

in vitro-Experimente unter Exposition mit
hochfrequenten elektromagnetischen Feldern
der Mobilfunkkommunikation
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Influence of RF-EMF on endothelial cells of the BBB?

BBB in vitro model (rat brain endothelial cells)

GSM & UMTS: 0.4, 1, 3 or 8 W/kg (72 h)

- differential gene expression (chip arrays)
- selection of BBB related candidates
- verification of protein expression (rt-PCR)
- identification / biochemical analysis of affected proteins

in vitro approach

- reduction of in vivo complexity
- precise determination of field parameters
- reproducible exposure conditions
- facilitated field and temperature monitoring

methods

- establishment and characterization of RBEC cultures as BBB in-vitro model
- design of exposure unit
- isolation of RNA from RBEC
- gene expression analysis
- rt-PCR analysis of regulated genes
- (protein analyses)



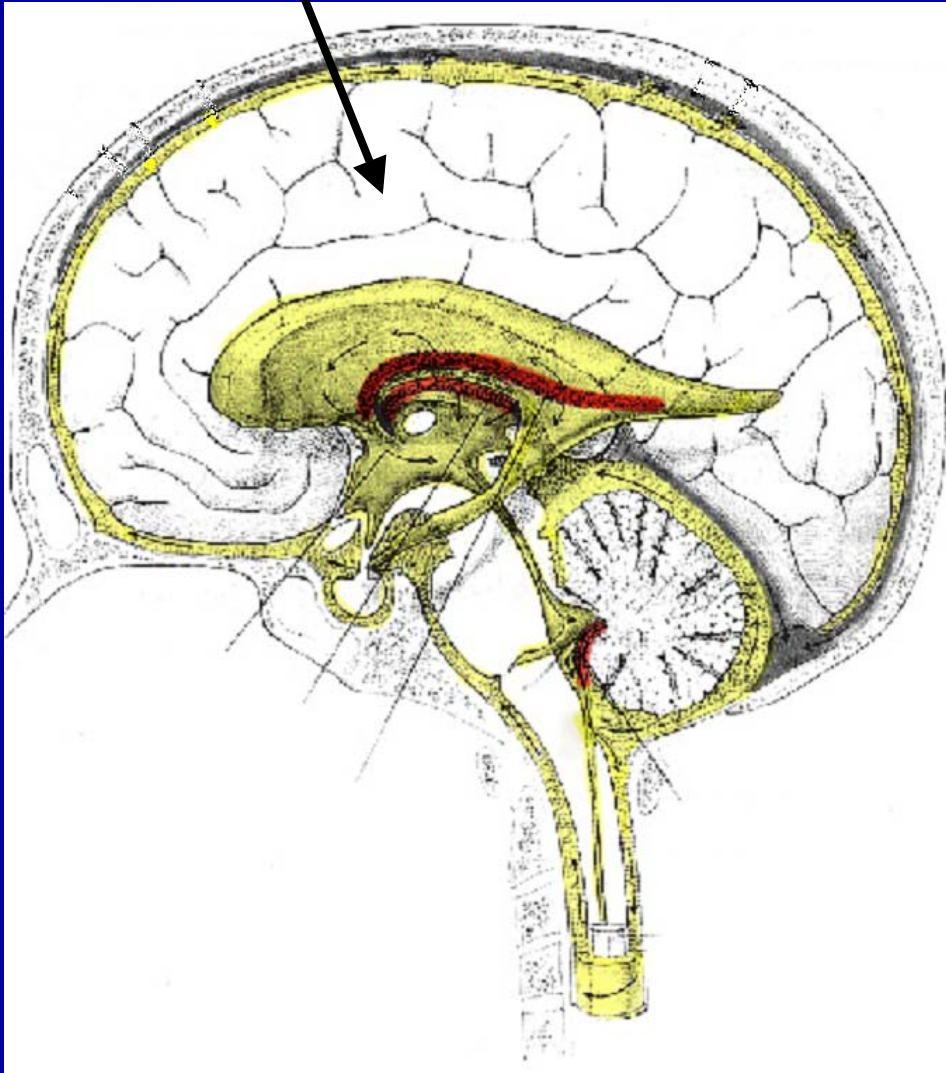
closed?



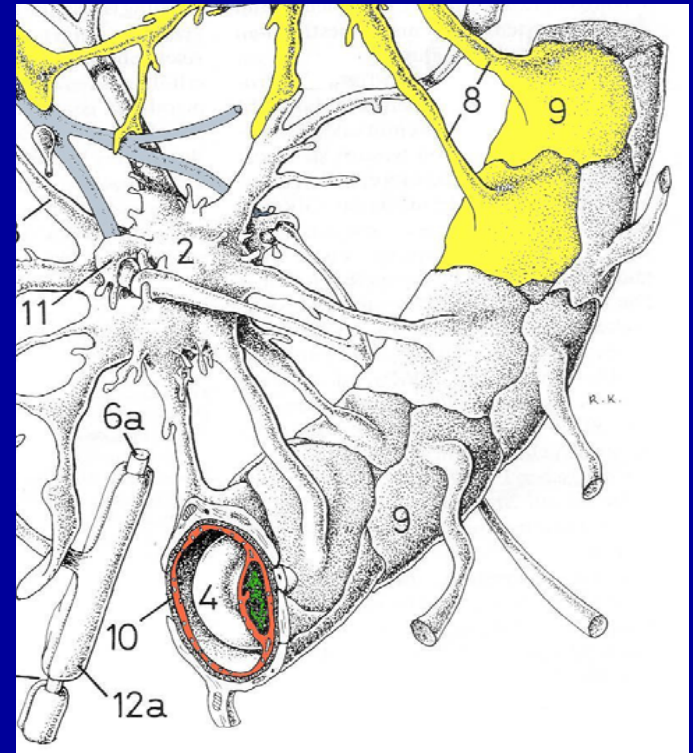
open?



