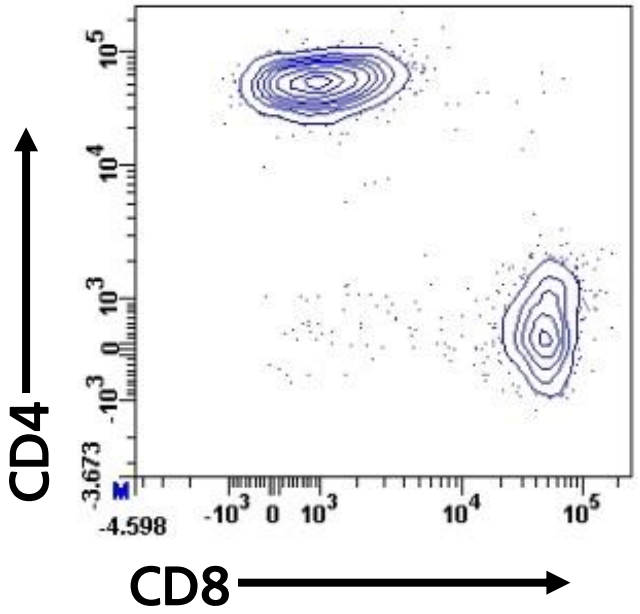
FACS sorting

8 Kanäle

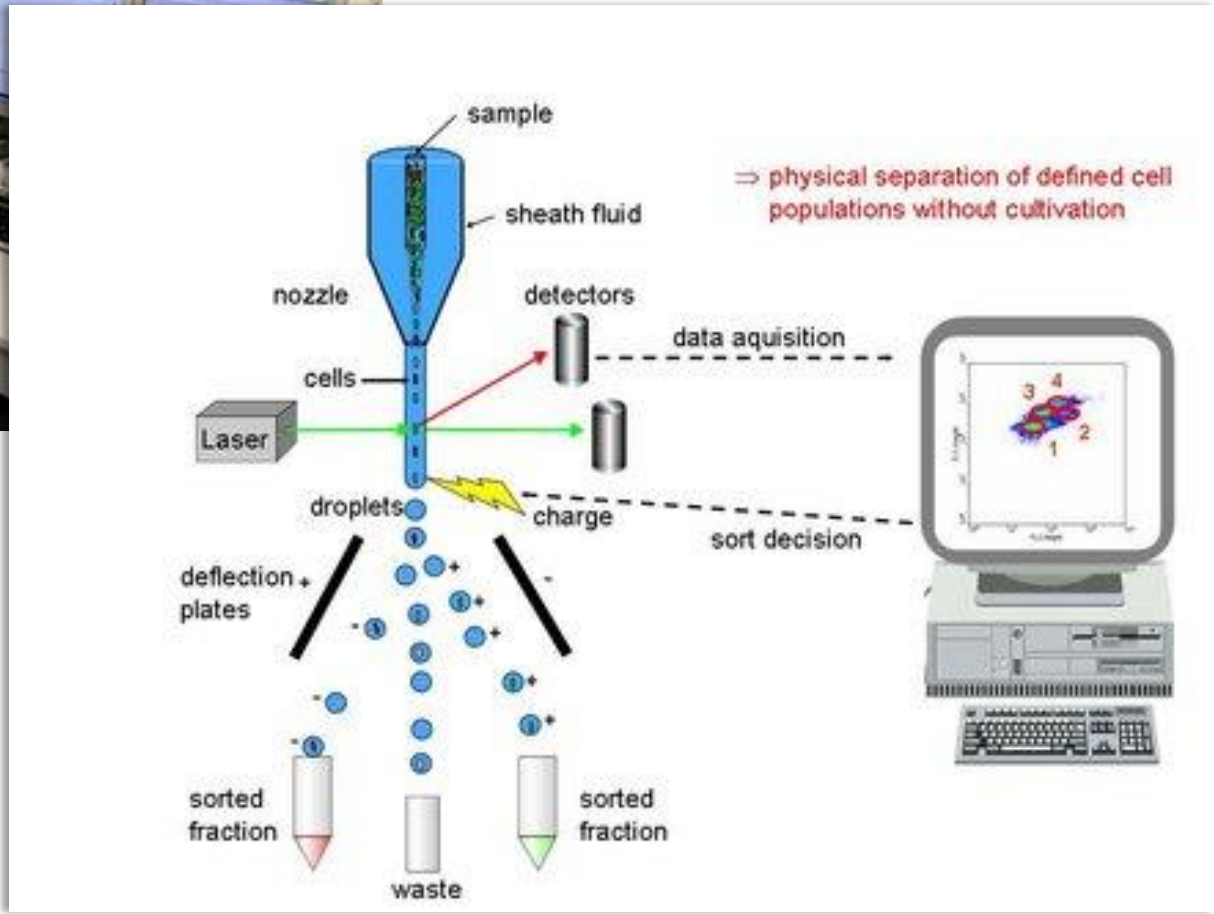
	CD11b	CD3	CD4	CD8
Myeloide Zellen	+	-		
T-Zellen	-	+		
T Helfer Zellen	-	+	+	-
Zytotoxische T Zellen	-	+	-	+







