Sub-cellular RF-field distribution & absorption depend on the dielectric properties of biological membranes

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4 topics:

- AC-electro-kinetic measurements of cell properties in the GHz-range (electro-rotation)
- Impedance characterization of blood- and brain-cell suspensions under EMF-exposition (membrane defects, ion leak)

- Search for special absorption mechanisms in cells (demodulation in biological membranes?)
- Exposition of neuronal networks on multi-electrode arrays of neuro-sensor chips to CW-, GSM-, and UMTS-fields (synchronisation effects)

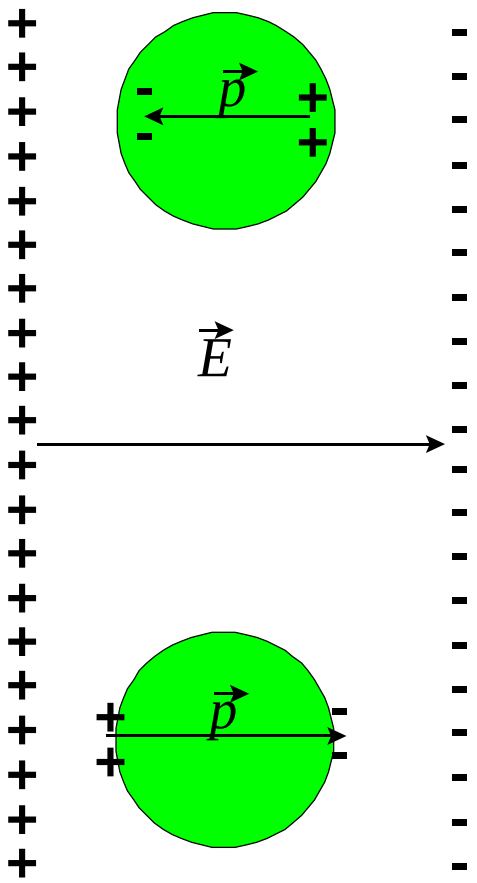
Philosophy for modelling cell & membrane properties

- Translate literature data on molecular properties into electric properties of subcellular compartments
- Put up geometrical model for cells/vesicles
- Calculate induced dipole moment, i.e. the Clausius-Mossotti factor (CMF)
- Check CMF by single particle AC-electrokinetic measurements (Alter suspension conditions to resolve certain dispersions)
- Consider **field strength and absorption** at exceptional model sites

Polarization of cells & particles

Object is

higher



or lower

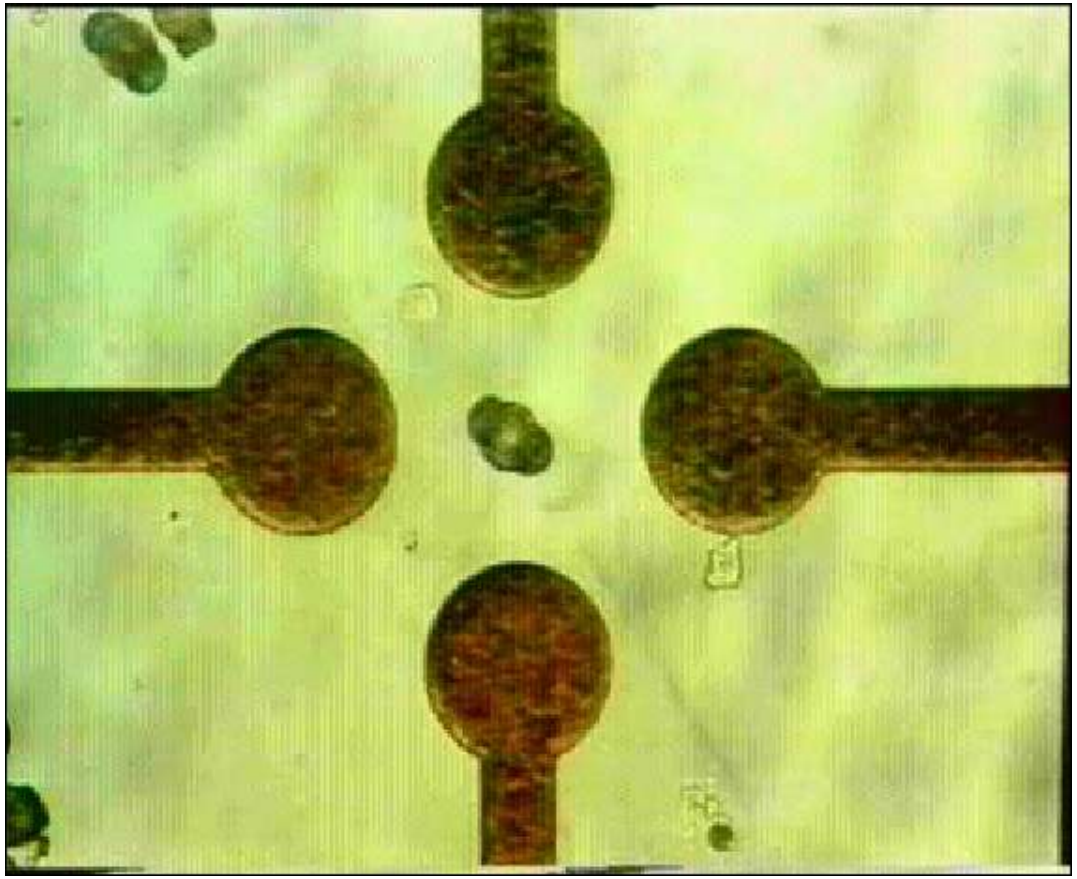
polarizable than surrounding medium.

Electrorotational spectrum of a pine pollen

10 kHz – 20 MHz

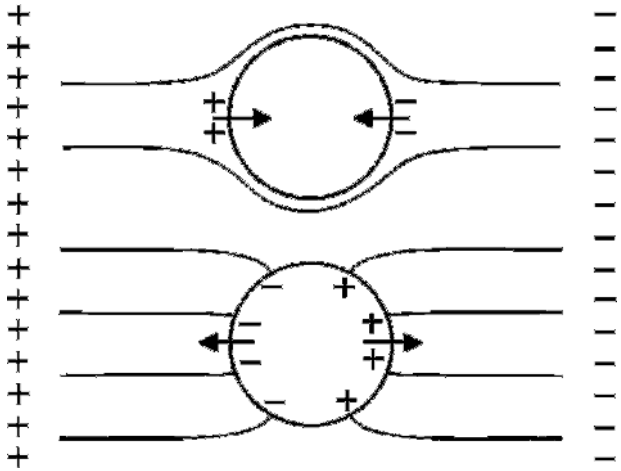
Gold on glass-chip

Tip to tip distance: 200µm



Impedance and AC-electrokinetics

Cell polarizability **Frequency**



lower

higher

than suspension medium

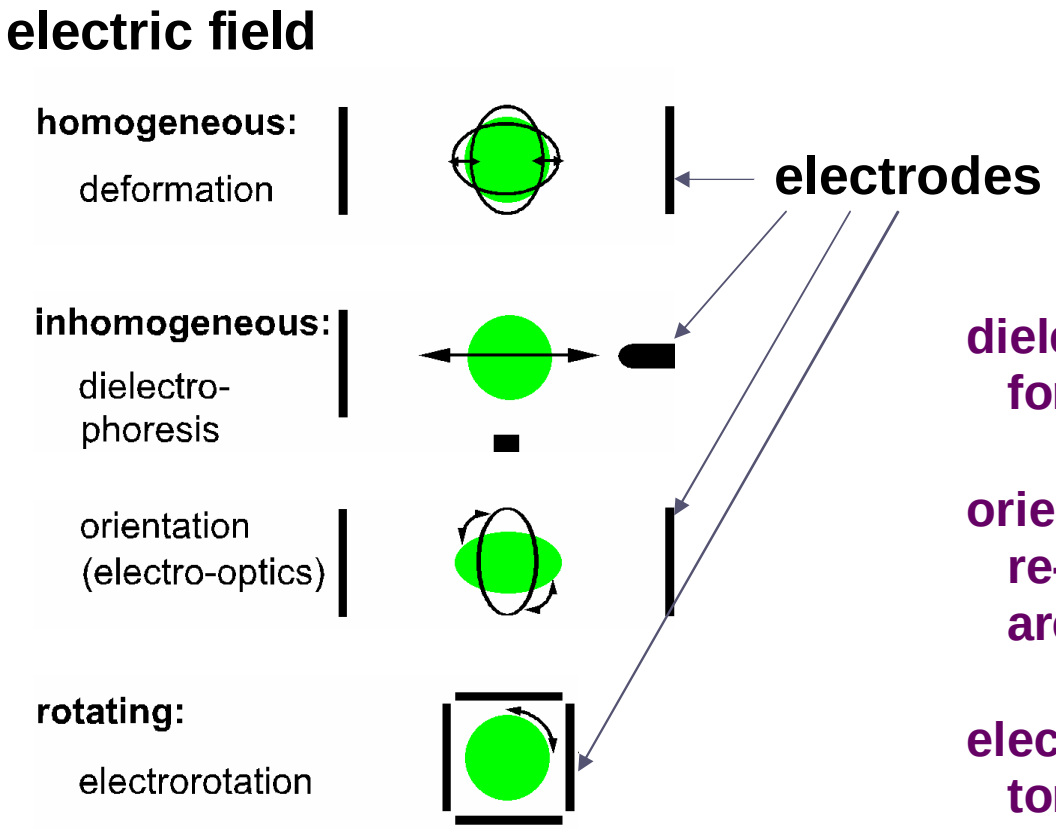
low
(or very high)

medium
(1-10 MHz)