The Blood-Brain Barrier (BBB)

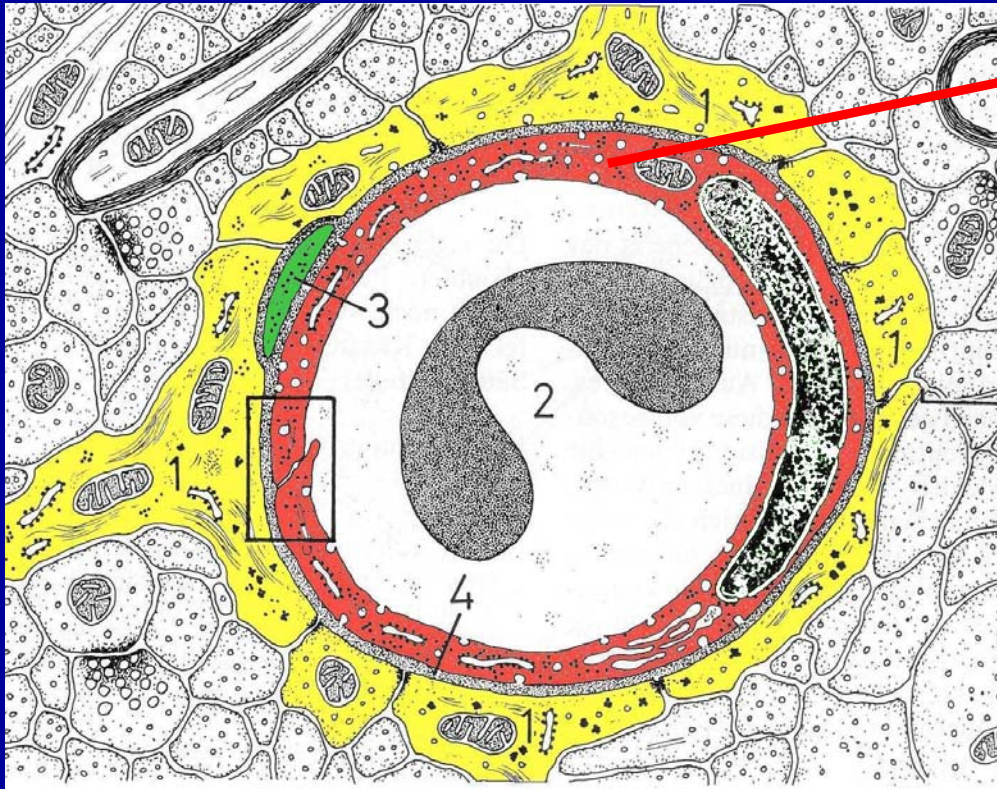


Endothelial cells of the cerebral capillaries form the permeability barrier



The BBB: Localization in vivo

Cross-section of a cerebral capillary



**Brain Capillary
Endothelial Cell
(BCEC) with**

 Tight
Junction (TJ)