FACS sorting

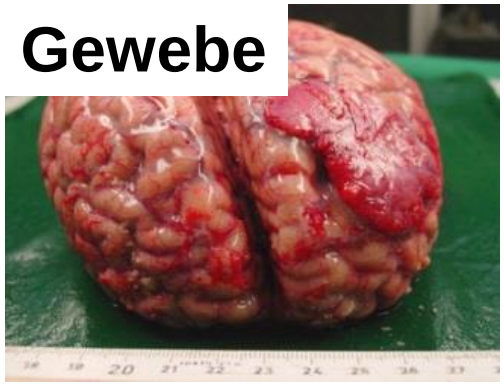


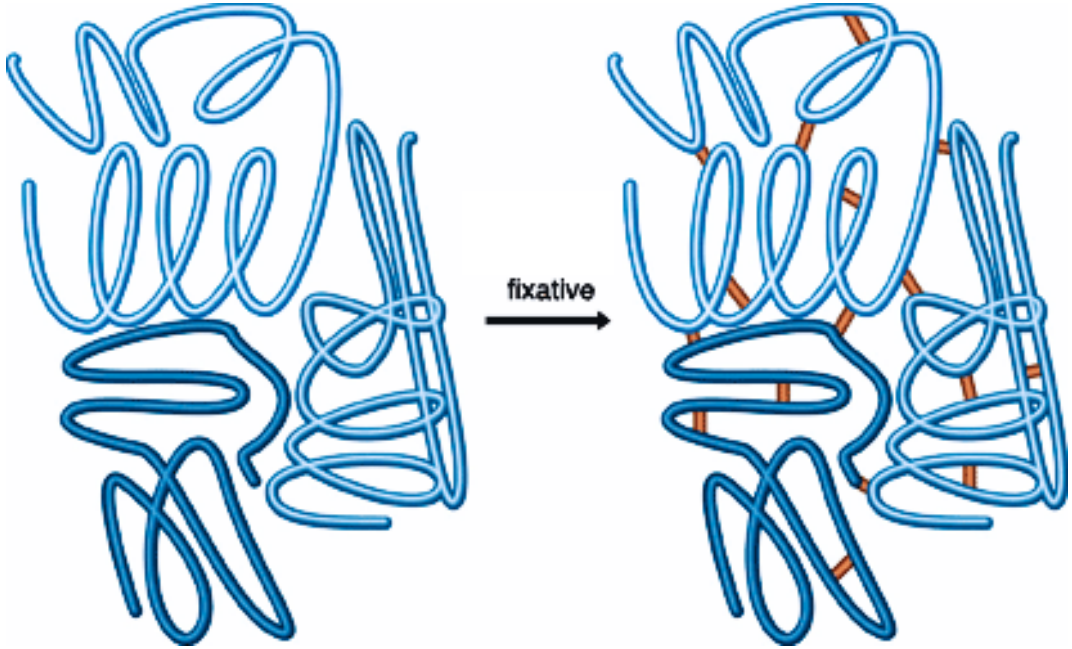
Fraunhofer IZI

Fixierung

Warum? To conserve!

Gewebe



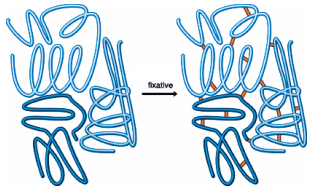


i) Proteolytische Enzyme

ii) Mikroorganismen

iii) Mechanische Struktur

Generelle Fixierungsverfahren



Physikalisch

- Trocknung
- Hitze
- Mikrowelle
- Gefrieren

Chemisch

- Immersion
- Injektion
- Auftropfen
- Perfusion

Fixierungssubstanzen

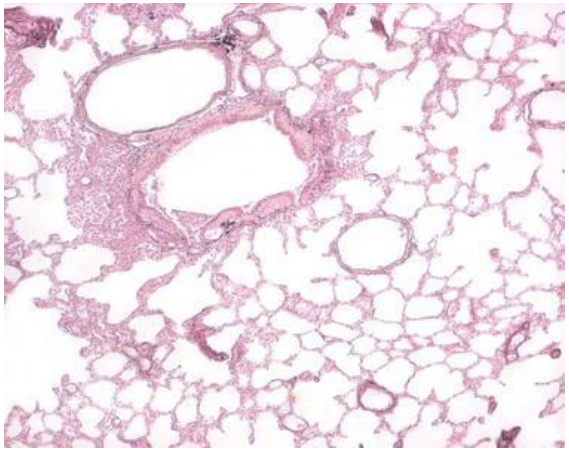
Formalin – Denaturierung und cross-linking

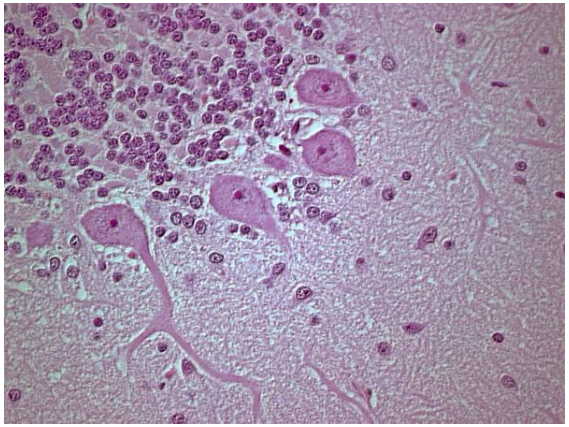
Glutaraldehyd - Denaturierung und cross-linking

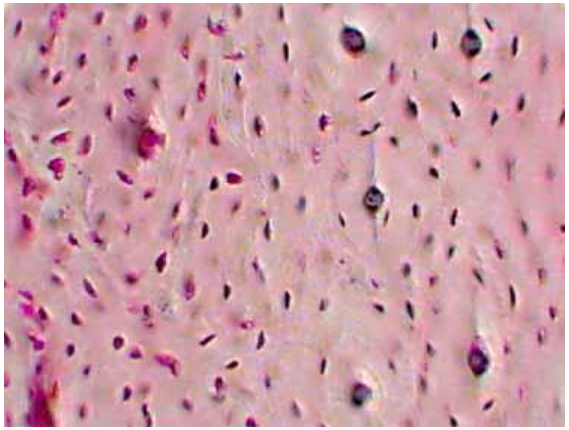
Ethanol/Methanol – Denaturierung und Wasserverdrängung

Schnittpräparation

Warum ? Konsistenz!

