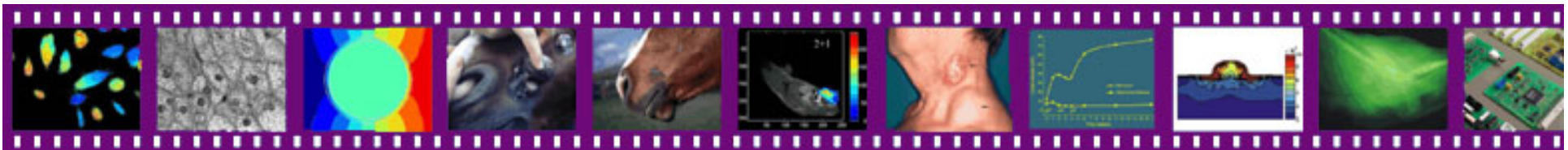


Elektroporacija u biologiji, biotehnologiji i medicini

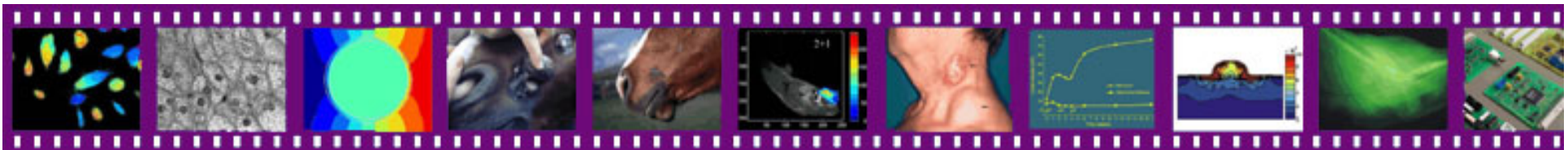


Damijan Miklavčič
University of Ljubljana
Faculty of Electrical Engineering
Ljubljana, SLOVENIA

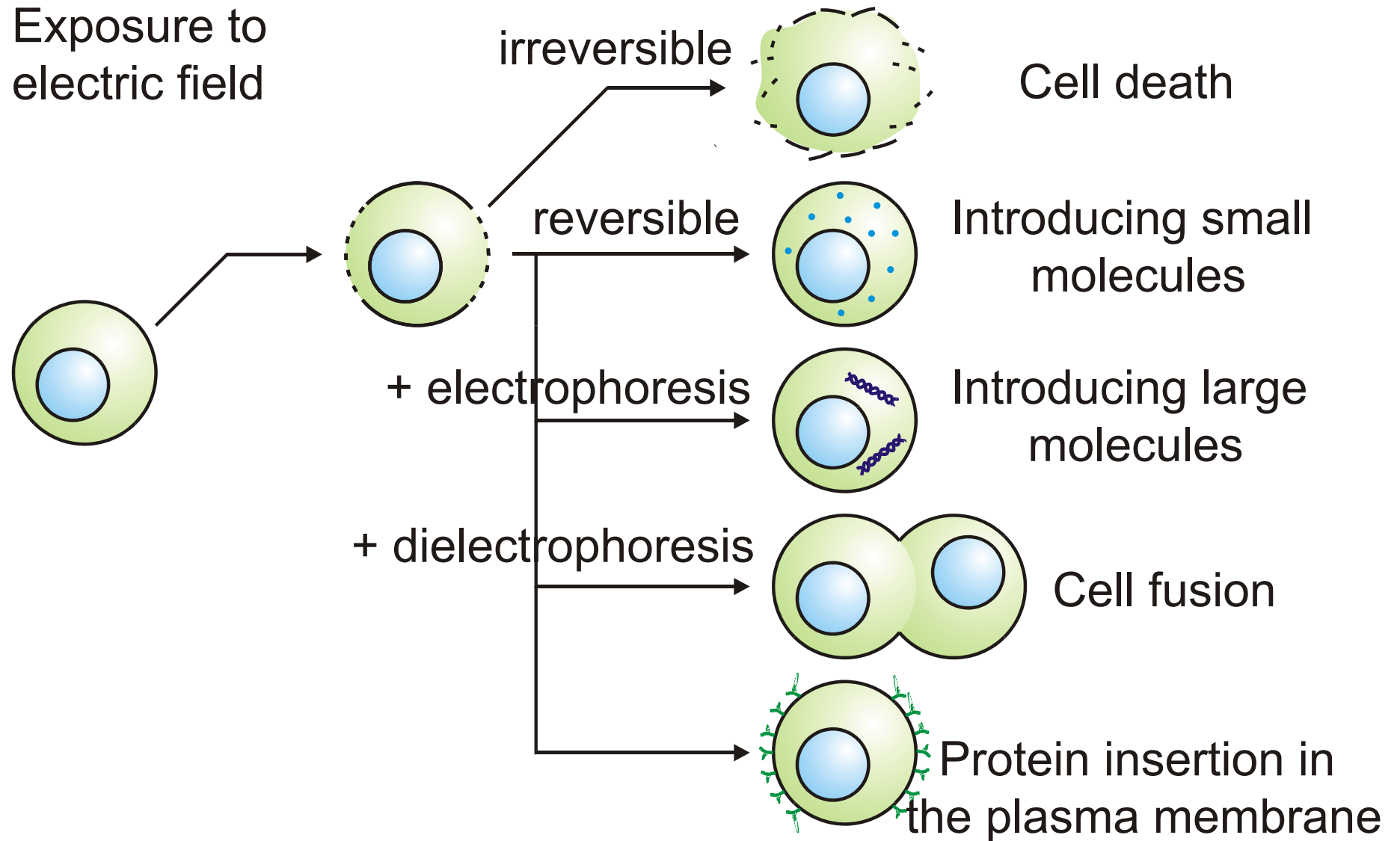


Aims and scopes

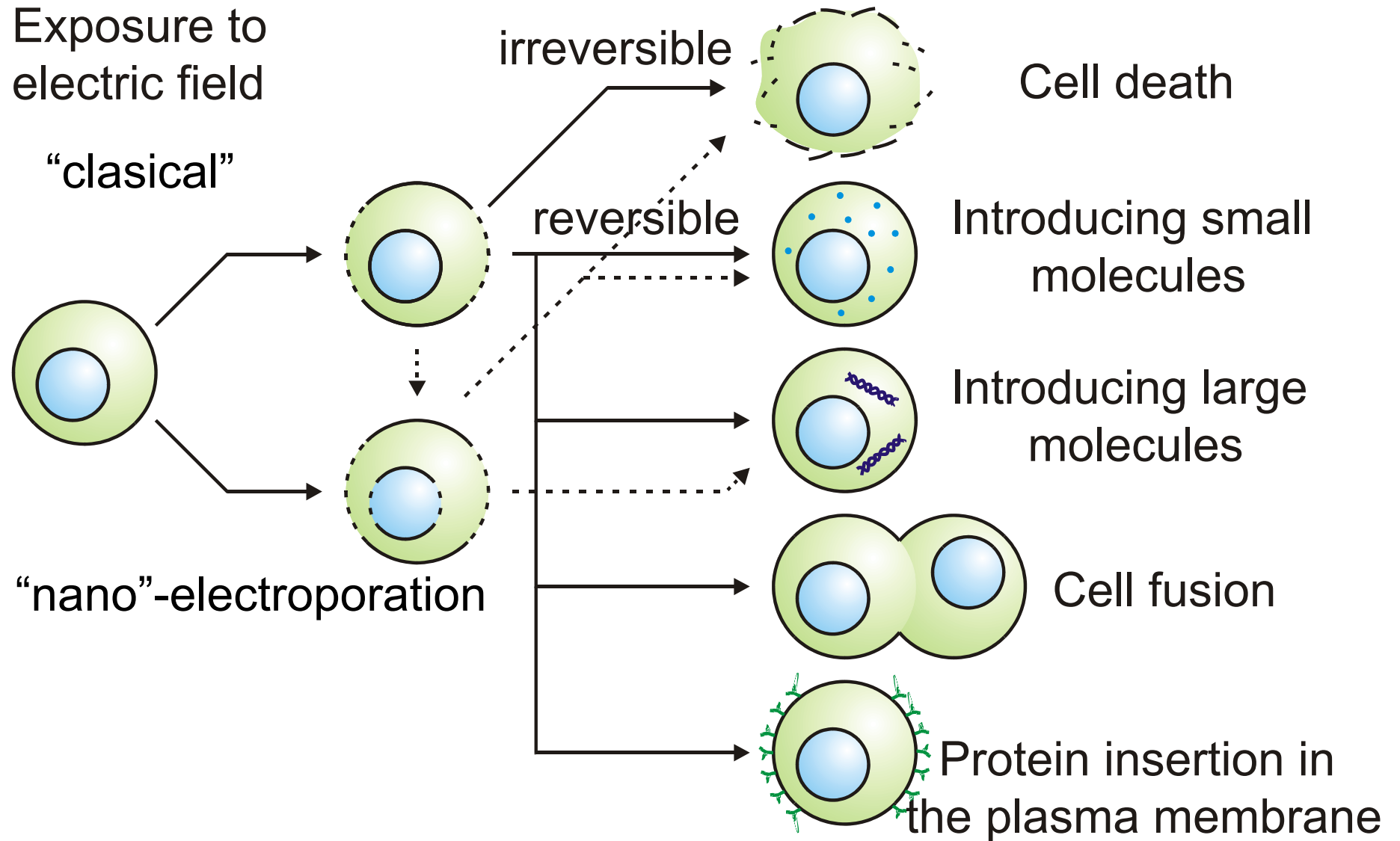
- What is electroporation?
- Use of electroporation in biology, biotechnology and medicine
- Electrochemotherapy
- Electrochemotherapy treatment planning



Applications of Electroporation



Applications of Electroporation



Patents and electroporation

- Electroporation is and active R&D field
- Number of patents/patent applications using keyword »**electroporation**« in PatentScope (<http://www.wipo.int/patentscope/search/en/search.jsf>)
 - Looking for keyword in abstract or description of patent application (EN_DE:electroporation OR EN_AB:electroporation) is **27 510** for 1999 to 2009 time span.

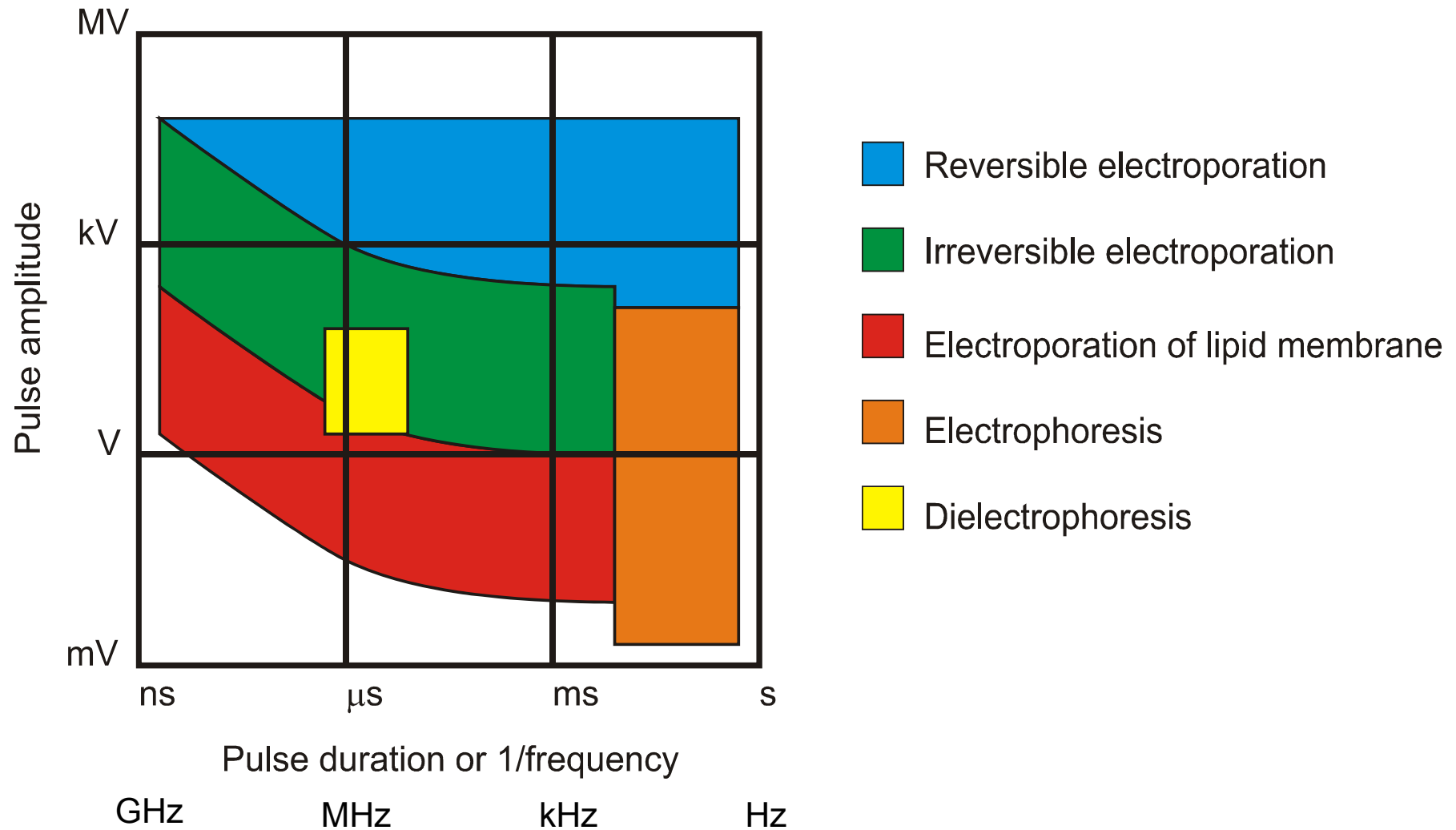
Electroporation based Technologies and Treatments

- Molecular cell biology research
- Electrochemotherapy
- Electrogenetherapy
- Gene expression silencing by siRNA
- Transdermal drug delivery
- Protein insertion into cell membrane
- Cell fusion
- Biotechnology
- Water and liquid food sterilisation
- Tissue ablation

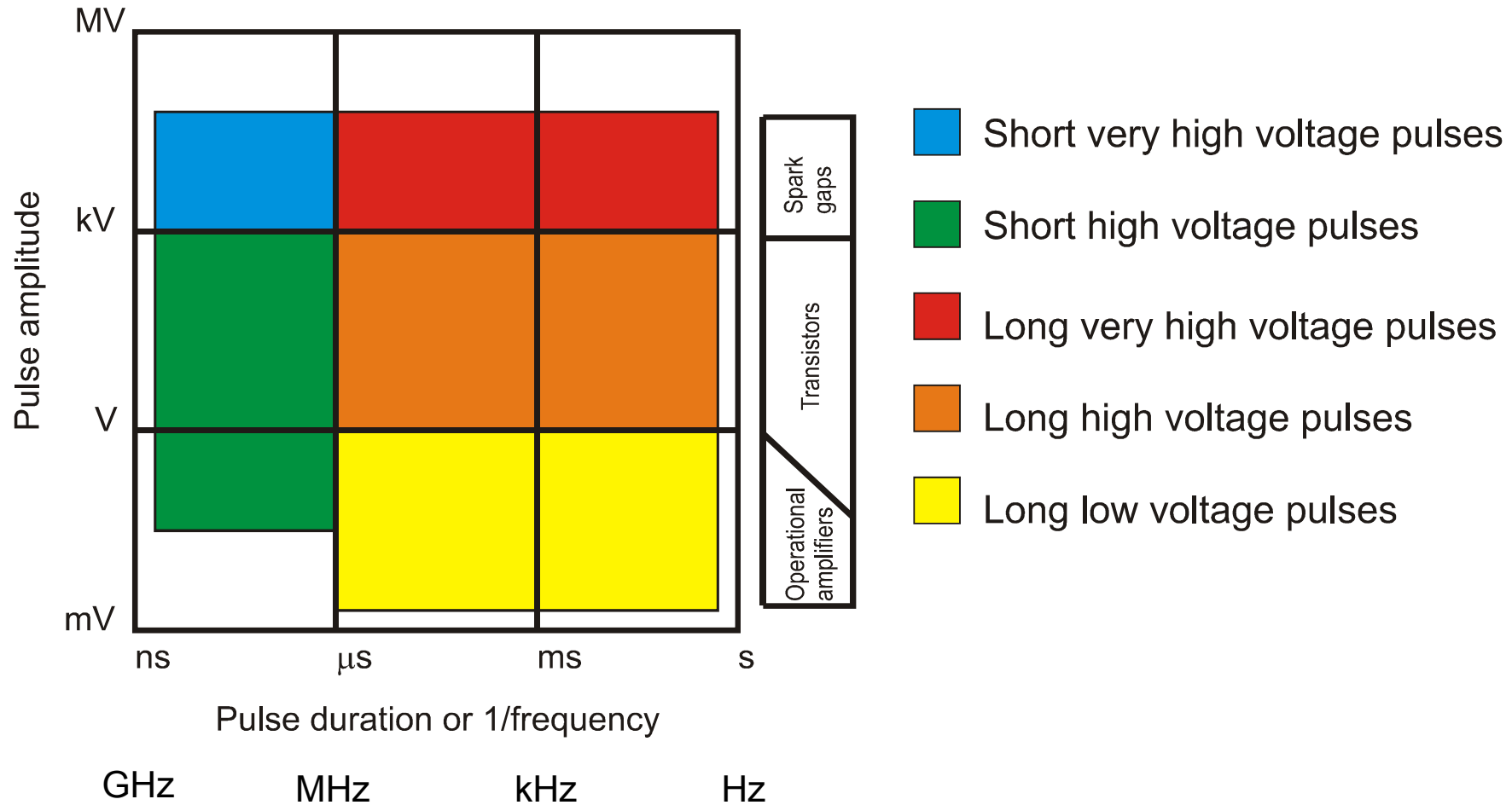
Electroporation based Technologies and Treatments

- Molecular cell biology research
- Electrochemotherapy ($8 \times 100 \mu\text{s}$, 1000 V)
- Electrogenetherapy ($1 - 20 \text{ ms}$, $< 500 \text{ V}$)
- Gene expression silencing by siRNA
- Transdermal drug delivery (200 V)
- Protein insertion into cell membrane
- Cell fusion
- Biotechnology
- Water and liquid food sterilisation (ms , 5 kV)
- Tissue ablation (ms , $> 2000 \text{ V}$)