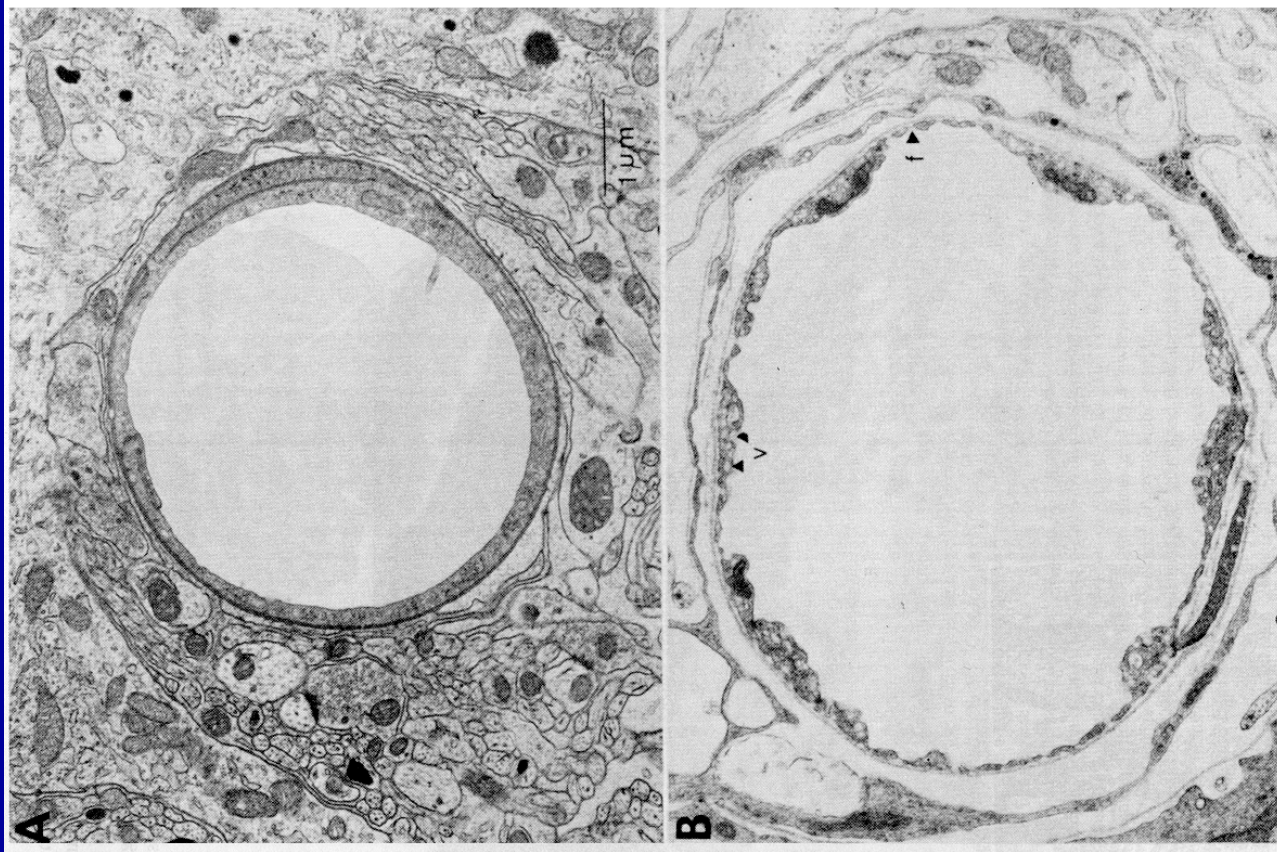
- 1 Astrocyte foot processes
- 2 Capillary lumen and erythrocyte
- 3 Pericyte
- 4 Basal lamina

(Krstić 1988)

Function of the BBB

- maintenance of a constant solute/ion environment: „homeostasis“ of the CNS
- essential for proper brain function
- control of substance flow between brain tissue and circulating blood
- controlled import of nutrients into the CNS
- protection against toxins

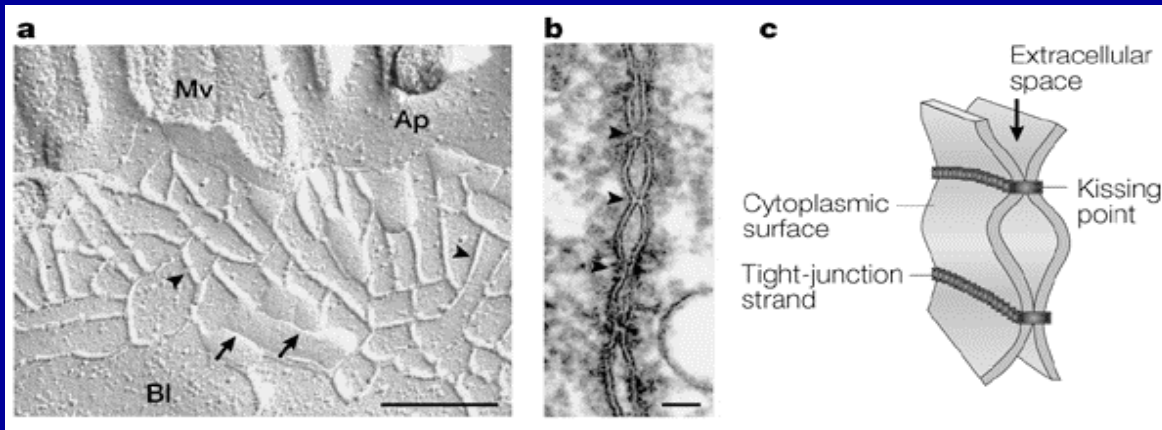
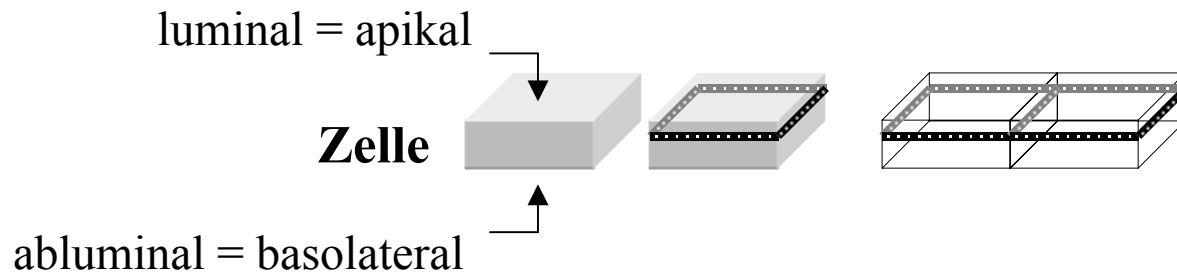
Special feature of the cerebral microvasculature: Tight Junctions