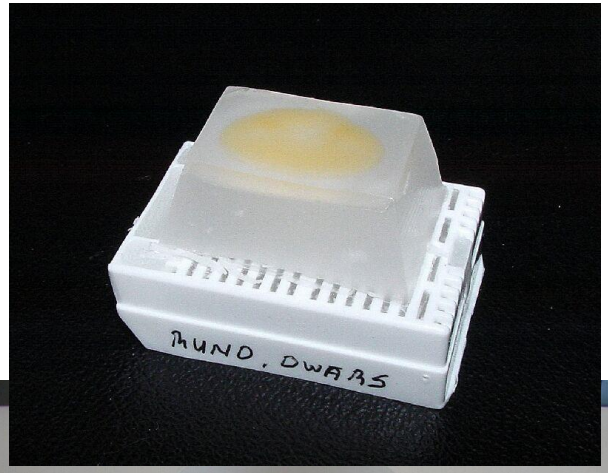


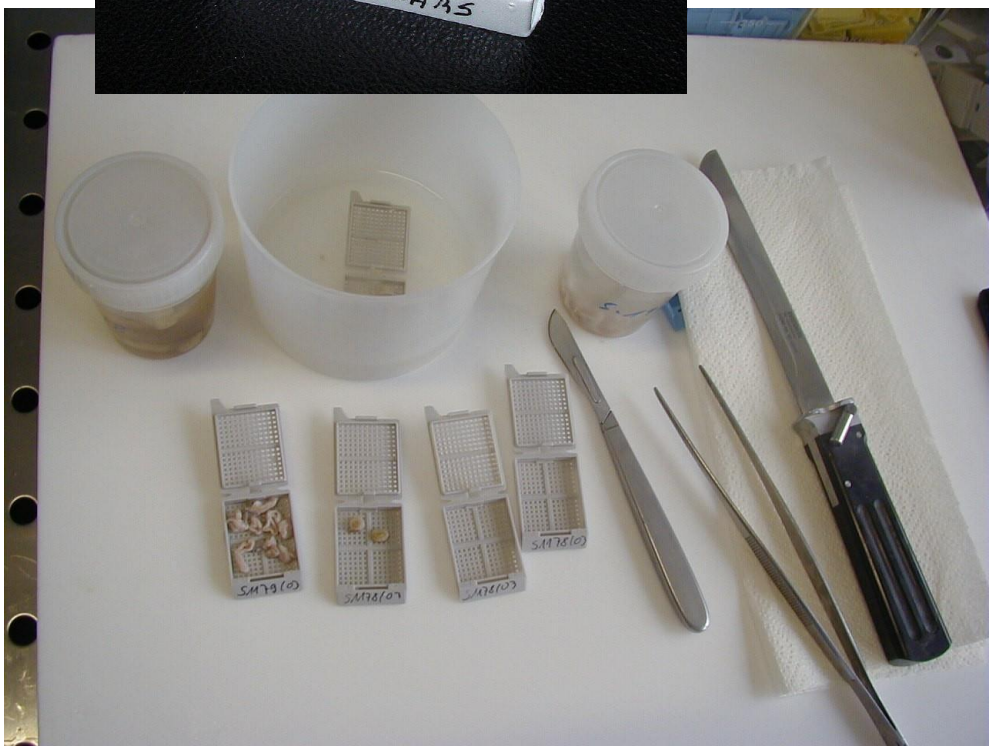


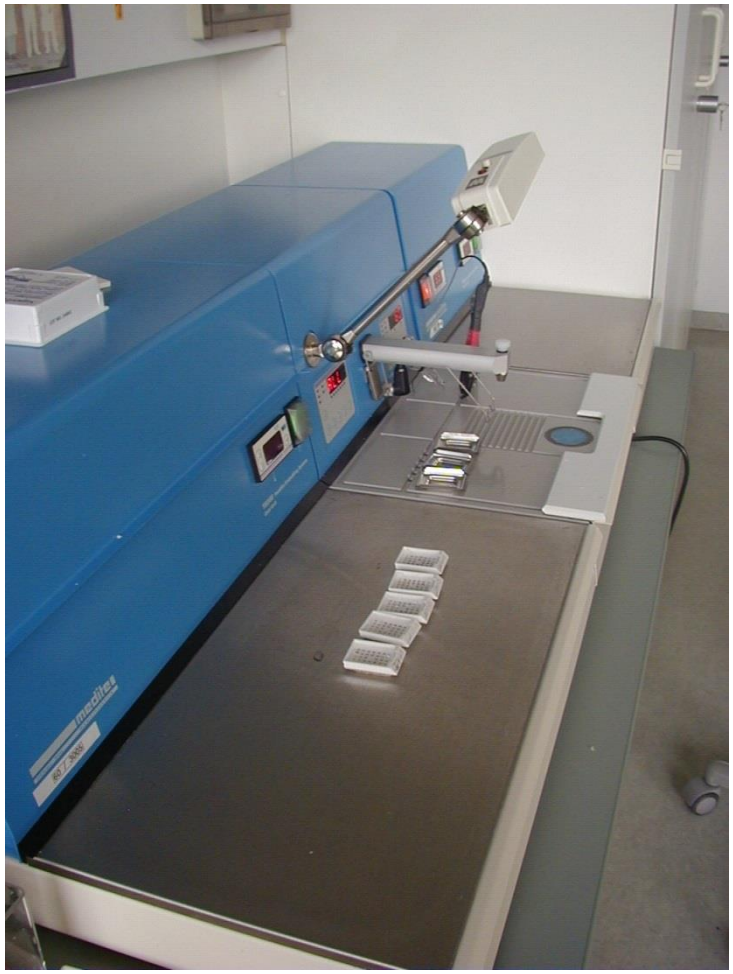


Nativ oder Paraffin/Kunststoff oder gefroren

Einbetten in Paraffin

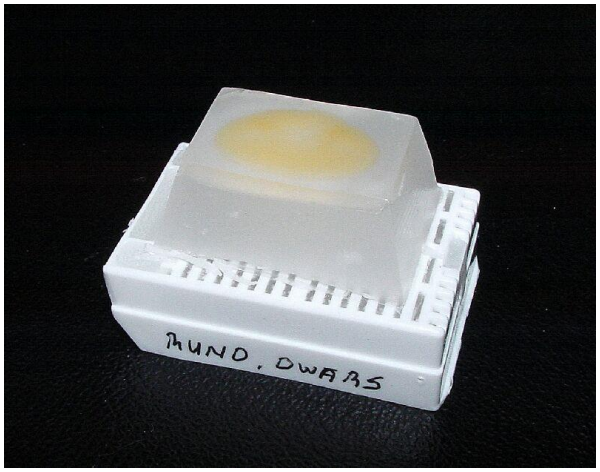






Einbetten in Paraffin

Auswaschen Leitungswasser	3h
50% Alkohol	2h
70% Alkohol	3h
96% Alkohol	4h
100% Alkohol	4h
100% Alkohol	4h
Methylbenzoat	2h
Methylbenzoat	2h
Xylol	2h
Xylol-Paraffin 1:1	1h
Paraffin 60°C	8h
Gesamtzeit	**47h**

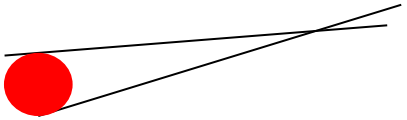


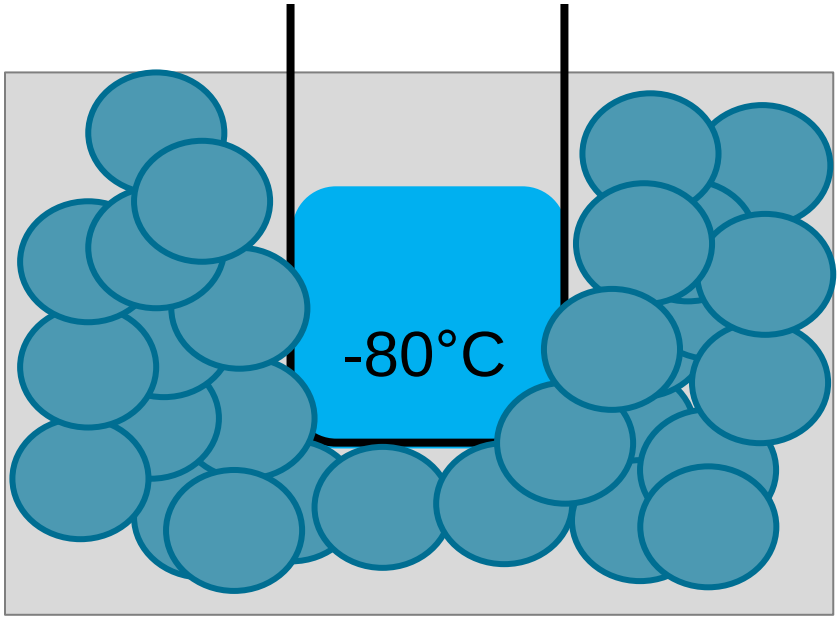
Einbetten in Paraffin

+ Fest bei RT
+ Unbegrenzt haltbar
+ sehr dünne Schnitte möglich
+ Serienschnitte möglich
+ Effiziente Verarbeitung, hohe Qualität

