Stochastic Approach for EM Modelling of Suspended Bacterial Cells with Non-Uniform Geometry & Orientation Distribution

Dominique Rauly*, Eric Chamberod**, Pascal Xavier*, Jean M.F. Martins*** ,
Jean Angelidis**** and Hakima Belbachir *****

AI. Introduction

Interactions between electromagnetic (EM) fields or currents and living cells constitute a recurrent subject of interest for several decades, and their effects have been profusely discussed [1], especially for the frequency bands currently encountered in everyday life of humans. In a more restrictive domain, large number of studies have been done, focused on EM modelling of a bacteria medium, in order to predict the EM field penetration and inner induced currents in living microorganisms. Relevant information to be collected deals with level and frequency of the EM signal that may affect the development of the considered cells. The former because non-thermal effects are nowadays suspected to exist, altering cell's metabolism, the latter because it is well known that the spectral response of the living cell impedance exhibits some dispersion in relation with cell membrane behaviour and shield effects [2].

It appears that these parameters may be strongly sensitive to shape, dimensions and orientation of bacterial cells in relation to the applied electric field orientation. The present paper aims to examine this problem and brings a streamlined lighting. This is done through the elaboration of a simplified equivalent electric circuit, supported by a prior COMSOL^{TM} analysis and a stochastic approach.

BII Analysis

The bacterium selected to test our approach is *Escherichia coli* DH5α. A first model, suitable for the required level of resolution, may be a 2 ± 1 µm long and 0.5 ± 0.2 µm wide cylinder filled with physiological fluid and surrounded by a dielectric membrane of thickness 50 nm. The medium is considered also as a physiological fluid. Figure 1 shows the COMSOL^{TM} model, with possible orientations for the external applied electric field, assuming that the field is provided through the application of an AC voltage between two plane parallel electrodes. A simplified equivalent circuit, similar to those explicited in the literature [2], can then be extracted from COMSOL^{TM} computation, where capacitance and resistor values depend on size and orientation (Fig. 2). Next, the equivalent circuit for the overall medium, containing the suspended bacterial cells, is deduced, taking into account the concentration and the orientation of bacteria, as well as the electrodes/medium interface.

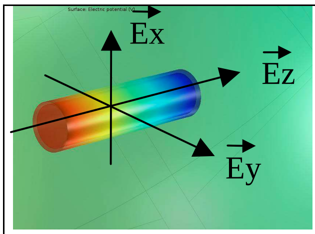

Figure 1

COMSOL^{TM} Model of E. Coli

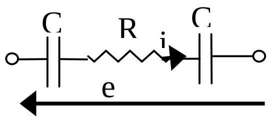

Figure2

Equivalent circuit for one bacterium, to be inserted in the overall circuit representing the bacterial medium. C and R values are orientation-dependent

CII Results

As an example of interesting result, Figure 3 shows the admittance of a bacterium longitudinally oriented with the electric field. It reveals the well-known shield effect at lower frequencies. The final paper will relate other relevant parameters as inner current in the micro-organism. Variations according to the size and orientation of bacteria will be detailed for both uniform and non-uniform media, supported by a stochastic analysis.

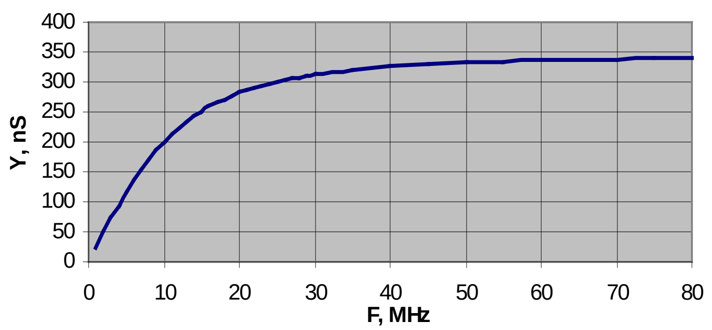

Fig. 3 – Frequency behaviour of the admittance of E. Coli

[1] M. Cifra, J.Z. Fields, A. Farhadi "Electromagnetic cellular interactions", Progr in Biophys. & Molec. Biology, 105 (2011) 223-246
[2] D.B. Kell, C.M. Harris "Dielectric Spectroscopy and Membrane Organization", Jal of Bioelectricity 4(2), (1985) 317-348

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Session : "Bioelectromagnetics"

Session Organizer : Professor Dr Carlos Martino.

Introduction

1. Background of the study :

- EM Decontamination

- Current requirements : Knowledge of effective parameters for EM decontamination in terms of :

  - Frequency Band

  - Required levels of the applied electrical signal

2. Objectives of the study ;

- Study of a suspended bacterial cells medium (*Escherichia coli* DH5α.) submitted to an external voltage between two parallel plates

- The medium is assumed to be water with 0.9% Na Cl - the bacteria could be longitudinally or transversally oriented , with regard to the applied voltage.

- Computation of the effective inner electrical current inside the bacterial cells, for various configurations in terms of size, orientation, concentration

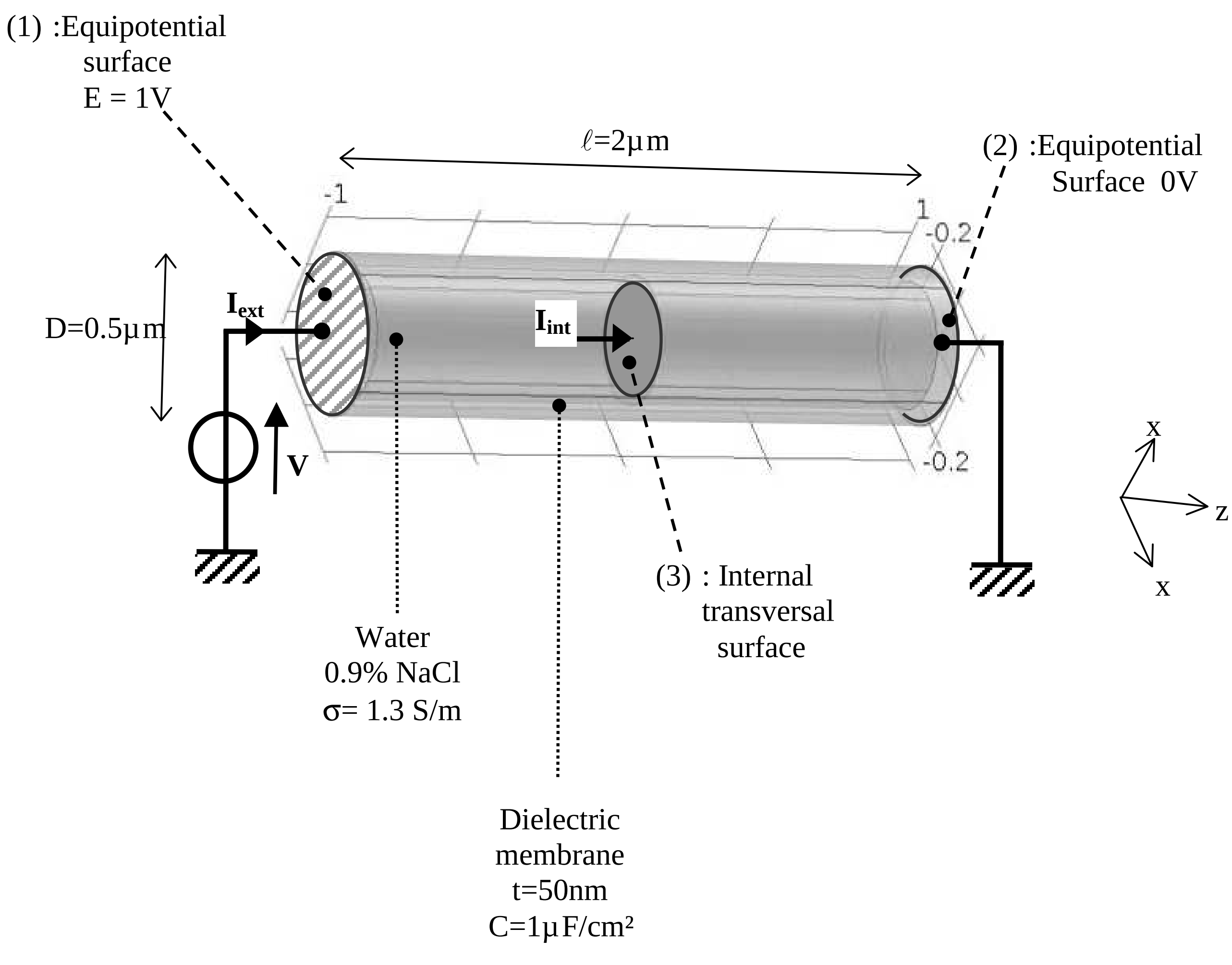
- Determination of the required levels of the applied electrical signal.

Preliminary Study

1. Bacterium alone, without surrounding medium :

a - Configuration of the study :

Longitudinal excitation (imposed potentials).



b - Computed physical quantities :

- Complex external admittance :

$$Y_{ext} = \frac{I_{ext}}{E} \quad [\Omega^{-1}]$$

with $I_{ext} = \int_{(1)} J_z dS$; Jz : computed longitudinal current density

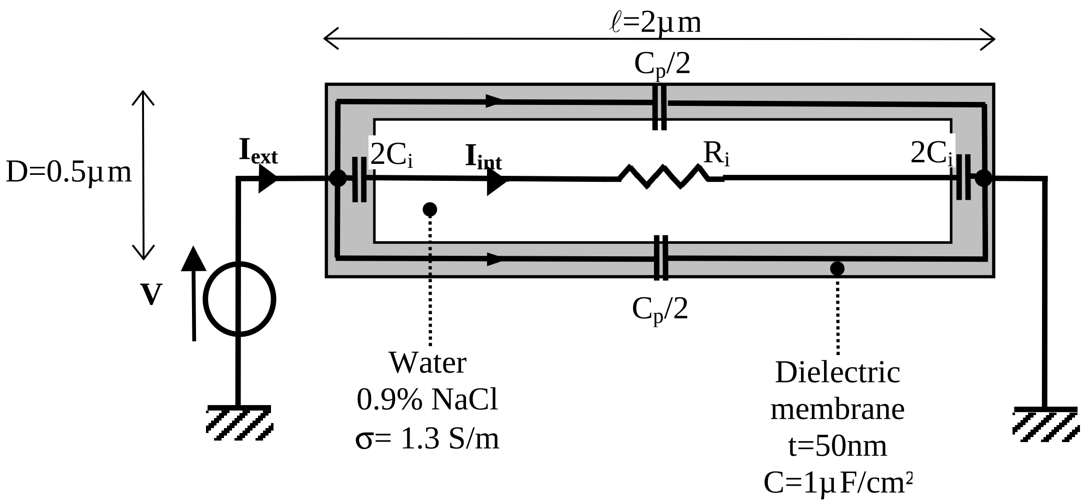
- Transconductance :

$$Y_{int} = \frac{I_{int}}{E} \quad [\Omega^{-1}]$$

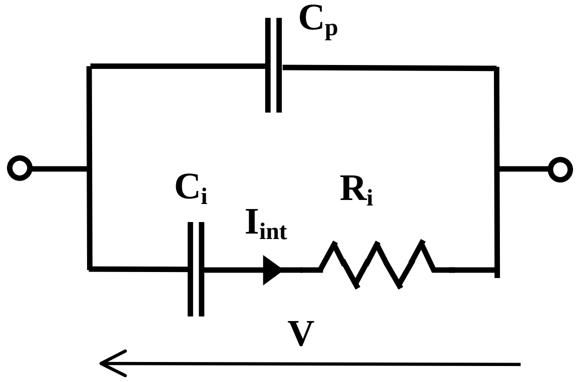
where : $I_{int} = \int_{(3)} J_z dS$: internal current; Jz : computed longitudinal current density

⇨ Y_{int} useful to predict internal currents
likely to cause disorders in the metabolism of the bacterium

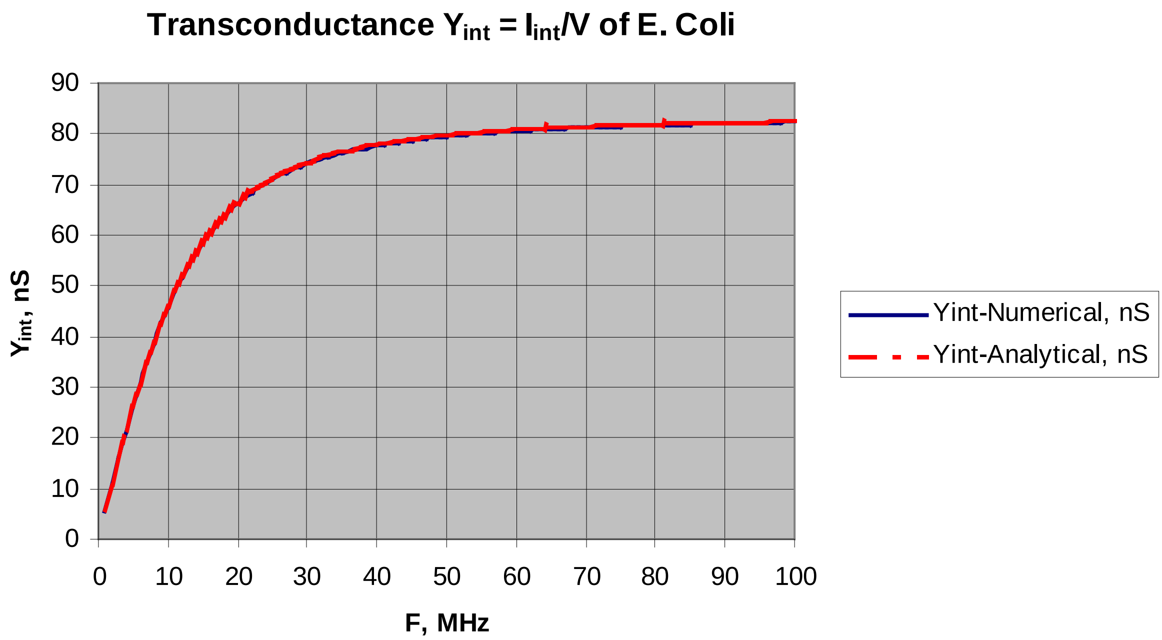
c - Proposed Equivalent network :