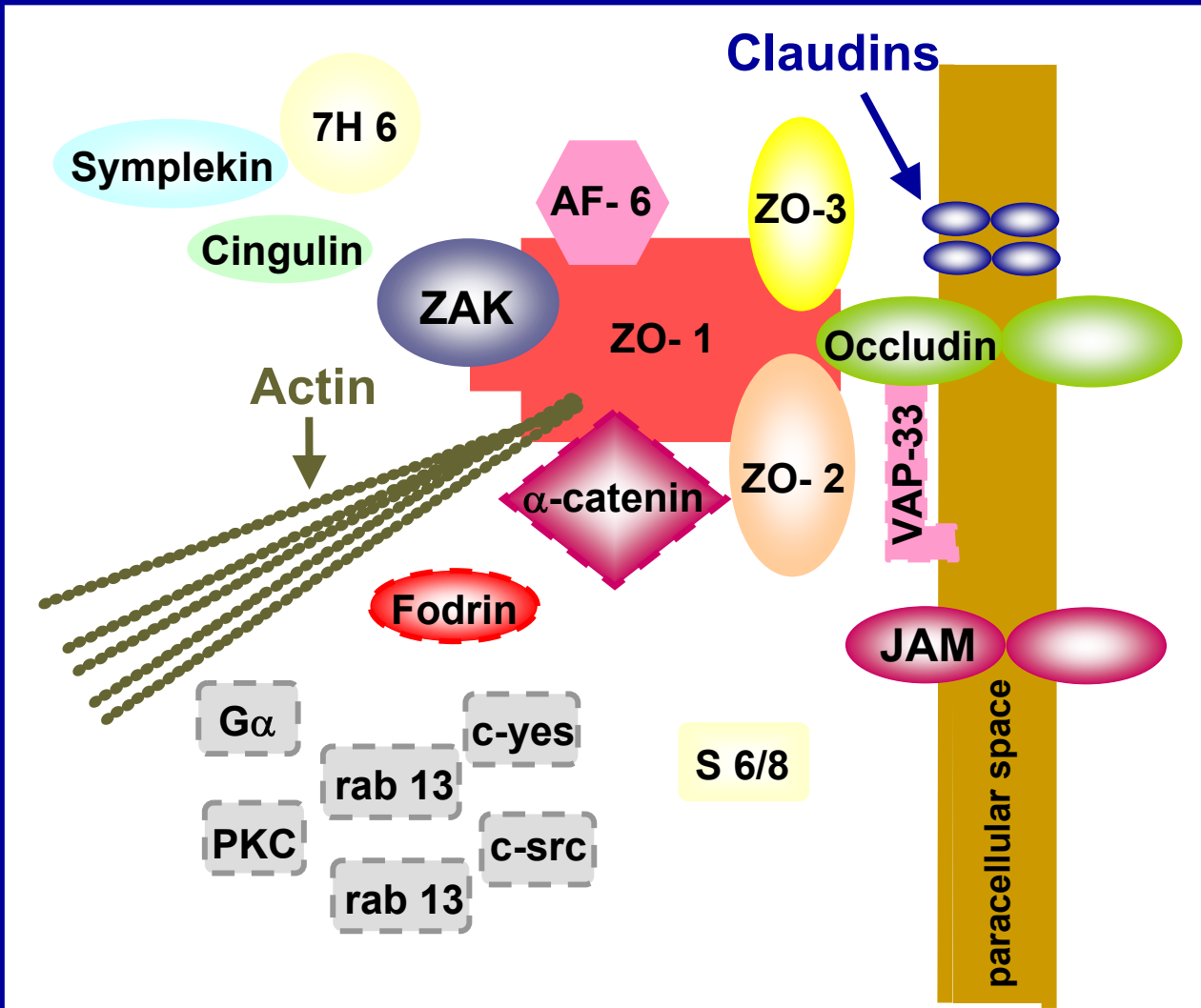
Tight endothelium

Leaky endothelium

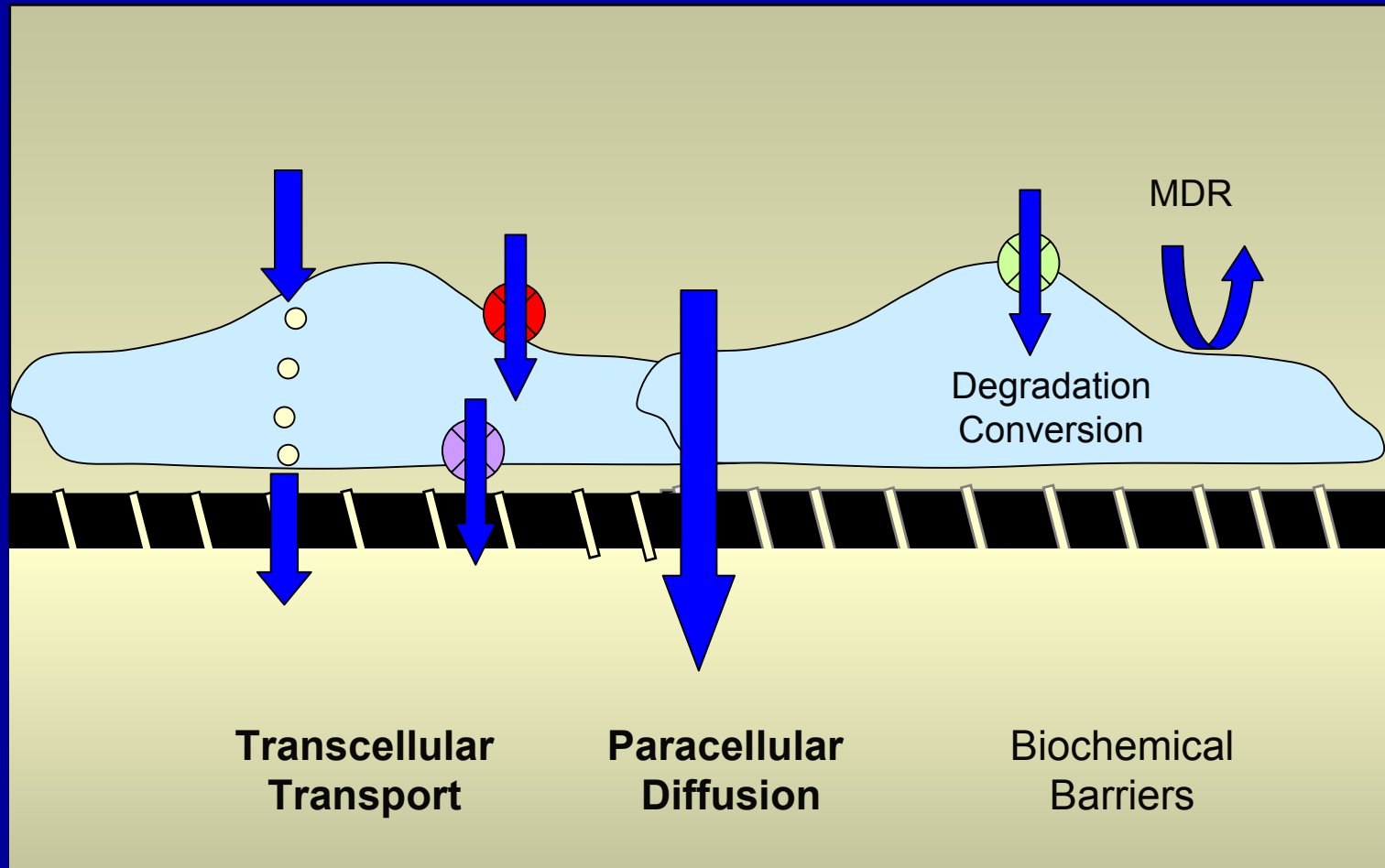
Tight junctions connect cells to build continuous monolayers



Proteins at tight junctions



Transport mechanisms at a cell monolayer



Rat brain capillary endothelial cells (RBEC)



removal of meninges

homogenizing

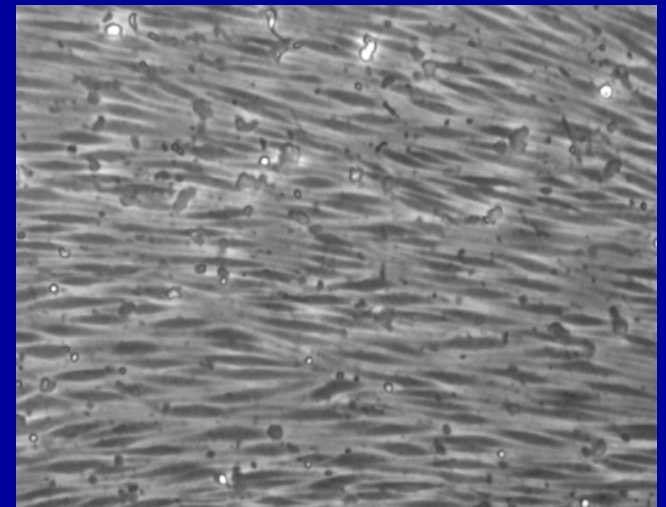