- Schrumpfung bis 20%
- Giftige Substanzen (Arbeitsschutz)
- Hohe Temperatur

Kryopräparation





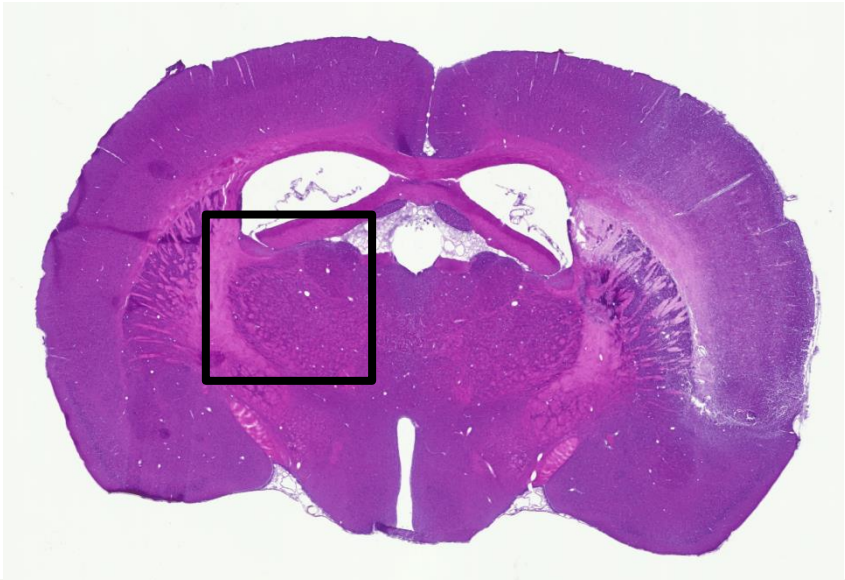
… daher schnell
und kleine Probe

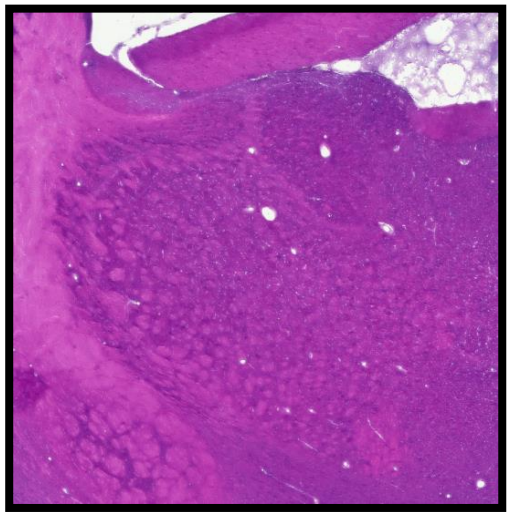
Kryopräparation

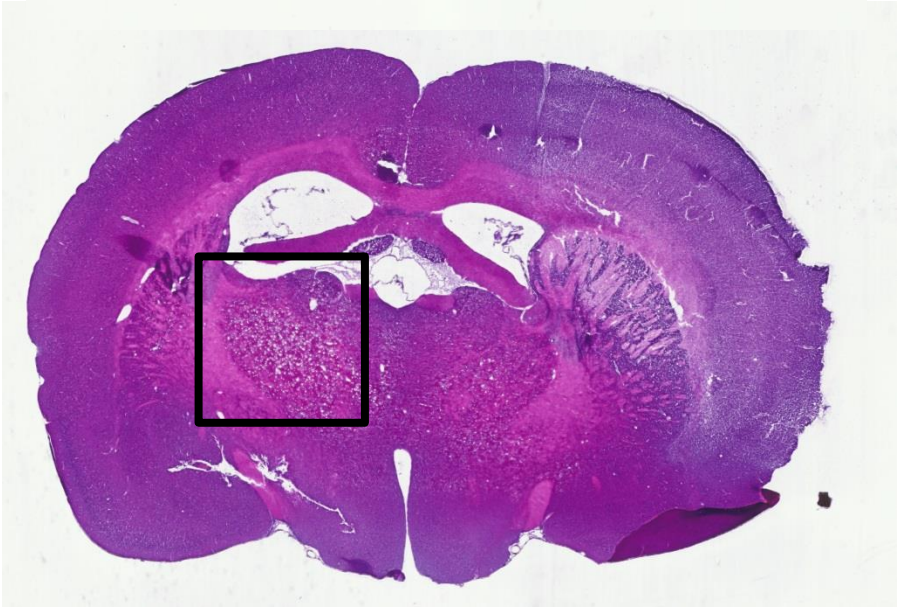
+ keine Schrumpfung

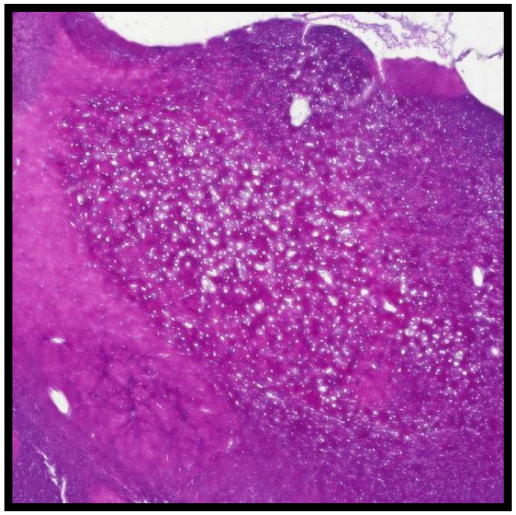
+ Gute Substanzerhalt

- Muss in Tiefkühler gelagert werden
- Begrenzte Haltbarkeit
- Fehlerquellen





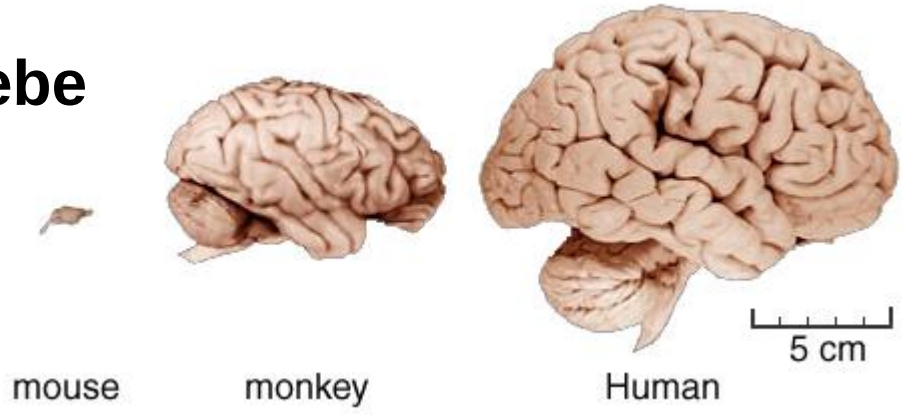




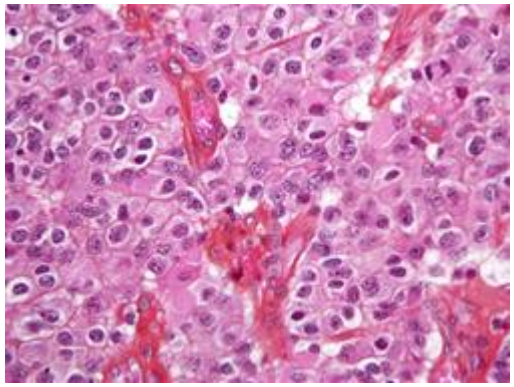