Cell polarizability determines Currents and Forces

AC-field induced force effects suitable for cell and particle characterization

AC-ELECTROKINETIC PHENOMENA depend on the induced dipole moment μ



dielectrophoresis:
force ~ $\Re(\mu)$

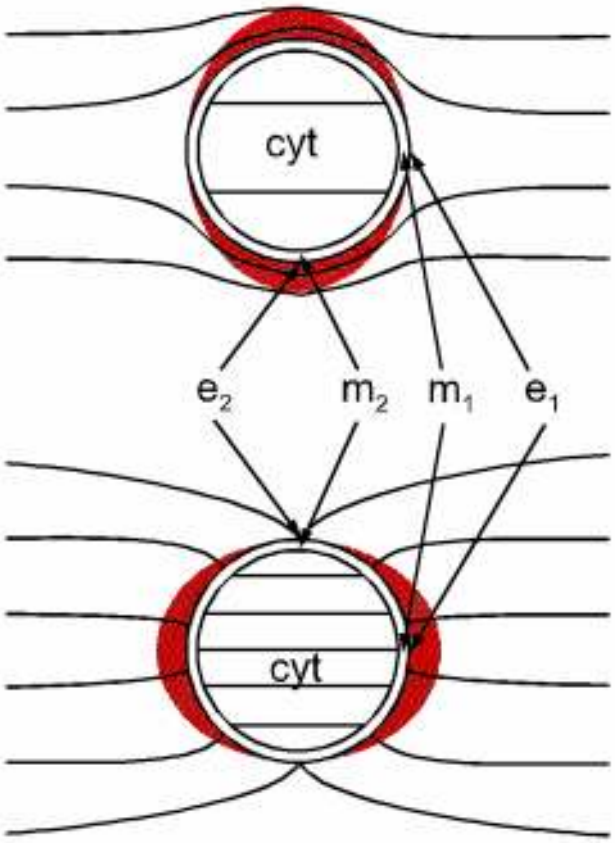
orientation:
re-orientation frequencies are function of $\Delta\Re(\mu)$

electrorotation:
torque ~ $\Im(\mu)$

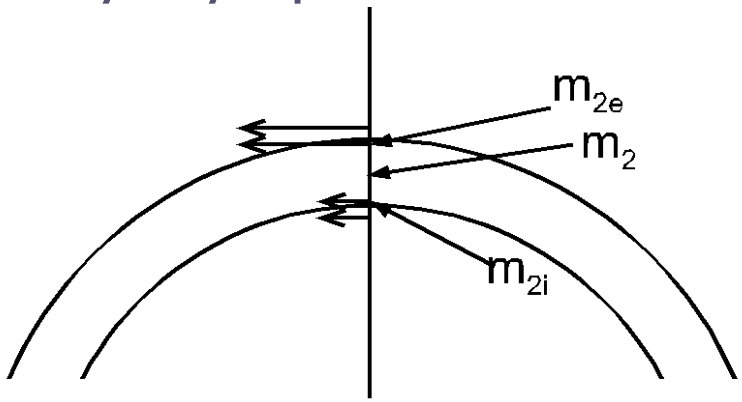
Theory describes relation of measurements & model

- AC-electrokinetic forces are related to induced dipole moment
- The dipole moment is described by the subcellular, frequency dependent field distribution (is given by the integral over the local field contributions in the cell volume)

Definition of exceptional sites in a single shell model



- e - external medium
- m - membrane
- cyt-cytoplasm



see: D. Wachner, M. Simeonova, J. Gimsa. 2002. Estimating the subcellular absorption of electric field energy: Equations for an ellipsoidal single shell model. Bioelectrochemistry. 56:211-213

1st problem: cytoplasm

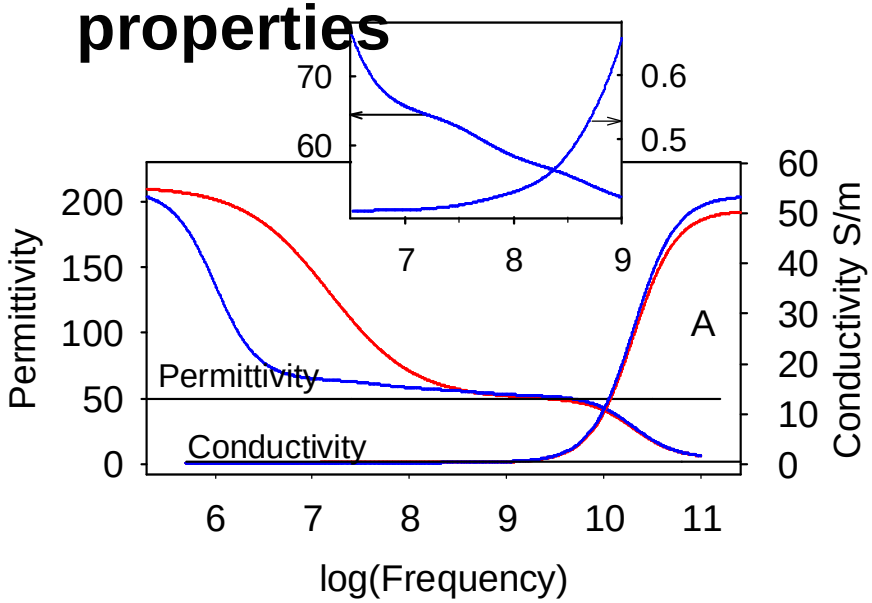
- Model: human red cells
  - homogeneous
  - abundance of hemoglobin
  - well investigated

Dielectric Properties of the Cytoplasm

Molecular components of the cytoplasm	f_j /MHz	Δε for different volume contributions (%) of a compartment		Extrapolated data for physiological concentrations: ε, and σ/ Sm^{-1}				Single cell dielectric spectroscopy: f_j/MHz, ε, and σ/ Sm^{-1}				
				Δε	Δσ	$\varepsilon_k(\omega)$	$\sigma_k(\omega)$	f_j	Δε	Δσ	$\varepsilon_k(\omega)$	$\sigma_k(\omega)$
Hb	1	15%	22%	35%		203.8	0.4				212	0.4
		63	89	145	0.01							
Hb side chains	50	22%Hb	26.6%Hb	35%Hb		58.8	0.41	15 α=0.5	162	0.14		
		4.5	5.4	7	0.02							
Hydration shell (0.25g/gHb)	500	100%		8.75%		51.8	0.43					
		55		4.8	0.27							
						47	0.7				50	0.54
Bulk water	20,000	85%		65%								
		55		42	52.7	5	53.4	20,000	45	49.9	5	50.4

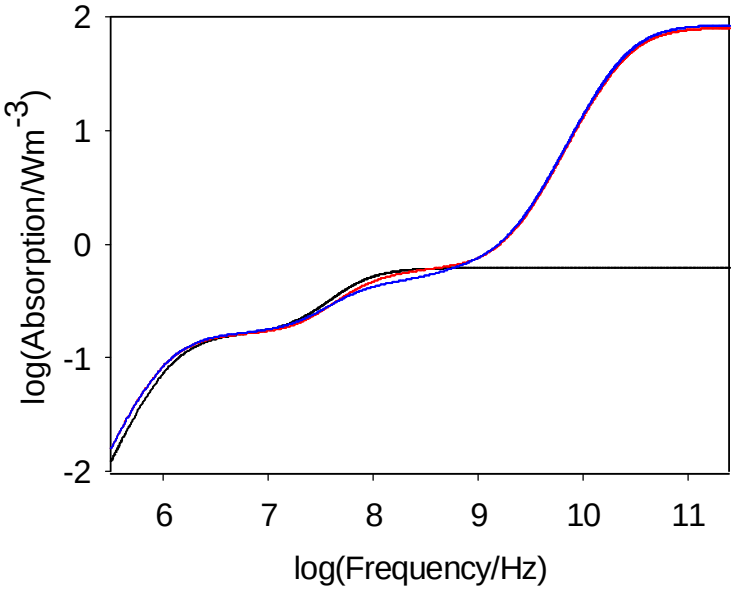
Cytoplasmic field absorption

Cytoplasmic properties



Resulting absorption

normalized to external field of 1V/m



Frequency independent properties

from data of Schwan et al. (impedance of Hb-Susp.)

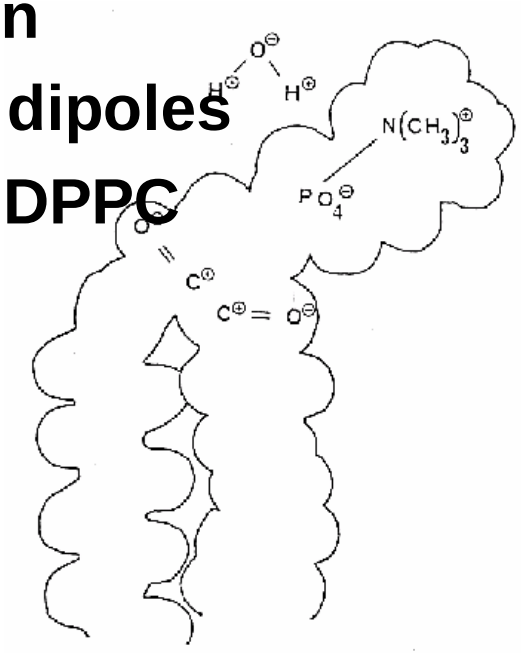
Gimsa et al. (single cell AC-electrokinetics)

2nd problem: lipid membrane

- Model: vesicles
  - homogeneous core
  - SUVs abundance of lipids
  - readily available