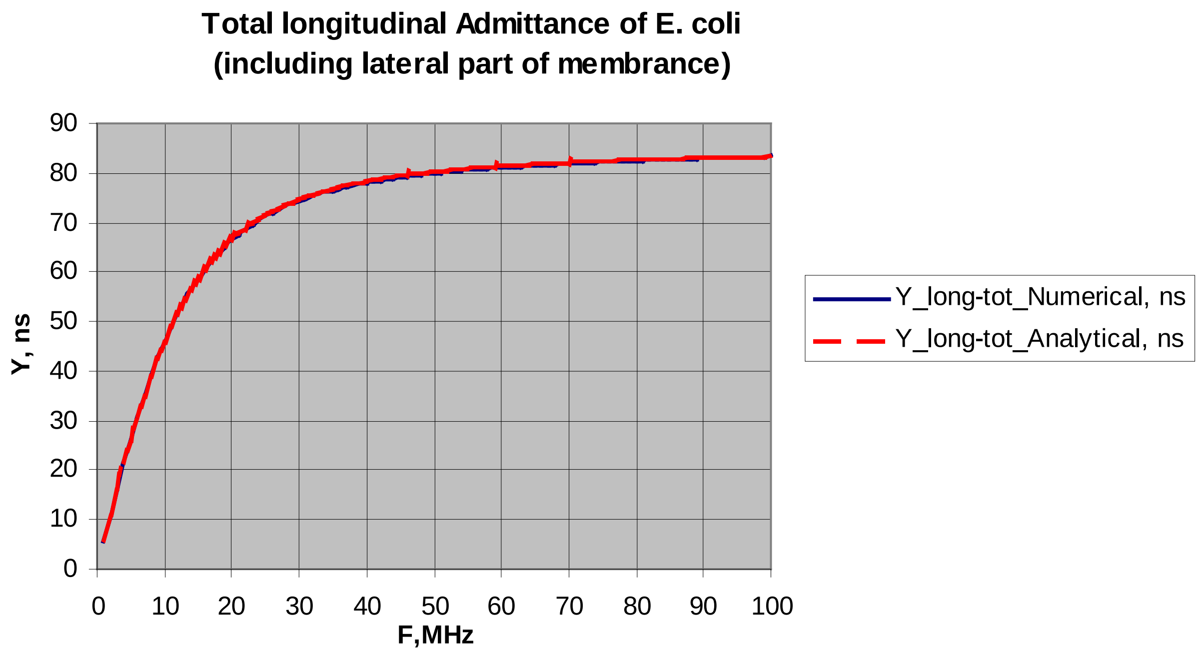


⇨



d - Numerical (COMSOL Multiphysics TM) and Analytical Compared Results:



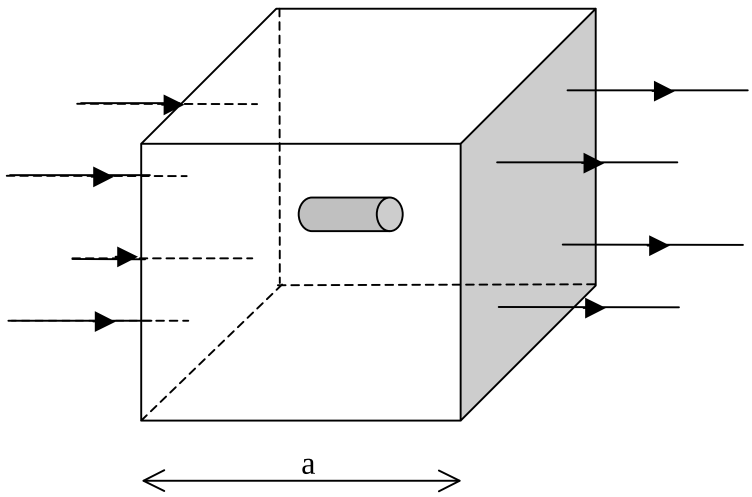


⇨ **Perfect fit with** :

- $R_i = 12\ M\Omega$
- $C_i = 0.87\ fF$
- $C_p = 1.5\ aF$

2. Determination of an Elementary Cubic Cell :

a Criteria :



- Full of water with 0.9% NaCl
- Contains one bacterium (longitudinally or transversally oriented)
- Suitable to be cascaded in 3 dimensions in order to shape the 3D macroscopic medium

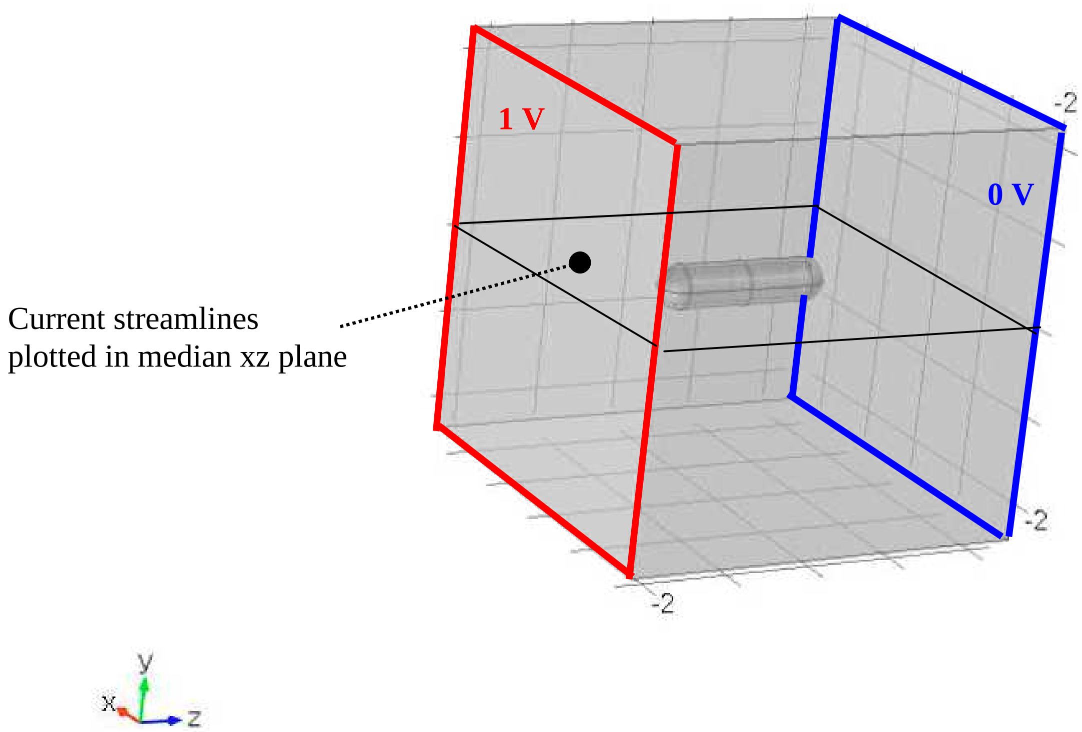
⇨ **Current lines entering in the input plane and emerging from the output plane must be parallel with uniform density.**

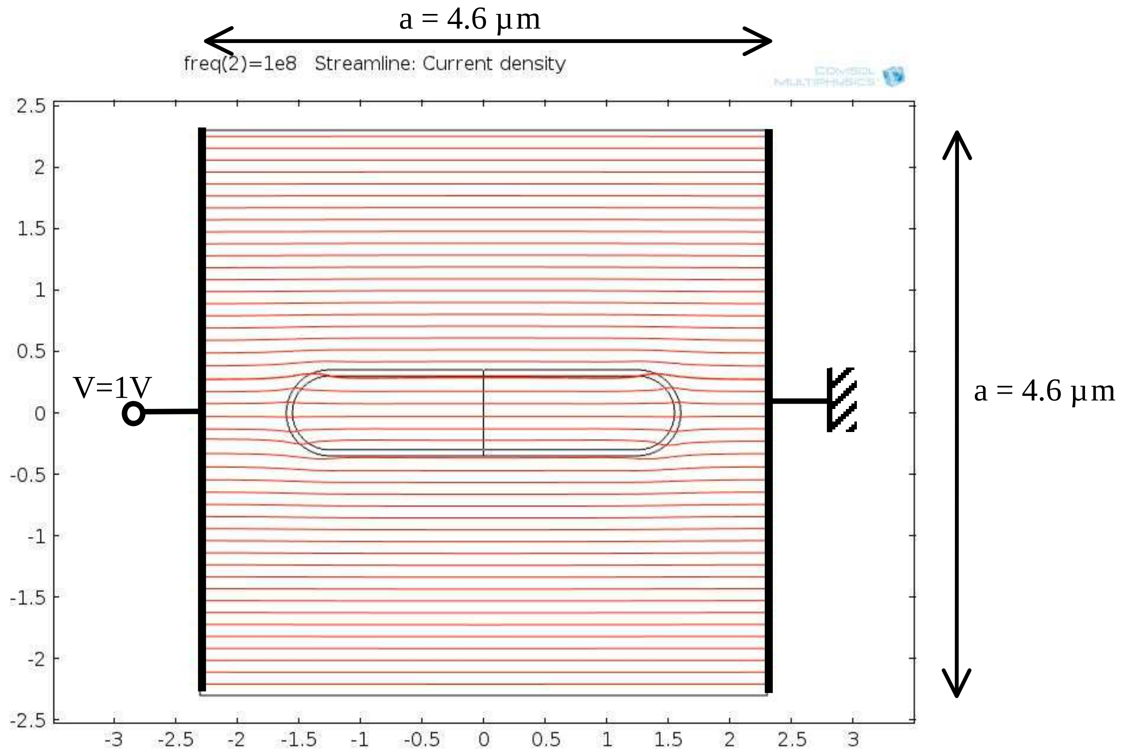
b Determination of minimal side a :

We examine the worst case, corresponding to the maximal size for the bacterium :
ℓ = 3µm, D = 0,7 µm

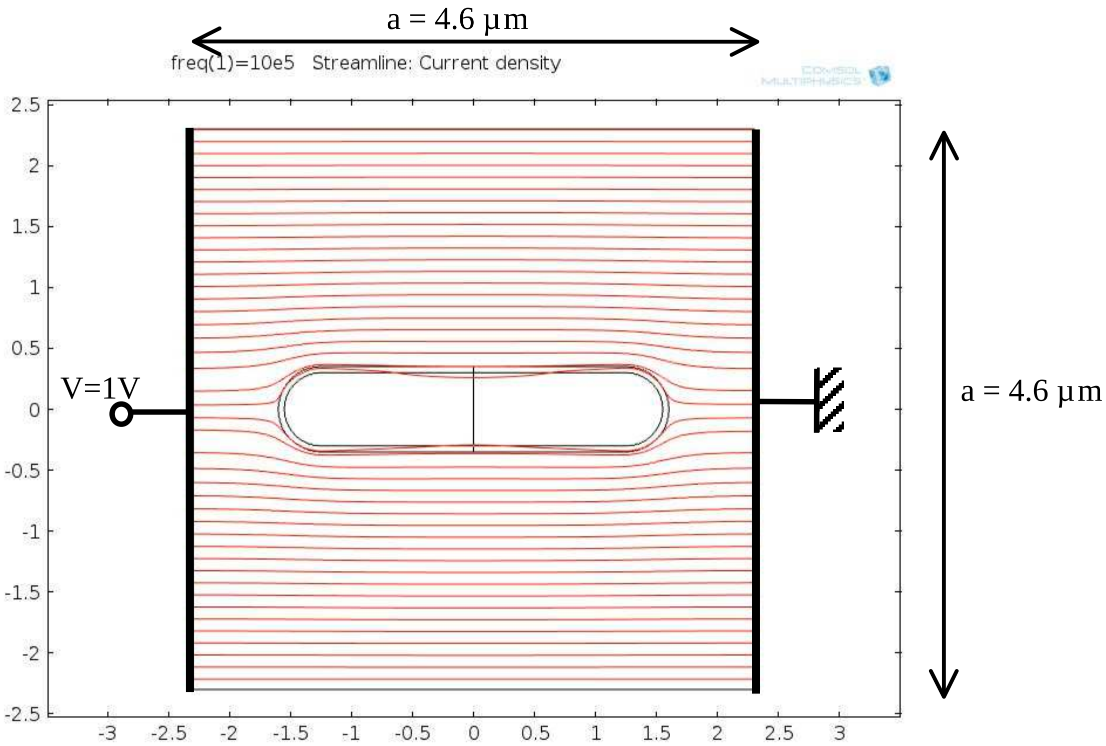
Longitudinal case :