FAIL

Fraunhofer IZI

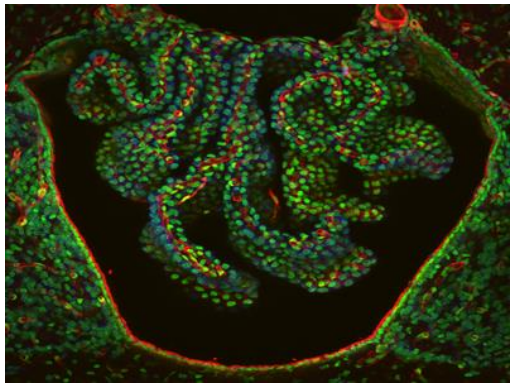
Gewebe



2P-Mikroskopie
200-800 µm

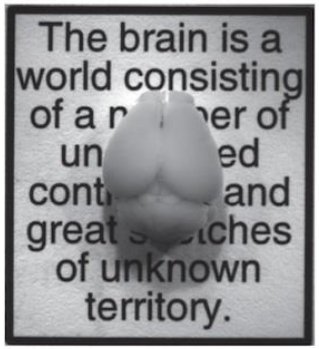


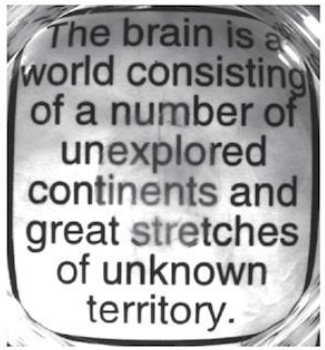
Morphologie
5 µm



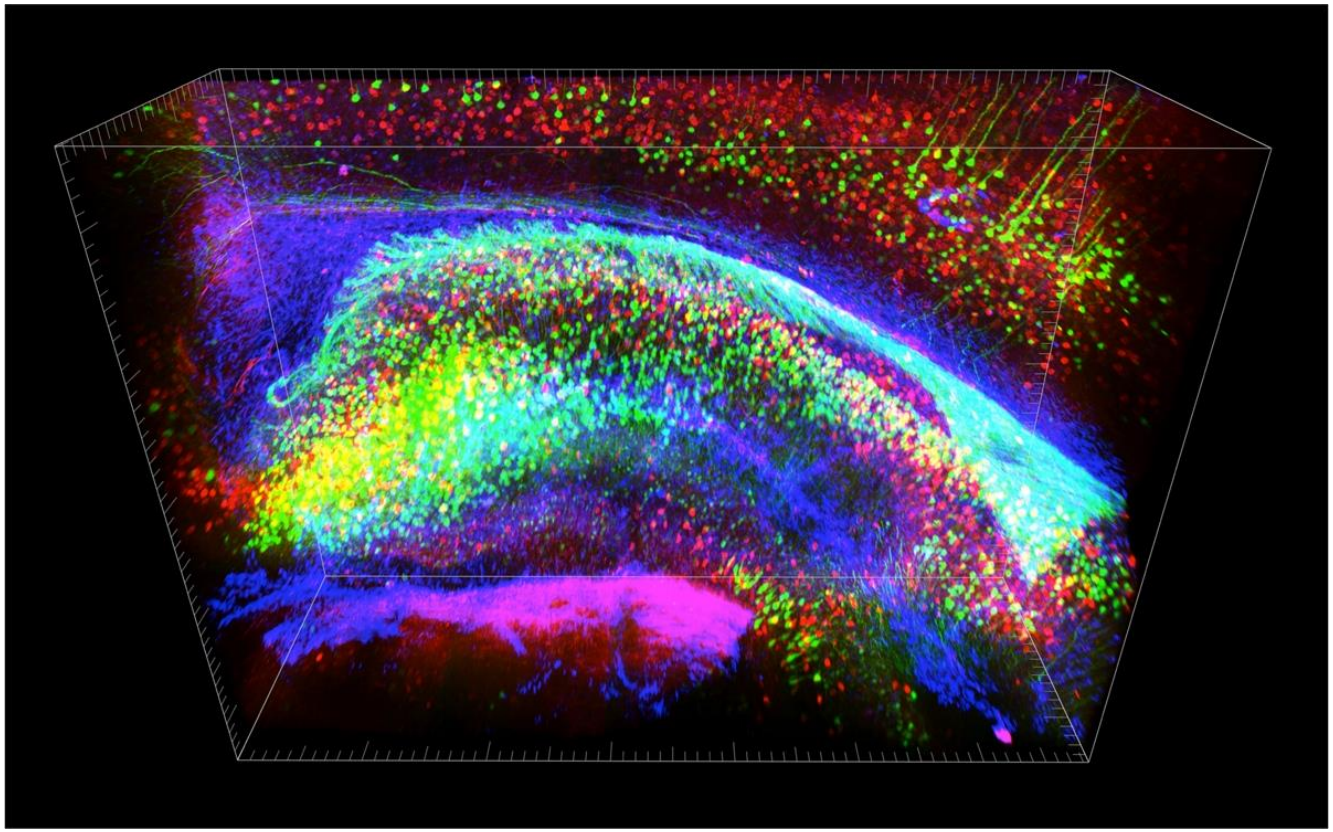
Konfokal-Mikroskopie
bis 50 µm

Fraunhofer IZI



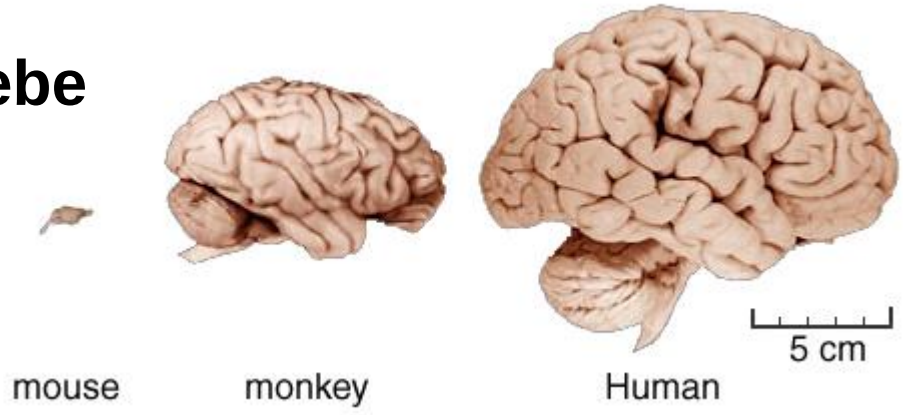


Karl Deisseroth

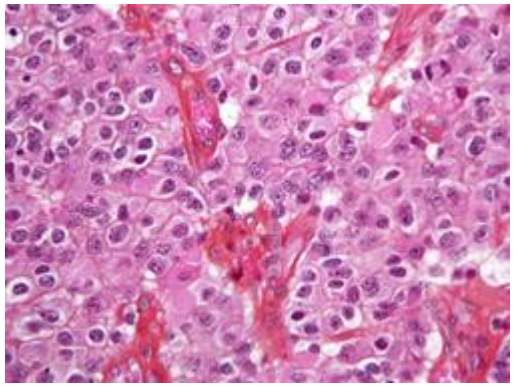


Fraunhofer IZI

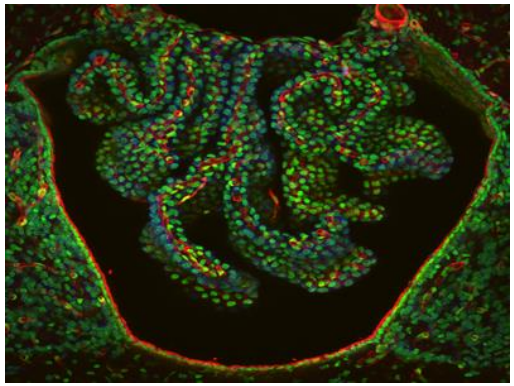
Gewebe



2P-Mikroskopie
200-800 µm