1st enzymatic digest

isolation of capillary vessels

2nd enzymatic digest

isolation of endothelial cells

sowing and culturing



RBEC monolayer

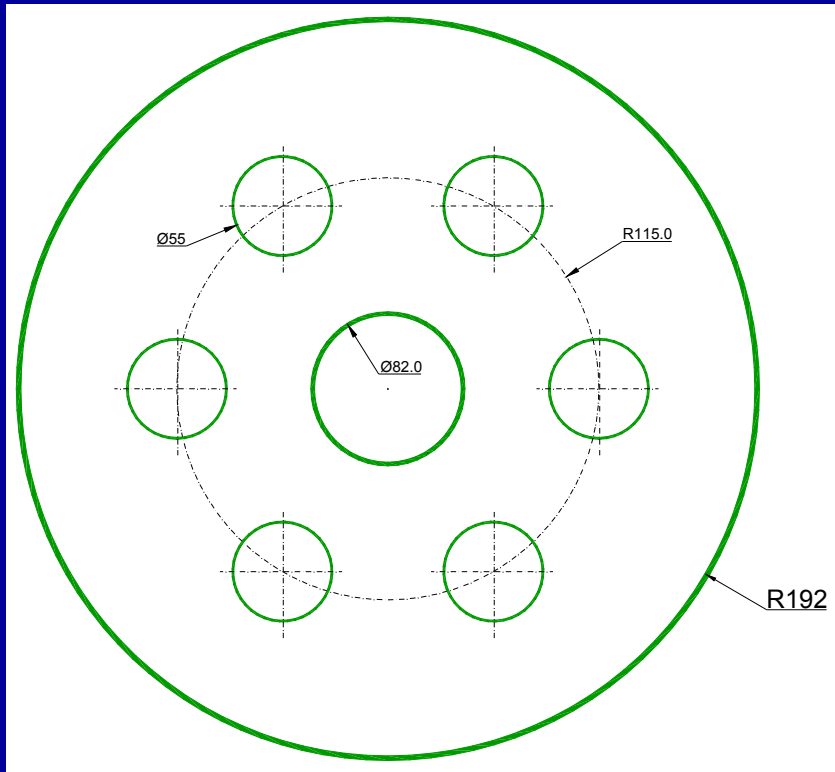
characterization of RBEC

- + squamous morphology
- + von-Willbrand-Factor-VIII
- + vimentin
- + tight junction proteins: ZO-1, occludin
- - smooth muscle actin, GFAP, CD11b
- ---> minimal cell contamination