Pulse amplitude and durations



Different principles of pulse generators and elements



Discharge of capacitor

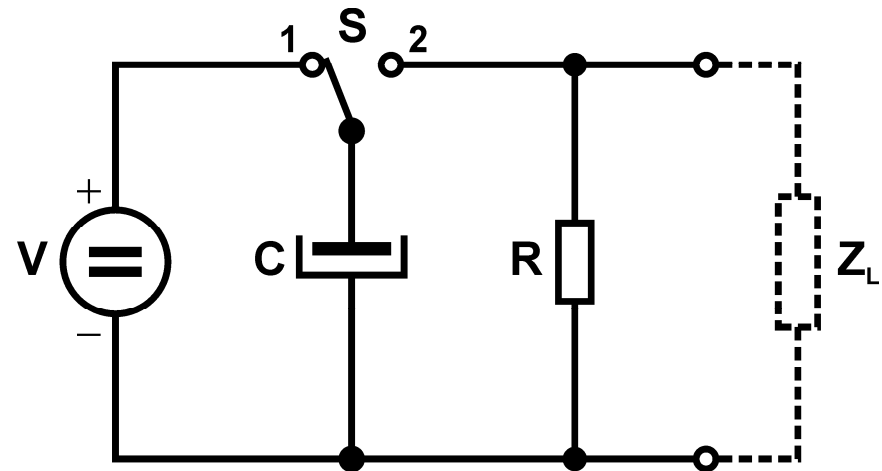
Generator of exponentially decaying pulses

Charge (S_1):

capacitor (C) charges to the preset voltage (V);

Discharge (S_2) – pulse delivery:

capacitor (C) discharges through the load (Z_L)



V - high-voltage power supply

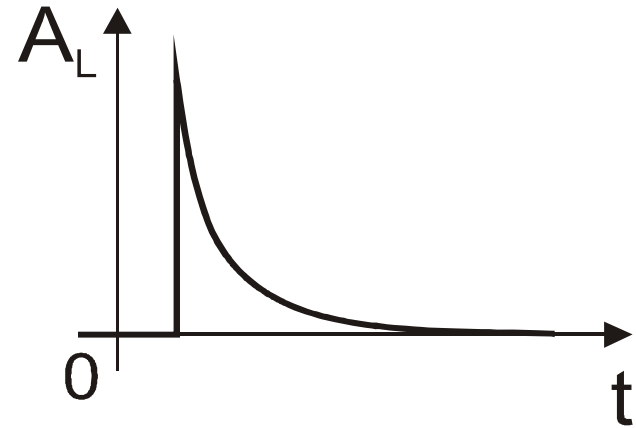
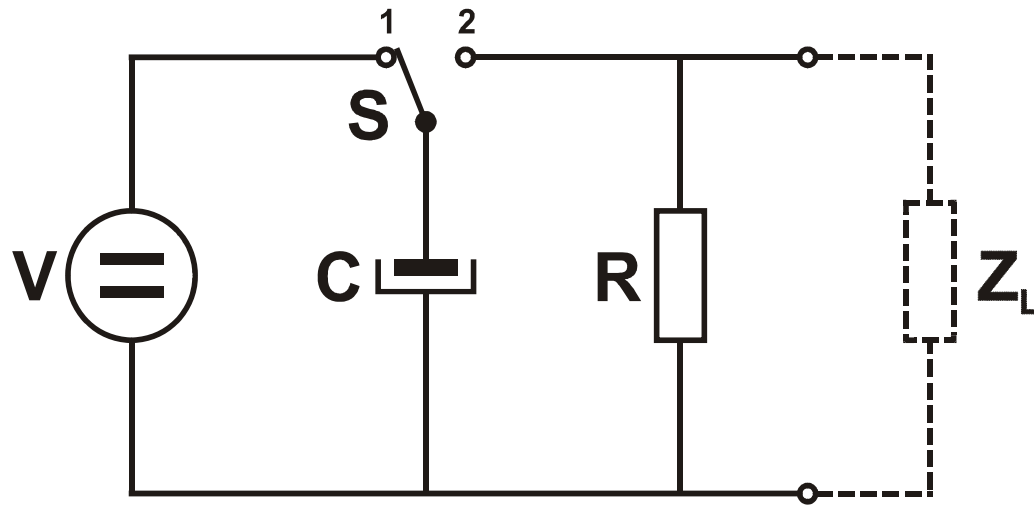
C – capacitor

S - switch

R - resistance (optionally)

Z_L - load

The current (I) is defined by the size of the load (Z_L).

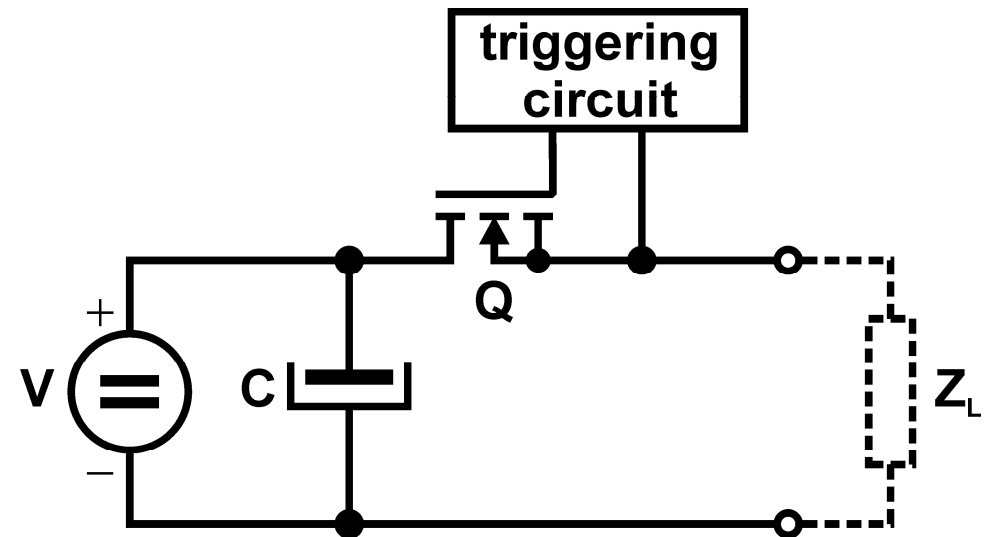


Switching high voltage power supply with power transistors

Generator of square wave pulses

The device consists of power supply part and pulse generator.

Triggering circuit generates low-voltage pulse, that opens transistor (Q) for the duration of the low-voltage pulse.

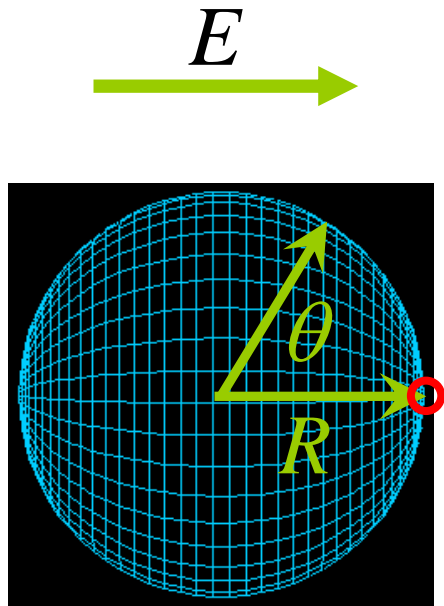


V - variable high-voltage power supply

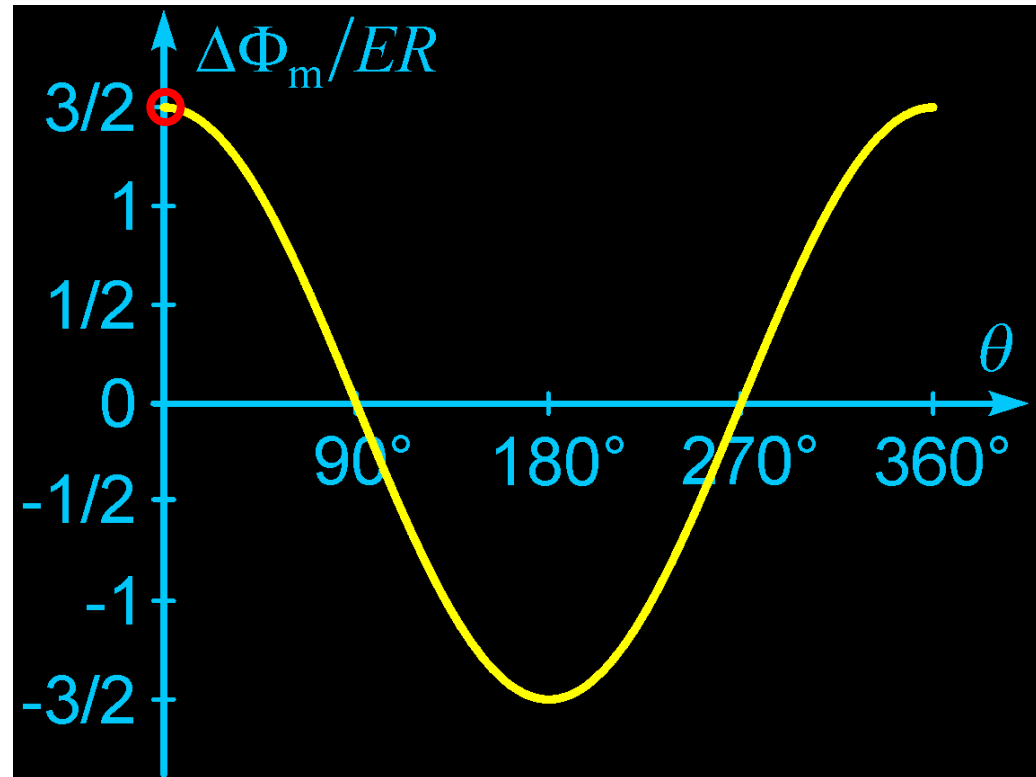
C - capacitor that stores energy required during the pulse

Q - transistor (e.g. fast power MOSFET or IGBT)

Induced Transmembrane Voltage

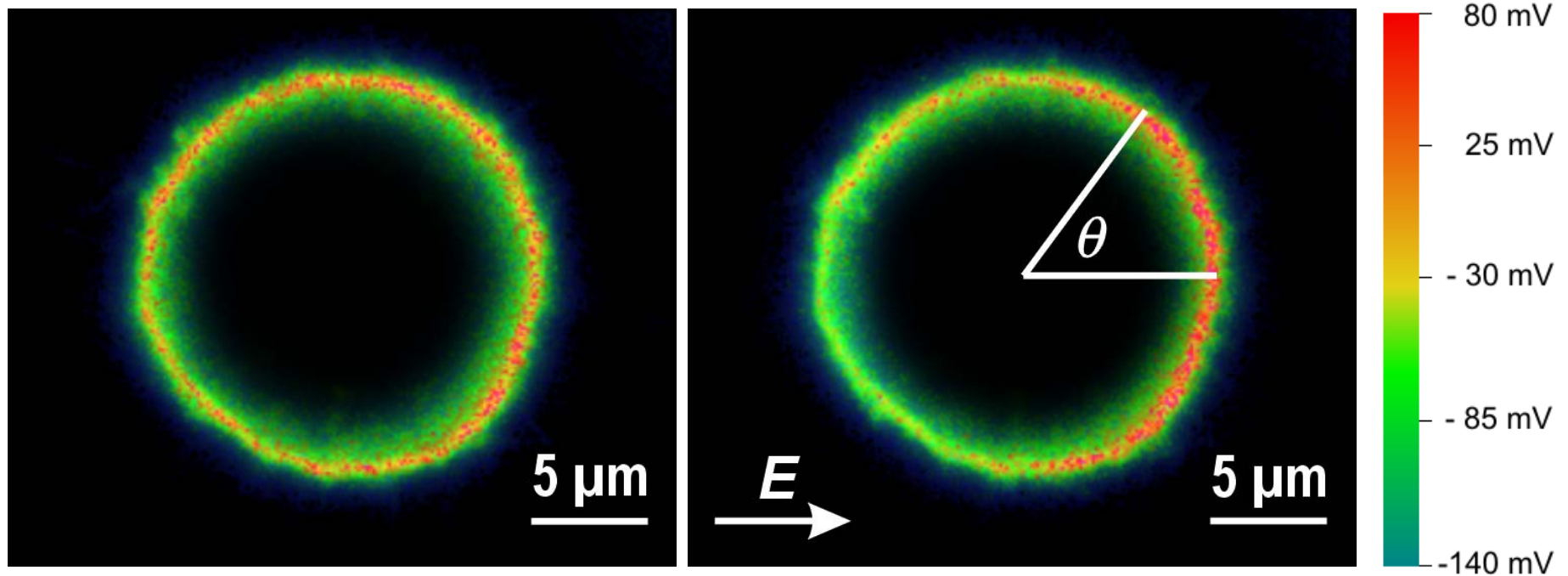


$$\Delta\Phi_m = \frac{3}{2} ER \cos \theta$$



More general analytical solution for spheroidal cells
T. Kotnik and D. Miklavčič. *Biophys. J.* **79**: 670-9, 2000

Cell exposed to $E \Rightarrow$ Induced TMV



Cells B16F1, dye ANEPPS

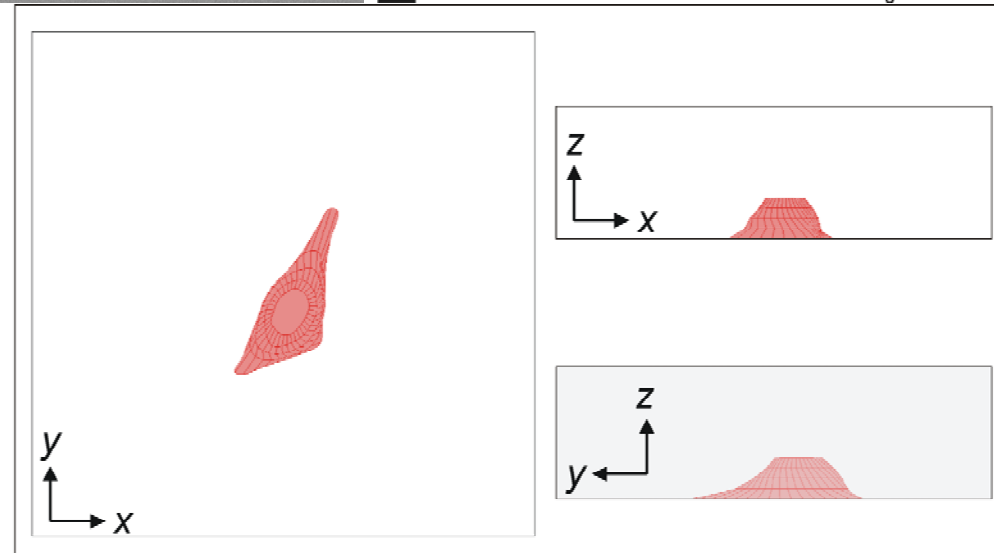
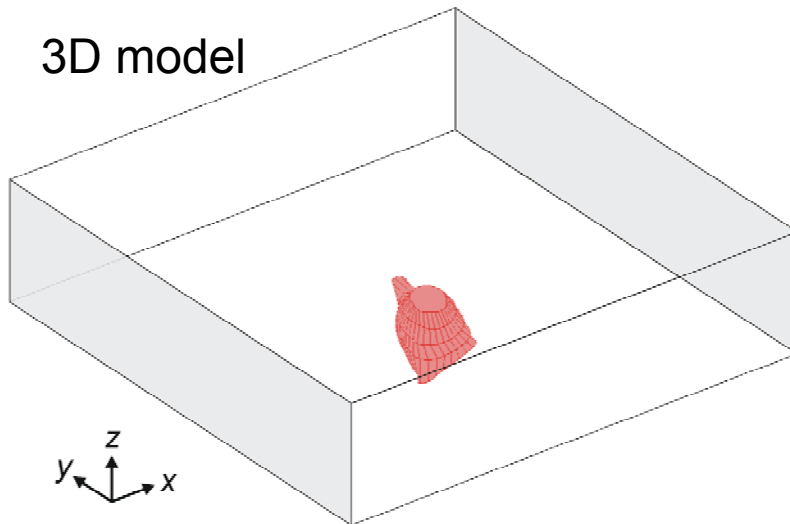
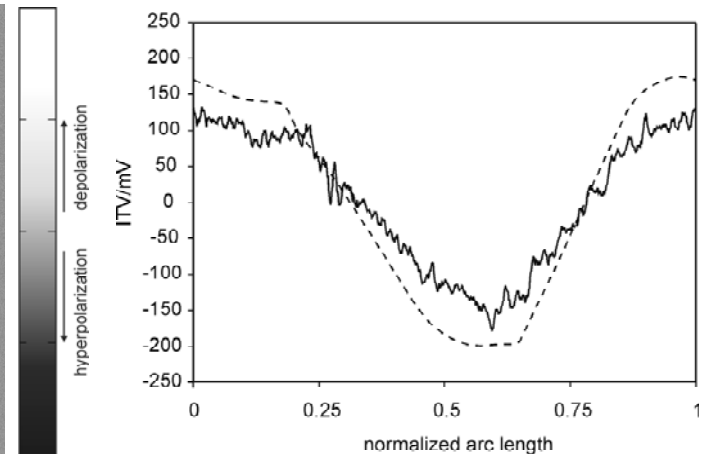
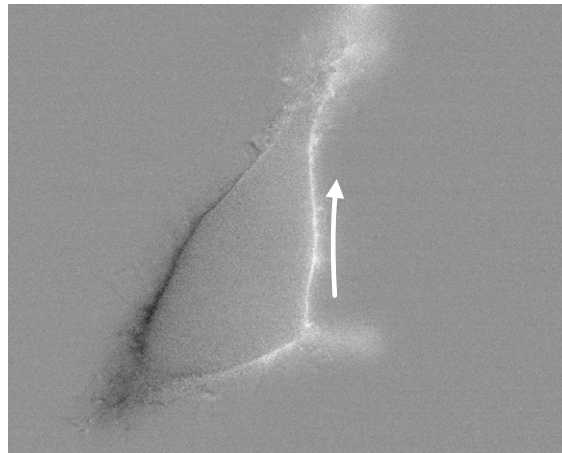
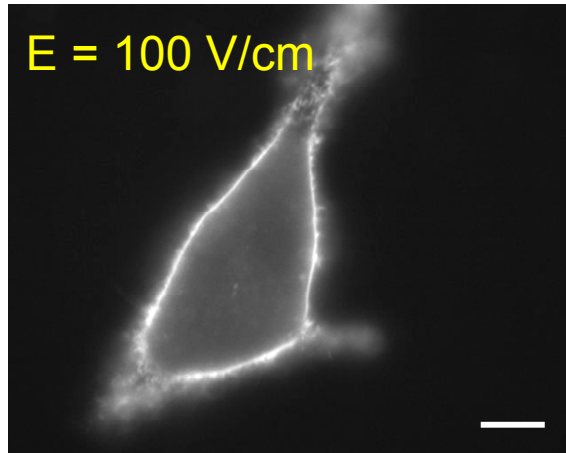
ex: 450 nm and 510 nm, em: >530 nm