Potentials imposed on xy planes ; Current streamlines plotted in xz plane





F = 100 MHz – Current streamlines pass through the bacterium

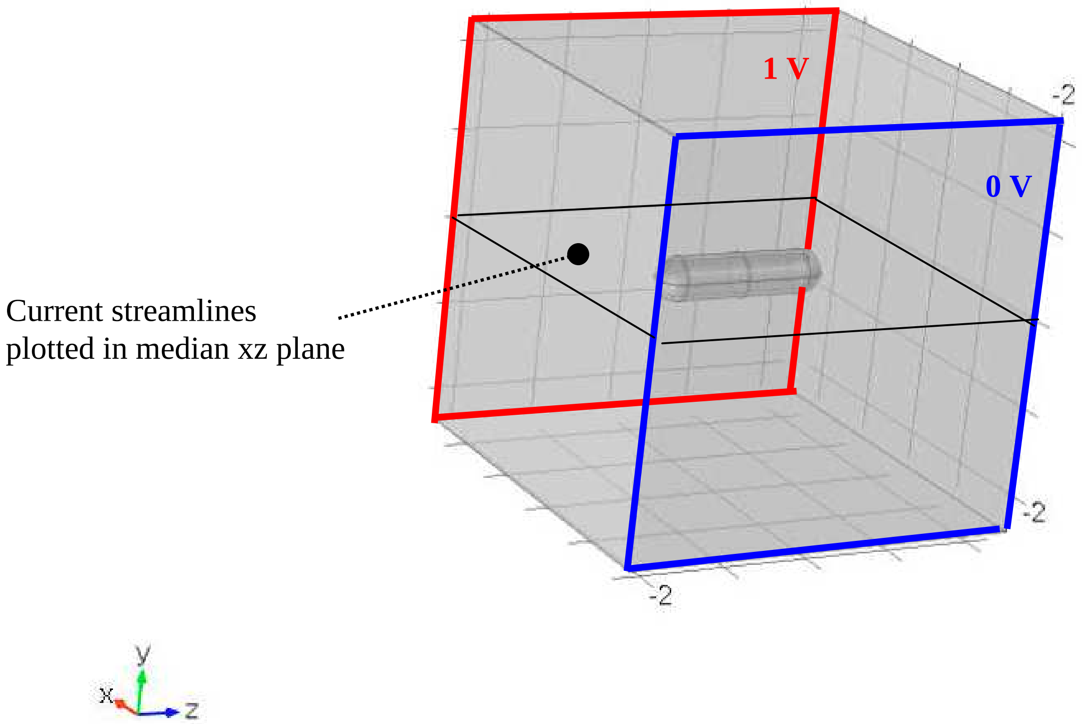


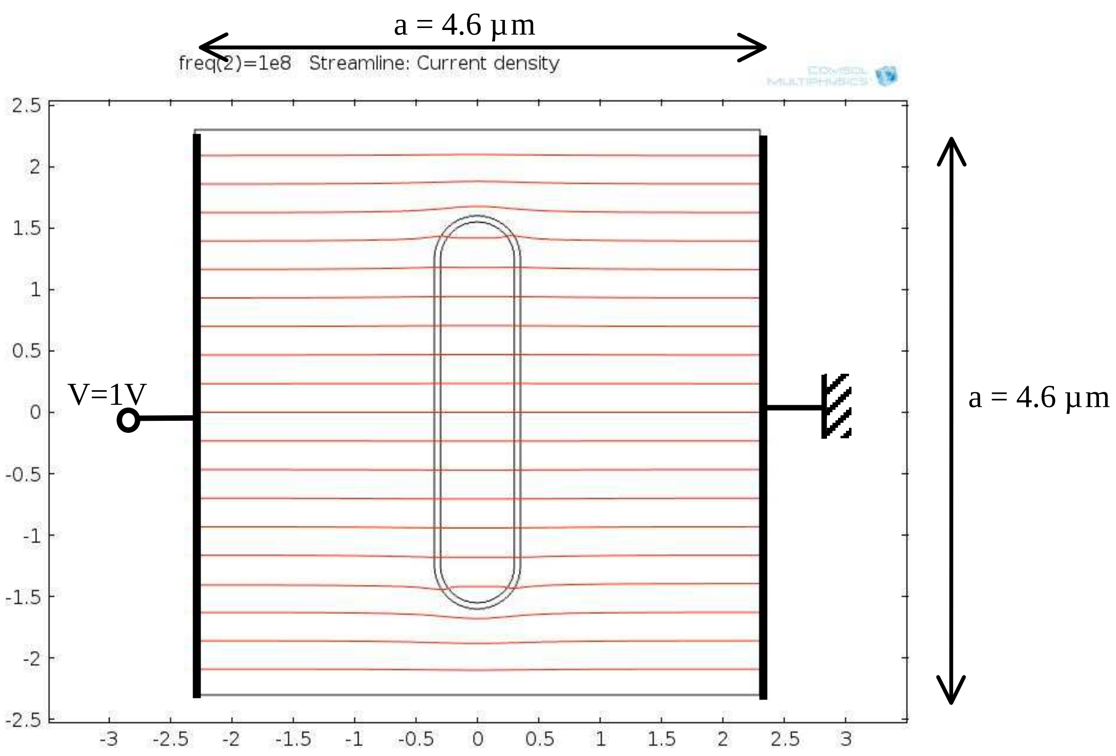
F = 1 MHz – Current streamlines bypass the bacterium

⇨ **Elementary cubic cell side a = 4.6 µm is suitable for longitudinal excitation**

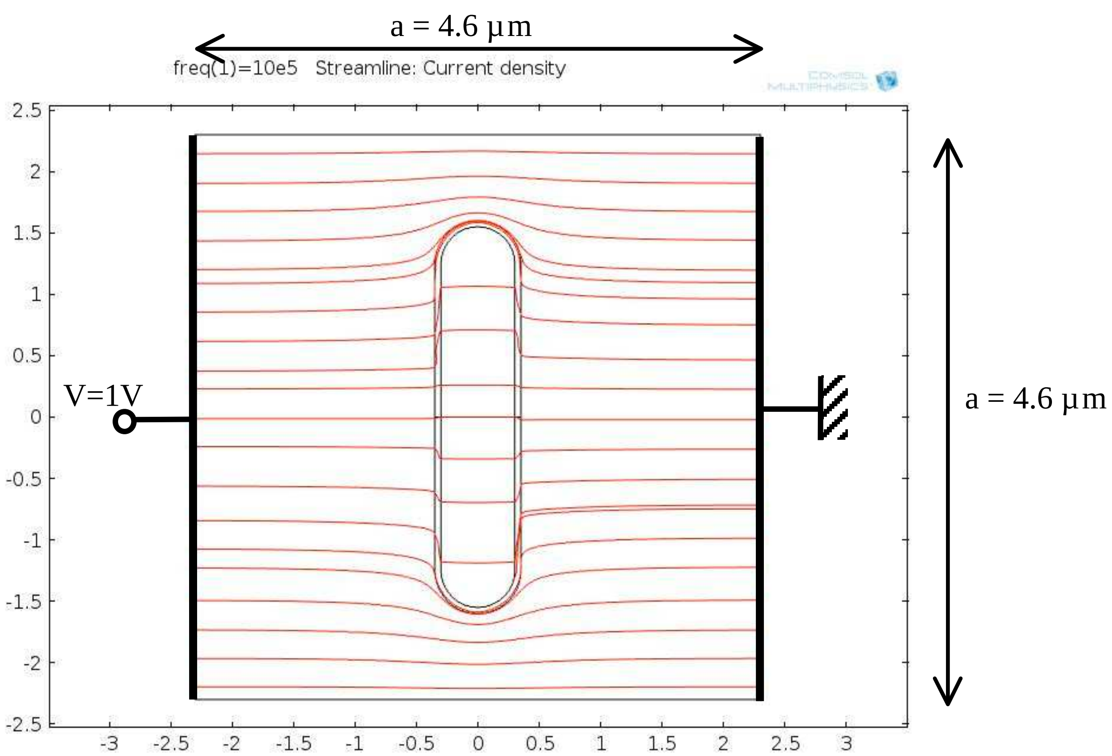
Transversal case :

Potentials imposed on yz planes ; Current streamlines plotted in xz plane



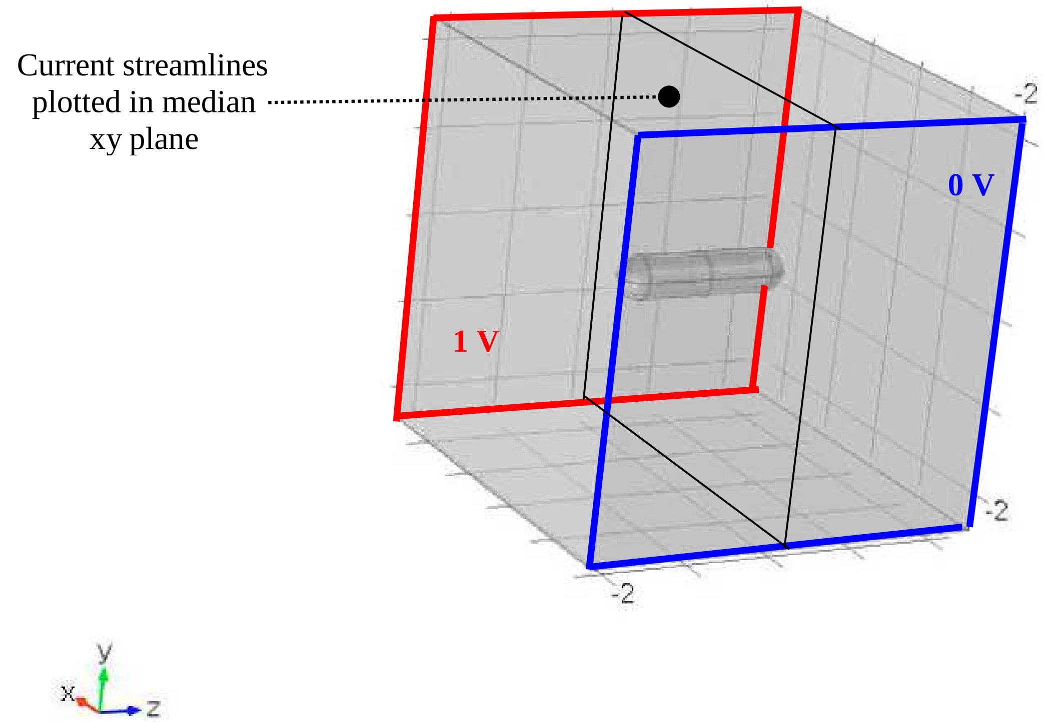


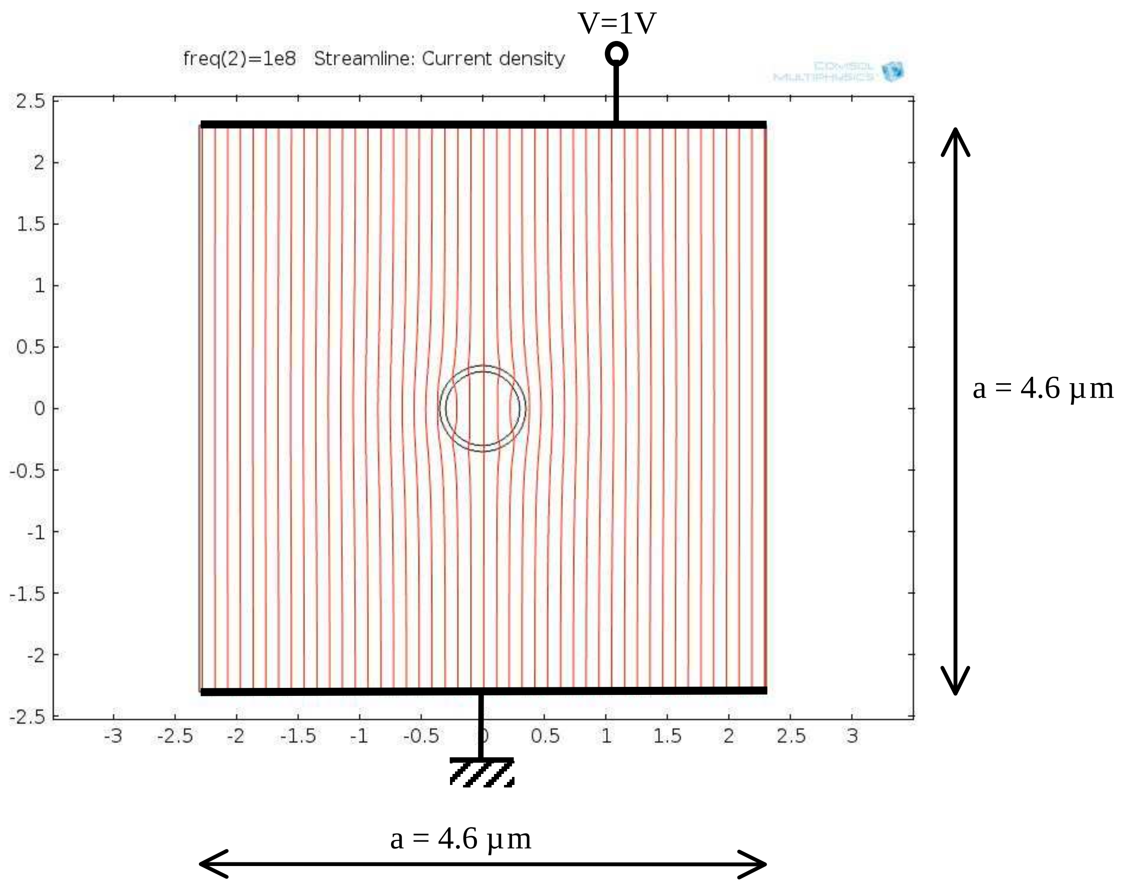
F = 100 MHz – Current streamlines pass through the bacterium