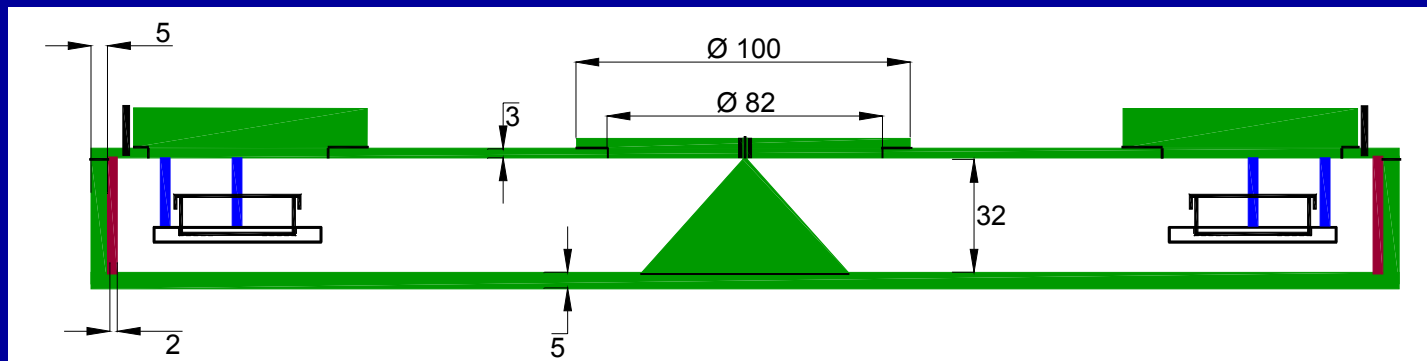
Exposure set-up

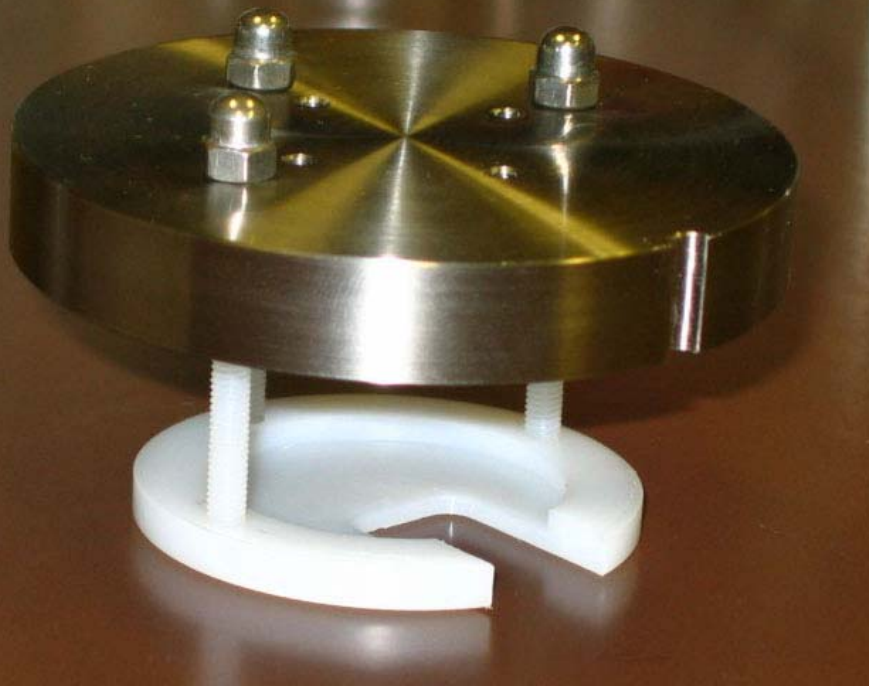
- two radial wave guides
 - 2 x 6 samples, thermistor probe, field antenna
 - parallel exposure & sham exposure
- generic UMTS signal
- generic GSM1800 signal
- permanent exposure
 - 3d duration
 - 4 different exposure levels (~0.4-8 W/kg)

assembly of radial waveguide



- 6 petridishes
- 40 cm diameter, 9 cm height
- sample holder centres petridishes
- temperature probe
- field probe