Induced Transmembrane Voltage

- Only exists as long as an external electric field is present
- Superimposes (adds) to the resting TMV
- Is position-dependent, hyperpolarizing the cell on one side and depolarizing it on the other
- Increases with the electric field strength
- Increases with cell size

Results - Irregularly shaped cells

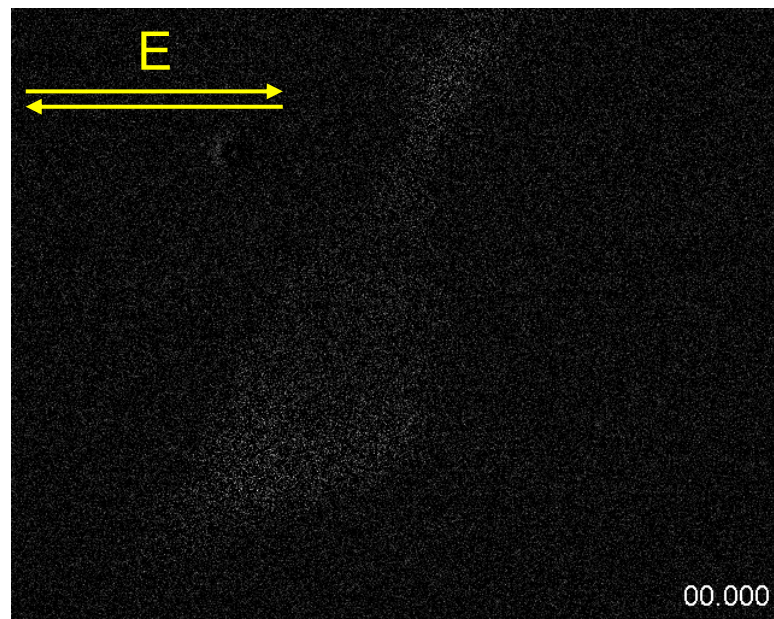
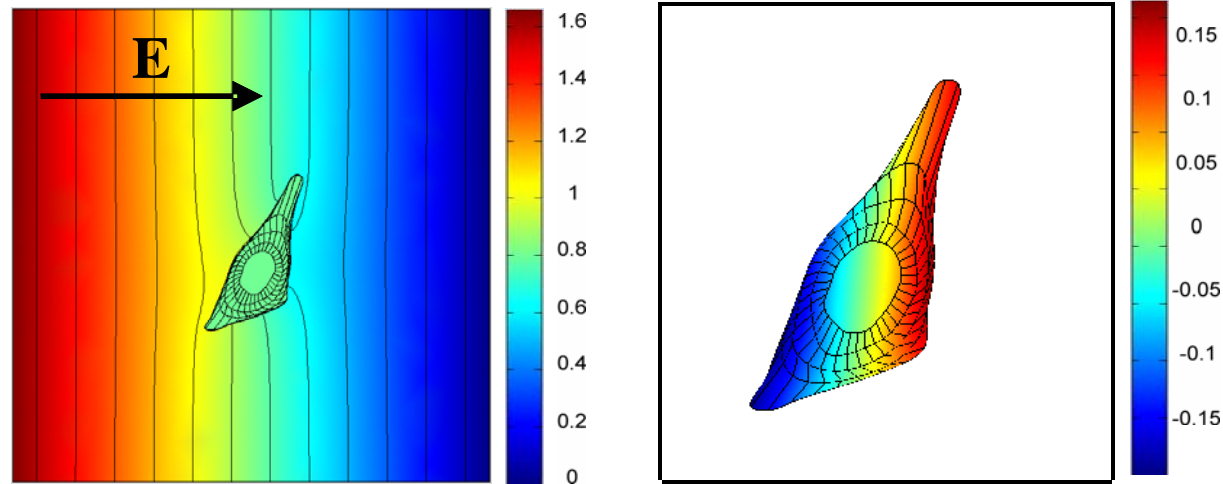
Induced transmembrane voltage



Pucihar *et al.*, Ann BME **34**: 642-52, 2006.

Results - Irregularly shaped cells

Calculations of ITV and membrane permeabilization



Electroporation observed by PI
 1×650 V/cm, $750 + 750$ μ s

Time [s]

Pucihar *et al.*, 2006
Ann BME **34**: 642-52,

Cytotoxicity of drugs can be increased by electroporation

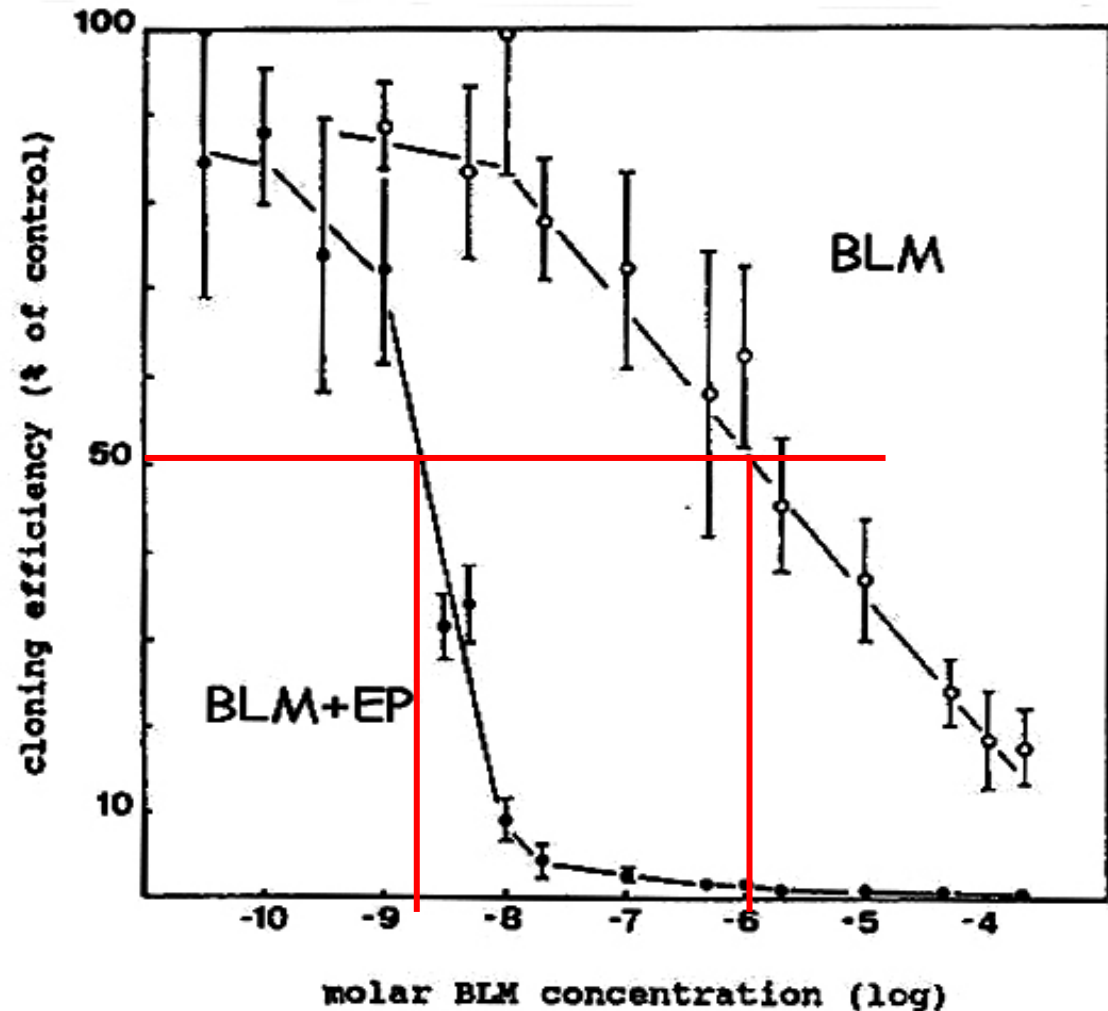
Drugs tested	Potentialiation
– Bleomycin	100-5000 -fold
– Cisplatin	3-80 -fold
– Carboplatin	10-13 -fold
– Methotrexate	
– Melphalan	
– Mithramycin	
– Actinomycin D	
– Adriamycin	
– Cyclophosphamide	
– Mitomycin C	
– Doxorubicin	
– 5-fluorouracil	
– Vinblastine	
– Vincristine	
– Paclitaxel	
– Taxotere	

Cytotoxicity of bleomycin *in vitro* is increased by means of electroporation

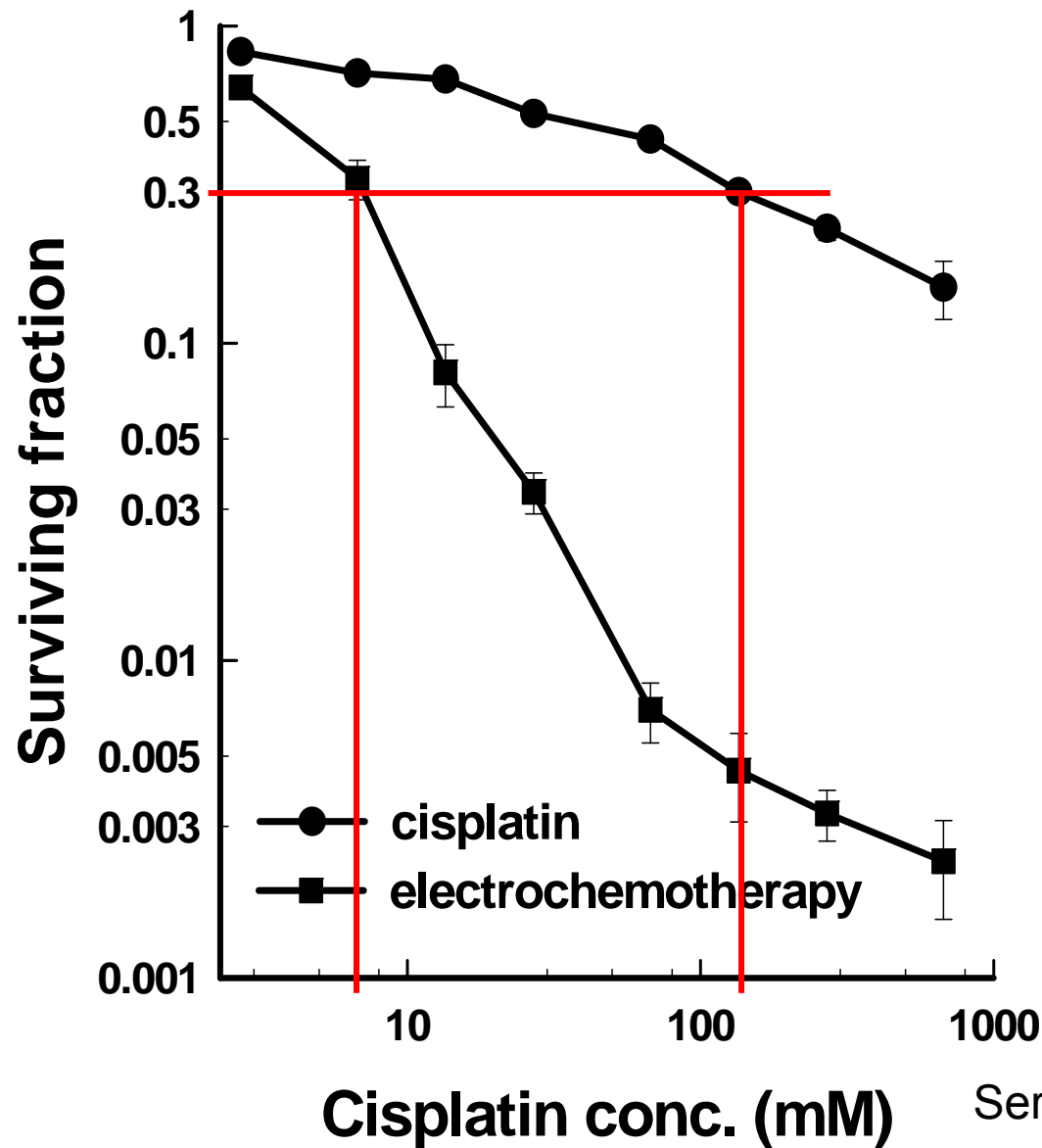
DC3F cells, a spontaneously transformed Chinese hamster lung fibroblasts.

Cell suspension was mixed with drug solution and immediately exposed to electric pulses.

Toxicity is potentiated ~1000 to 10 000 fold.



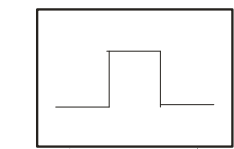
Malignant melanoma B16 cells *in vitro*



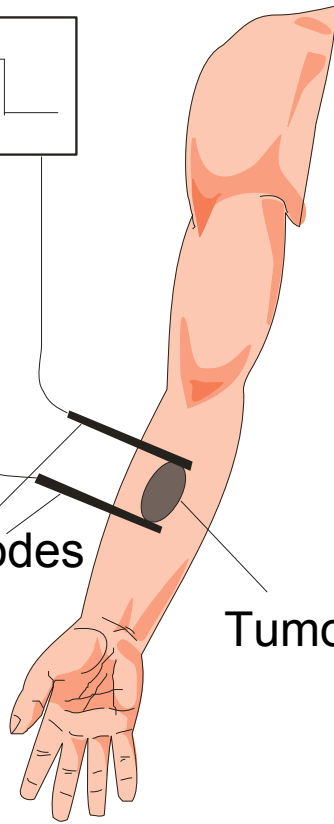
Serša, Miklavčič, Čemažar,
Cancer Res. 1995

Electrochemotherapy = = chemotherapy + electroporation

Electric pulse generator

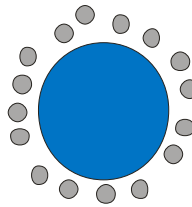


Electrodes

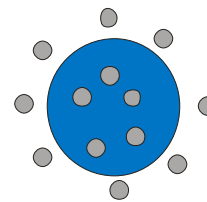


Tumour

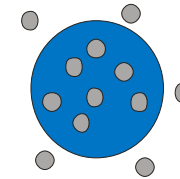
Chemotherapeutic surrounds the cells



Increased membrane permeability allows access to the cytosol



Pores reseal

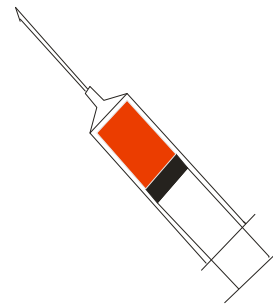


time

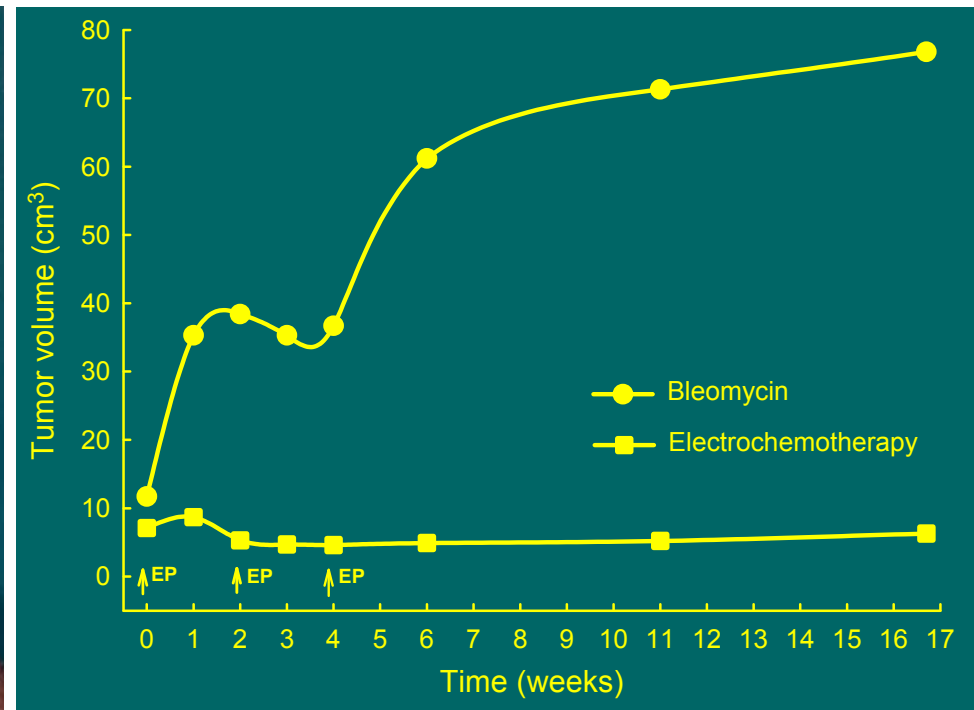
Electric pulse application



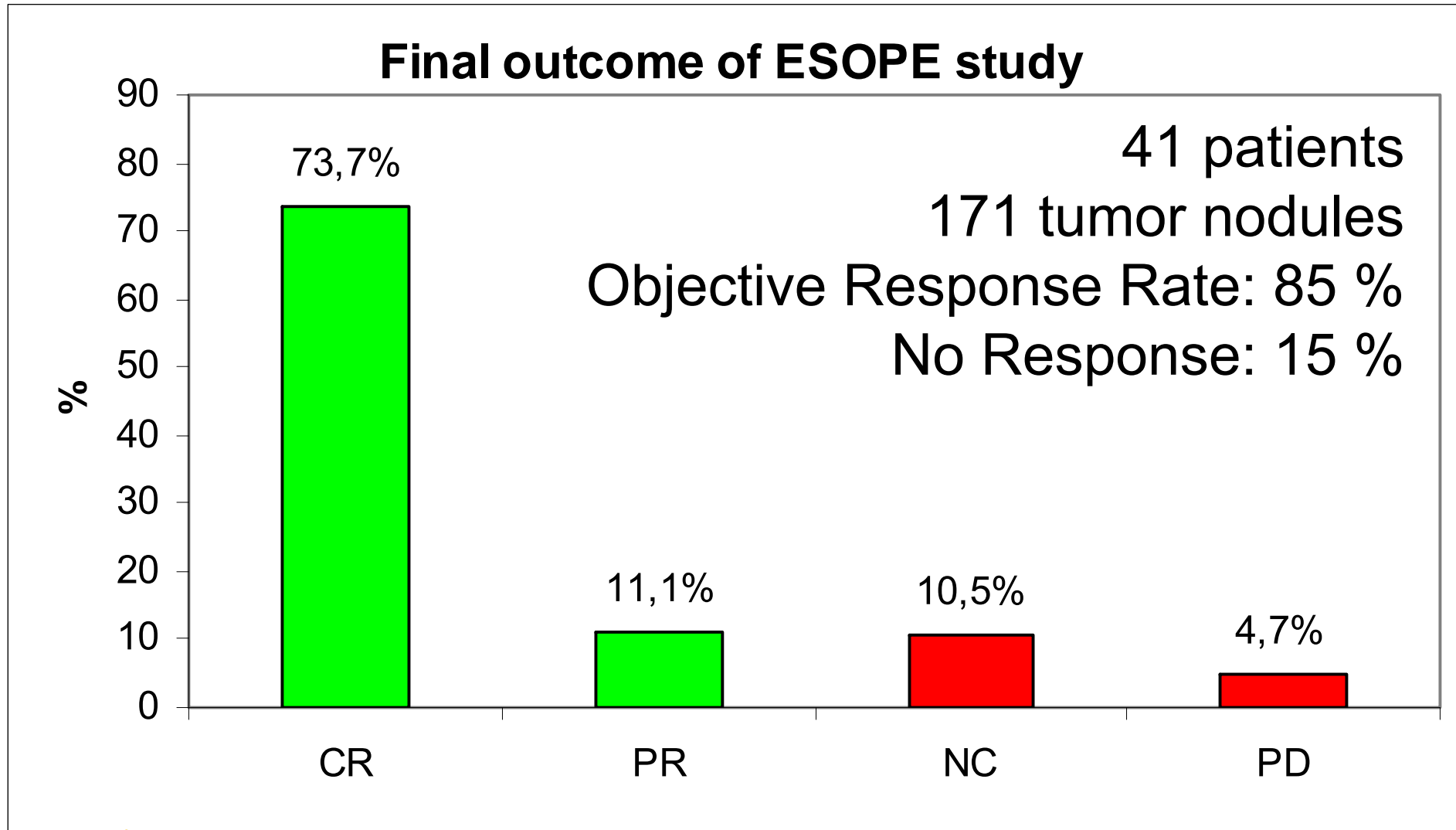
Injecting chemotherapeutic



Hypernephroma metastases treated by ECT with bleomycin given intravenously



ESOPE project - results



Play DVD!