Lipid structure

Orientation of dipoles in DPPC



Gawrisch et al., Biophys.J., 1992, 61:1213-1223

Neutron & x-ray diffraction

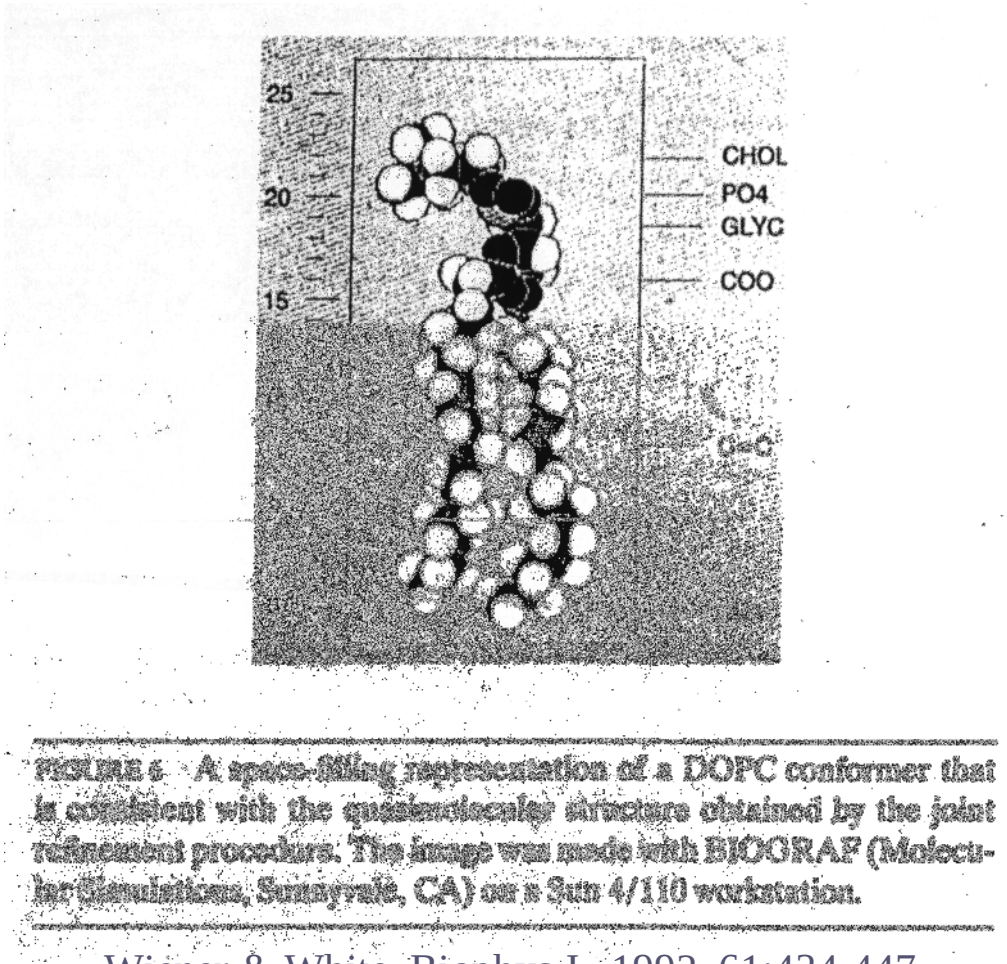
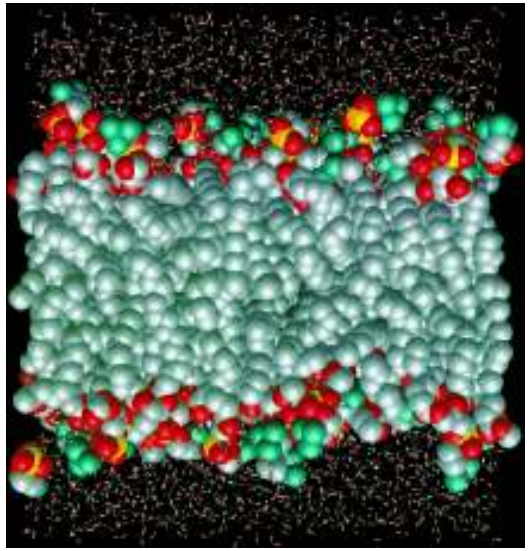


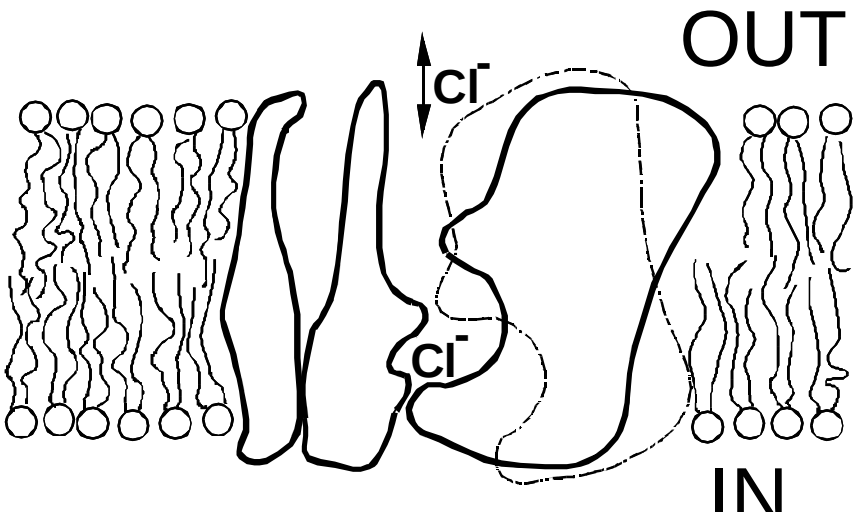
FIGURE 6 A space-filling representation of a DOPC conformer that is consistent with the quasimolecular structure obtained by the joint refinement procedure. The image was made with BIOGRAF (Molecular Simulations, Sunnyvale, CA) on a Sun 4/110 workstation.

Wiener & White, Biophys.J., 1992, 61:434-447

Biological membranes

border, confinement, controlled exchange
=> special electric properties





Molecular dynamics simulation (CPK-model)

128 Lipid- (DPPC) & 3910 Water molecules

Water: stick models, H: white, O: red, P: yellow,

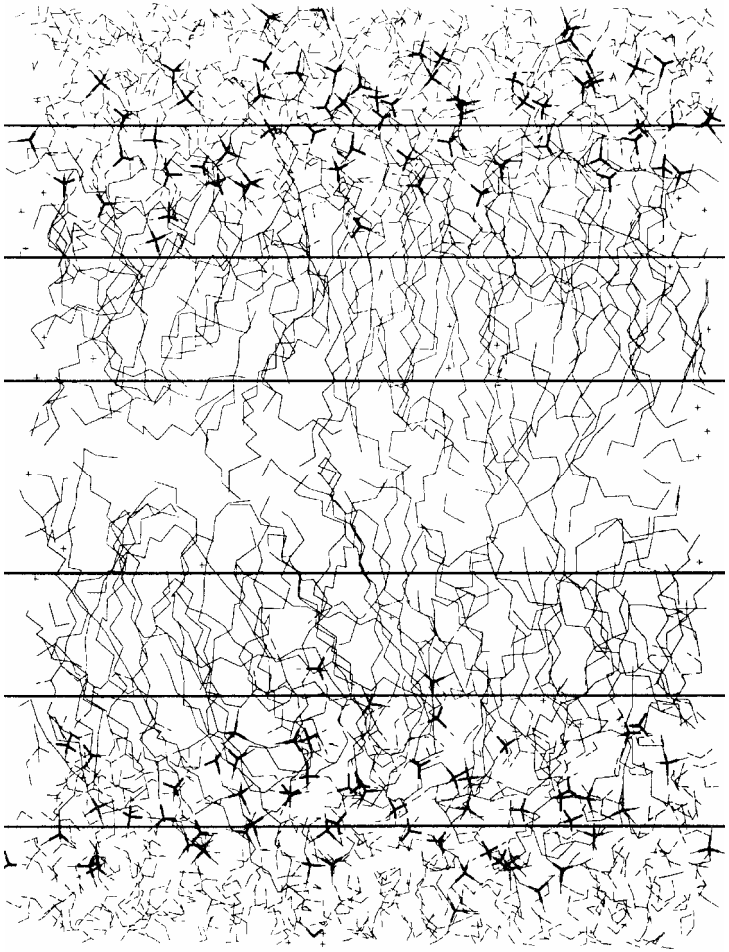
C: grey N: dark blue, Methyl groups of choline (3 per N): green (Tielemann et al. BBA 1997, 1331:235-270)

Band 3

Anion exchanger protein

(Gimsa und Ried 1995, Mol. Membr. Biol. 12:247-254)