mounting of
petri-dishes into
the waveguide



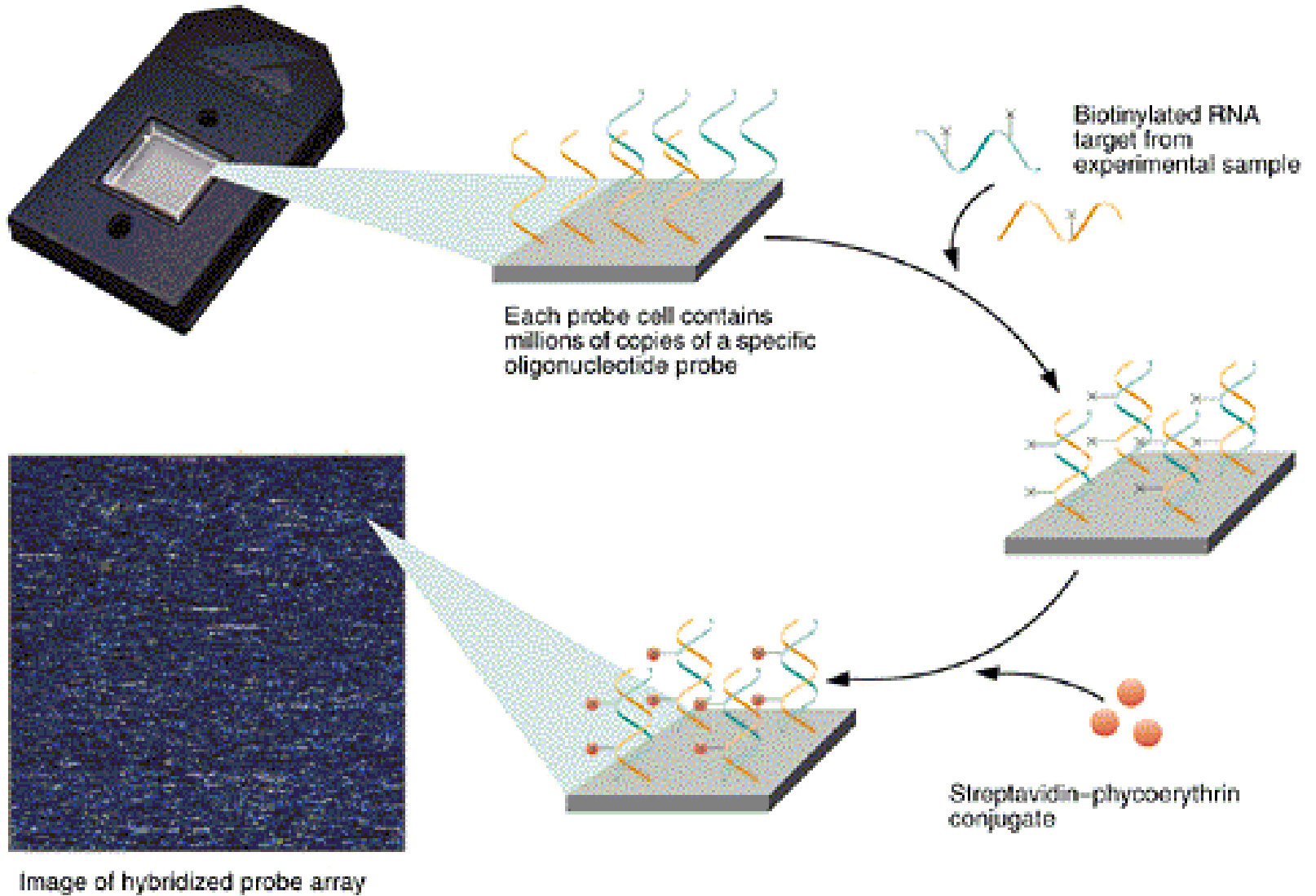
Exposure setup:



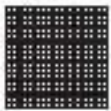
- amplifier
- signal generator
- wave guides
- samples
- incubator

chip microarrays



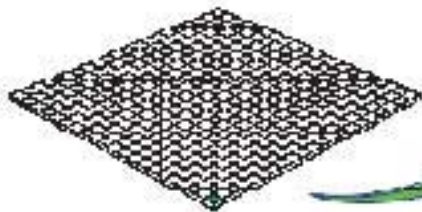
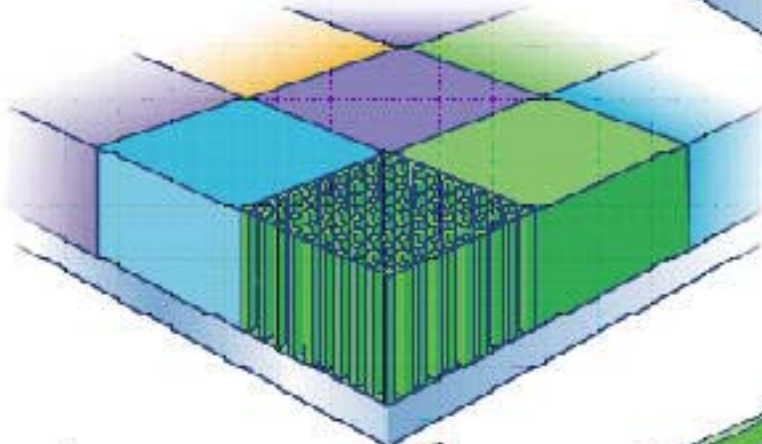