Morphologie
5 µm



Konfokal-Mikroskopie
bis 50 µm

Fraunhofer IZI

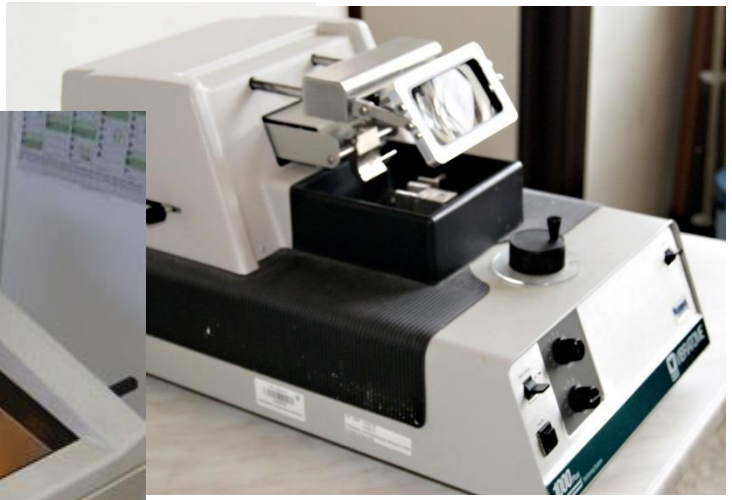
Gewebe schneiden

Freihand

Mikrotom

Vibratom

Kryostat



Fraunhofer IZI

Vibratom

+ Für Frischgewebe oder lebendes Gewebe

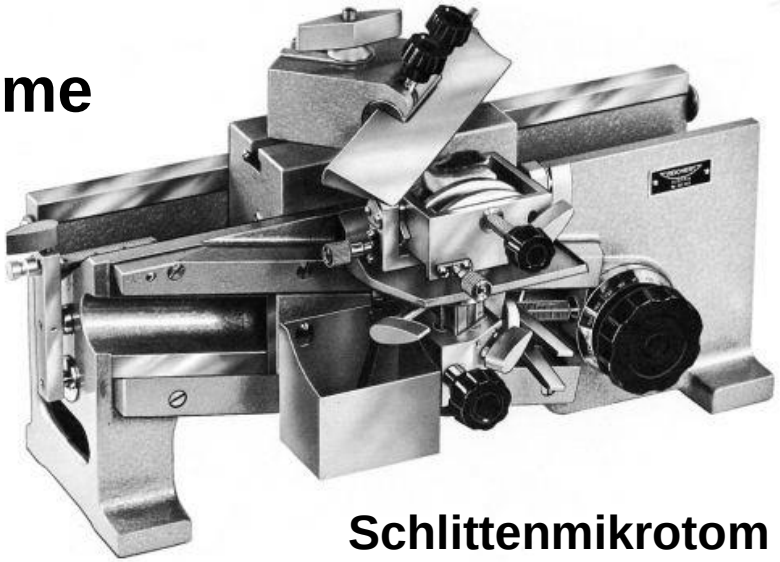
+ Keine Paraffin-Einbettung nötig

+ weniger Autofluoreszenz, weniger Artefakte

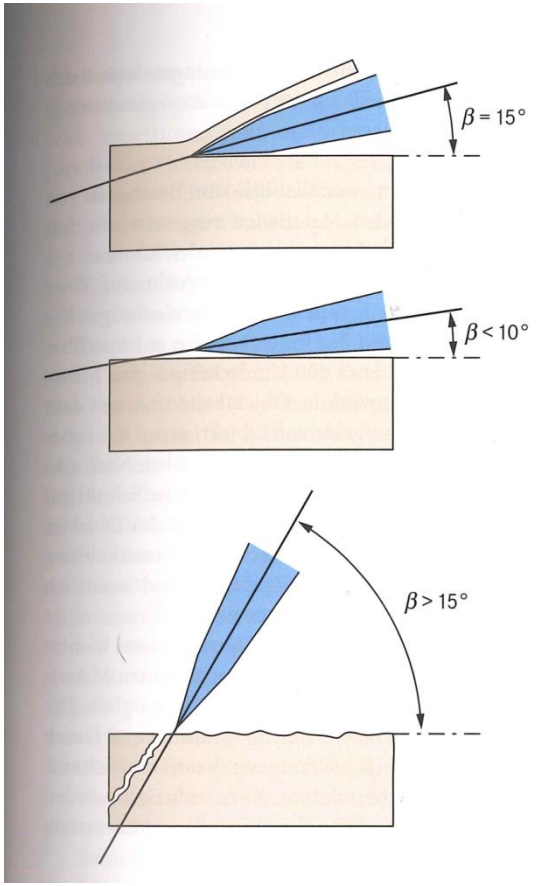
- schwierig

- Dickere Schnitte

Mikrotome



Schlittenmikrotom



Rotationsmikrotom

Mikrotome

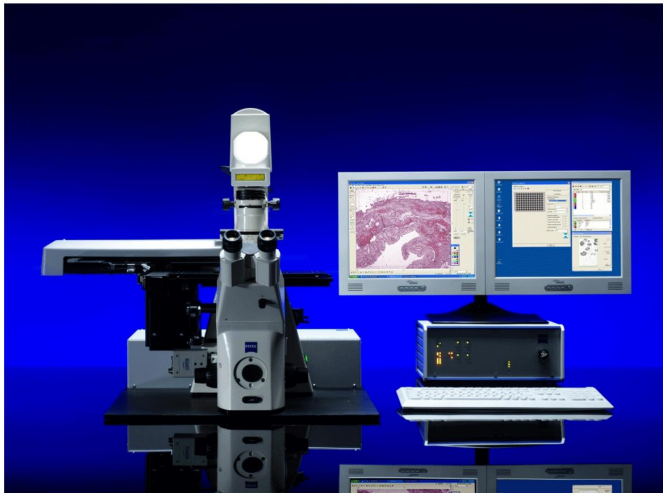