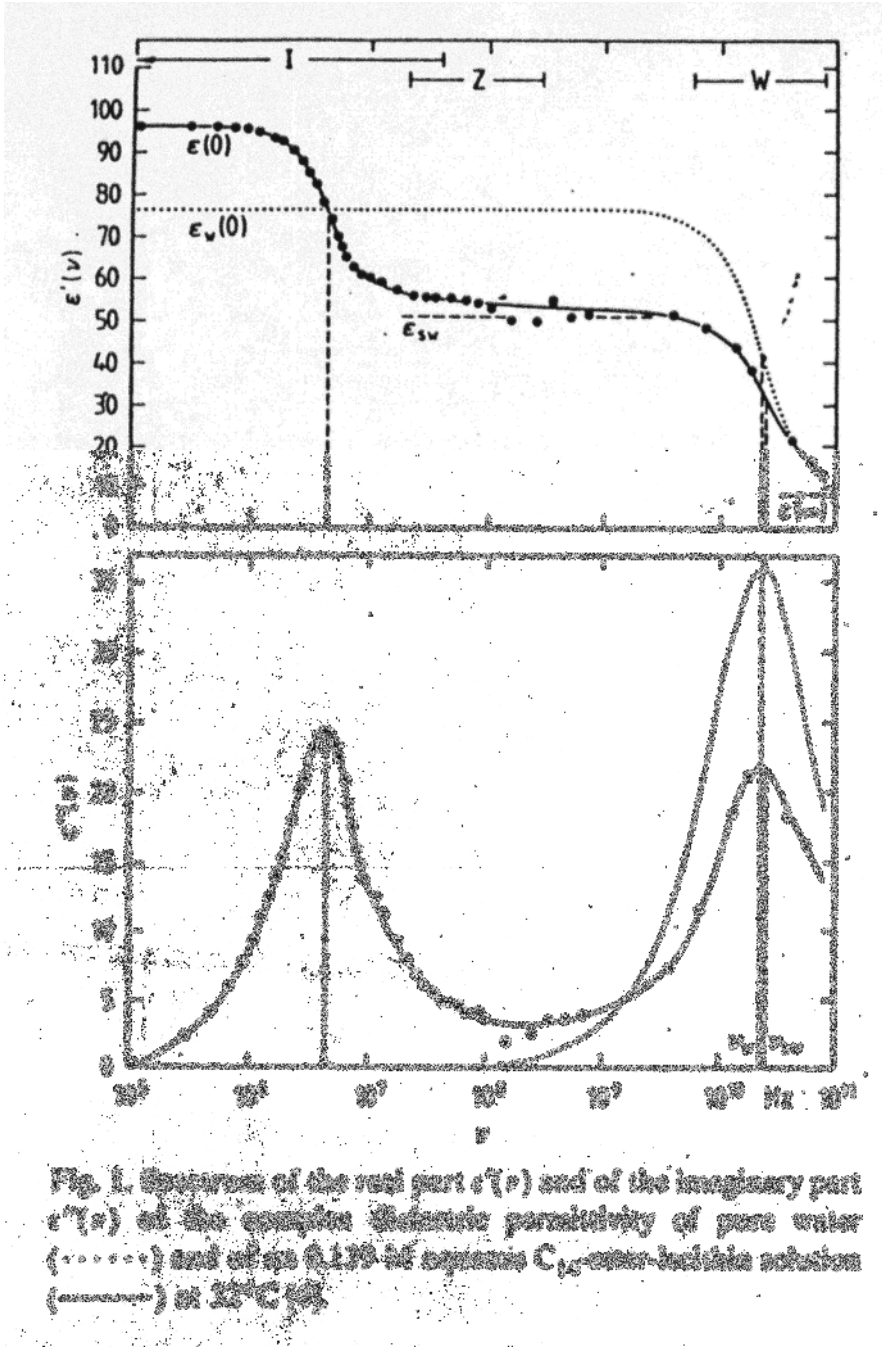
Membrane lipid organization



Tieleman et al., BBA 1997, 1331:235-270

Dispersion of water & aqueous lecithin solution

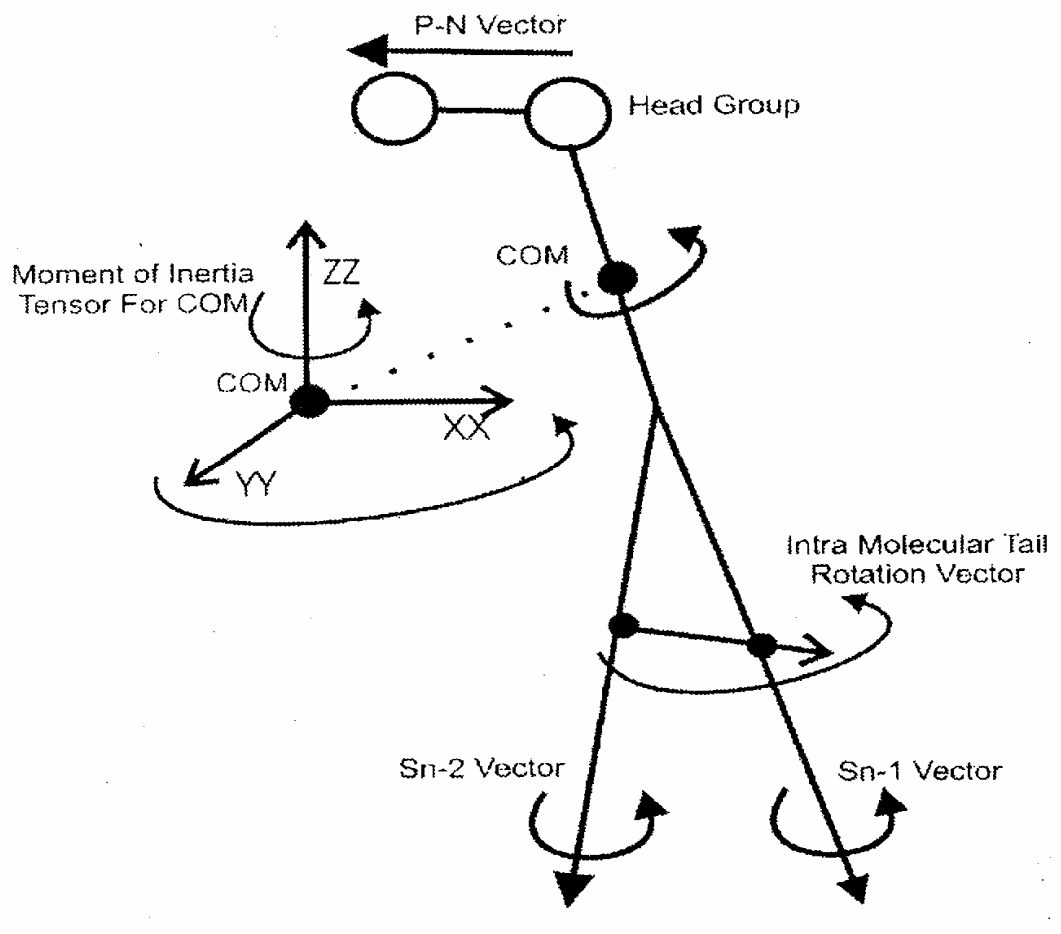
Pottel et al., Biopys. Biochem. 1984, 19:233-244



Question:

- Do mechanisms other than the classical structural dispersion or Debye dispersions exist in the system?

Rotational freedom of a membrane lipid molecule



Diffusion constants of lipids

Molecules:

- rotation $D_{\parallel} = 3.2 \cdot 10^9$ rad^2/s
- wobbling $D_{\perp} = 12 \cdot 10^9$ rad^2/s

Headgroups:

- rotation $D_{hg} = 2.2 \cdot 10^9$ rad^2/s
- tilting = ?

Tails:

- rotation $D_{tail} = 25 \cdot 10^9$ rad^2/s

Moore et al., Biophys. J. 1981, 5:2484-2494

Dipole moment of lipids

- **Data of Frischleder & Peinel; Chem. Phys. Lipids 1982, 30:121-158**
- **note:** 1D=3.3*10^{-30} Asm

PE (phosphatidylethanolamine):

- μ_{norm}= -5…0 D (perpendicular to membrane surface)
- μ_{tang} = 2…23 D (parallel to membrane surface)
- μ_{tot}= 2…24 D (μ_{tot} =(μ_{norm}^2 + μ_{tang}^2)^0.5)

PC (phosphatidylcholine):

- μ_{norm}= -9.5… 3 D (neg. sign - positive charge at surface)
- μ_{tang} = 17.5…24 D
- μ_{tot} = 18.5…25 D

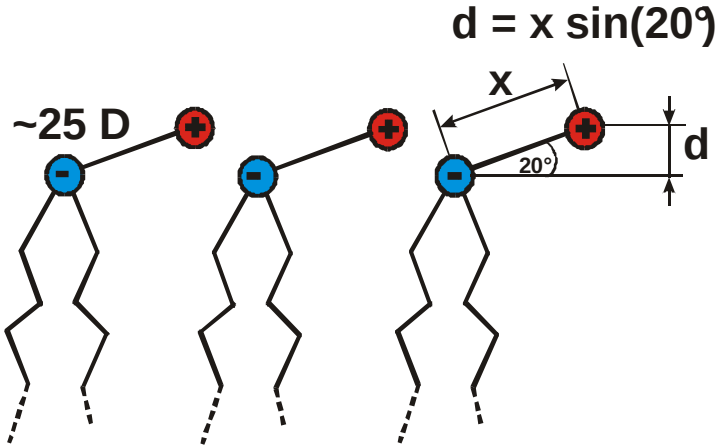
Hydration shell of head group: μ_{norm}= 5…10 D

Permittivity of head group region

Estimation:

$$C_{\perp} = \frac{Q}{\Delta\Psi} = \varepsilon_{\perp}\varepsilon_0 \frac{A}{d}$$

$$\varepsilon_{\perp} = \frac{Qd}{\varepsilon_0 A \Delta\Psi}$$



Tilt angle: ~1°/100 mV
(Sargent et al. 2001. Biophys. J. 81:1823-1824)

μ_{total}~ 25D, 0.48<A<0.75 nm^2
(Adam, Läuger, Stark: Biophysik)

$$\varepsilon_{\perp} = \frac{\mu \sin(1°)}{\varepsilon_0 A * 100mV} = 2.1 - 3.3$$

Bound water contributions missing!

Lipid dispersions

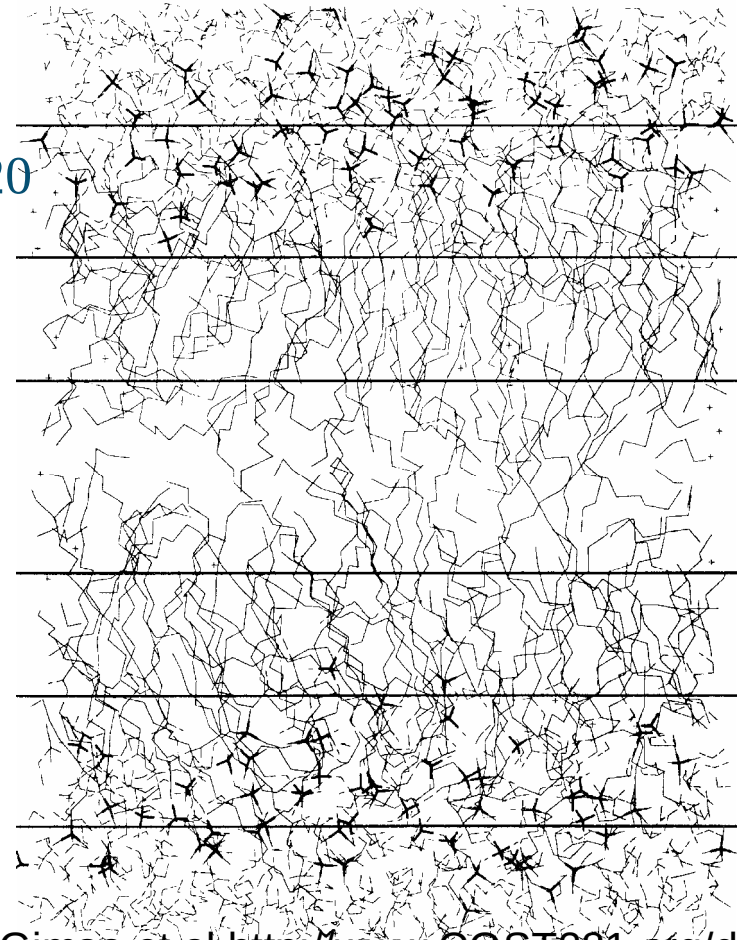
DC-permittivity

ϵ_{norm} ϵ_{tang}

60

10..30 40..120

2...3



Bound water layer

Polar lipid headgroups

Hydrophobic lipid chains

Low density interior

Hydrophobic lipid chains

Polar lipid headgroups

Bound water layer

for references see: see: Gimsa et al.http://www.COST281.org/documents.php;
Simeonova and Gimsa. J. Phys.: Condens. Matter. 2005 (17): doi:10.1088/0953-8984/17/0/000