Sersa G, Miklavcic D.

Electrochemotherapy of tumours (Video Article). *J. Visual Exp.* 22: 1038, 2008.

DOI [10.3791/1038](https://doi.org/10.3791/1038)

Clinical electrochemotherapy

Cliniporator™, is **now in use in 53 institutions** throughout Europe:

- Italy (23)
- Spain (5)
- Germany (2)
- Sweden (3)
- Great Britain (2)
- Greece
- Portugal
- Hungary
- Slovenia
- France
- Denmark
- Ireland

	Number of patients	Number of clinical centres
2006	130	
2007	300	37
2008	600	53
2009	1000	100

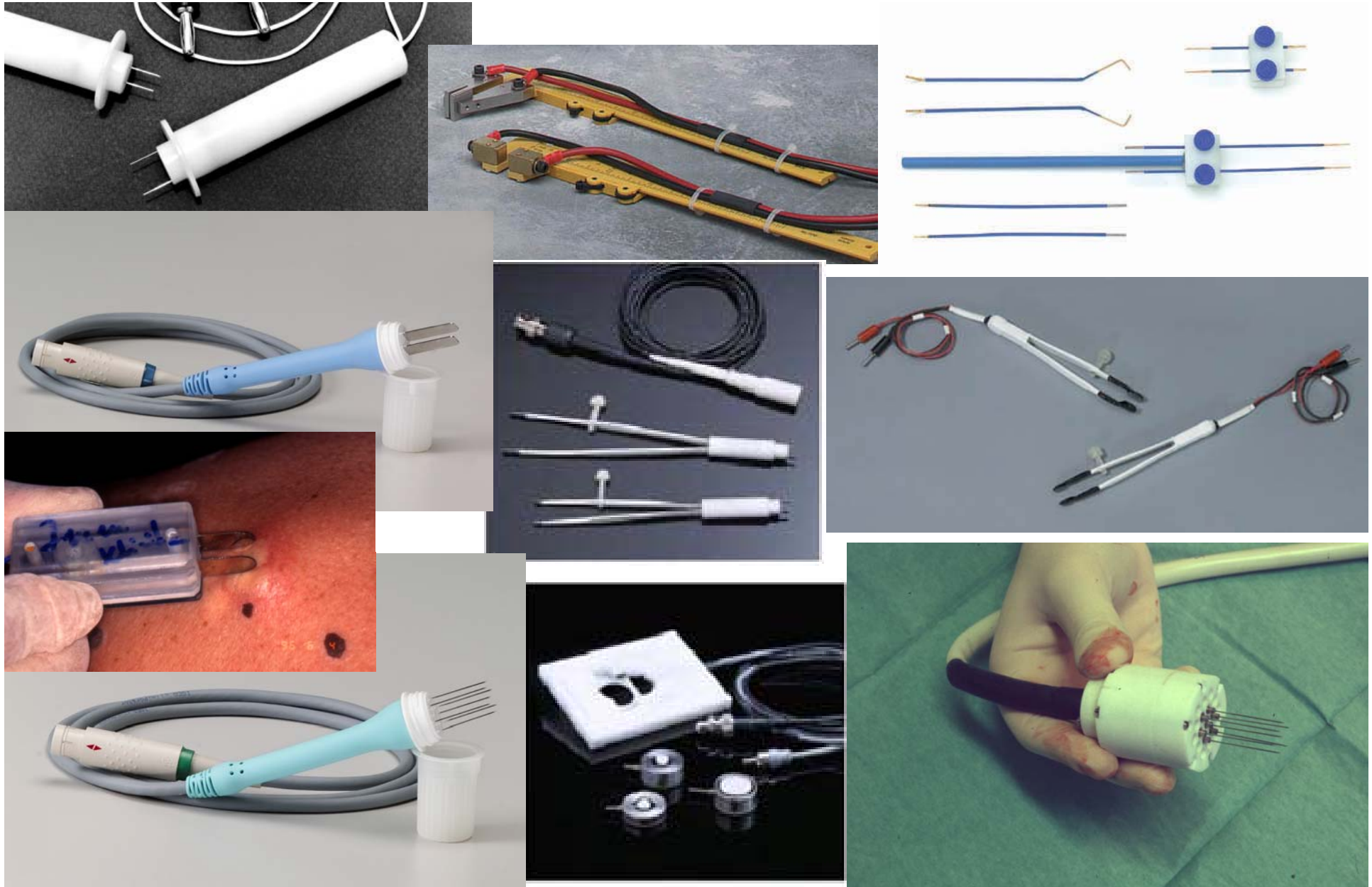


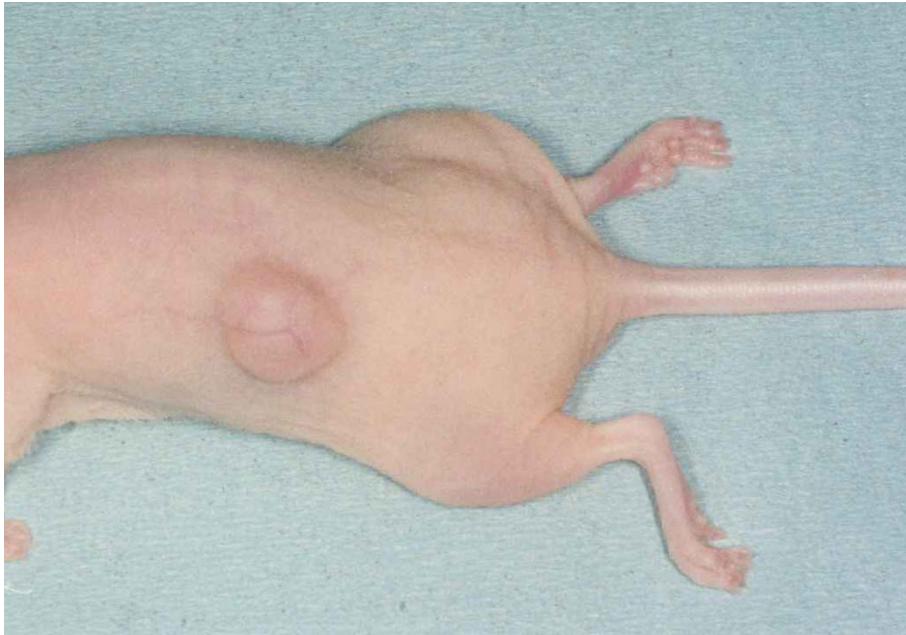
IGEA, Carpi
January 2006

Delivery of electric pulses in patients



Electrodes used for *in vivo* electroporation

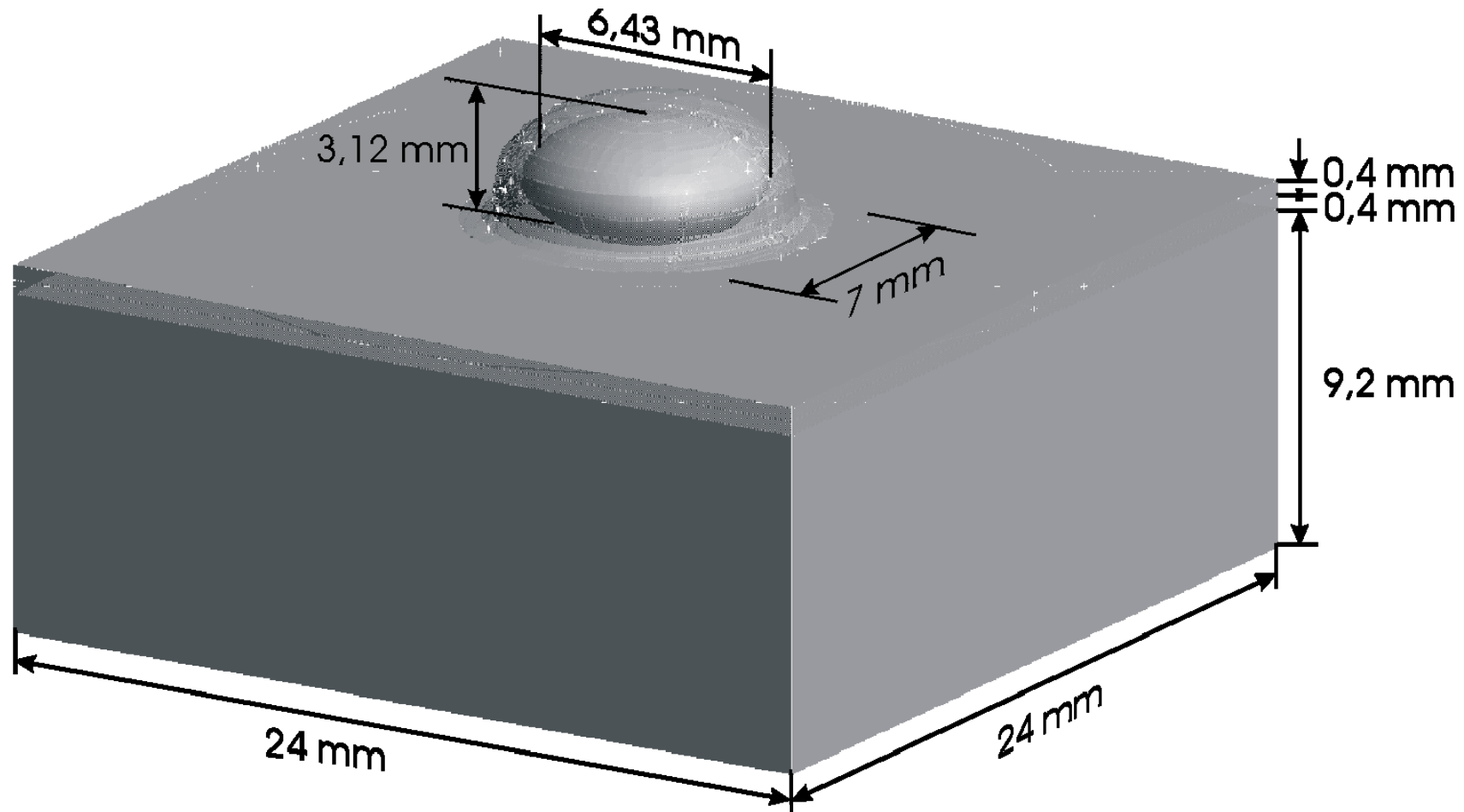




Serša et al. Bioelectrochem. Bioenerg. 39: 61-6, 1996
Miklavčič et al, Eur. J. Cancer Suppl. 4: 45-51, 2006

Subcutaneous tumor model

Model is composed of: skin, fat, tumor and muscle.



Electrodes are modeled as boundary condition.
Electrode separation is 8 mm.

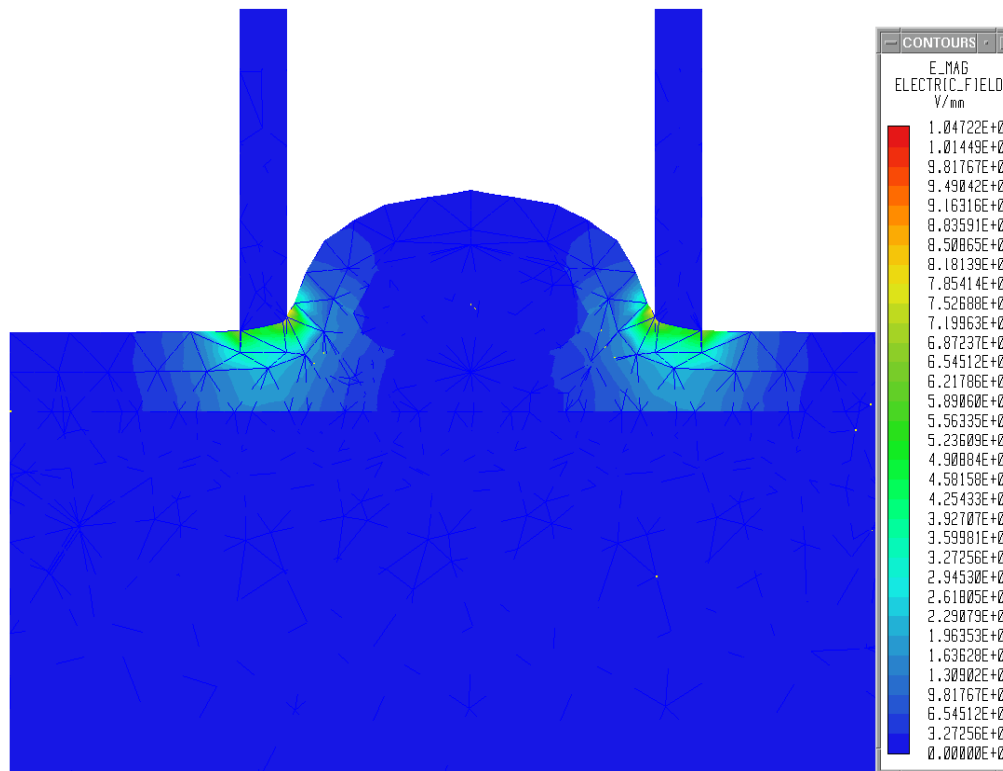
Tissue passive electrical properties

		Conductivity (S/m)
TUMOR		0.22 – 0.4
FAT		0.02 – 0.04
MUSCLE	transversal	0.04 – 0.14
	longitudinal	0.3 – 0.8
SKIN (DRY)		0.00002 – 0.0002
SKIN (WET)		0.0003 – 0.2 (?)
<i>stratum corneum</i>		0.0000125
LOWER LAYERS		0.227
BONE		~ 0.006

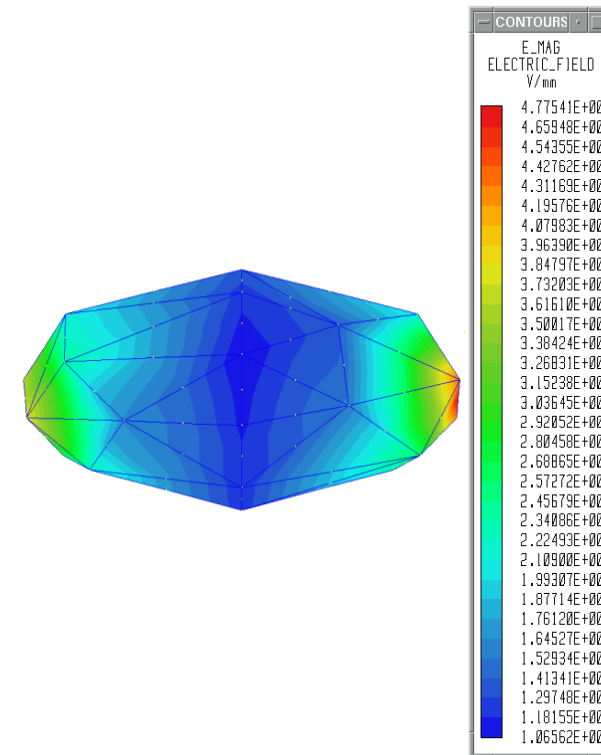
Results of modeling for s.c. tumor

Electric field distribution in tumor before permeabilization, plate electrodes, 8 mm distance, applied voltage 100V.