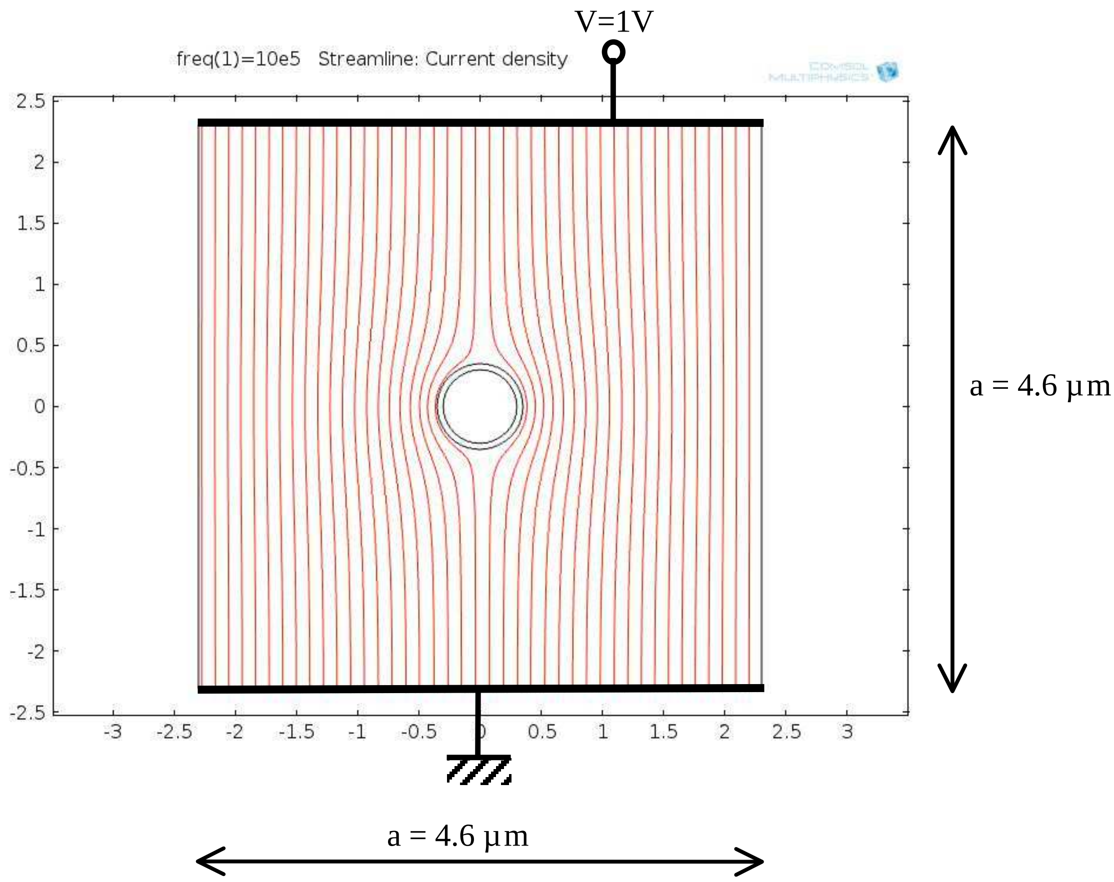
F = 1 MHz – Current streamlines partially bypass the bacterium

Potentials imposed on yz planes ; Current streamlines plotted in median xy plane





F = 100 MHz – Current streamlines pass through the bacterium

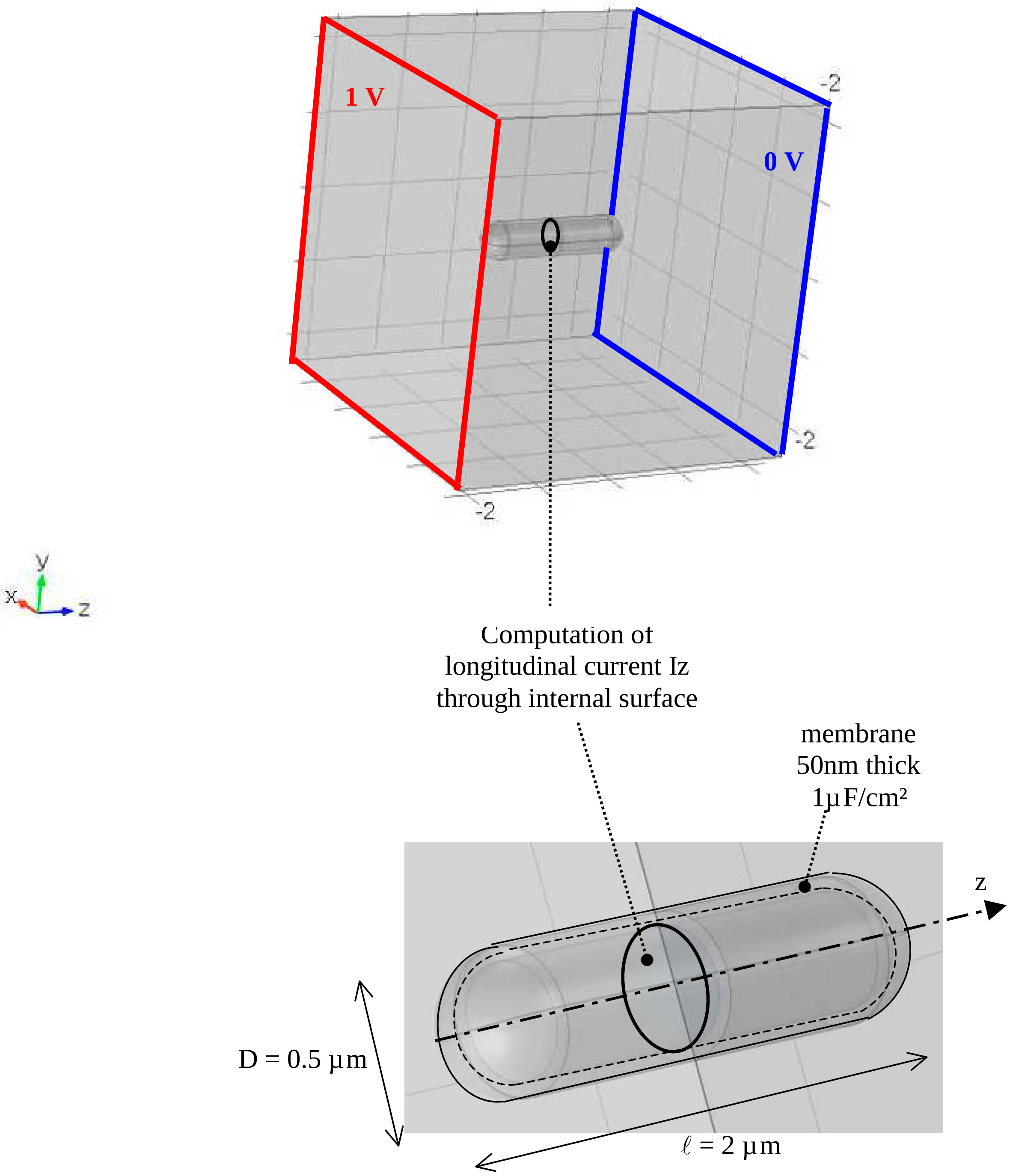


F = 1 MHz – Current streamlines bypass the bacterium

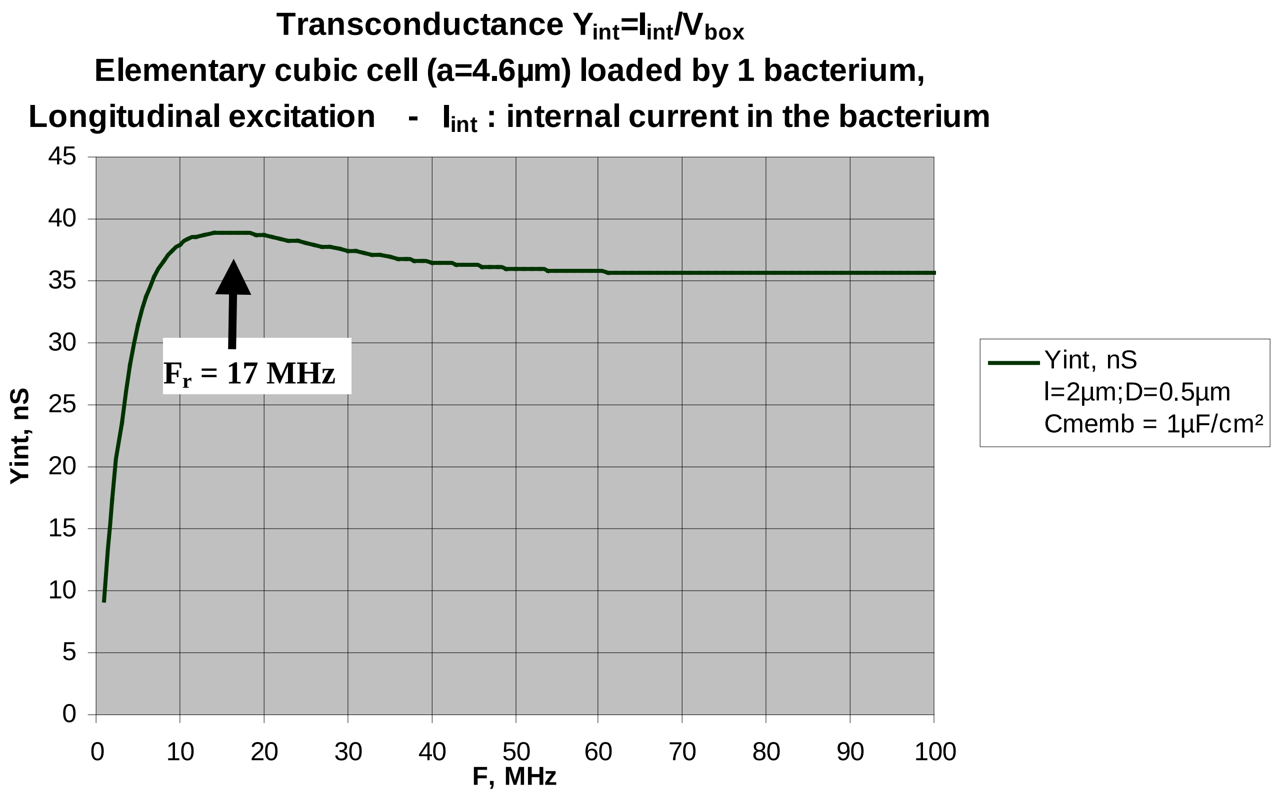
⇨ **Elementary cubic cell side a = 4.6 µm is suitable for transversal excitation**

Study of Longitudinal Excitation

3. Standard case :



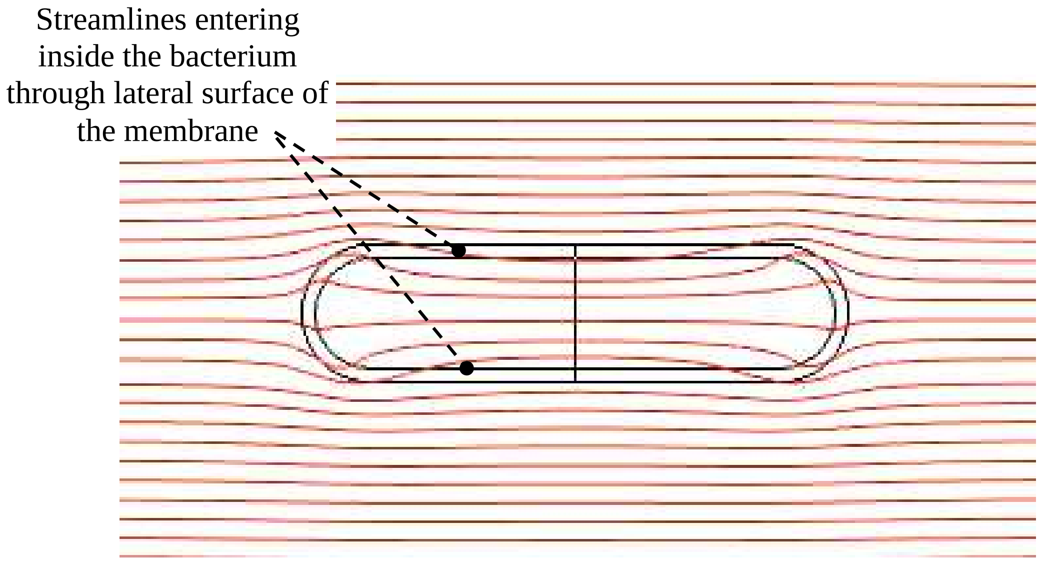
a - Numerical result (COMSOL Multiphysics):



⇨ High-pass behaviour due to the well known shield effect of the membrane

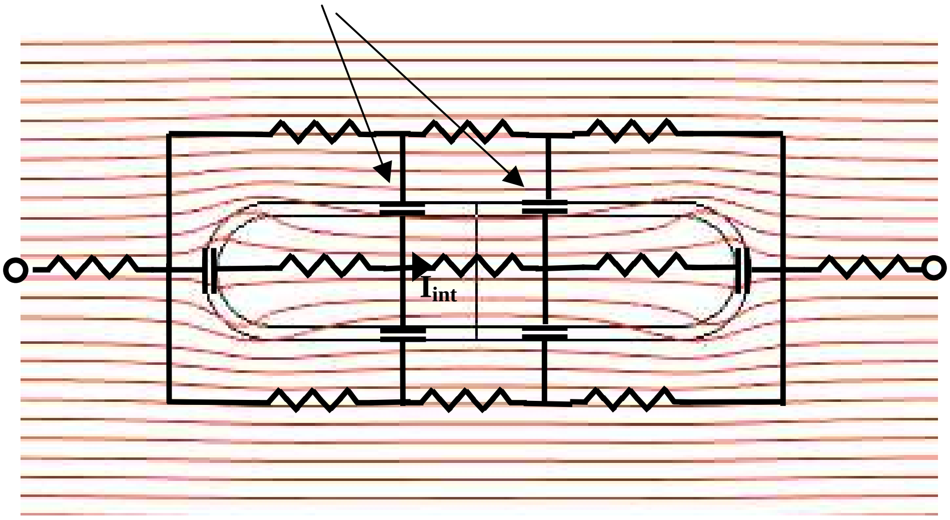
⇨ Appearance of a resonant frequency F_r = 17 MHz

b - Physical interpretation and equivalent network:

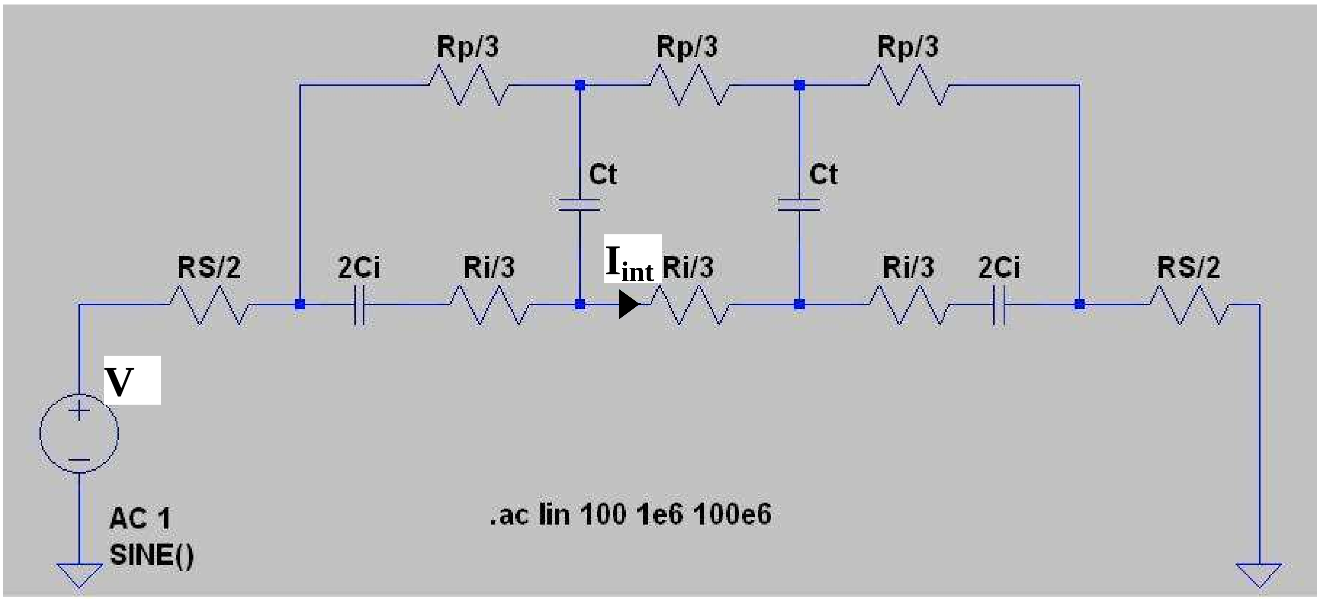


Current streamlines @ F_r = 17 MHz

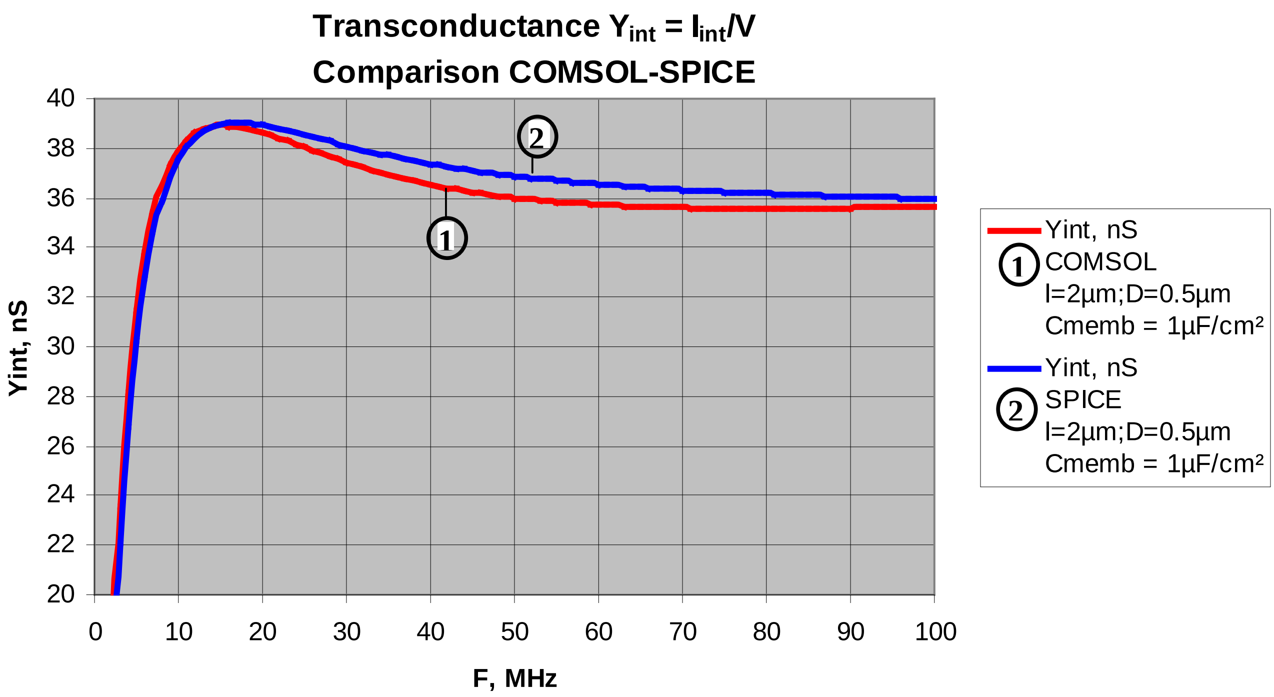
⇨ Transversal capacitor C_t must be added in the equivalent network to describe lateral charges transfers through the membrane in AC mode.



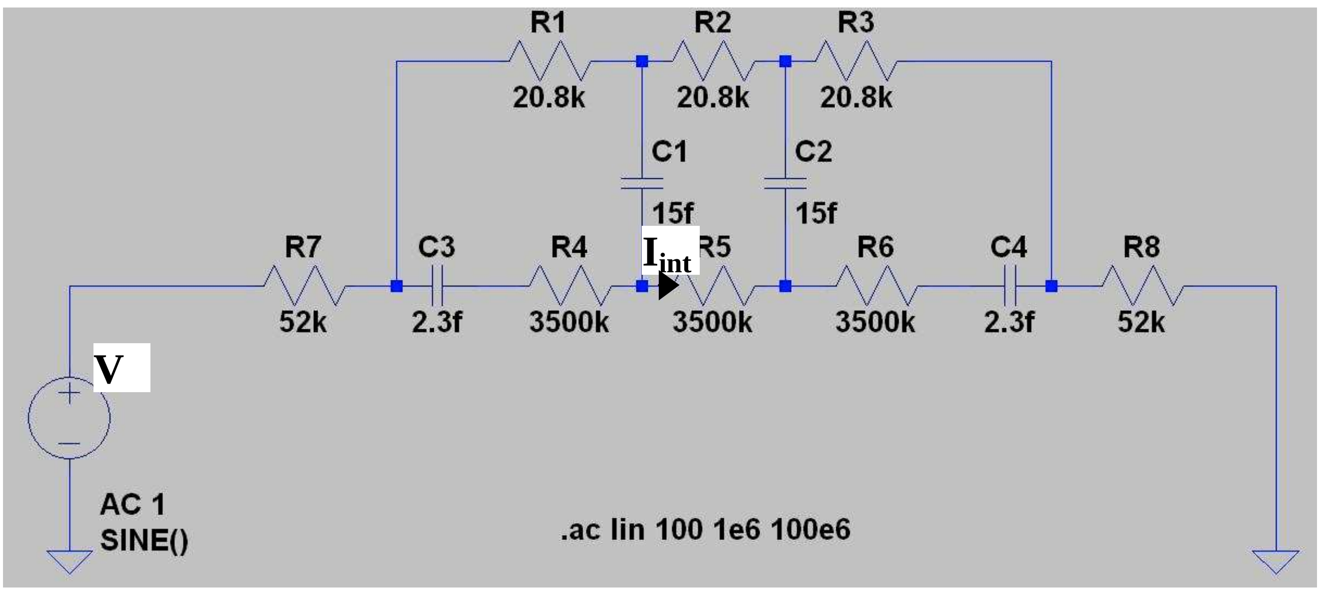
⇨ Final equivalent network, suitable for longitudinal excitation (to be analysed by means of SPICE software):



c - **Identification & extraction of element's values of the equivalent network:**



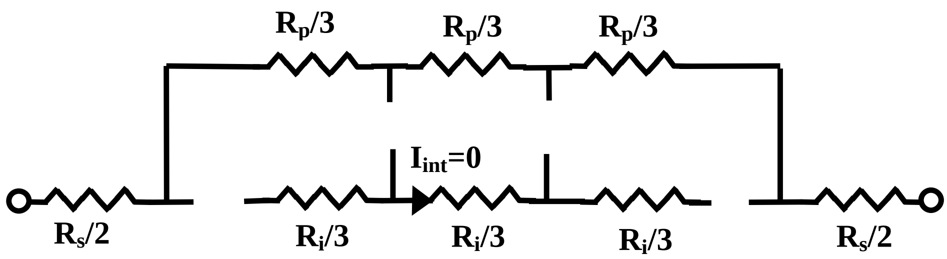
⇨ Result of identification iterative processus:



	Bacterium in elementary cubic cell a = 4.6 µm (realistic shape)	**Bacterium alone (cylindric shape)**
Ri	10.5 MΩ	12 MΩ
Rs	104 kΩ	-
Rp	62.4 kΩ	-
Ci	1.15 fF	0.87 fF
Ct	15 fF	-

d **Particular cases :**

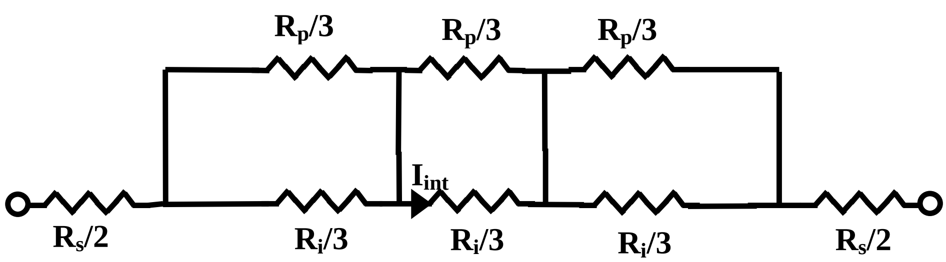
- **Frequency F➜ 0**



⇨ The membrane acts as an open circuit (shield effect) ➪ $I_{int} = 0$

⇨ **Y_{int} = 0**

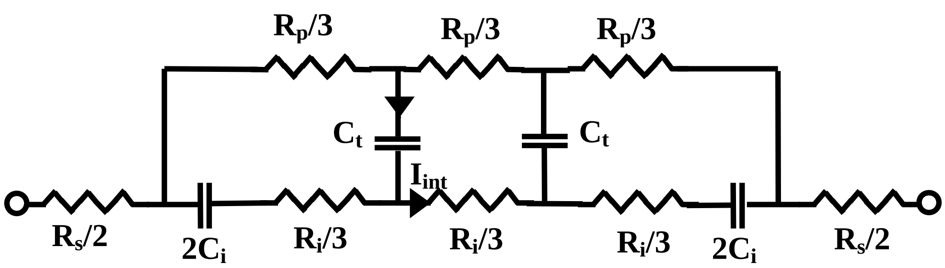
- **Frequency F➜ ∞**



⇨ The membrane acts as a short circuit (shield effect no longer effective)