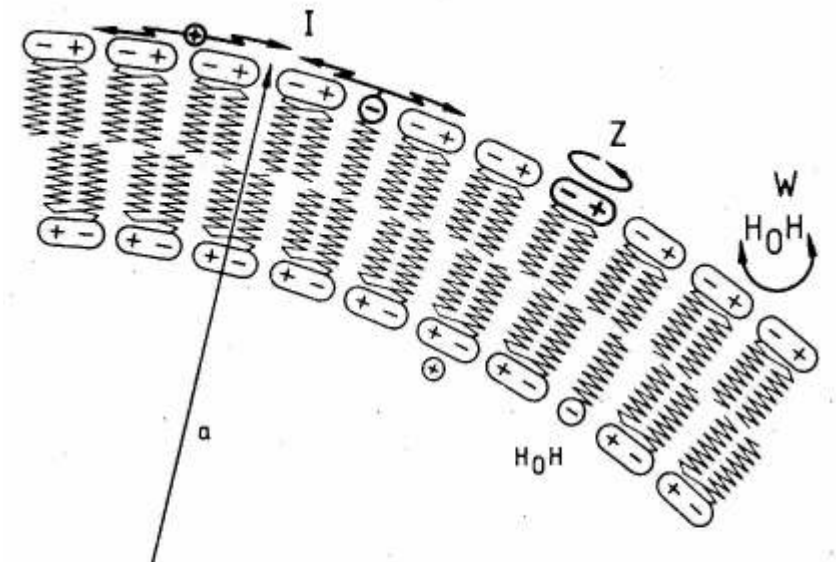
Water diffusion

bulk: $D = 8 \cdot 10^{-5}$ cm^2/s

at the interface: $D = 4 \cdot 10^{-5}$ cm^2/s

Buuren et al., Coll. Surf. 1995, 102:143-157

Surface conductance



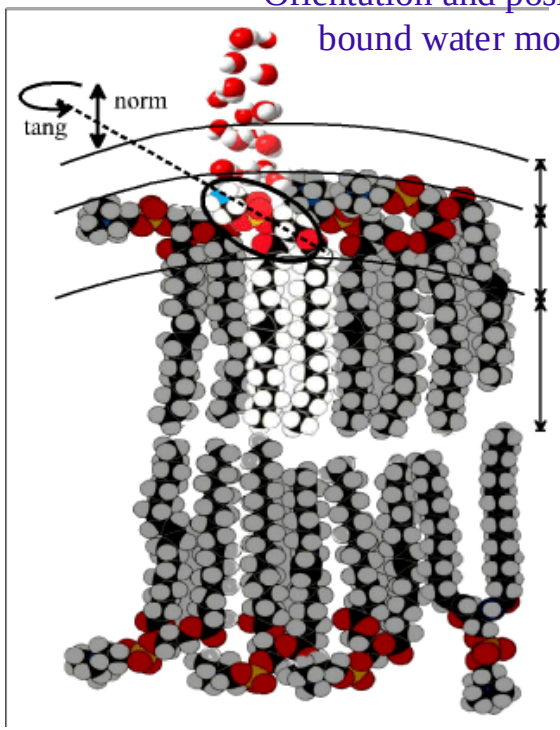
Numerical modeling

CPK model of a phospholipid membrane

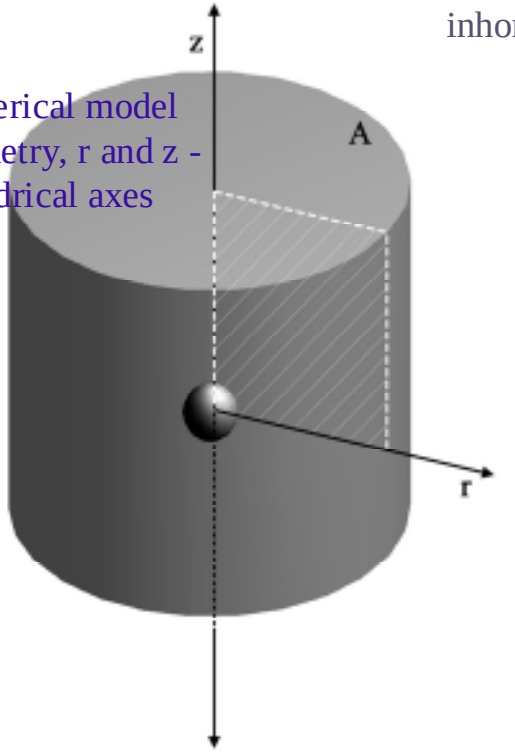
elliptic contour: lipid headgroup with degrees of orientational freedom

Solutions were obtained in the two dimensional axis symmetric module of FEMLAB©

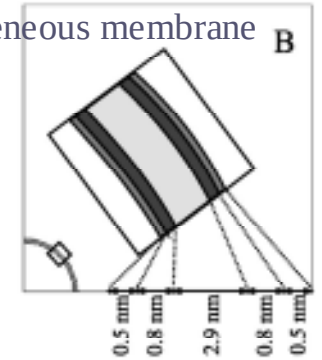
Orientation and position of bound water molecules



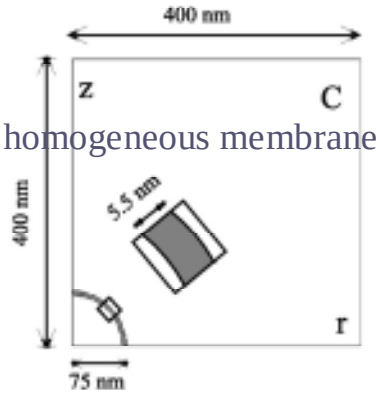
Numerical model geometry, r and z - cylindrical axes



inhomogeneous membrane



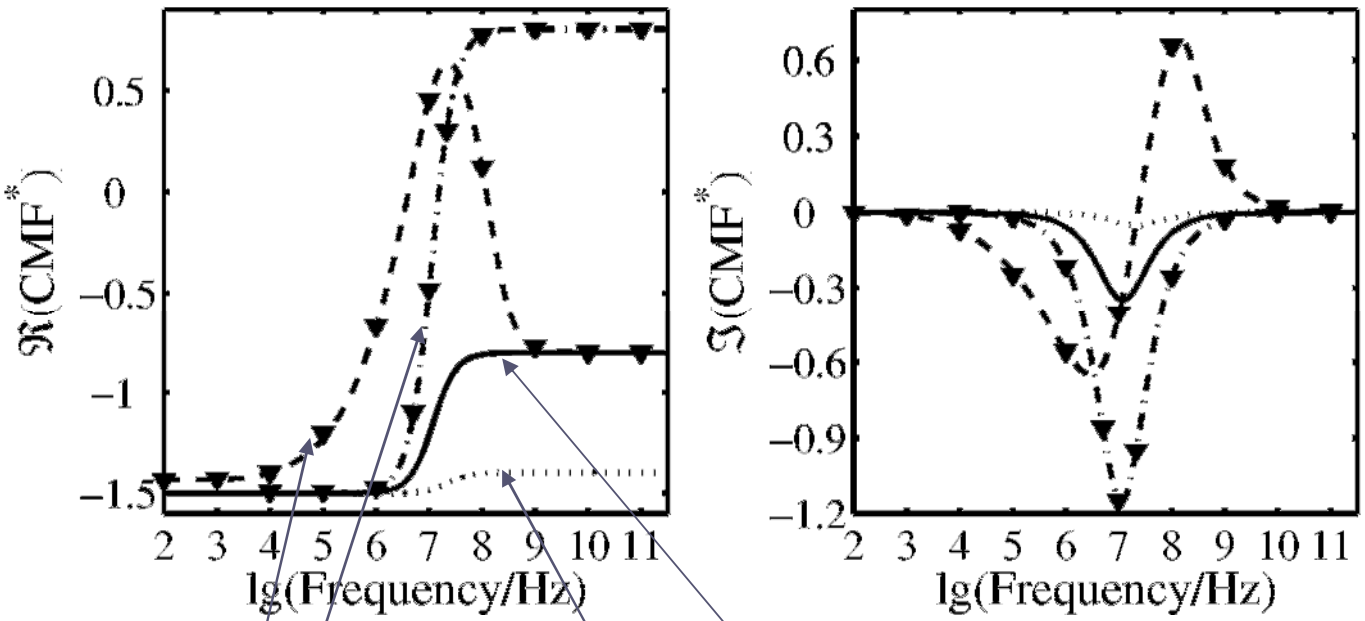
homogeneous membrane



Anisotropy

Induced dipole moment: comparison of analytical solutions (lines) with FEM solutions (triangles) for anisotropic models

Model: diameter 75 nm, $\sigma_{ext} = \sigma_{int} = 0.1$ S/m, $\varepsilon_{ext} = \varepsilon_{int} = 78$



1. Isotropic homogeneous model (dotted)
2. Single shell model with:
   a) homogeneous isotropic membrane (continuous) and
   b) anisotropic membrane:
      dashed-dotted: permittivity, $\varepsilon_{norm} = 3.7$; $\varepsilon_{tang} = 3700$
      dashed: conductivity, $\sigma_{norm} = 7*10^{-7}$ S/m; $\sigma_{tang} = 7$ S/m

reference values

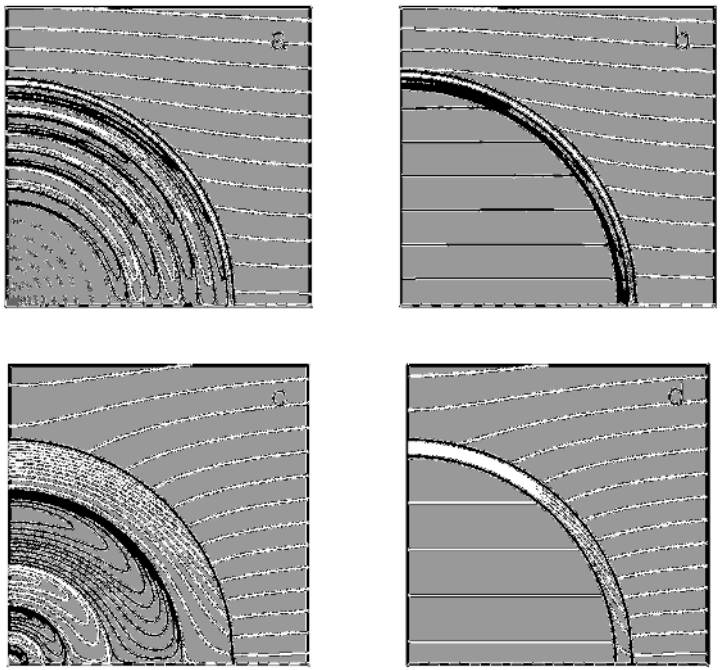
Potential distribution (real part) within anisotropic objects

homogeneous with membr. props. | single-shell model

10 *MHz*

field

1 *GHz*



white: positive equipotential lines
dark: negative equipotential lines
dashed lines are at 0 V

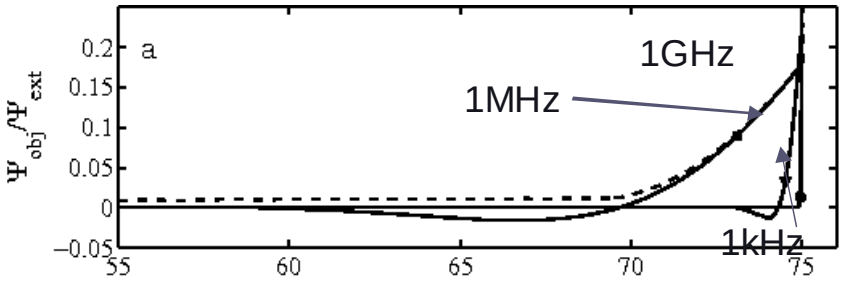
Please note that the distances between the isopotential lines are not to scale (compare to right figure).