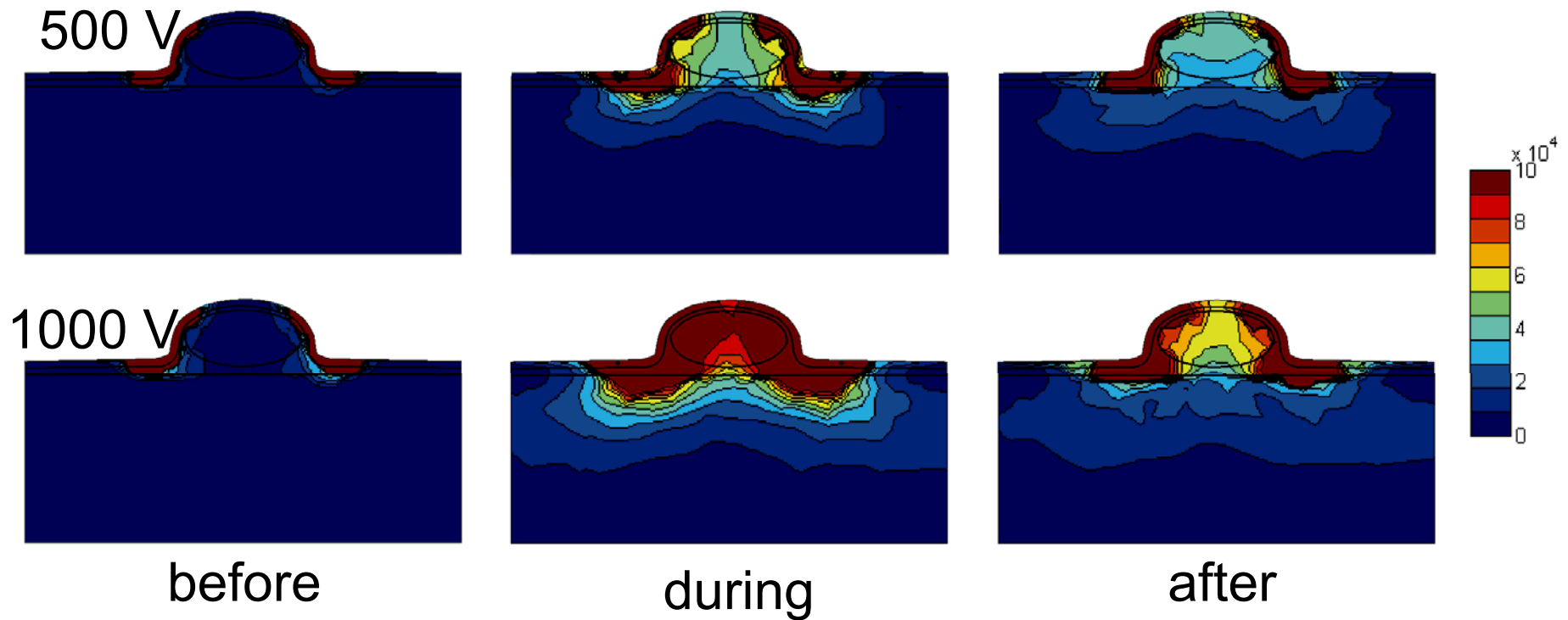
whole model, section plane: XY

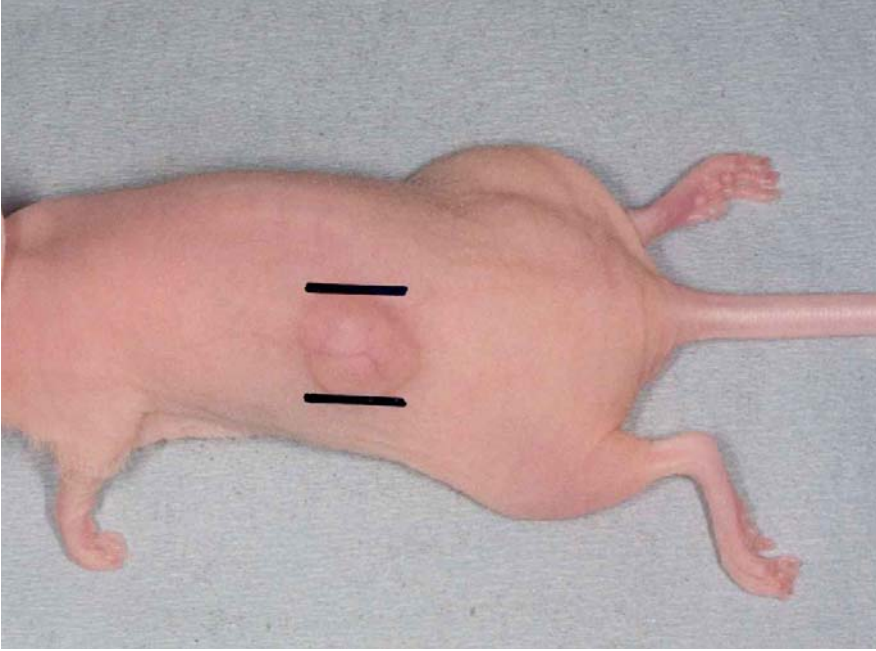


tumor

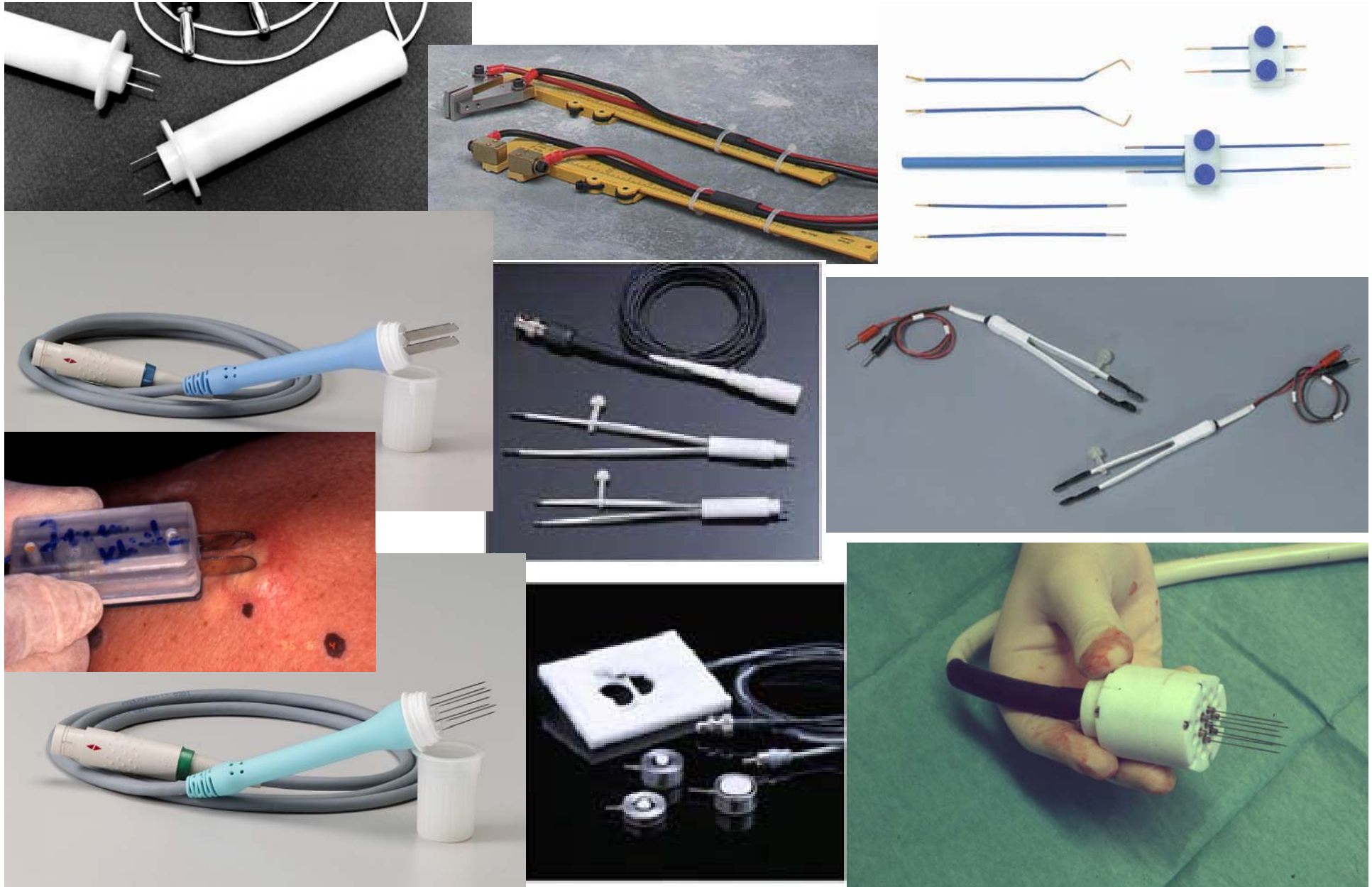


Electroporation of s.c. tumor - electric field (V/m)



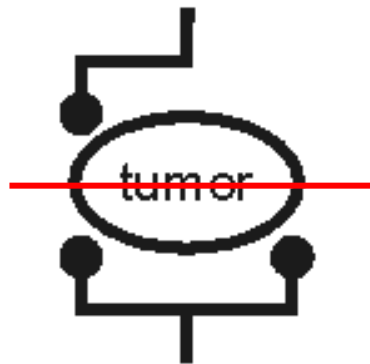


Electrodes used for *in vivo* electroporation

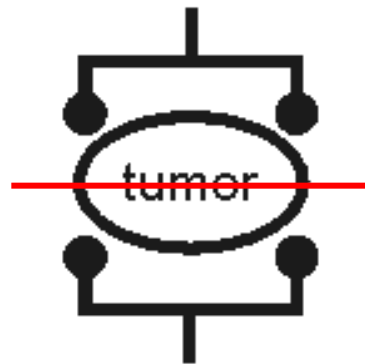


What kind of electrodes to use?
And how to place them with respect
to the target tissue?

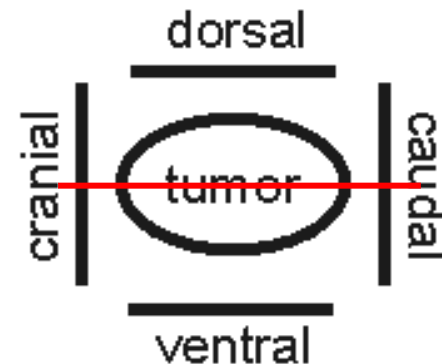
Correlation between electrochemotherapy effectiveness and electric field distribution in experimental tumour model



2+1

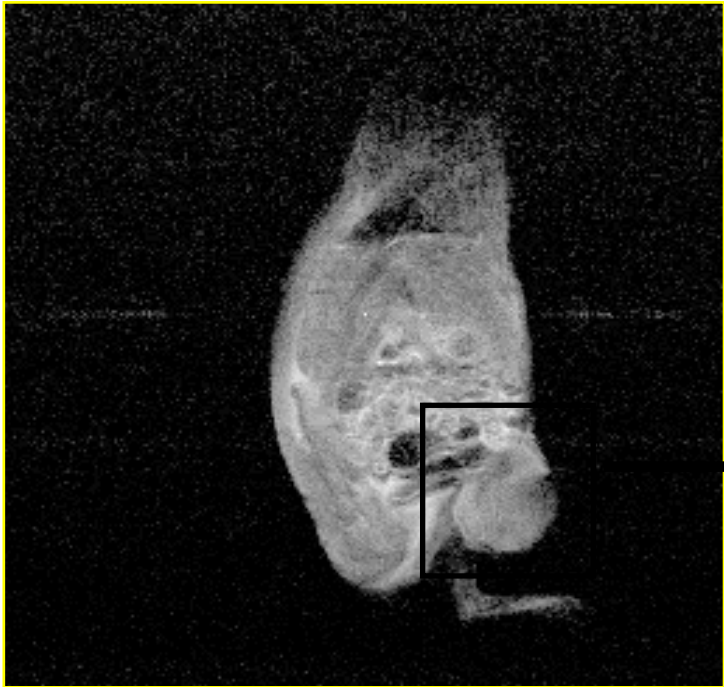


2+2



DV, CC
4+4

proton weighted image



flat/flat electrode configuration



MRI Bruker Biospec system (2.35 T)

equiped with Oxford gradient coils and a saddle radiofrequency coil

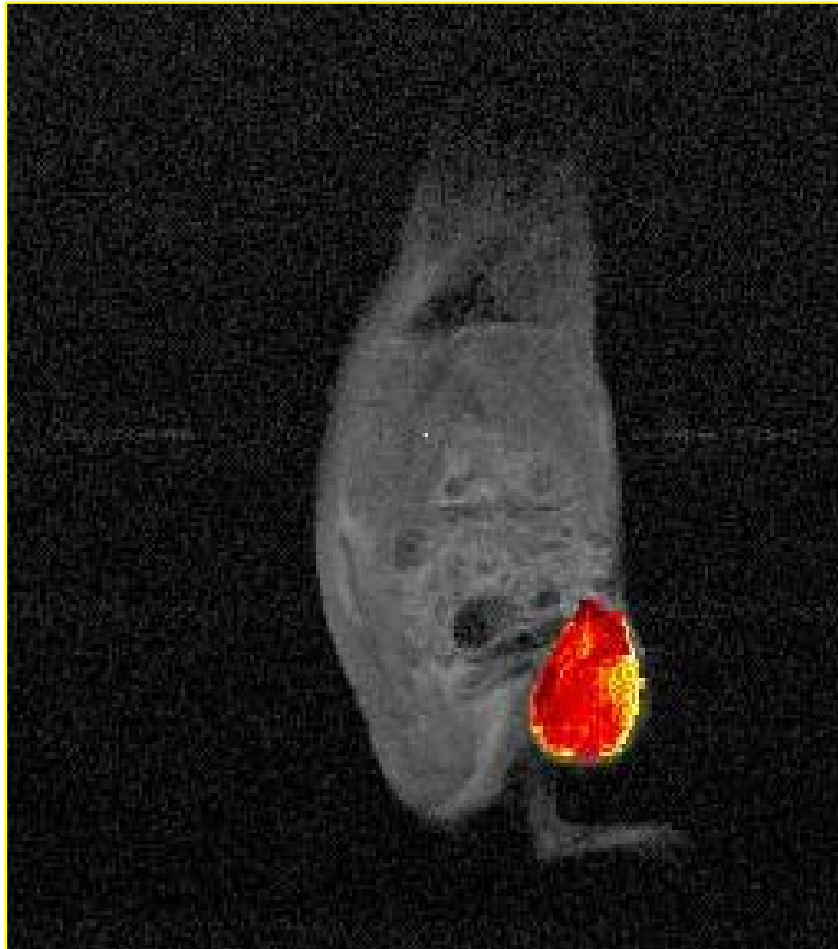
Parameter settings:

TR 600 ms, TE 25 ms, matrix 256x256,

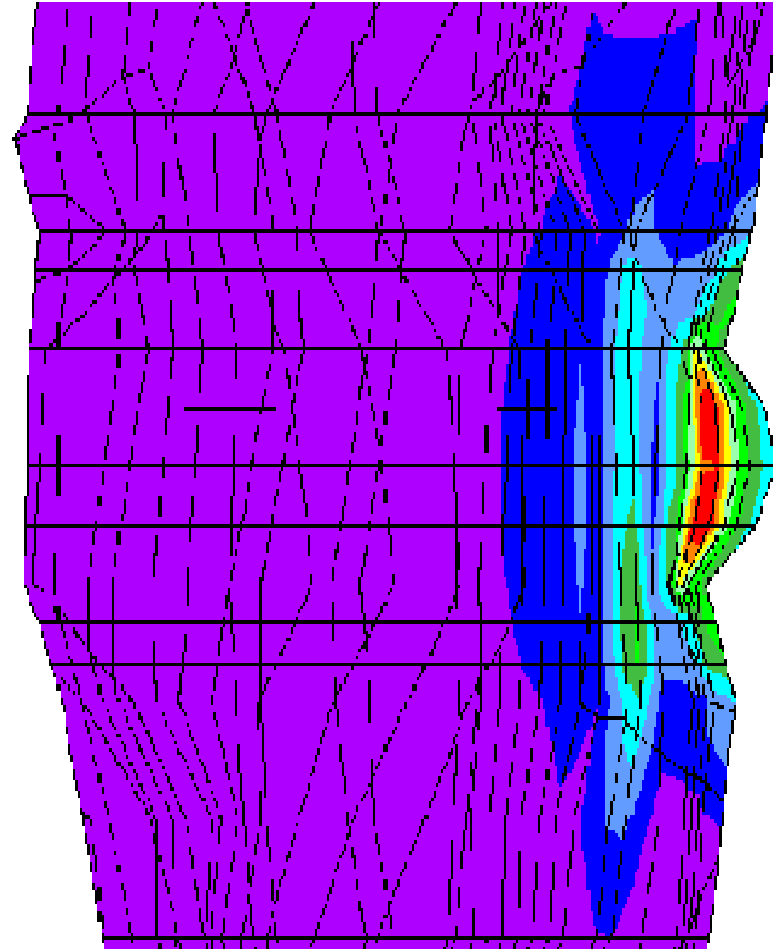
slice thickness 2 mm, FOV 8 cm, applied voltage 50 V,

duration of current pulses T_c 10 ms and total imaging time 4 min.