1.28 cm

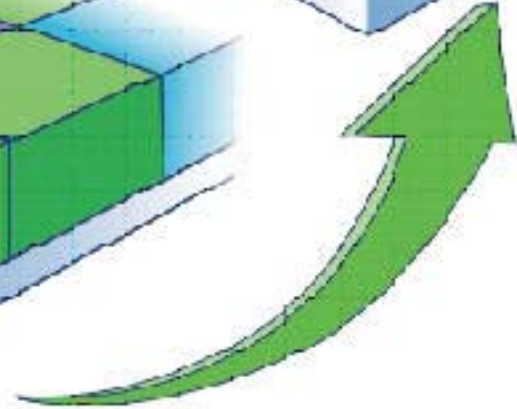


1.28 cm

Actual size of
GeneChip[®] array



500,000 locations on each GeneChip[®] array

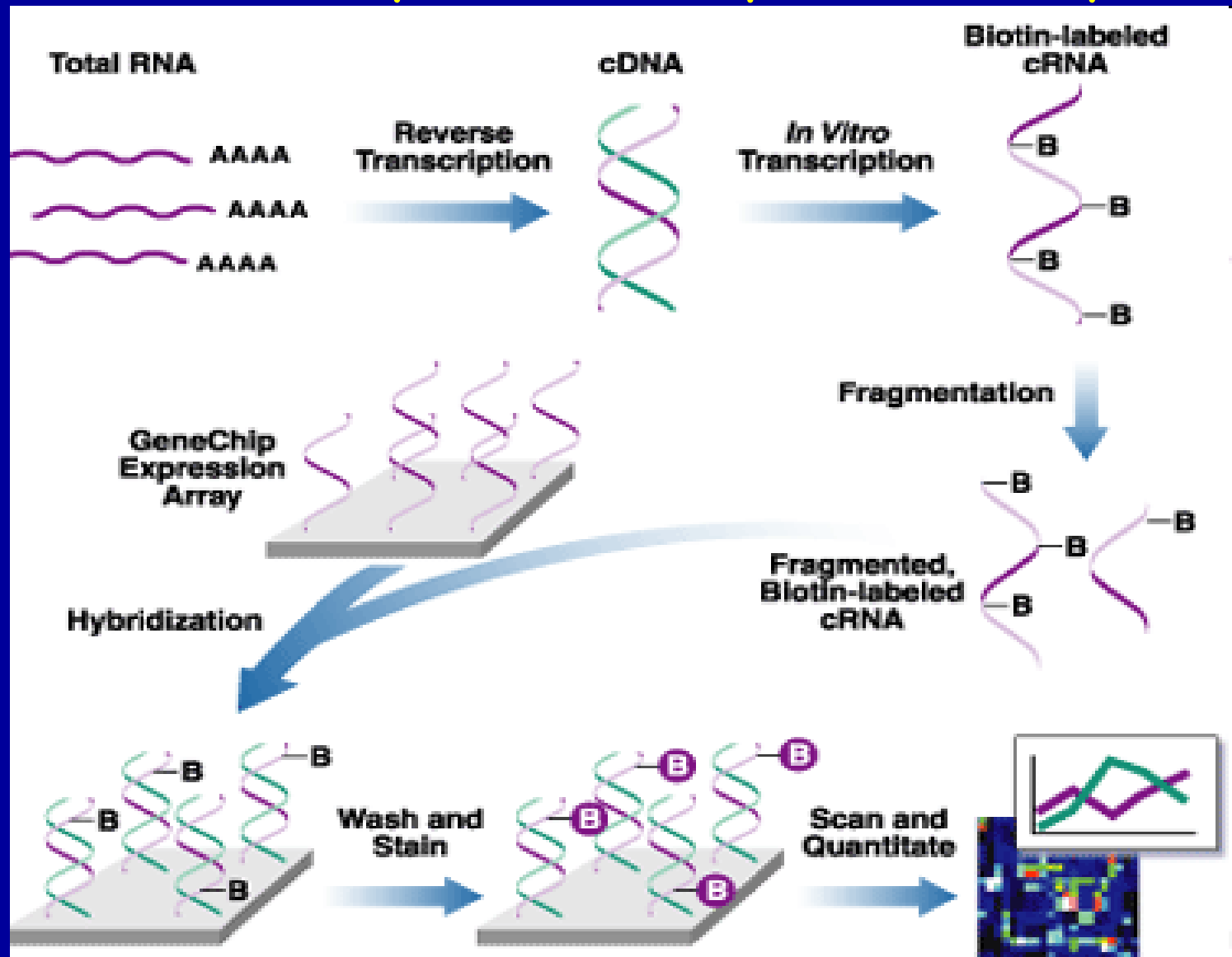


Millions of DNA strands built up in each location



Actual strand = 25 base pairs

RNA-analysis with chip-microarrays



protocol for chip-arrays

- RNA isolation from RBEC: *Qiagen RNeasy kit; store@ -70°C*
- quality control: *Agilent Bioanalyzer*
- *RNA-conc: min. 1µg/µL*
- *reverse transcription RNA -> cDNA*
- *in vitro transcription cDNA -> cRNA+biotinlabelling*
- *fragmentation of cRNA*
- *hybridization: Affym. GeneChip® Rat Genome 230 2.0 Array*
- *washing and staining*
- *array scanning*
- *evaluation: Genedata Expressionist 2.0 pro*

summary

- ✓ establishment of an isolation method for RBEC
 - ✓ characterization of RBEC
 - ✓ installation of exposure device and determination of field parameters
 - ✓ RNA isolation
-
- exposure of RBEC
 - chip-arrays for differential gene expression
 - identification of protein targets