Object diameter 75 nm

$\sigma_{ext}=\sigma_{int}=0.1$S/m, $\varepsilon_{ext}=\varepsilon_{int}=78$, $\varepsilon_{mem}=3.7$

Anisotropic membrane conductivity:

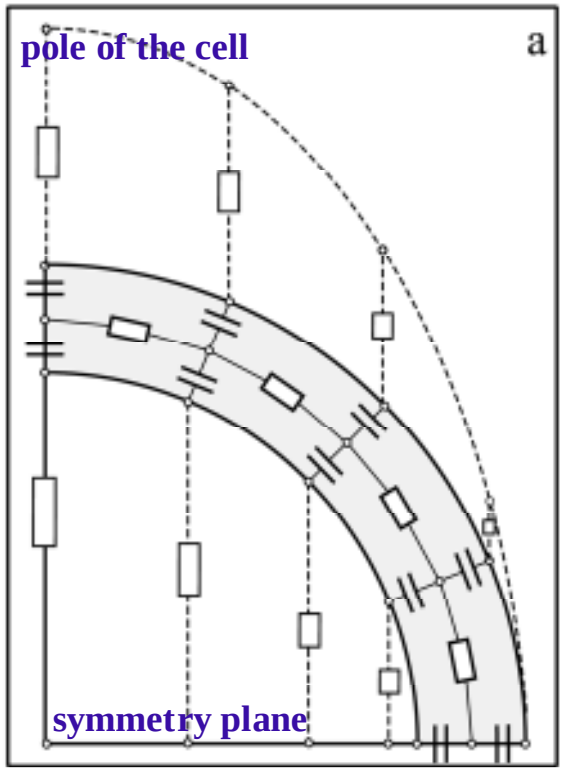
$\sigma_{norm}= 7*10^{-7}$ S/m, $\sigma_{tang}= 7$ S/m



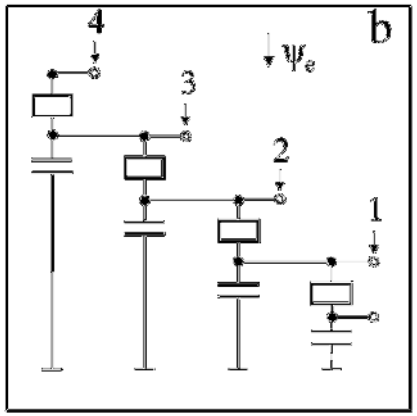
Potential along the polar radii of the homogeneous (continuous) and the single shell (dashed) models at:
1 kHz (circle)
1 MHz (triangle)
1 GHz (square)

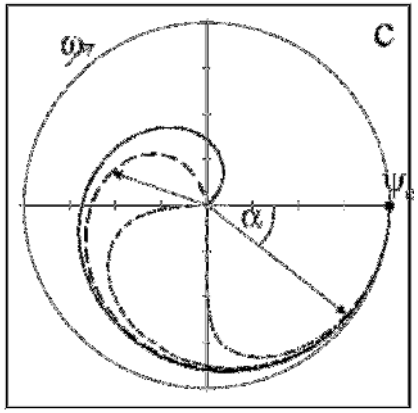
Qualitative explanation

Single-shell model with membrane of enhanced tangential conductivity (grey area). The external side of the membrane is charged by the external potential via the resistivity of the external medium, whereas the inner side is grounded (0 V) to the equatorial plane by the core resistivity.



RC-phase shifter, producing a phase shift of more than 270°



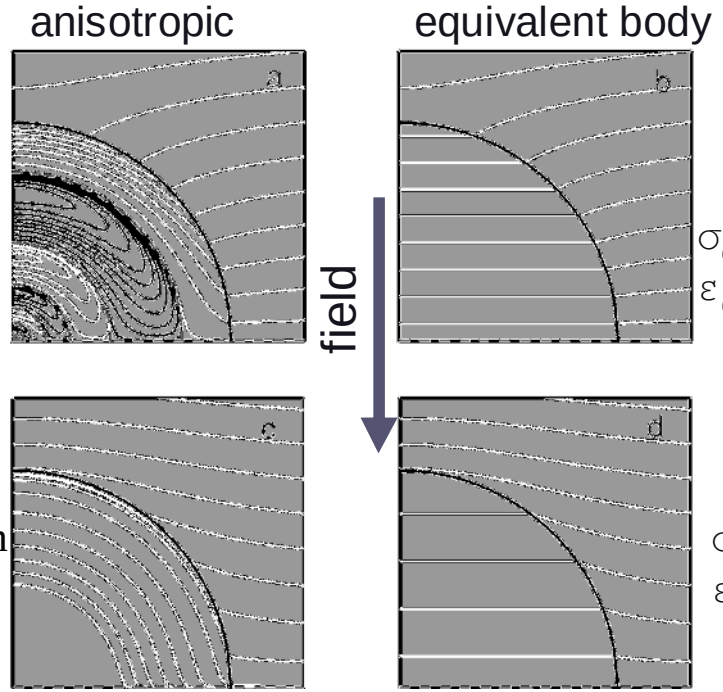


Phase-shift (angle, α) of the potential, created by one (-.-.-), two (- - -), three (_ _ _ _) or four (____) RC-pairs is presented in a polar plot.

Please note the logarithmic axes scaling.

3rd problem:
Can the anisotropic properties be detected by electric measurements?

Potential distribution (real part) for anisotropic objects and their Maxwellian equivalent bodies



$\varepsilon = 3.7$
$\sigma_{norm} = 7*10^{-7}$ S/m
$\sigma_{tang} = 7$ S/m

$\sigma_{equiv} = 1.18$ S/m
$\varepsilon_{equiv} = 20.086$

$\sigma_{norm} = 7*10^{-7}$ S/m
$\varepsilon_{norm} = 3.7$
$\varepsilon_{tang} = 3700$

$\sigma_{equiv} = 1.5*10^{-5}$ S/m
$\varepsilon_{equiv} = 163.63$

diameter: 75 nm, 1GHz
$\sigma_{ext} = 0.1$S/m, $\varepsilon_{ext} = 78$

white: positive equipotential lines
dark: negative equipotential lines
dashed lines are at 0 V

Please note that the distances between the isopotential lines are not to scale

Please note the identity of the external potential distribution of the anisotropic objects and their equivalent bodies!

Frequency dependence of the conductivities (right scale) and permittivities (left scale) of the equivalent bodies of different vesicle models

diameter: 75 nm, $\sigma_{ext} = \sigma_{int} = 0.1$S/m, $\varepsilon_{ext} = \varepsilon_{int} = 78$, $\varepsilon_{norm} = 3.7$, $\sigma_{norm} = 7*10^{-7}$ S/m

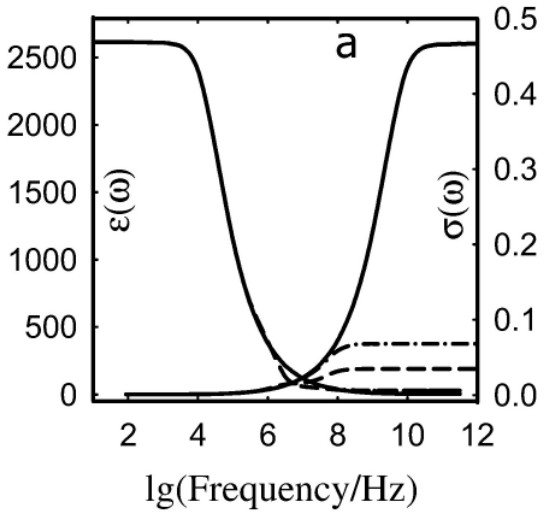
Model features:

- homogeneous (_____)
- single-shell model (-.-.-)
- three-shell model (- - - -)

with either anisotropic conductivity (a) or anisotropic permittivity (b)

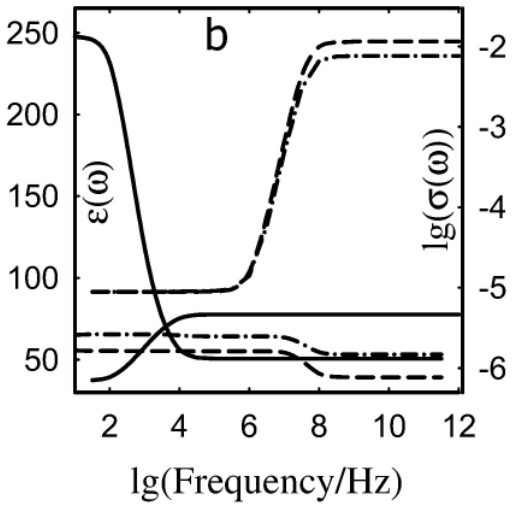
anisotropic conductivity

$\sigma_{tang} = 0.7$ S/m



anisotropic permittivity

$\varepsilon_{tang} = 370$



Anisotropies: consequences

- The surface polarization of radially-anisotropic spherical objects is equivalent to the polarization of their homogeneous isotropic (Maxwellian) equivalent bodies
- Dielectric characterization methods are based on surface polarization. The anomalous potential distribution within the volume of the objects remains hidden in the relaxations-like behavior of the frequency-dependence of the conductivity and permittivity of their equivalent bodies
- This equivalence may result in erroneous interpretations of experimental data and may explain why anisotropy effects have rarely been detected

- Even though dispersion processes in anisotropic (biological or colloidal) objects are based on the structure of the objects, they possess properties that are qualitatively different from common structural dispersions:
  - in practice, the anisotropic properties are introduced by the molecular structure of the objects;
  - anisotropic properties generate an electric "fine-structure" in homogeneous media
- Electric anisotropies may lead to strong inhomogeneities in the potential and field distributions of homogeneous media