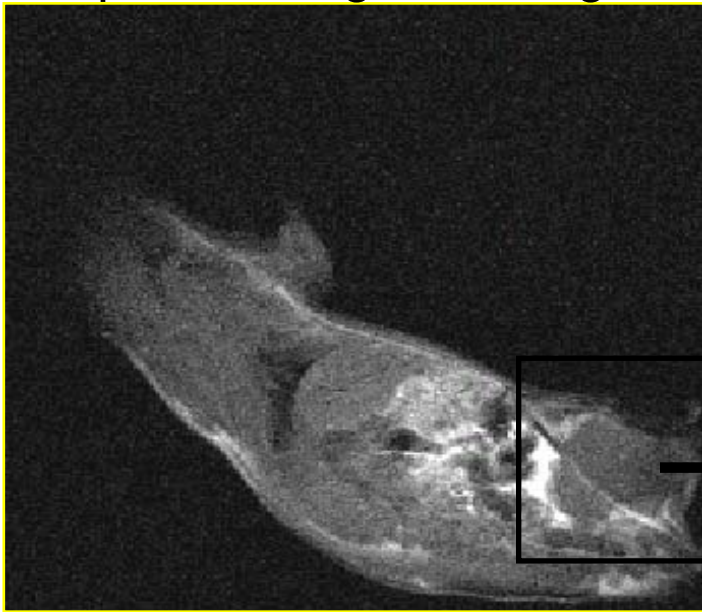
current density image



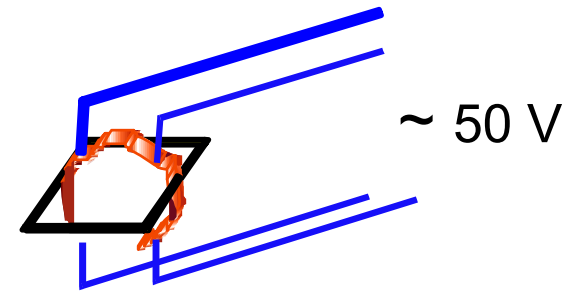
3D finite element model



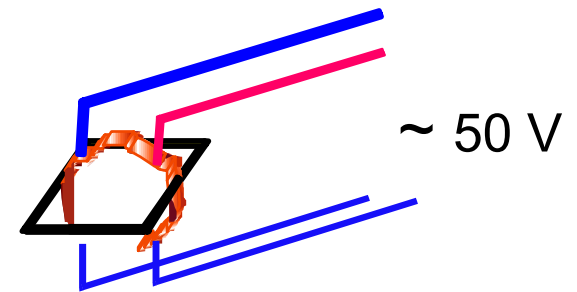
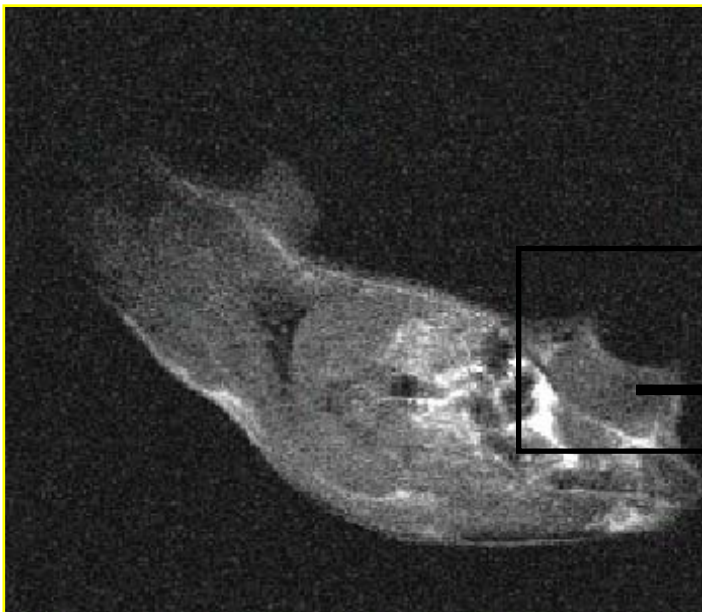
proton weighted images



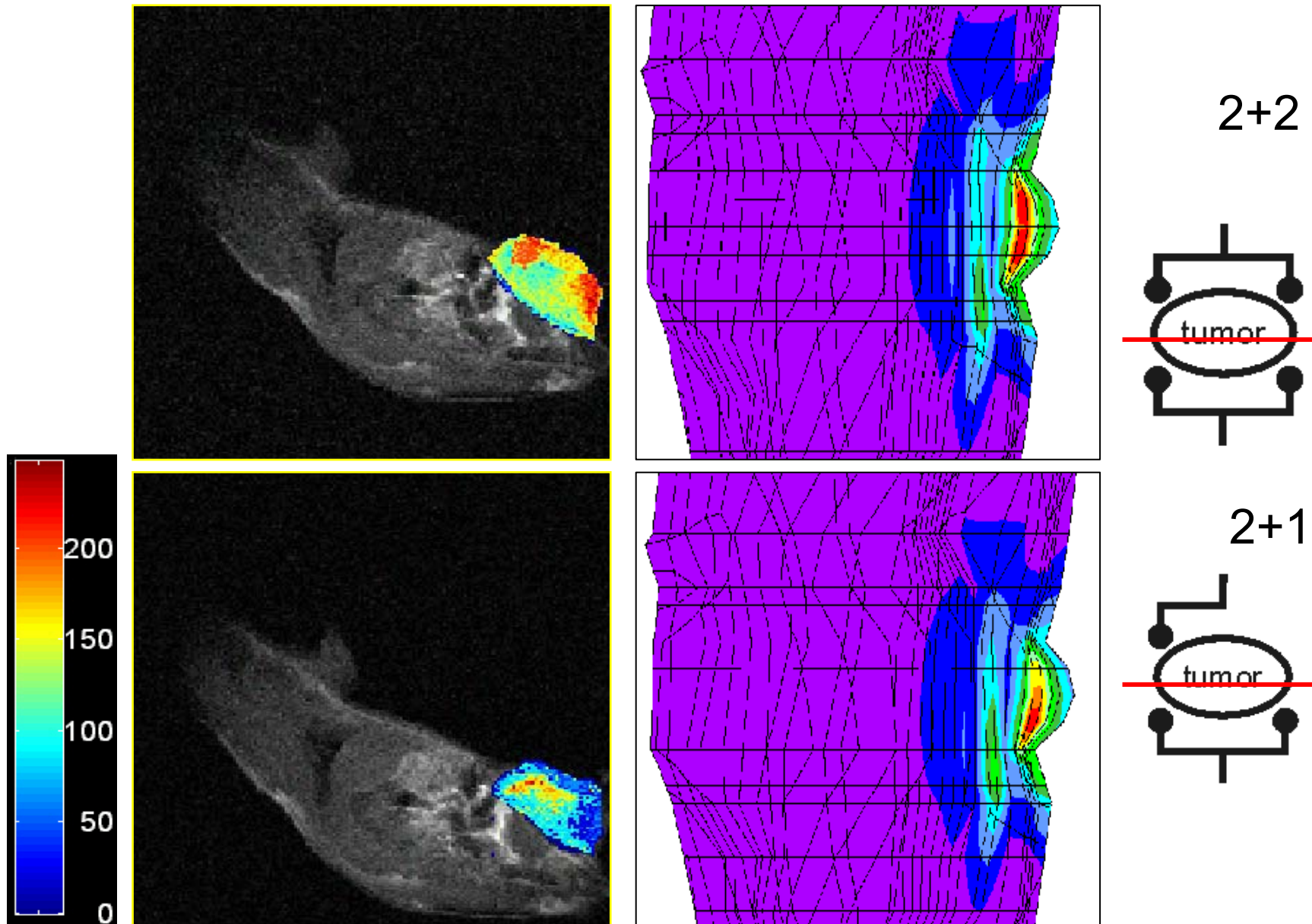
2+2 electrode configuration



2+1 electrode configuration



Current density imaging



Complementary approach

Electrochemotherapy is a local treatment of tumours.

Electrochemotherapy = Chemotherapy + Electroporation

Electrochemotherapy effectiveness is measured as:

- tumor growth delay
- complete responses

Electrochemotherapy effectiveness can be used as an indicator of *in vivo* cell/tissue permeabilization.

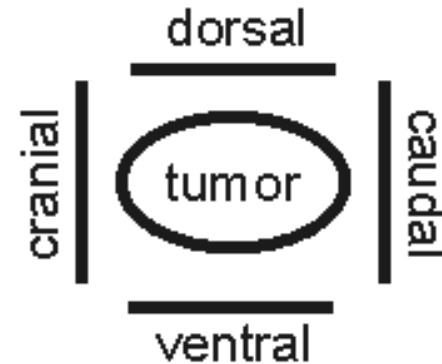
Correlation between electrochemotherapy effectiveness
and
electric field distribution in experimental tumour model



2+1



2+2

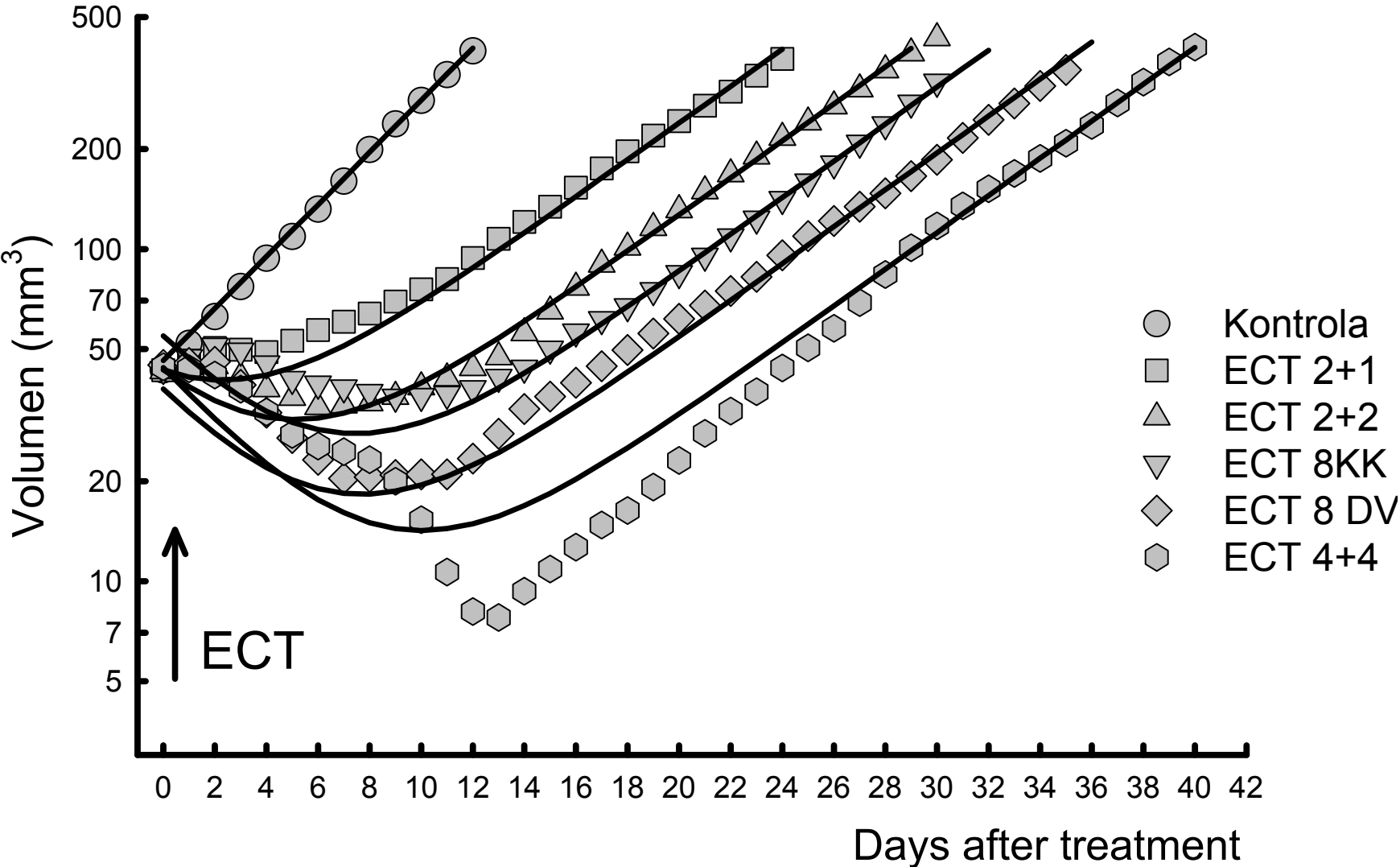


DV, CC
4+4

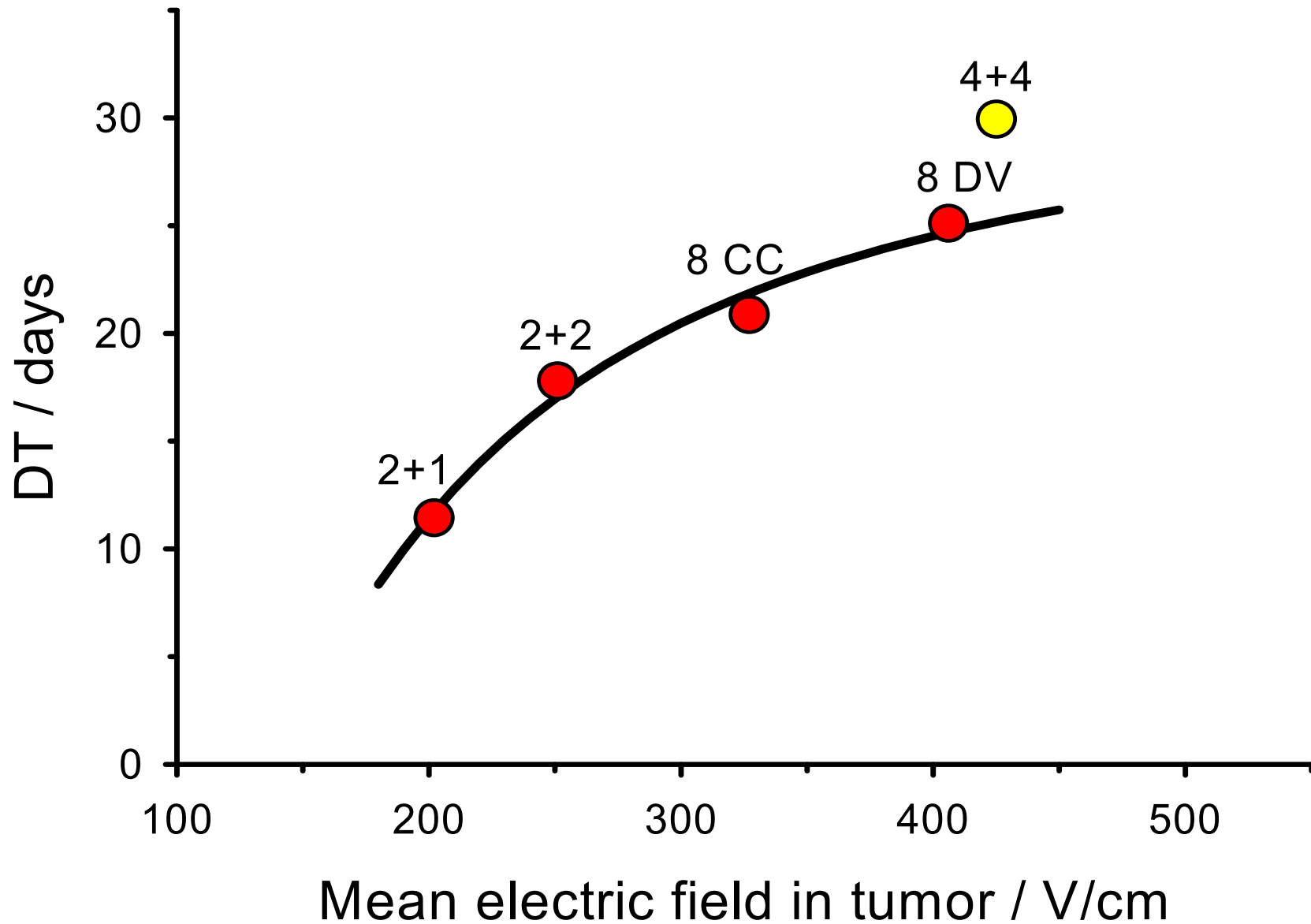
MATERIALS AND METHODS

- A/J mice (10-12 weeks old),
- fibrosarcoma SA-1,
- ECT:
 - 100 μg bleomycine i.v.,
 - 8 pulses, 100 μs , 1 Hz,
 - electrodes: 2+1 and 2+2 (1300 V, 5.5 mm),
 - plate (1040 V, 8 mm).
- numerical model:
 - Finite Element Method (EMAS, Ansoft),
 - Miklavčič *et al.*, Biophysical J. 74: 2152-2158, 1998.

Results: experimental on animal tumor model



Correlation between electric field and ECT effectiveness



Anti-tumor effectiveness of ECT with electrodes in different orientations and changing of the electrodes, i.e. 4+4 electrode configuration.

ECT	E_{mean} inside tumour (V/cm)	growth delay (days)
cranial/caudal	327	17.10±1.60
dorsal/ventral	406	21.35±1.94
4+4	425	26.17±2.32

Serša et al., Bioelectrochem. Bioenerg. 39: 61-66, 1996.

Šemrov & Miklavčič, Methods in Molecular Medicine 37: 63-81, 2001

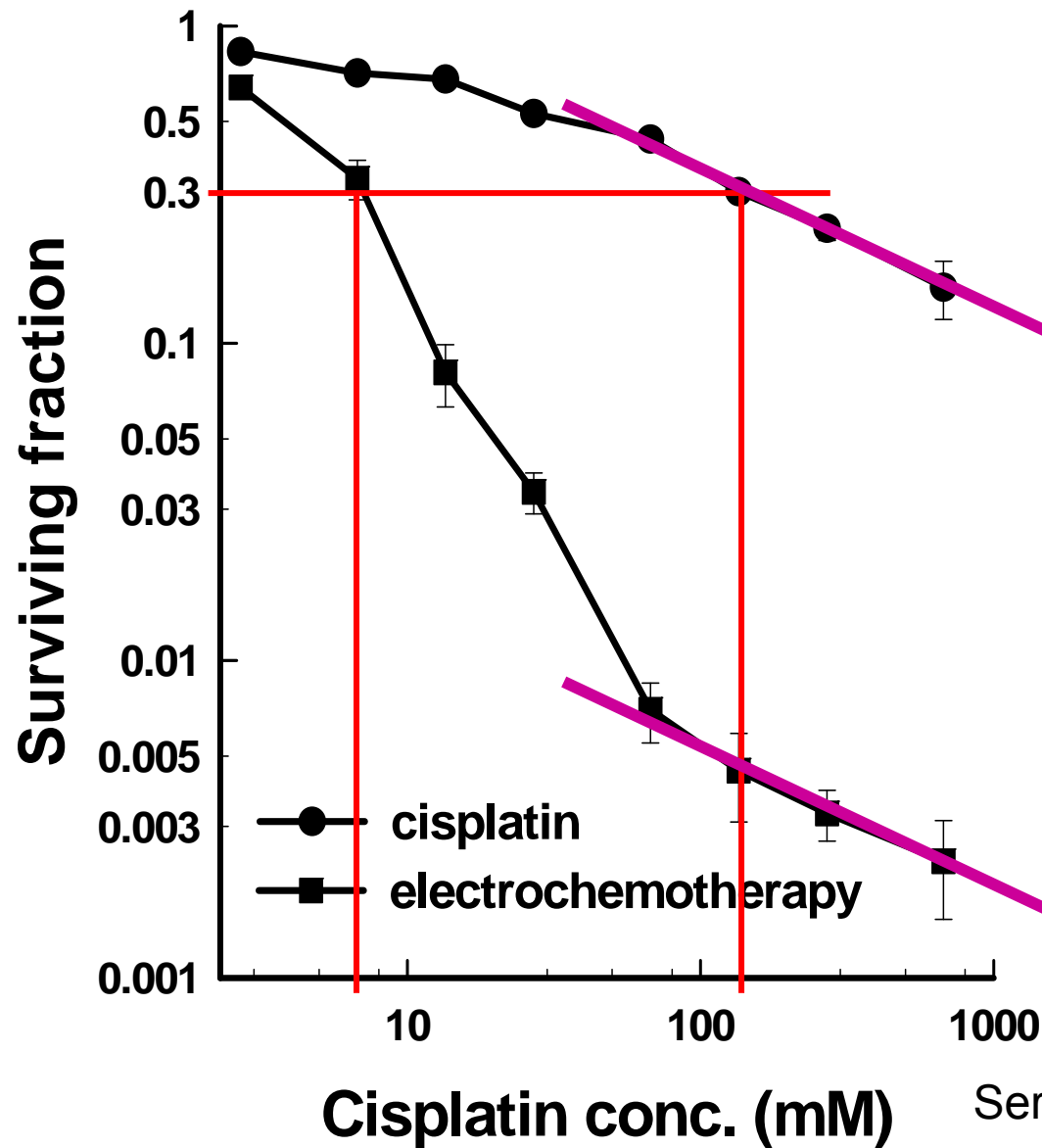
Summary:

- Good correlation between experimental results and numerical model.
- Changing electrode orientation improves electric field distribution.
- Electric field distribution depends on electrode design.