⇨ **Y_{int} converges to a value depending on R_p, R_s, R_i**

- **Intermediate Frequency**



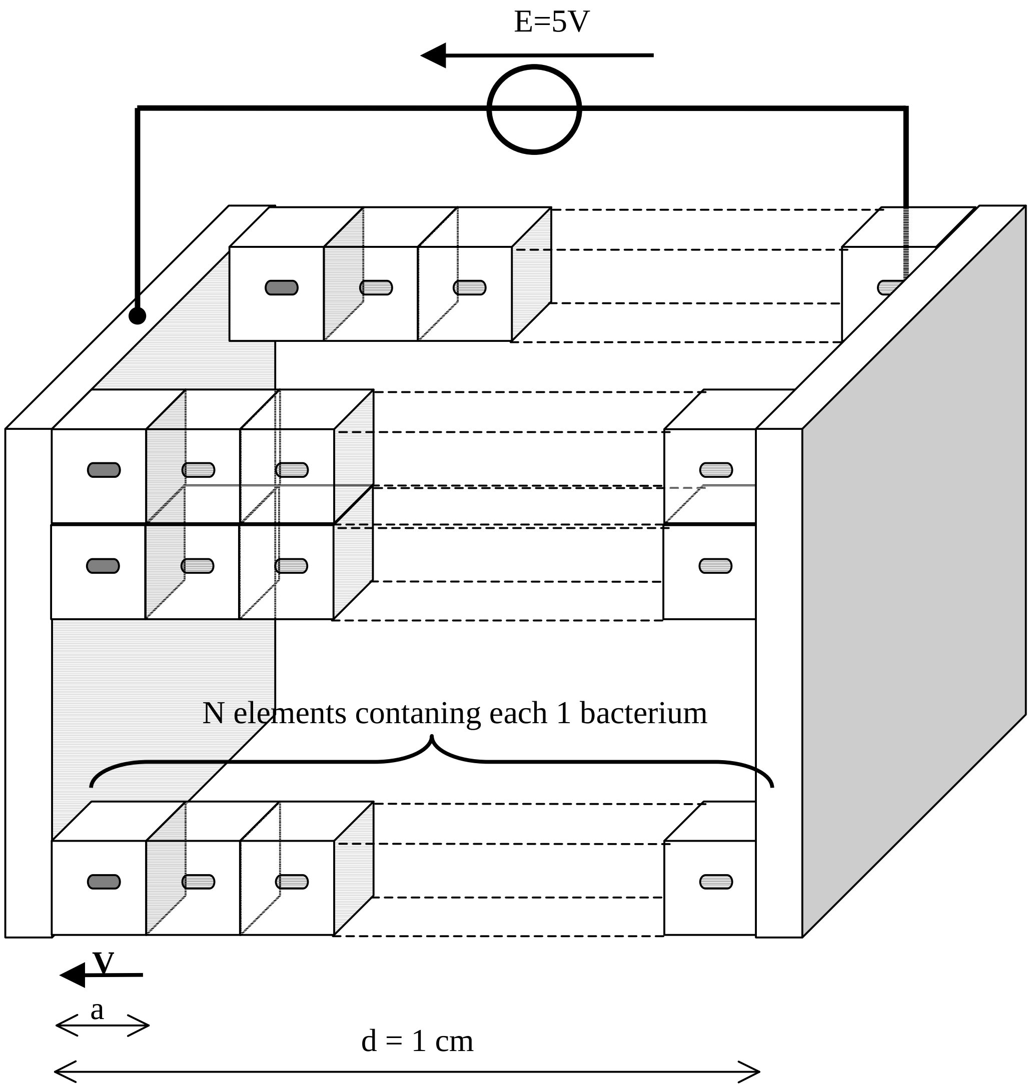
⇨ Internal current I_{int} is mainly supplied by current in capacitors C_t (>>C_i)

⇨ **Y_{int} is maximal for a resonant frequency F_r**

e – Macroscopic scale:

<u>Example of Setup :</u>

- Medium of suspended bacterial cells
- Located between two parallel plate electrodes
- Separated by a distance d =1cm
- Submitted to a voltage E = 5V



Equations :

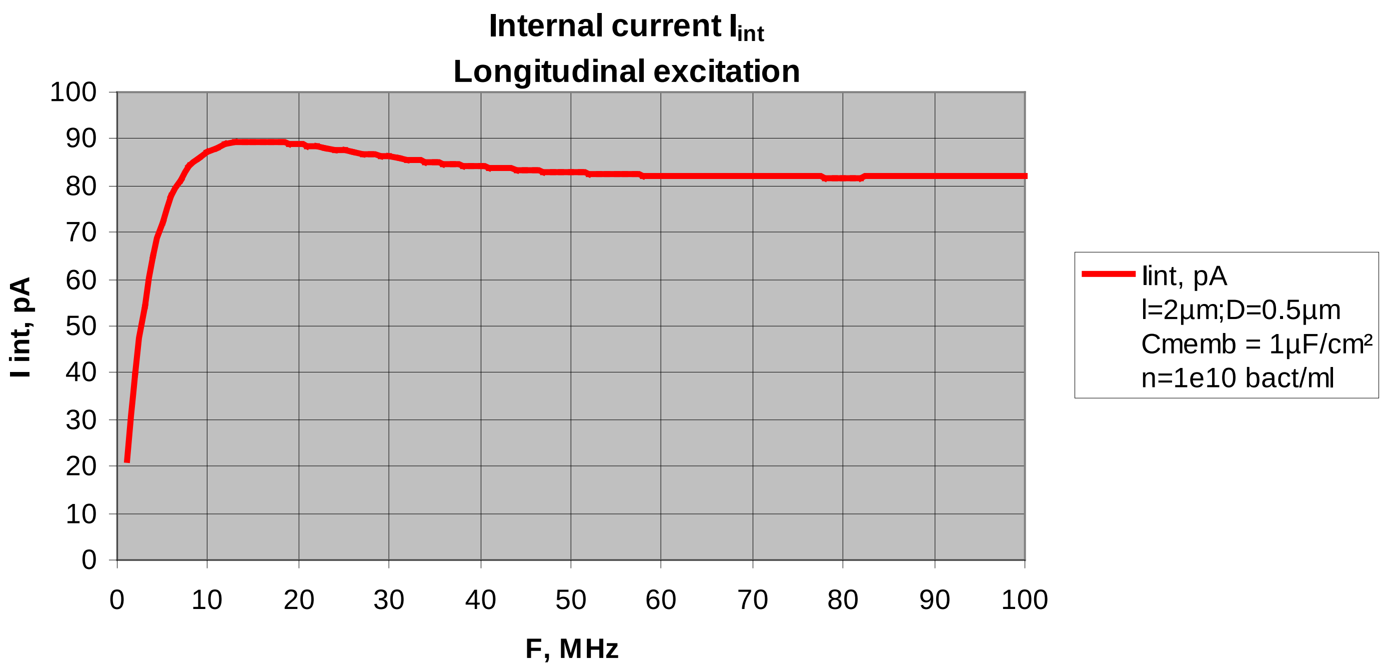
- $I_{int} = Y_{int} \cdot V$: Internal current in each bacterial cell :

where :

- Y_{int} : Computed Transconductance
- $V = \frac{E}{N}$: Voltage applied on one cubic element
- $N = \frac{d}{a}$: Number of elements contained in a d-long current tube
- $a = \frac{1}{n^{1/3}}$: Side of a cubic element depending on concentration n [bact/m^3]

for n = 10^{10} bact /ml ➪ a = 4.6 µm

Results :

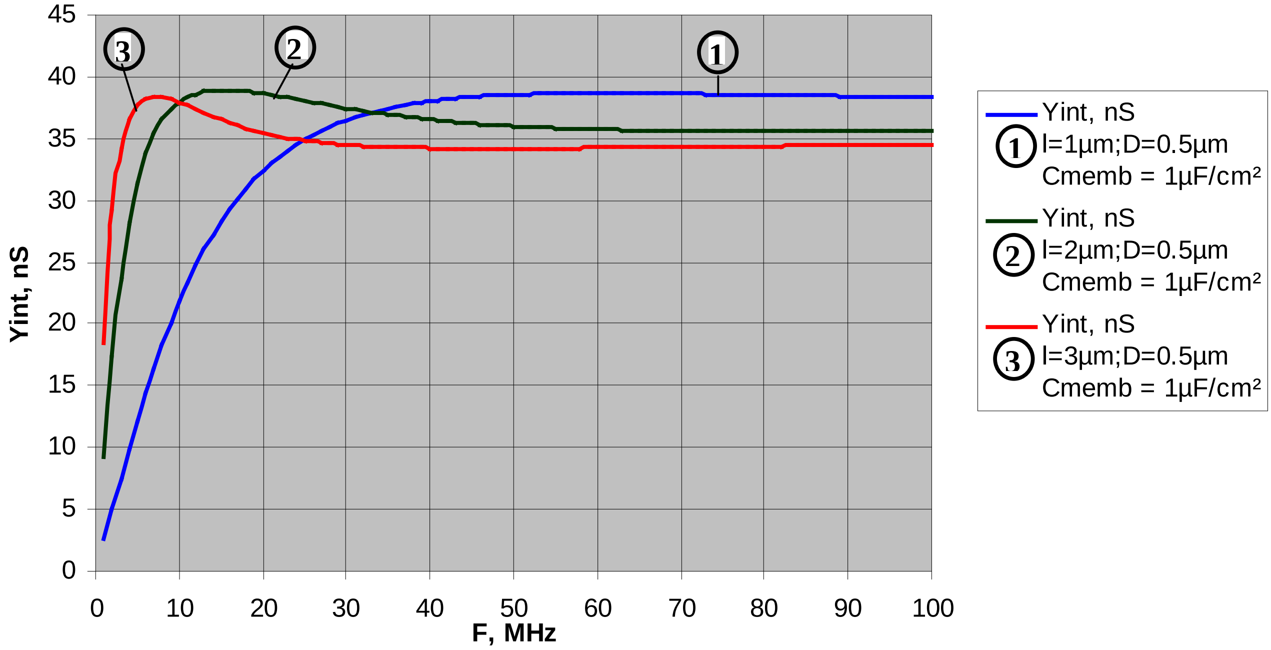


⇨ Order of magnitude of the maximal value to be compared with the current due to charges transfers during the metabolism of the living cell

4. Influence of Bacterium's length :

We assumed that 1µm < ℓ < 3 µm

Transconductance $Y_{int}=I_{int}/V_{box}$
Elementary cubic cell (a=4.6µm) loaded by 1 bacterium,
Longitudinal excitation - I_{int} : internal current in the bacterium

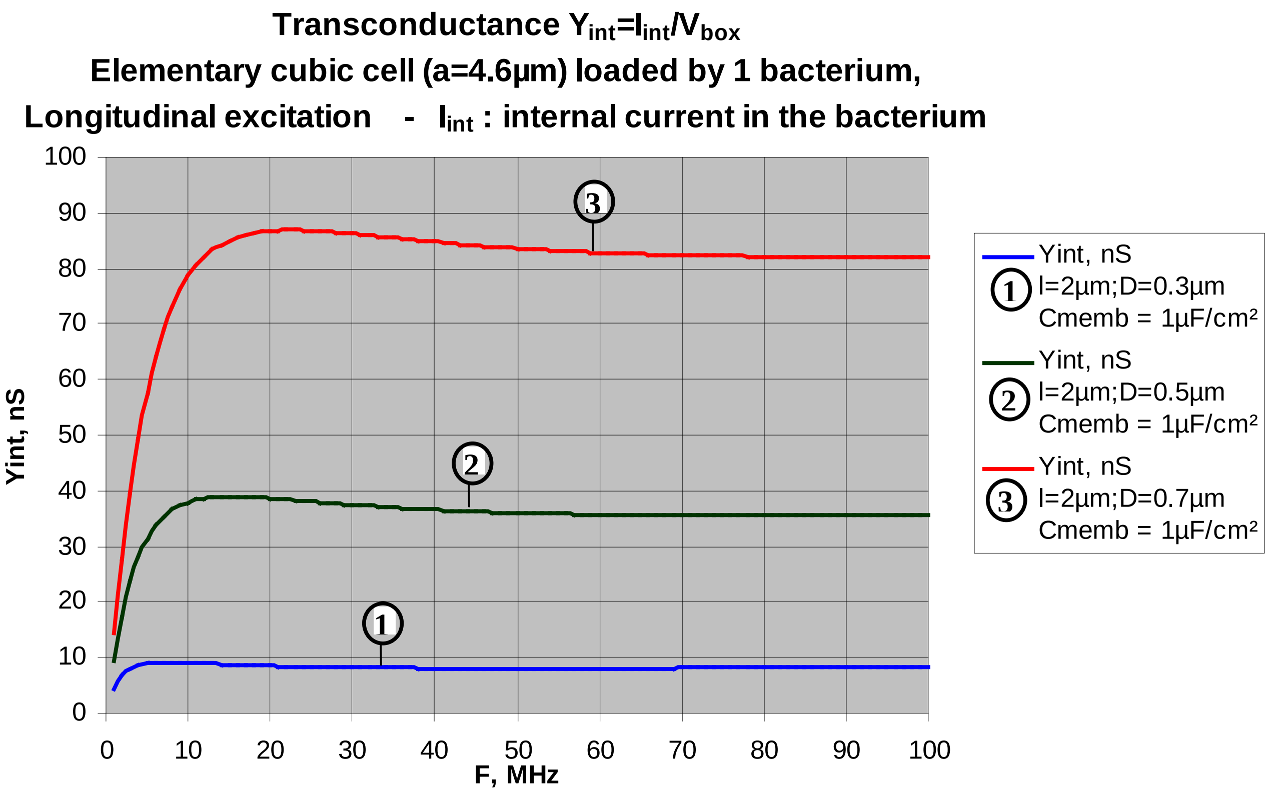


⇨ Resonance frequency is lowered when length increases, mainly due to the increased resistance R_i:

Bacterium's length	Frequency resonance
1 µm	63 MHz
2 µm	17 MHz
3 µm	8 MHz

5. *Influence of Bacterium's diameter :*

We assumed that 0,3µm < D < 0,7 µm



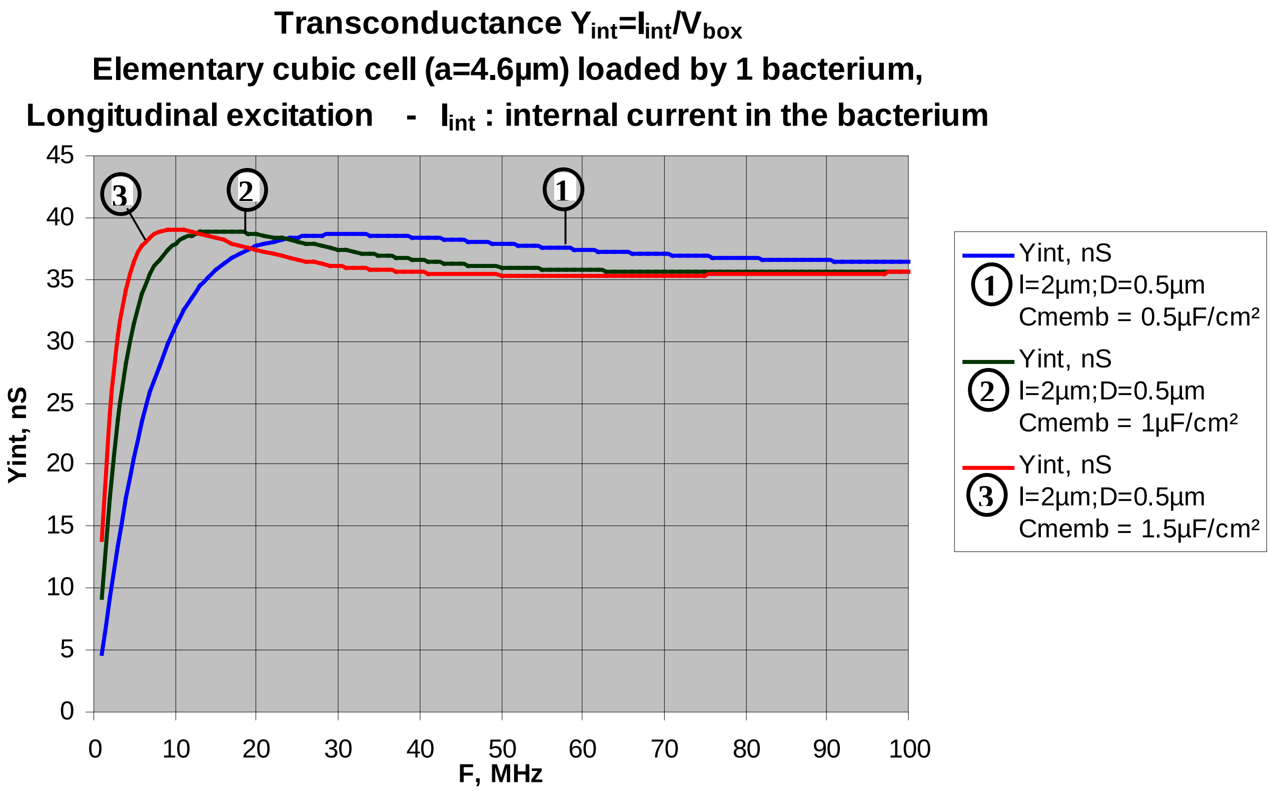
⇨ Resonance frequency is increased when diameter increases, mainly due to the lowered resistance R_i:

⇨ High frequency Y_∞ is increased when diameter increases, mainly due to the lowered resistance R_i:

Bacterium's diameter	Frequency resonance
0,3 µm	10 MHz
0,5 µm	17 MHz
0,7 µm	23 MHz

6. *Influence of membrane surface capacitance :*

We assumed that 0,5 µF/cm² < C < 1,5 µF/cm²

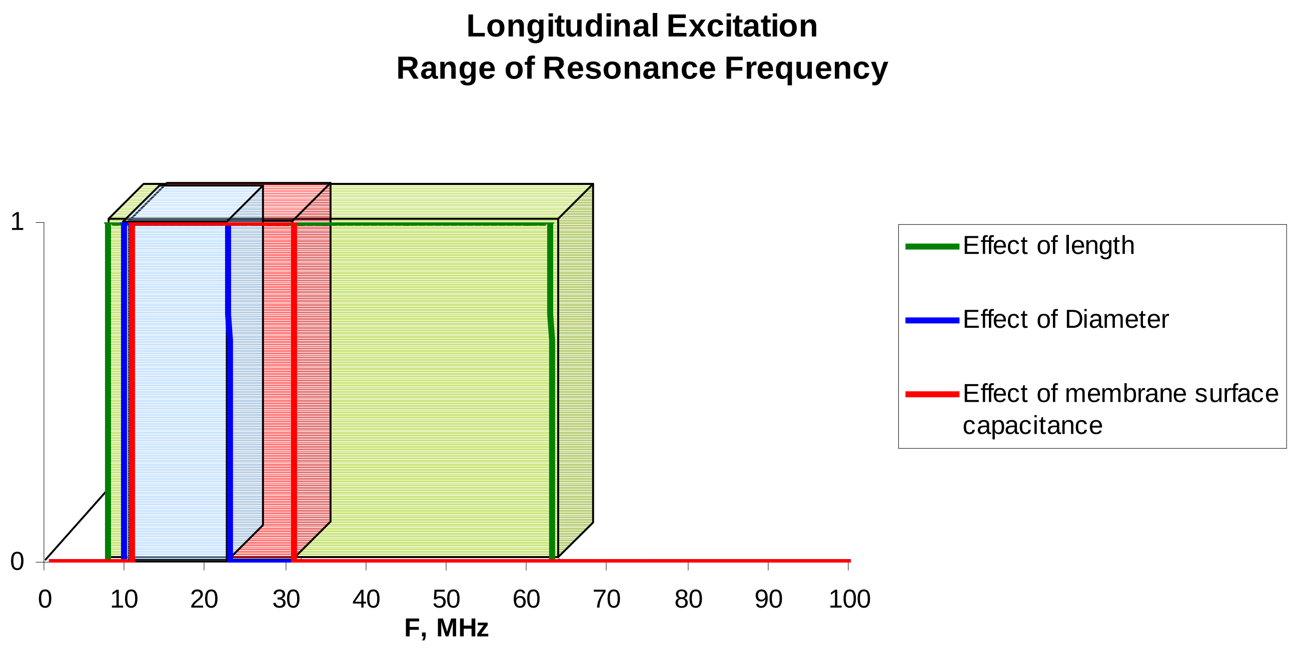


⇨ Resonance frequency is lowered when membrane surface capacitance increases, mainly due to the increased capacitance C_i:

Membrance surface capacitance	**Frequency resonance**
0,5 µF/cm²	31 MHz
1 µF/cm²	17 MHz
1,5 µF/cm²	11 MHz

7. Stochastic approach :

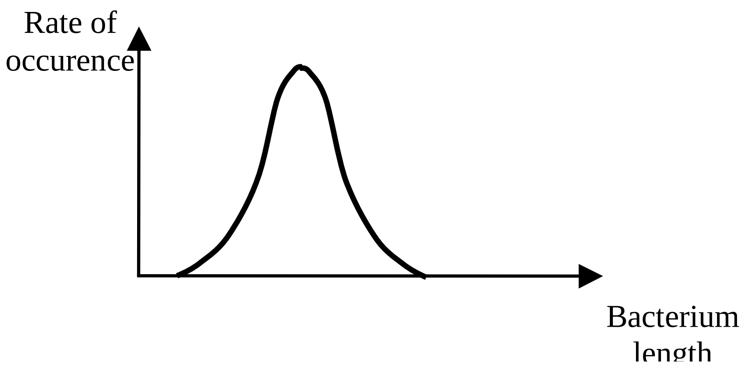
First step : Uniform Distribution of Probability for Parameters



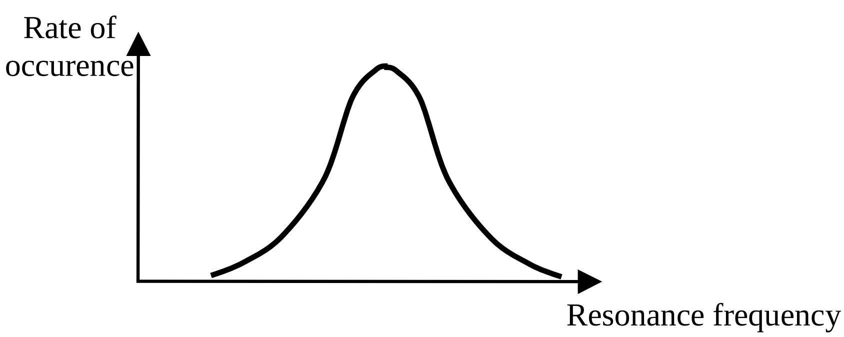
⇨ The effect of length could be the most significant

Further study :

Distribution law of length values (from biological data)



⇨ Distribution of Resonance Frequencies

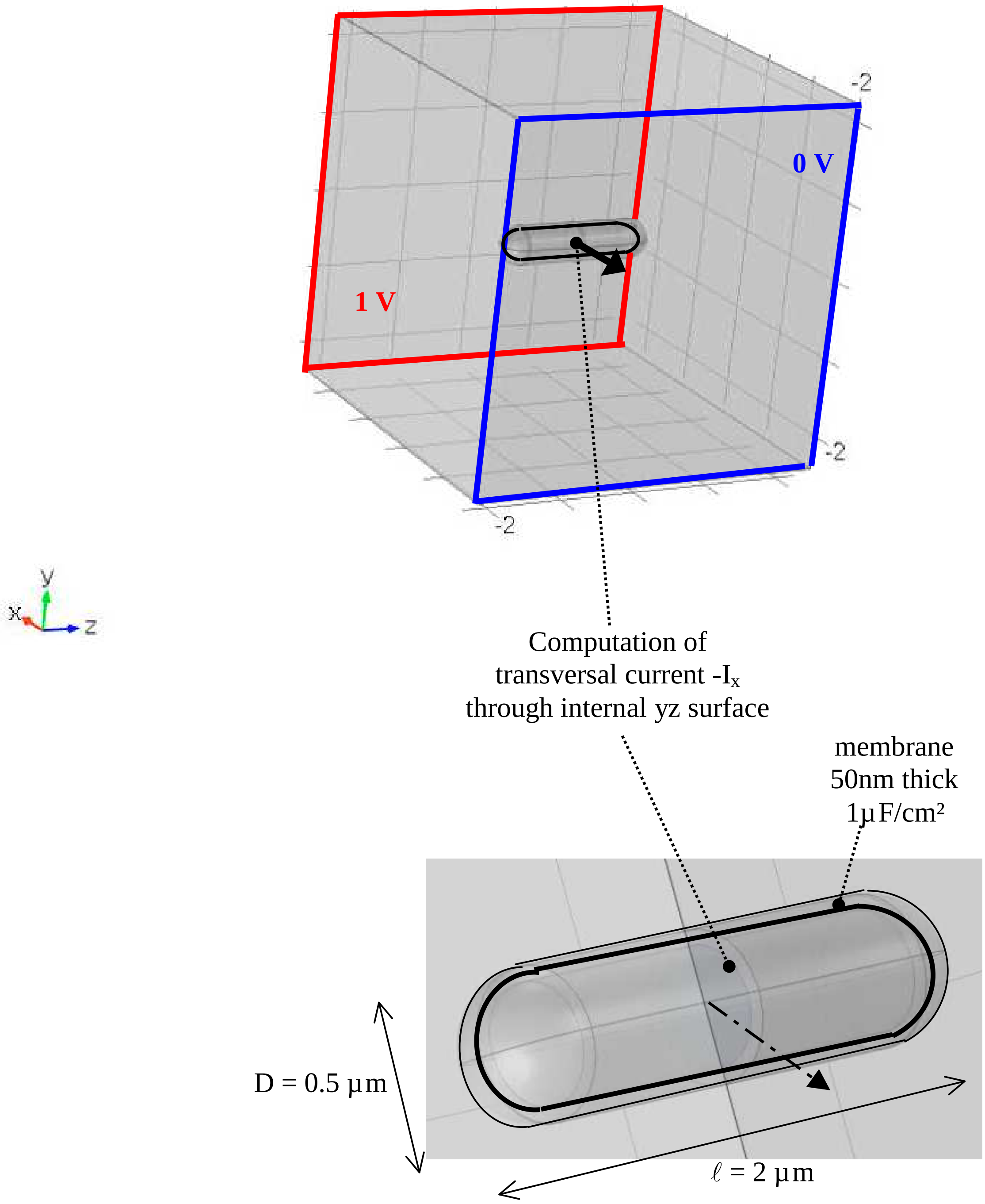


Study of Transversal Excitation

1. Configuration :

Potentials imposed on yz planes ;

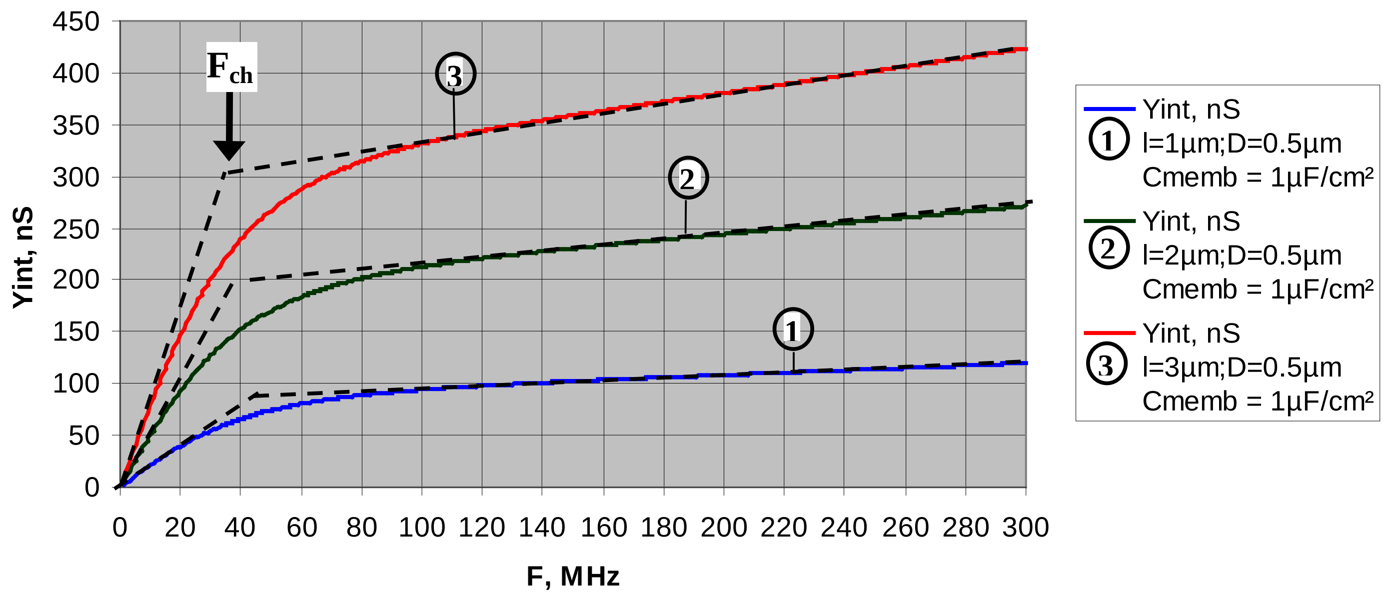
Computation of Current Ix through the internal yz surface