Lipid dispersions

DC-permittivity

ϵ_{norm} ϵ_{tang}

60

10..30 40..120

2...3

Dispersions

f_{char} ϵ_{inf}

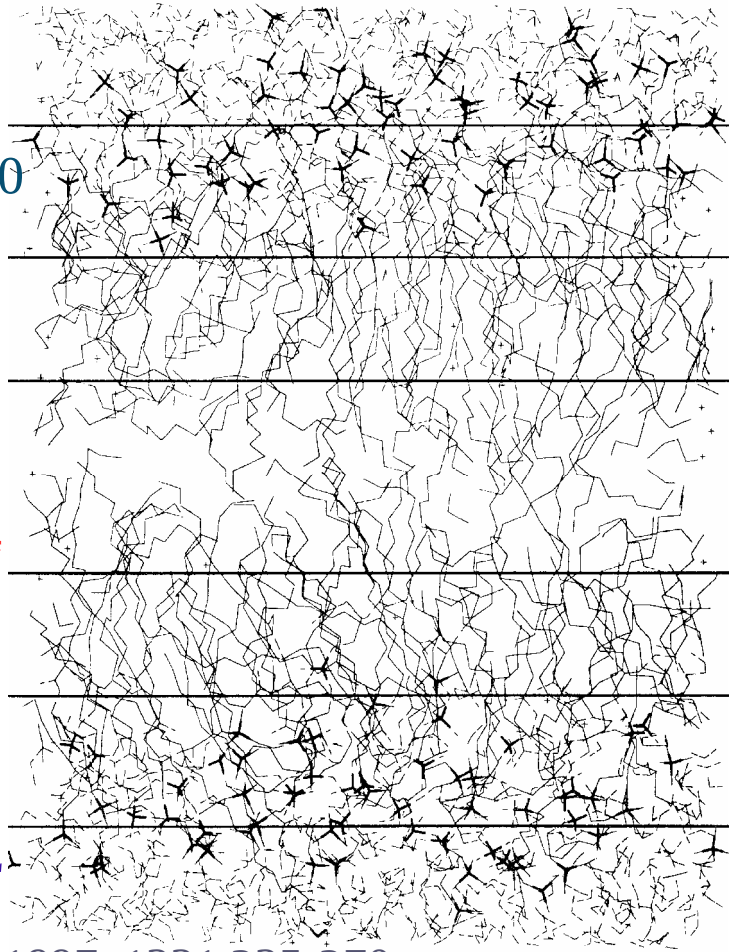
? ?

42..66..350MHz

~2 ~2

200MHz...2GHz

~2 ~2



Bound water layer

Polar lipid headgroups

Hydrophobic lipid chains

Low density interior

Hydrophobic lipid chains

Polar lipid headgroups

Bound water layer

Tieleman et al., BBA 1997, 1331:235-270

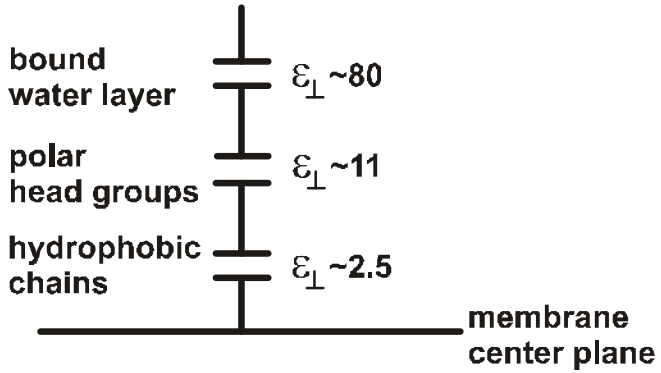
for references also see: Gimsa et al.http://www.COST281.org/documents.php;
Simeonova and Gimsa. J. Phys.: Condens. Matter. 2005 (17): doi:10.1088/0953-8984/17/0/000

4th problem: membrane proteins

- Model: red cells?
  - well known composition
  - readily available

Estimating the specific membrane capacitance

	thickness, d	$\epsilon_\perp$
bound water	0.5 nm	80
head group	0.8 nm	11
lipid chain	1.3 nm	2.5



$$C^A_{mem} = \frac{C}{A} = \frac{\epsilon_\perp \epsilon_0}{d}$$

$$\frac{1}{C^A_{mem}} = \frac{1}{C^A_{bw}} + \frac{1}{C^A_{hg}} + \frac{1}{C^A_{lc}}$$

$$\frac{1}{C^A_{mem}} = \frac{d_{bw}}{\epsilon_{bw}\epsilon_0} + \frac{d_{hg}}{\epsilon_{hg}\epsilon_0} + \frac{d_{lc}}{\epsilon_{lc}\epsilon_0}$$

C^A_{mem} =1.48 μF/m^2
(0.74μF/m^2 for the complete lipid membrane)

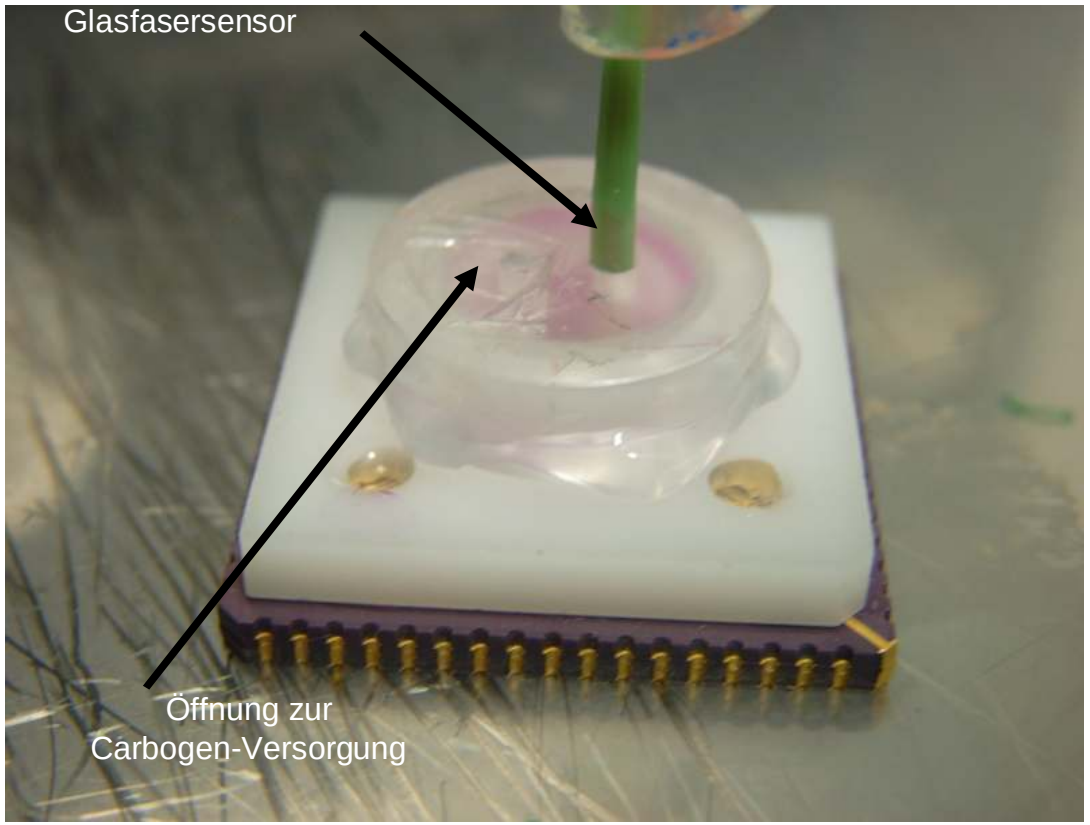
The human red cell has a capacitance of about 1μF/m^2 at a lipid area portion of about 89% leading to about **4.2μF/m^2** for the protein regions.

Summary/Conclusions

- Molecular properties strongly influence the absorption in various cell compartments
- Anisotropy effects are probably underestimated:
  - Possible anomalous potential distributions within the membrane or the volume of the objects remain hidden in the relaxations-like behavior of the frequency-dependence of the conductivities and permittivities of the equivalent bodies
- Hidden "anisotropy dispersions" may result in erroneous data interpretations
- Membrane anisotropy may induce higher local currents leading to a higher energy dissipation
- Dispersion of the permittivity anisotropy will lead to conductivity anisotropy
- All anisotropies are probably dispersed above 500 MHz
- Averaging absorption over the membrane layers may lead to a higher energy dissipation (up to 10 times) than averaging over membrane properties
- In layered membrane models the absorption in the outermost layer is dominating
- Effects of membrane proteins on membrane-field distribution remain to be elucidated

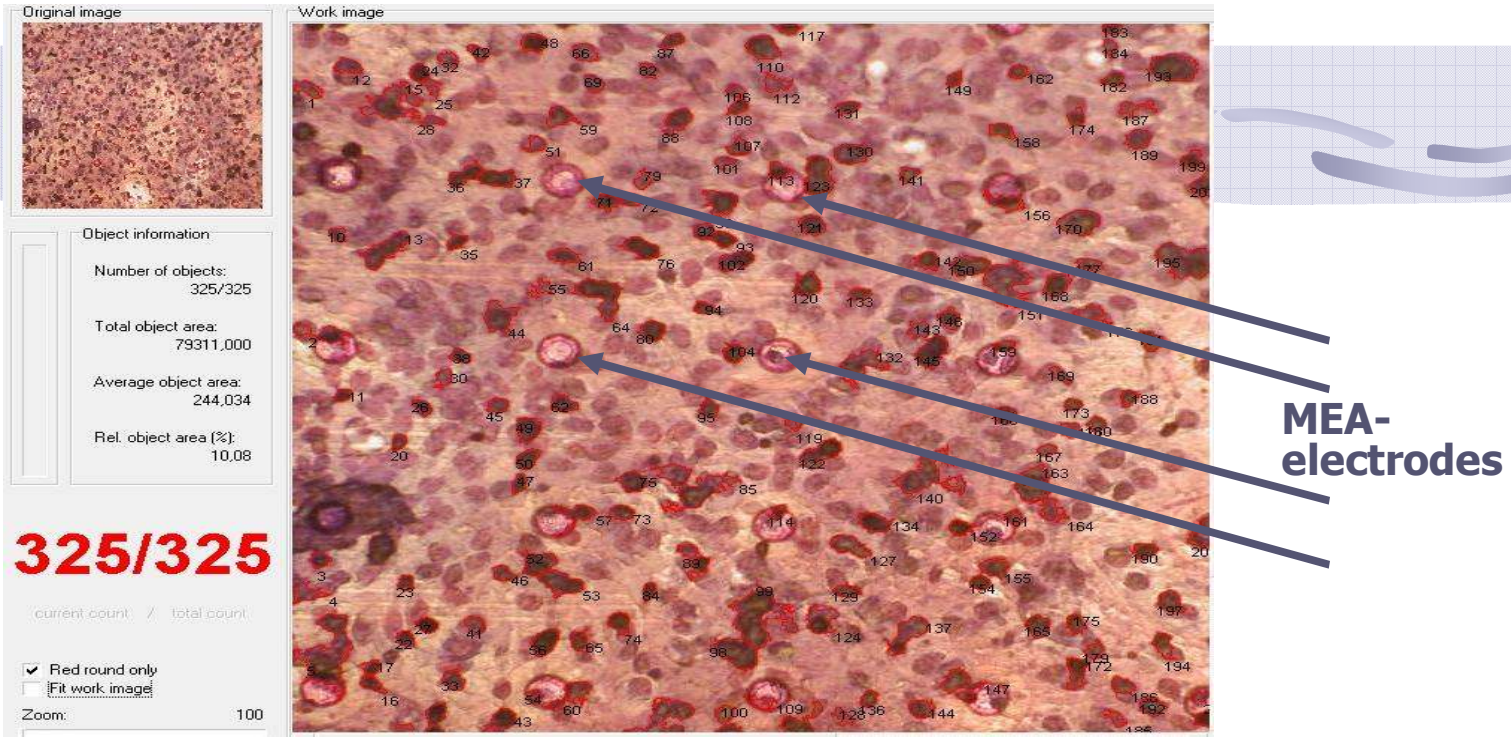
Outlook:
Neuro-sensor-chips
in wafe-guide
(CW, GSM, UMTS)