Summary:

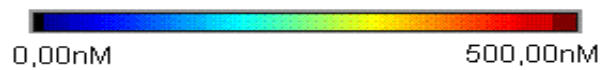
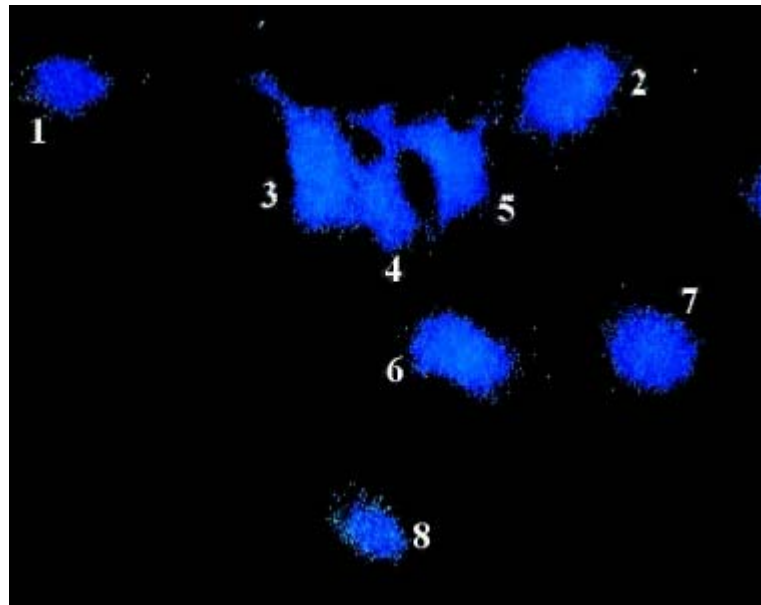
- Good correlation between experimental results and numerical model.
- Changing electrode orientation improves electric field distribution.
- Electric field distribution depends on electrode design.

Malignant melanoma B16 cells *in vitro*



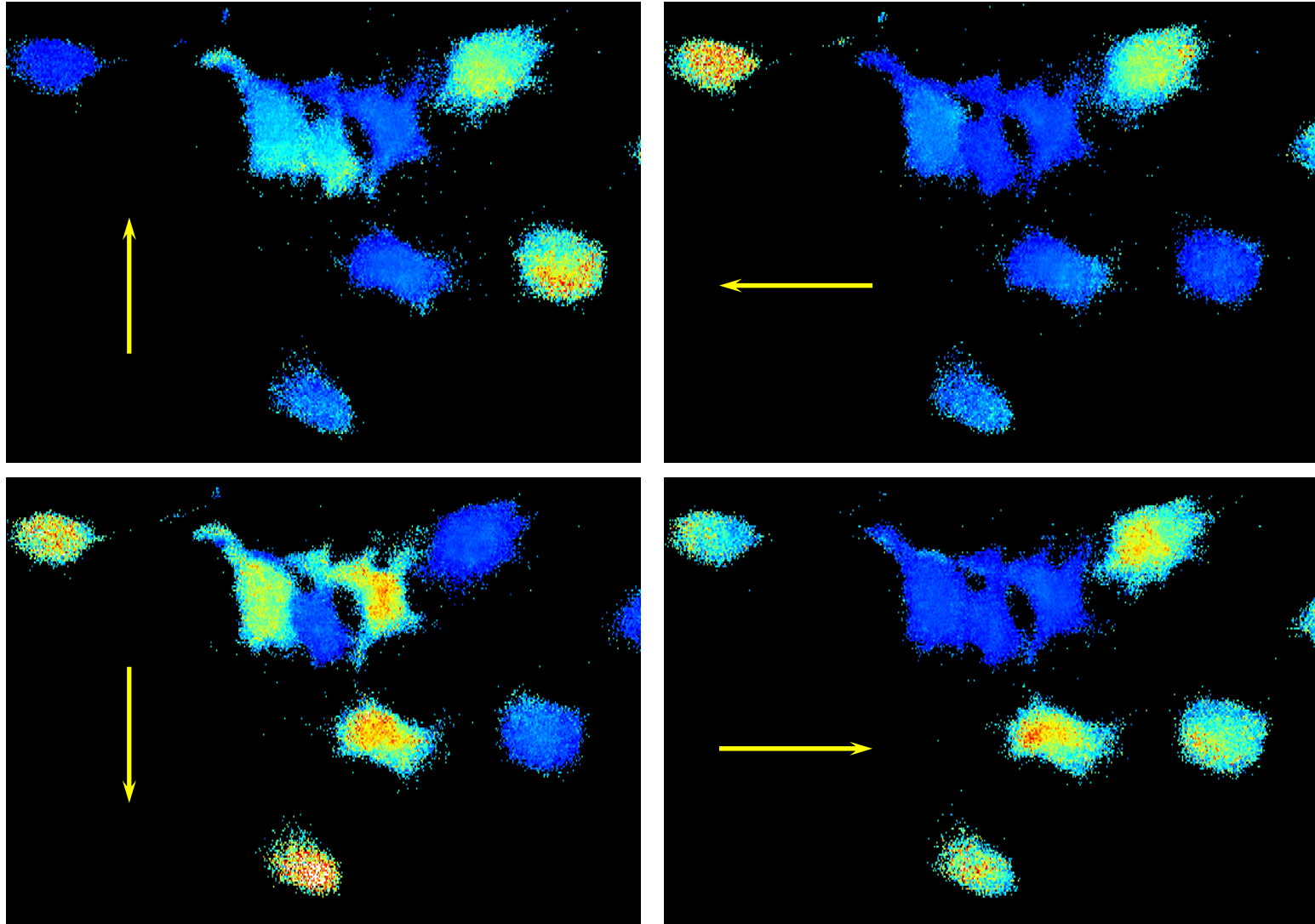
Serša, Miklavčič, Čemažar,
Cancer Res. 1995

Experiment: dm171104-7; Changing field orientation: 450 V, 100 μ s, N=1



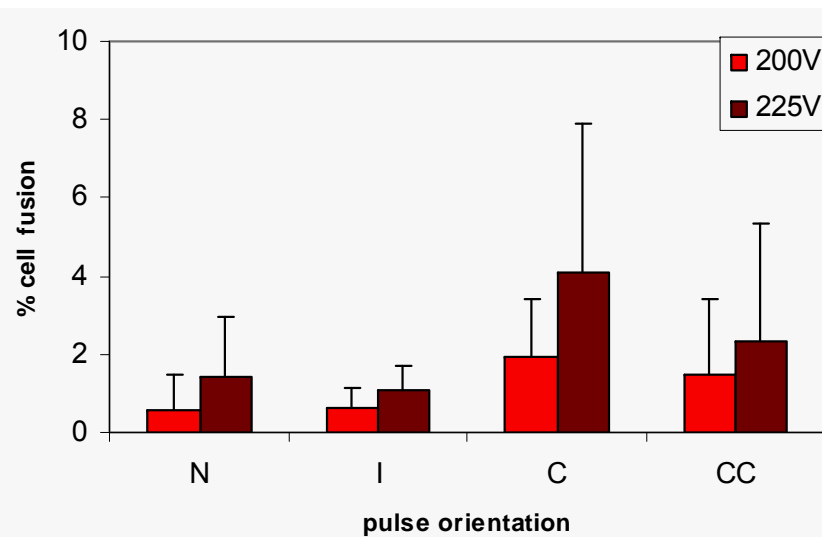
	↑	←	↓	→	Σ
1	○	●	●	●	●
2	●	●	○	●	●
3	●	○	●	○	●
4	●	○	○	○	●
5	○	○	●	○	●
6	○	○	●	●	●
7	●	○	○	●	●
8	○	○	●	●	●
Σ	4/8	2/8	5/8	5/8	8/8

Experiment: dm171104-7; Changing field orientation: 450 V, 100 μ s, N=1

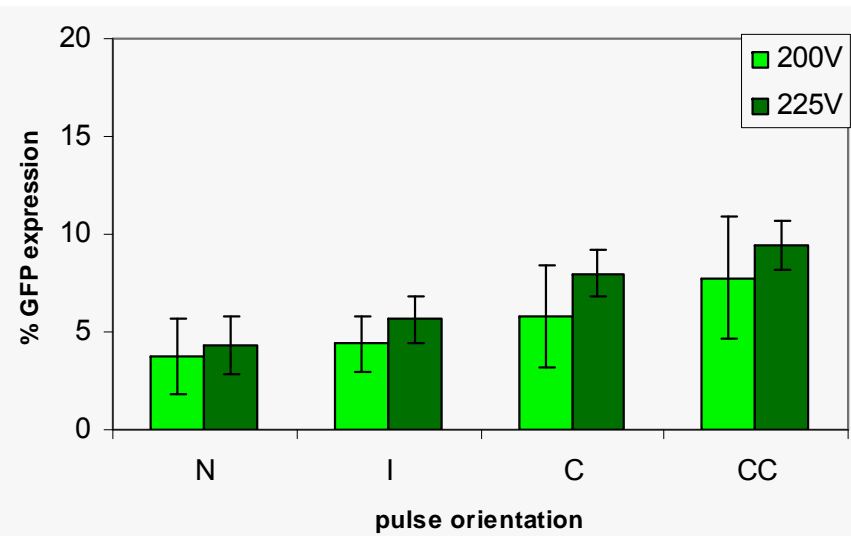


Changing E-field orientation

Cell fusion



DNA transfection



Summary:

- Good correlation between experimental results and numerical model.
- Changing electrode orientation improves electric field distribution.
- Electric field distribution depends on electrode design.

Choice of electrodes with respect to target tissues location

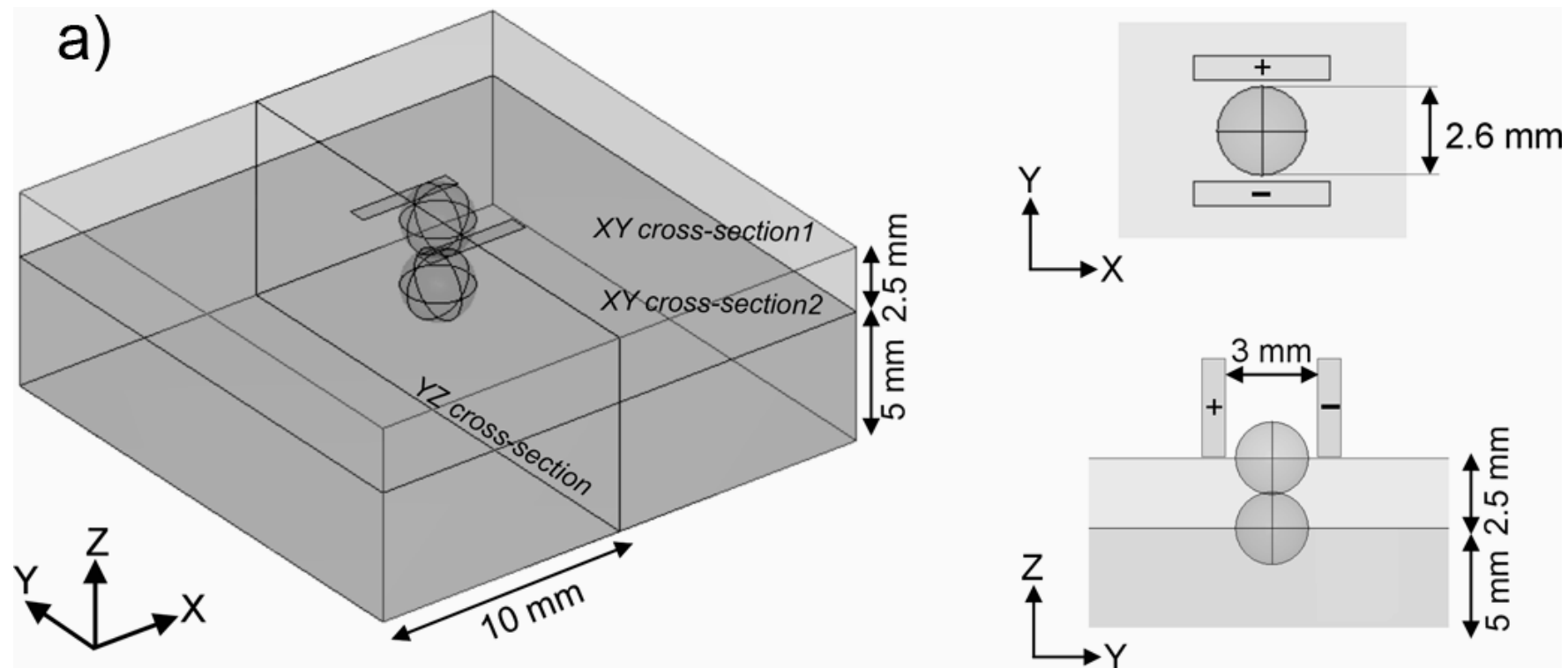
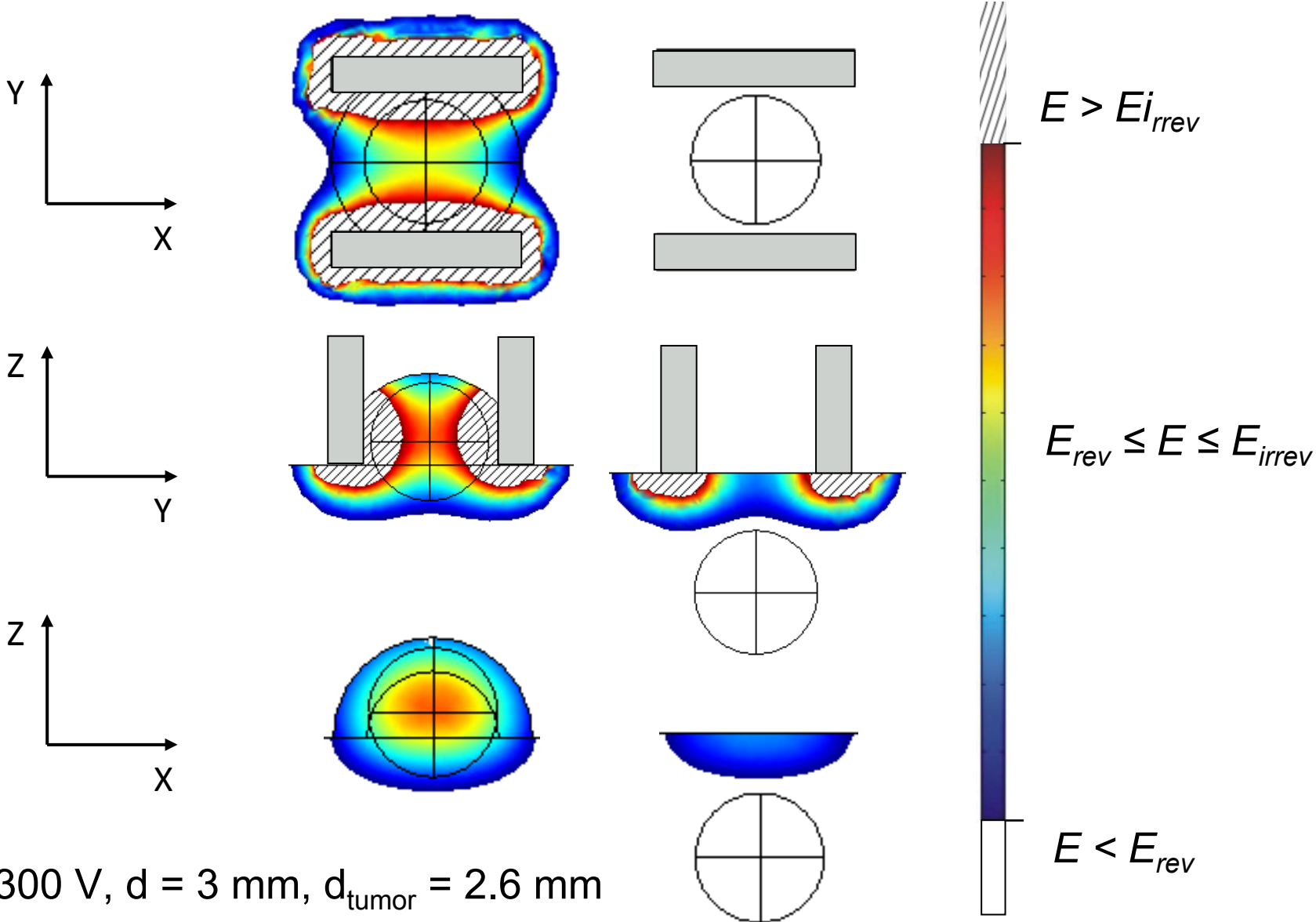
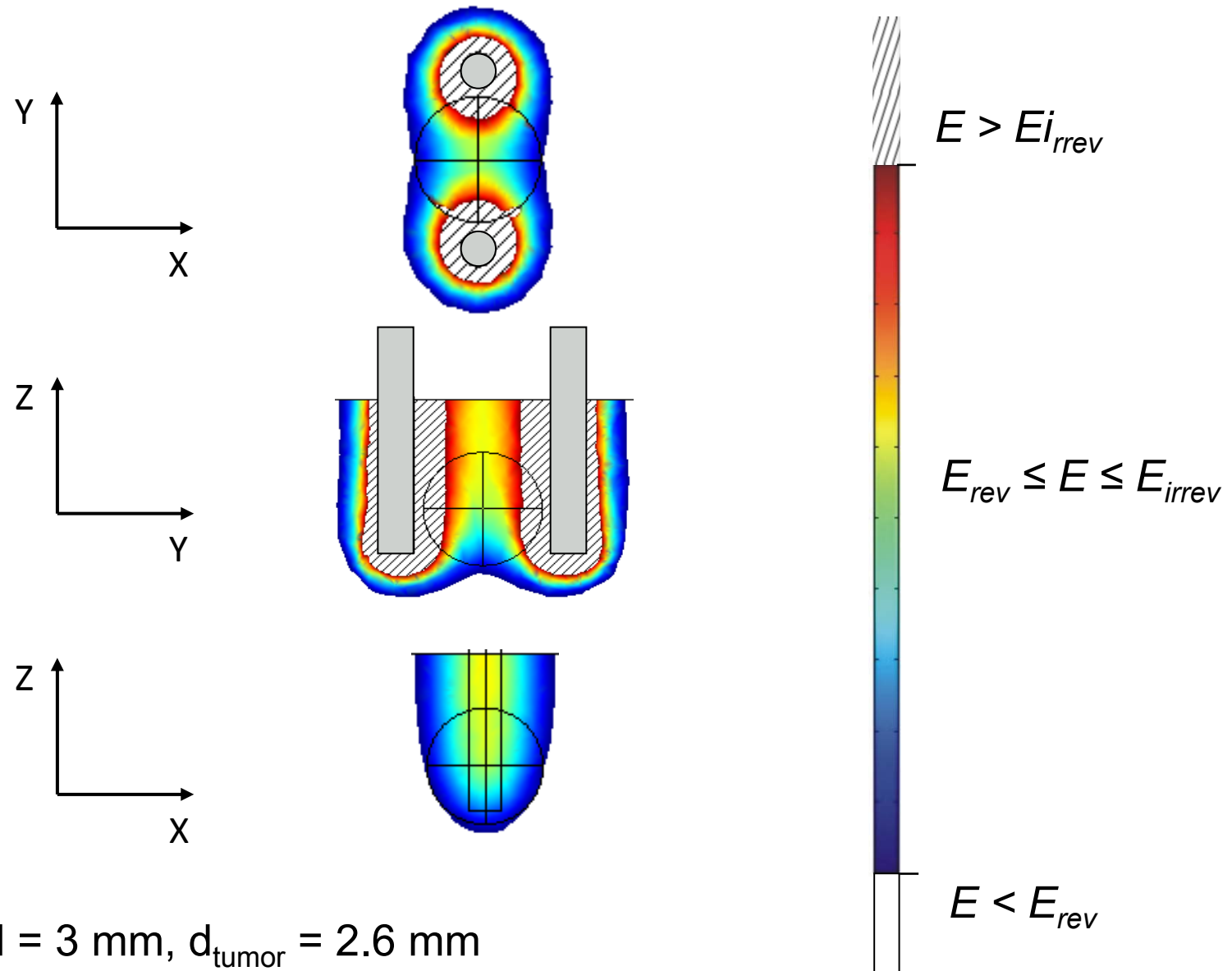