2. *Influence of Bacterium's length :*

We assumed that 1µm < ℓ < 3 µm

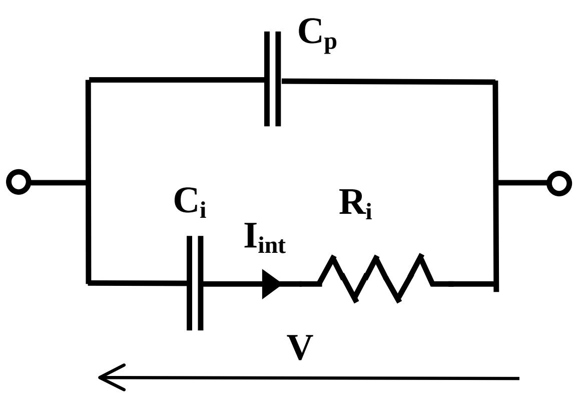
Transconductance $Y_{int}=I_{int}/V_{box}$
Elementary cubic cell (a=4.6µm) loaded by 1 bacterium,
Transversal excitation - I_{int} : internal current in the bacterium



⇨ The resonance no longer occurs.

⇨ This could be explained because transversal capacitors Ct become negligible in transversal mode

⇨ Equivalent network similar to that of preliminary study (bacterium alone):



But with very slow convergence when F → ∞ due to weak value of C_p

⇨ Y_{int} increased as the lenght increases, due to augmentation of transversal surface.

We specify a “Characteristic Frequency” F_{ch}, corresponding to the intersection point between both initial and final slopes (valid in the range 0-300 MHz):

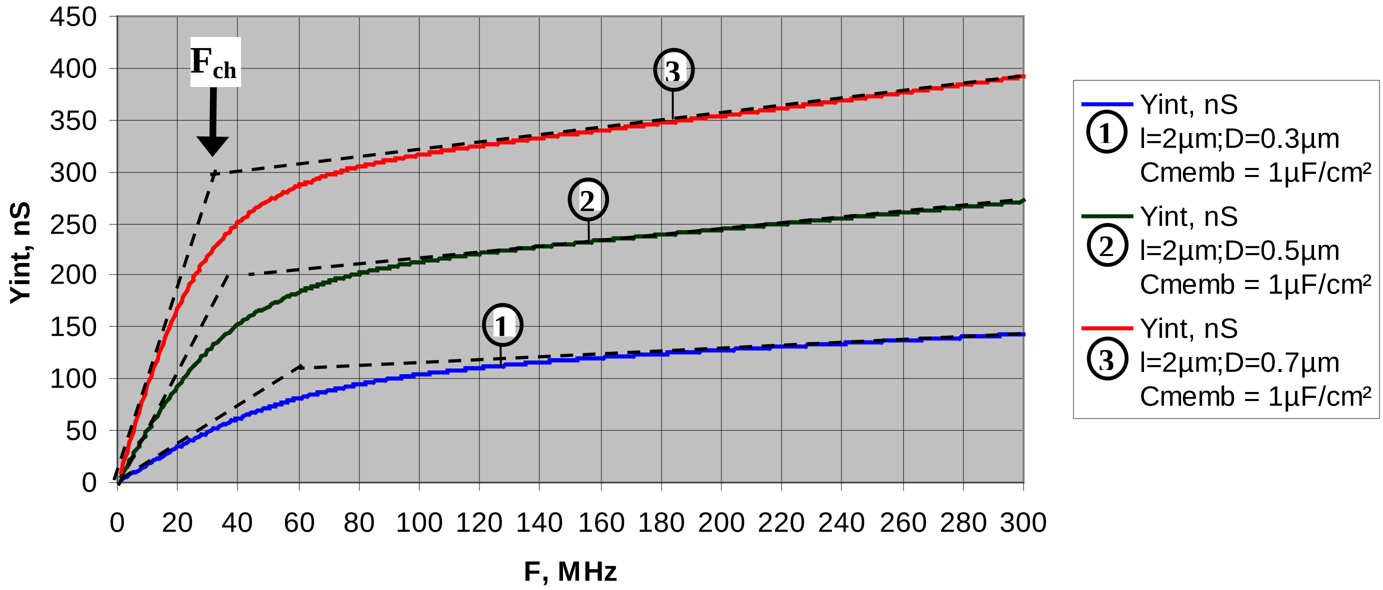
Bacterium’s length	Characterisitic Frequency
1 µm	45 MHz
2 µm	38 MHz
3 µm	35 MHz

⇨ F_{ch} is lowered as the lenght increases, due to augmentation of resistance Ri.

3. Influence of Bacterium’s diameter :

We assumed that 0,3µm < D < 0,7 µm

Transconductance $Y_{int}=I_{int}/V_{box}$
Elementary cubic cell (a=4.6µm) loaded by 1 bacterium,
Transversal excitation - I_{int} : internal current in the bacterium



⇨ Y_{int} increased as the diameter increases,
mainly due to augmentation of transversal surface.

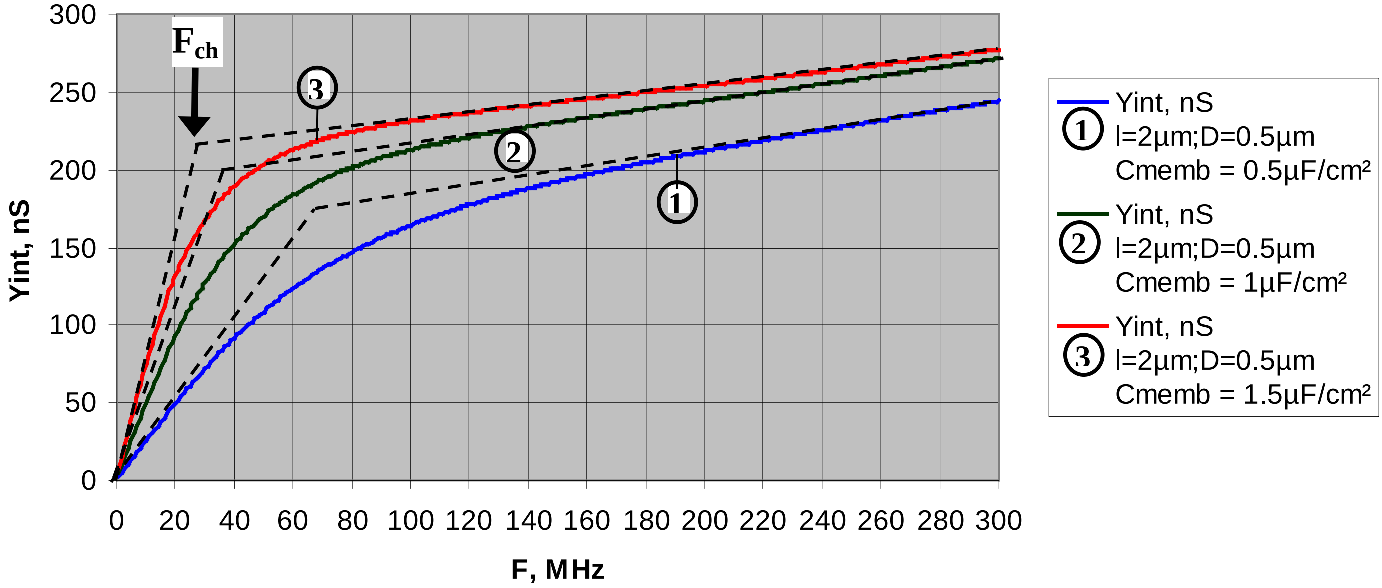
⇨ F_{ch} is lowered as the diameter increases,
mainly due to augmentation of capacitance Ci.

Bacterium's diameter	Charactrisitc Frequency
0,3 µm	60 MHz
0,5 µm	38 MHz
0,7 µm	32 MHz

4. Influence of membrane surface capacitance :

We assumed that 0,5 µF/cm² < C < 1,5 µF/cm²

Transconductance $Y_{int}=I_{int}/V_{box}$
Elementary cubic cell (a=4.6µm) loaded by 1 bacterium,
Transversal excitation - I_{int} : internal current in the bacterium

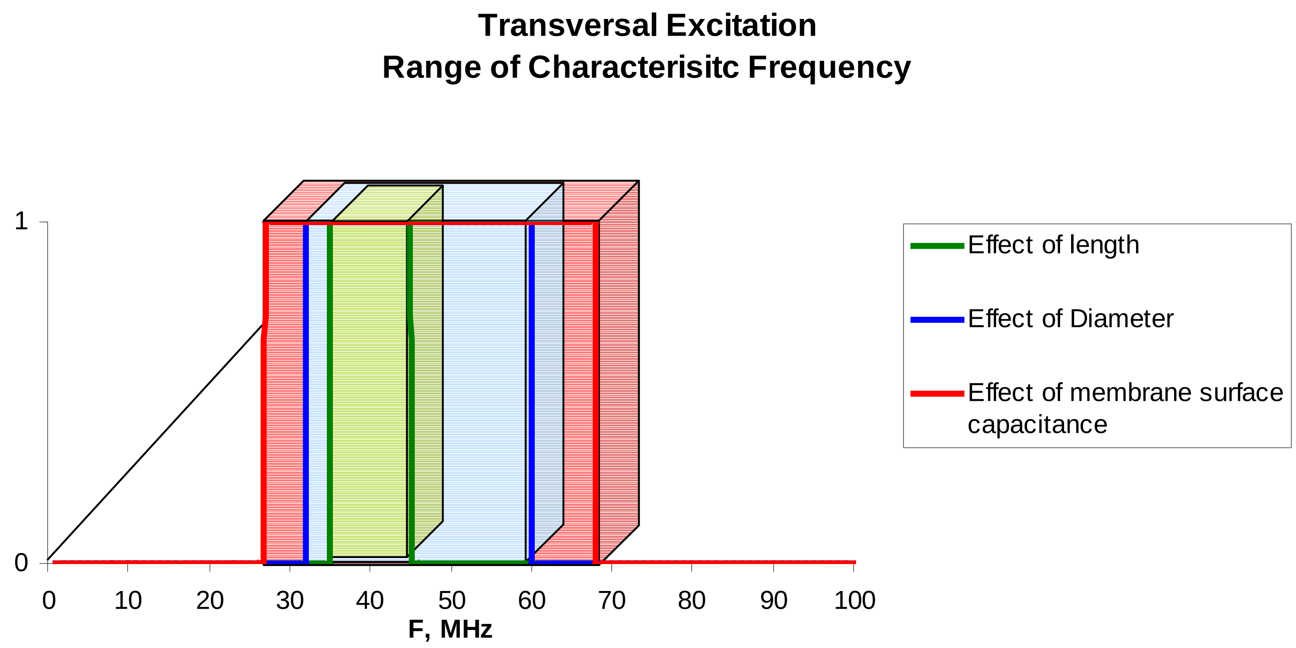


⇨ F_{ch} is lowered as the membrane surface capacitance increases, mainly due to augmentation of capacitance Ci.

Membrance surface capacitance	Characterisitc Frequency
0,5 µF/cm²	68 MHz
1 µF/cm²	38 MHz
1,5 µF/cm²	27 MHz

5. Stochastic approach :

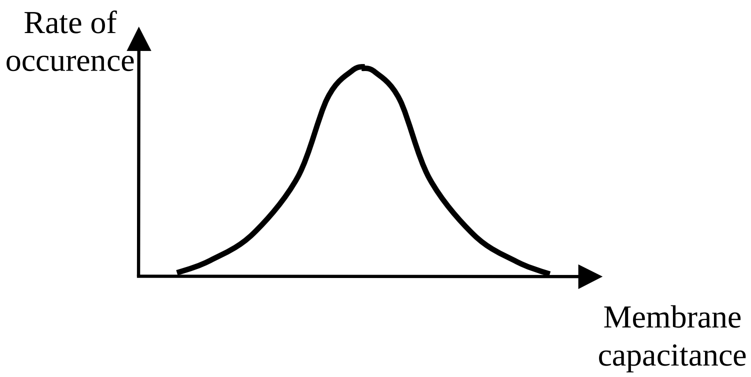
First step : Uniform Distribution of Probability for Parameters



⇨ The effect of membrane surface capacitance could be the most significant

Further study :

Distribution law of membrane capacitance values (from biological data)



⇨ Distribution of Characteristics Frequencies