Kryostat – für gefrorene Gewebe

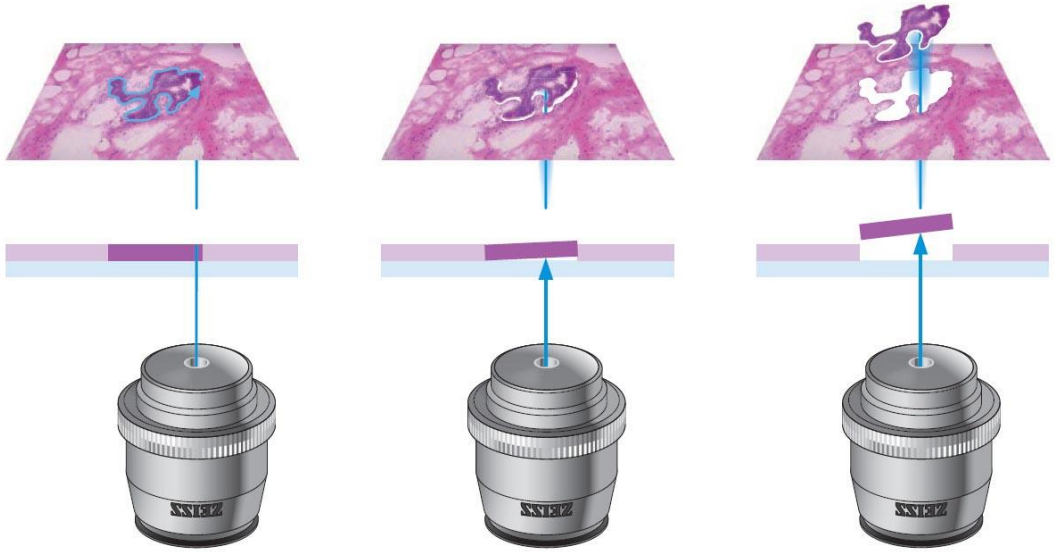
Kryostat – für gefrorene Gewebe

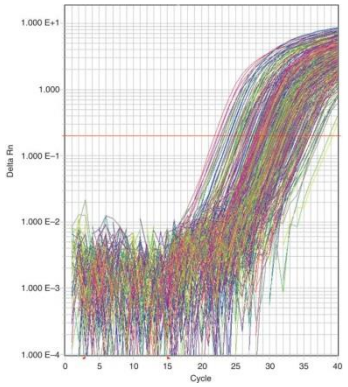
Cryostat Cutting

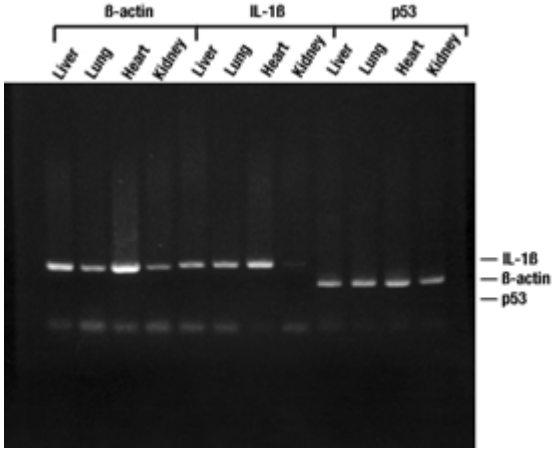
(to Keane, of course)

Laser capture microdissection



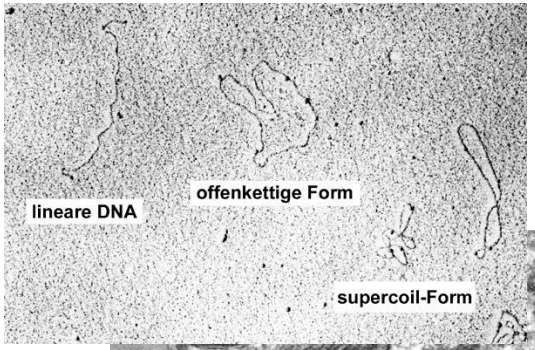




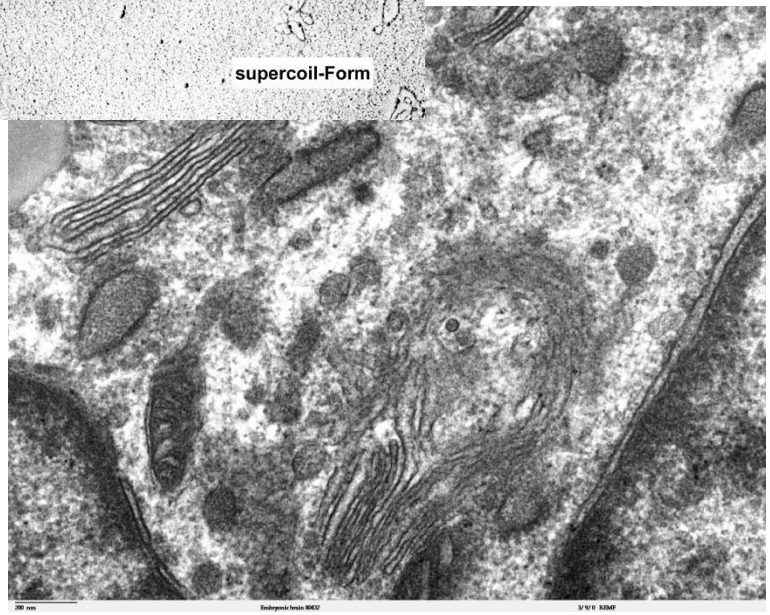


Fraunhofer IZI

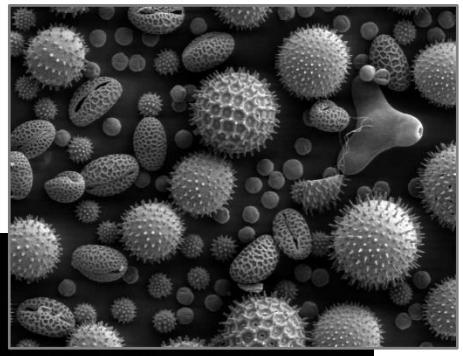
ElektronenMikroskopie



TEM



REM



Fraunhofer IZI

Besondere Anforderungen TEM:

Vakuumfest = Wasserfrei

Thermische Stabilität

Elektronendurchlässigkeit

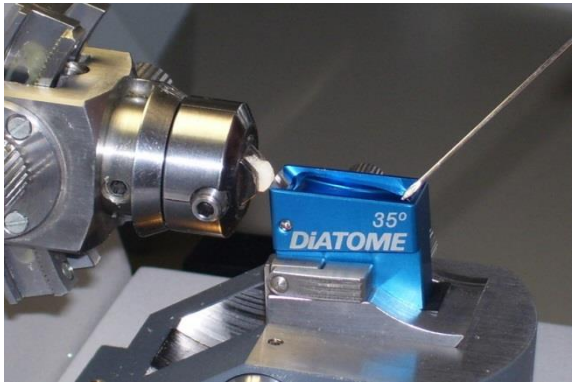
Kontraste

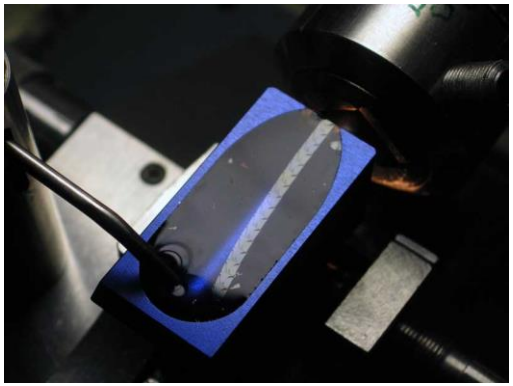
Entwässern

Einbetten

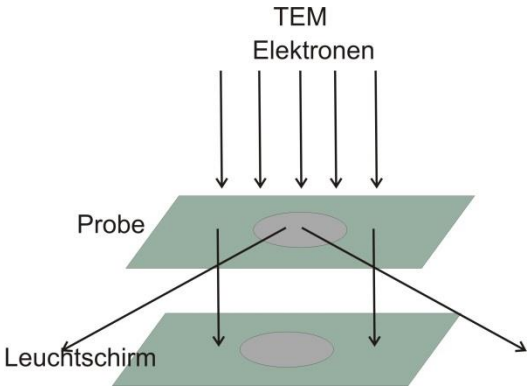
Schneiden

Kontrastieren

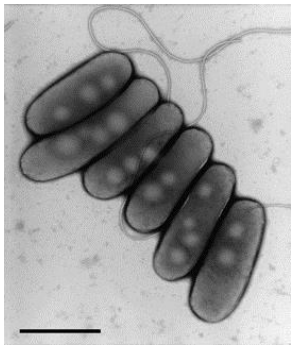


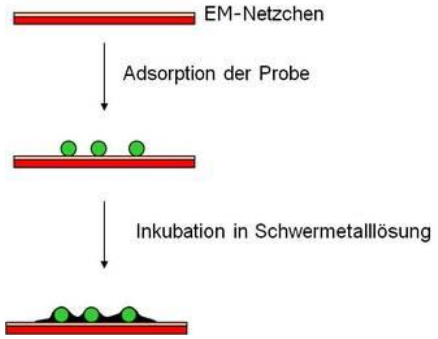


Kontrastierung

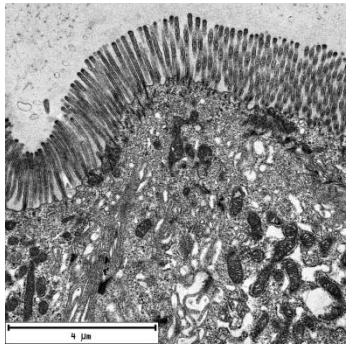


Negativ-Kontrastierung



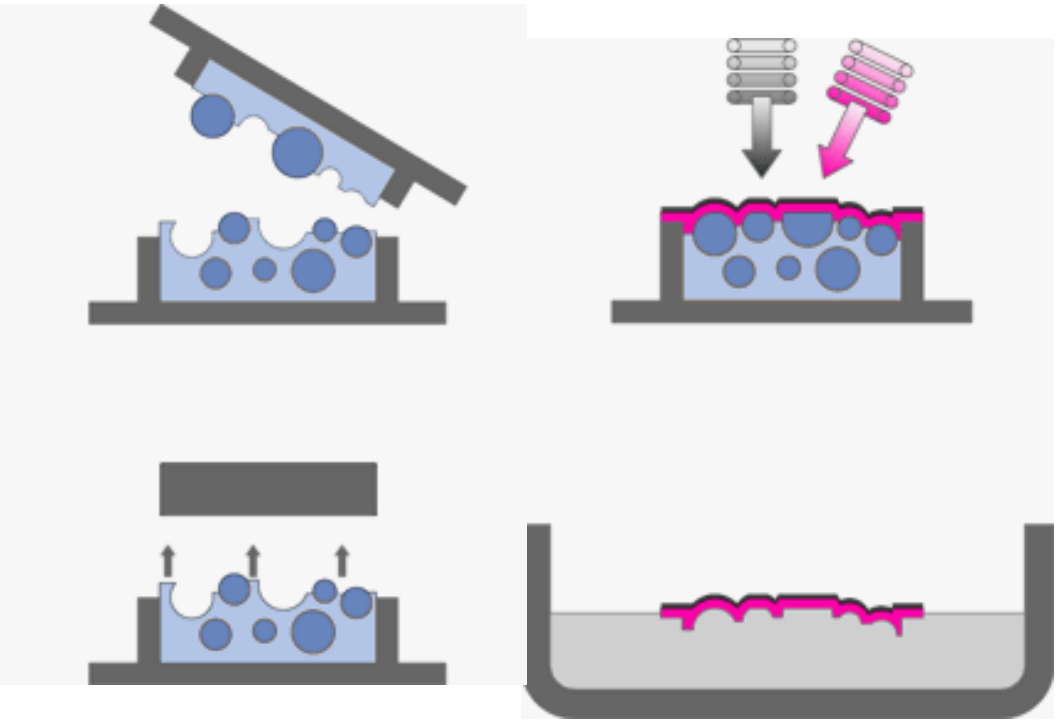


Positiv-Kontrastierung



Gefrierbruchreplika

Anordnung + Verteilung von Membranproteinen



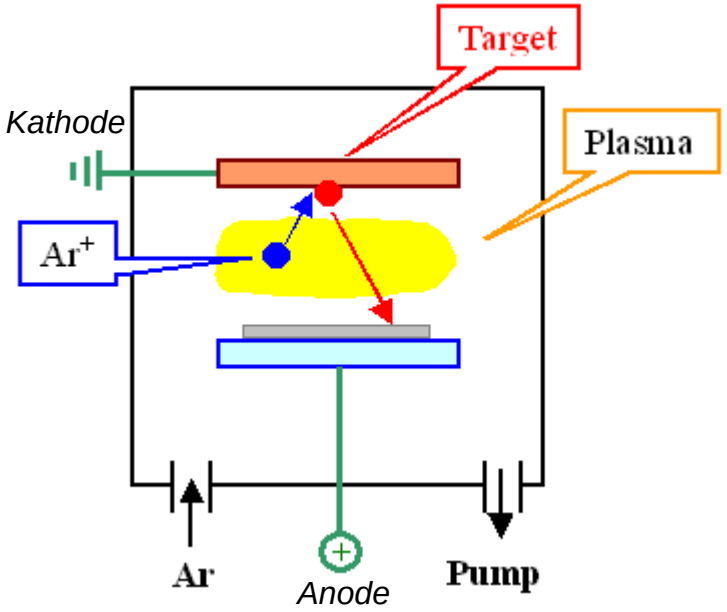
Besondere Anforderungen REM:

i) Gereinigte Oberfläche (Pressluft, Lösungsmittel)

ii) Vakuum-stabil

iii) Leitfähige Oberfläche (Sputtern)

- Stabilität gegen Elektronenbeschuss
- Reduziert Aufladung
- Verbessert Emission der Sekundärelektronen
- Verringert Eindringtiefe des Elektronenstrahls




PAUSE