Neuro-sensorchip



Neuro-sensorchip (Micronas & Chair of Biophysics, 25 mm x 25 mm) with teflon cell-trough and plexi-glass cap with probe-bores. Left bore covered by semi-permeable membrane to hinder medium evaporation and allow for CO_2-O_2 exchange. Right bore (diameter 1.9 mm) for glass-fiber temperature sensor.

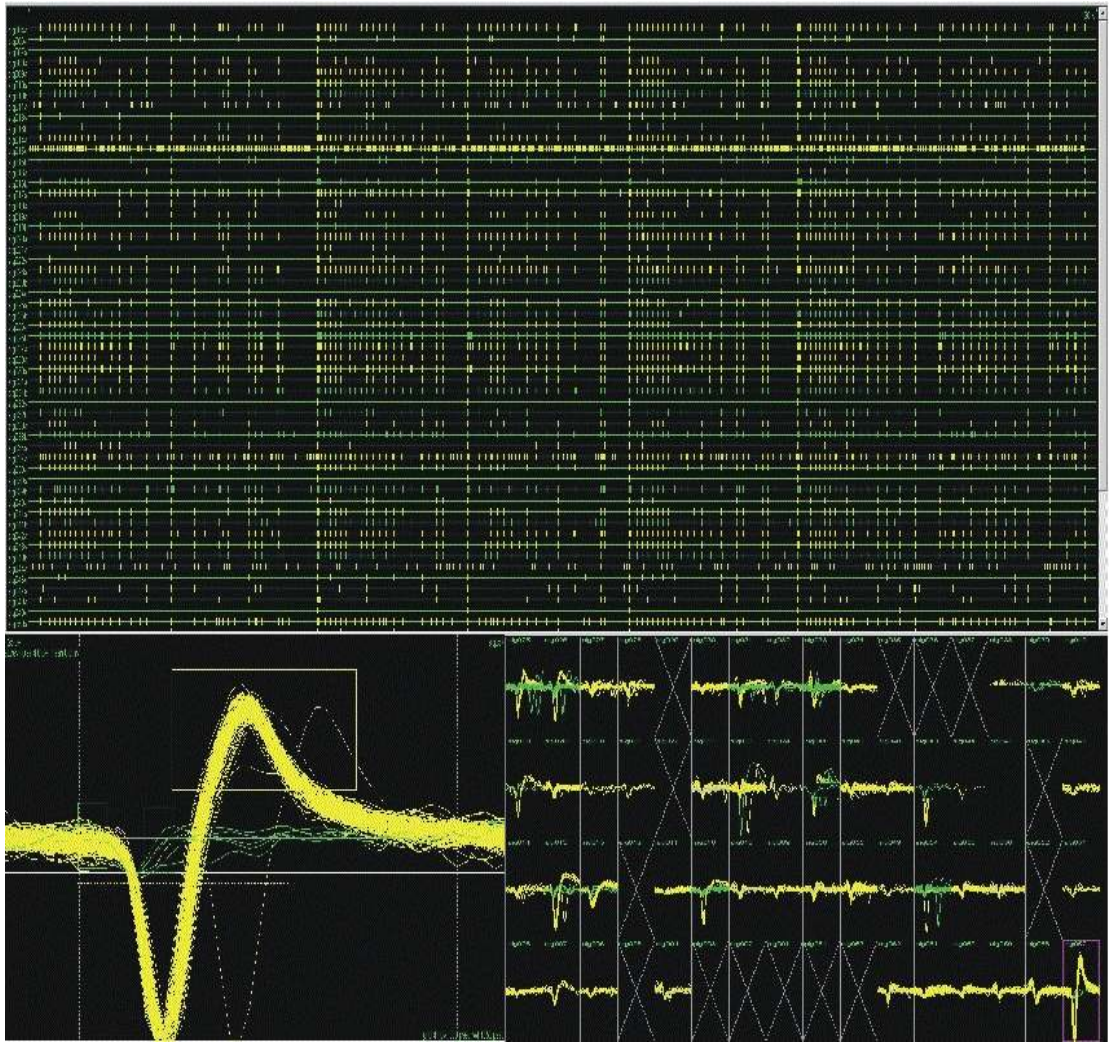
Image of primary neurons on MEA



Increased cell-gain by an improved protocol for neuronal primary cells: Image of a neuronal net-work at the surface of a micro-electrode array (MEA). 325 cells out of 665 seed-cell survived after 6 weeks.

Action potentials from neuro-sensorchips

58 MEA-electrodes, signals of 57 active units



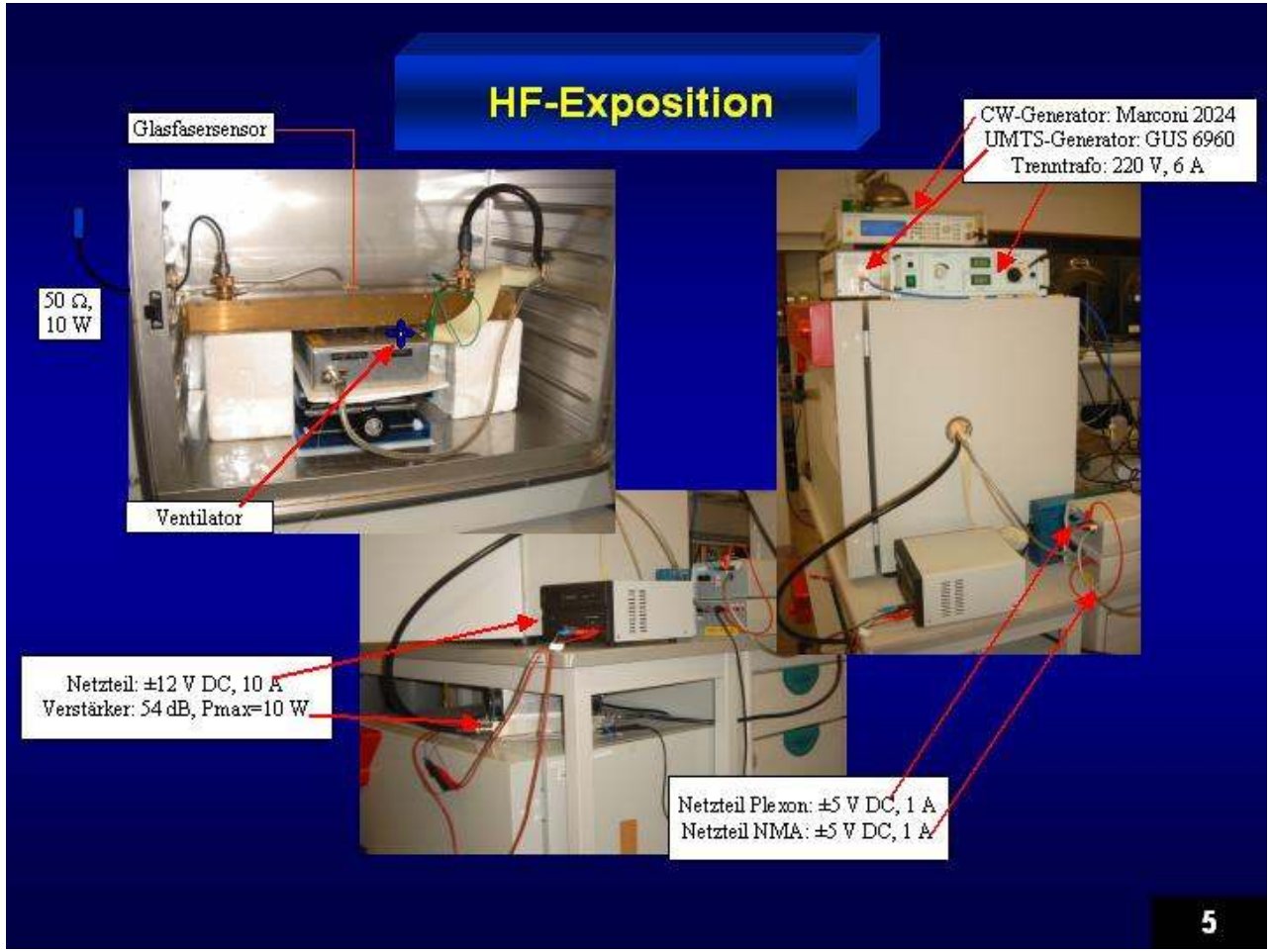
Top: 57 spike traces (80 s)

Lower left: Wave form of a single unit

Lower right: Wave forms of all units. Green traces correspond to a 2nd signal from a single electrode. Amplitudes correlate to the spatial distance of an active neuron to the pick-up electrode. Xed boxes mark signal-free electrodes.

Software: MEA Server (Plexon Inc., Dallas, TX, USA; Version Oct. 2005).

Exposition setup for neuro-sensorchips



Components for neuro-chip exposition by CW-EMF at 2 GHz (Marconi 2024) & generic UMTS-fields (GUS 6960 S) at 1.966 GHz. Upper l.: wave-guide in incubator; upper r.: incubator; bottom.: power supplies

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