Plate electrodes for cutaneous or sc. tumor



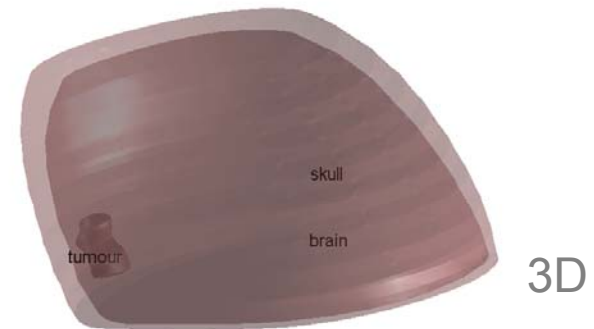
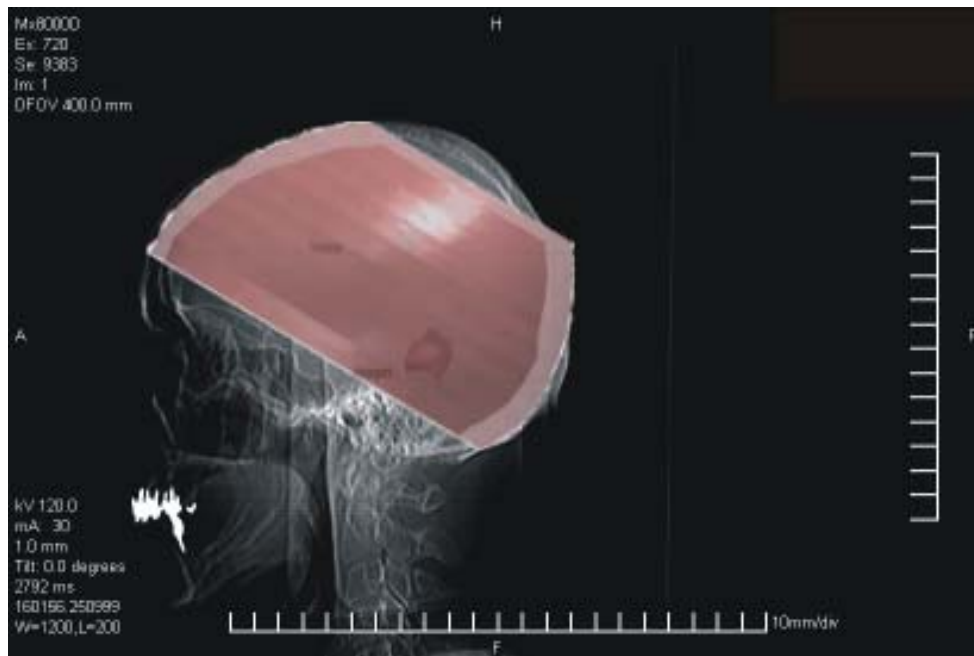
$U = 300 \text{ V}, d = 3 \text{ mm}, d_{\text{tumor}} = 2.6 \text{ mm}$

Needle electrode for cutaneous tumor



$U = 300 \text{ V}$, $d = 3 \text{ mm}$, $d_{\text{tumor}} = 2.6 \text{ mm}$

3D geometry of brain, skull and tumour

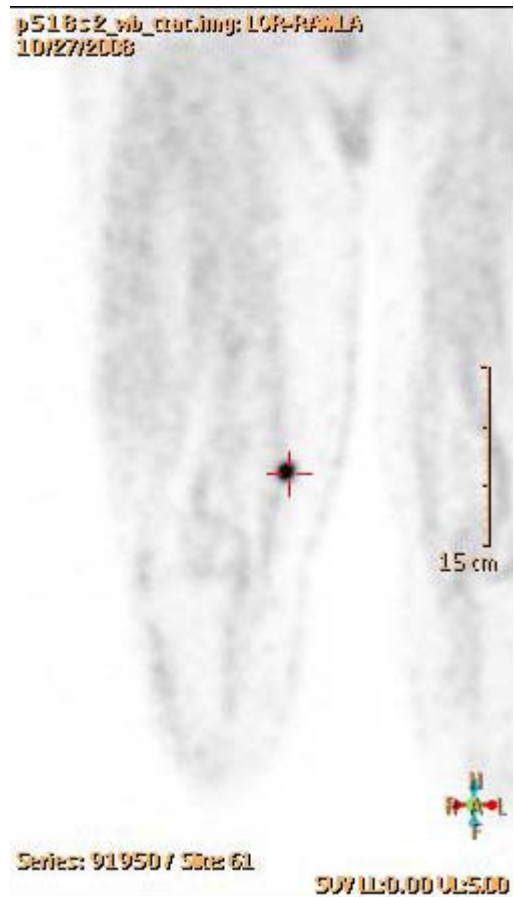


Targetting specific tissue –
treatment planing

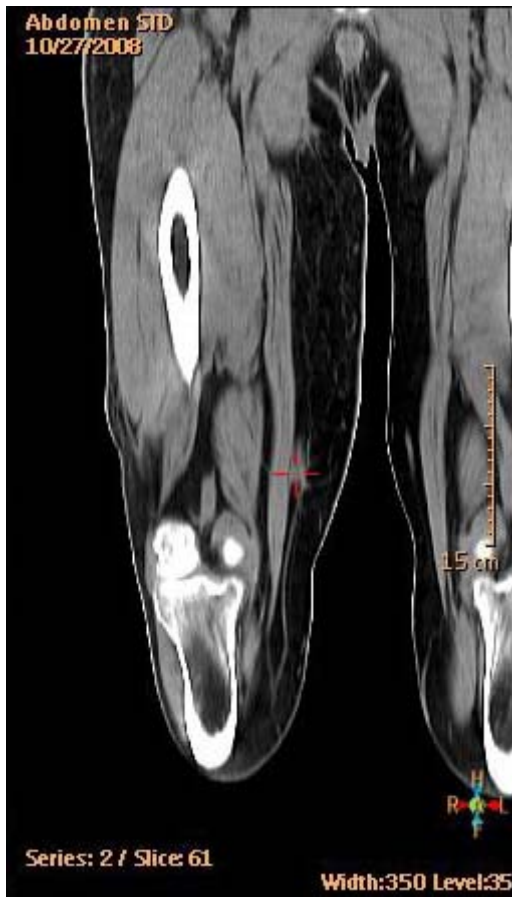
Deep tumor treatment by ECT

- Patient: M, 54 years
- Tumor: disseminated malignant melanoma
- Tumor size: 20 x 16 x 14 mm
- Location: thigh, 18 mm below the skin
- Electrodes: needle, 3 cm tip not insulated, insulated, diameter 1.8 and 1.2 mm
- Cliniporator VITAE (IGEA, Carpi, Italy)
 - 8 pulses, 100 μ s, max 3000 V, max 50 A, repetition frequency 4 Hz, 6 channels

Tumor location



+



=

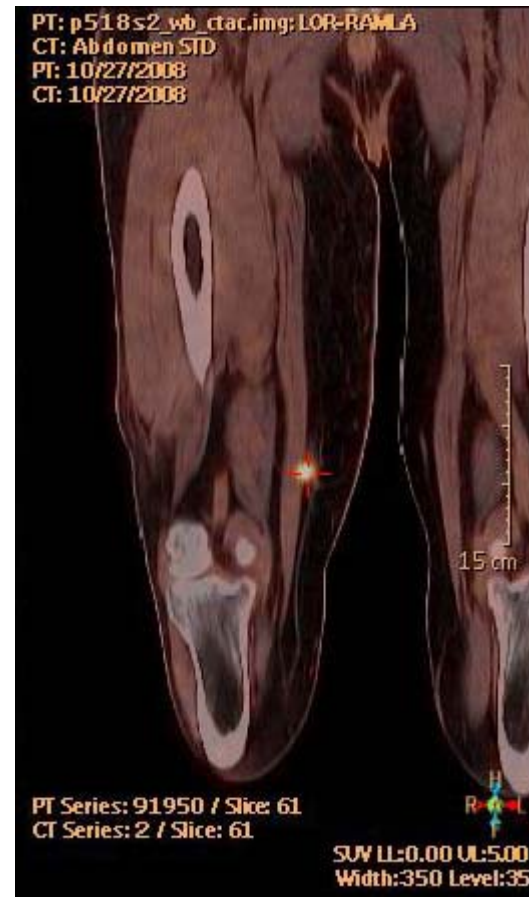
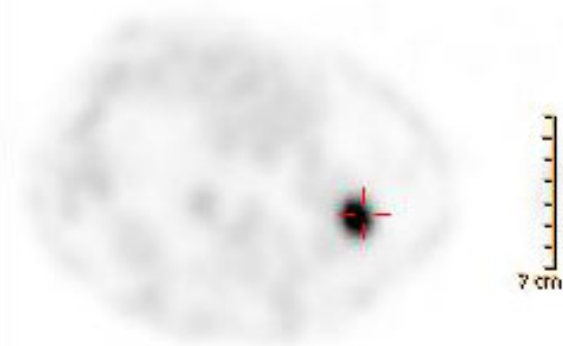


Image segmentation

PET + CT

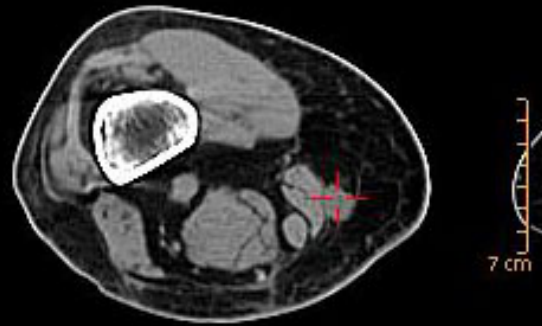
p518s2_wb_ctac.img: LDR-RAMLA
10/27/2008



Series: 91950 / Slice: 302

SUV LL:0.00 UL:5.00

Abdomen STD
10/27/2008



Series: 2 / Slice: 302

Width:250 Level:25

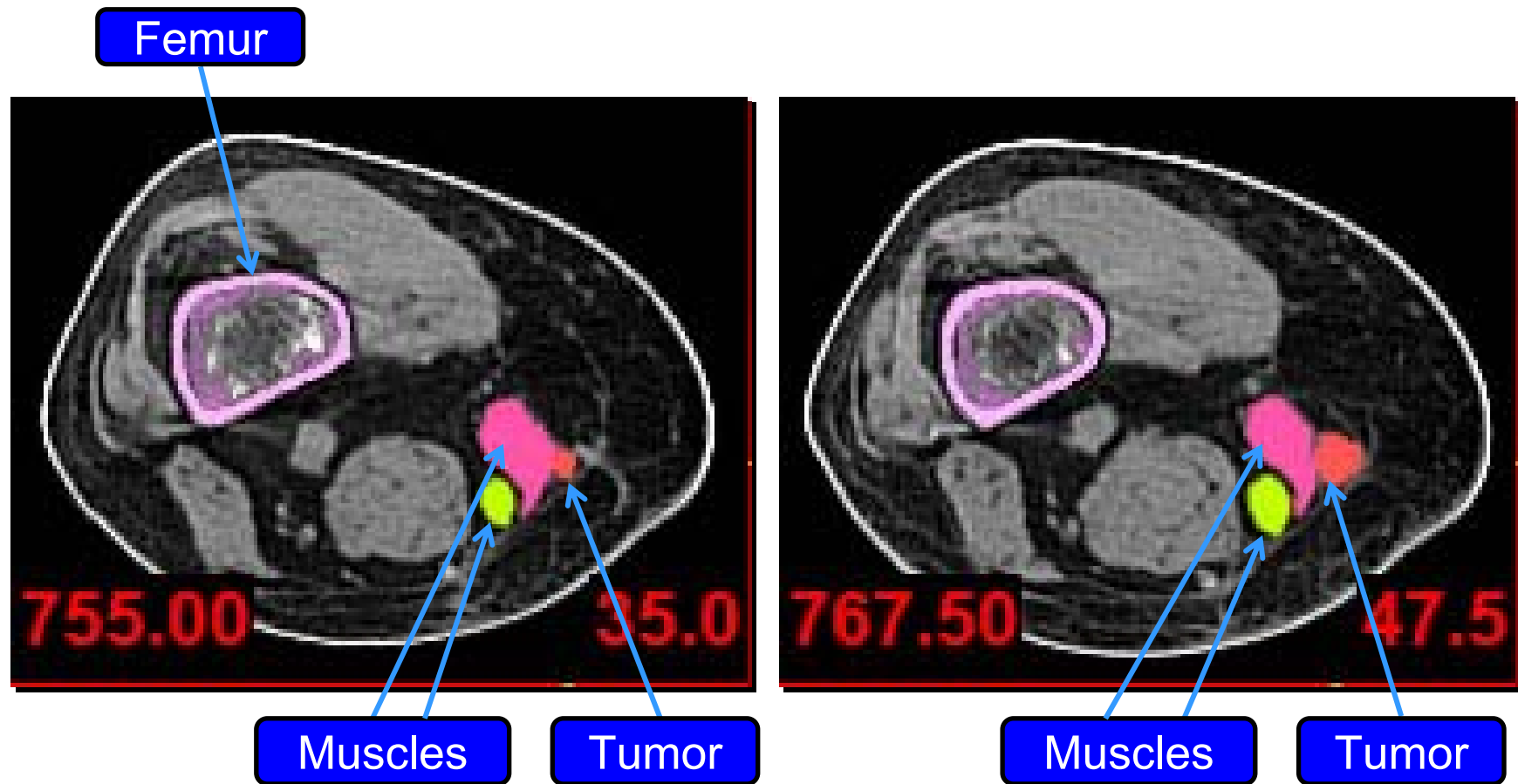
PT: p518s2_wb_ctac.img: LDR-RAMLA
CT: Abdomen STD
PT: 10/27/2008
CT: 10/27/2008



PT Series: 91950 / Slice: 302
CT Series: 2 / Slice: 302

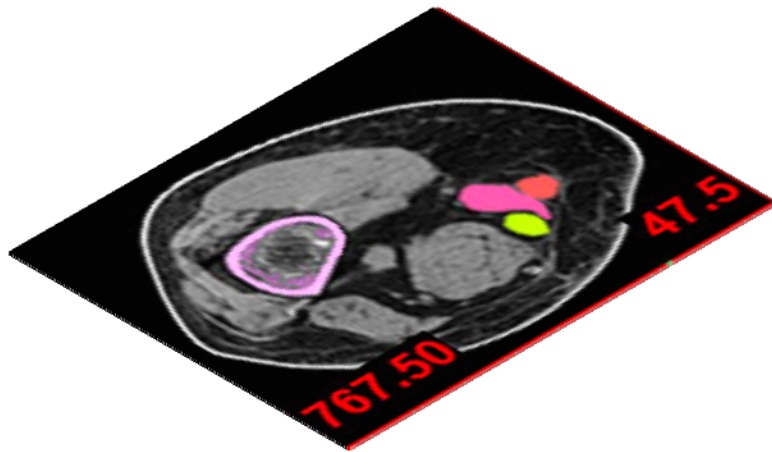
SUV LL:0.00 UL:5.00
Width:350 Level:35

Image segmentation



Building a 3D FEM model for optimisation of electrode placement

Segmented images



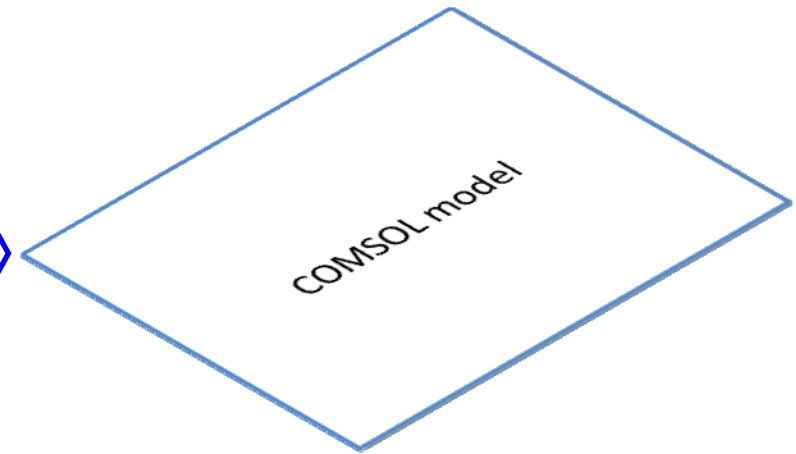
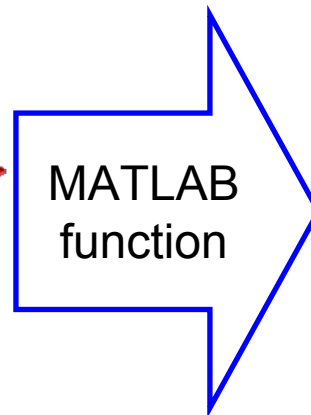
Conductivity:

Tumor: 0.2 S/m

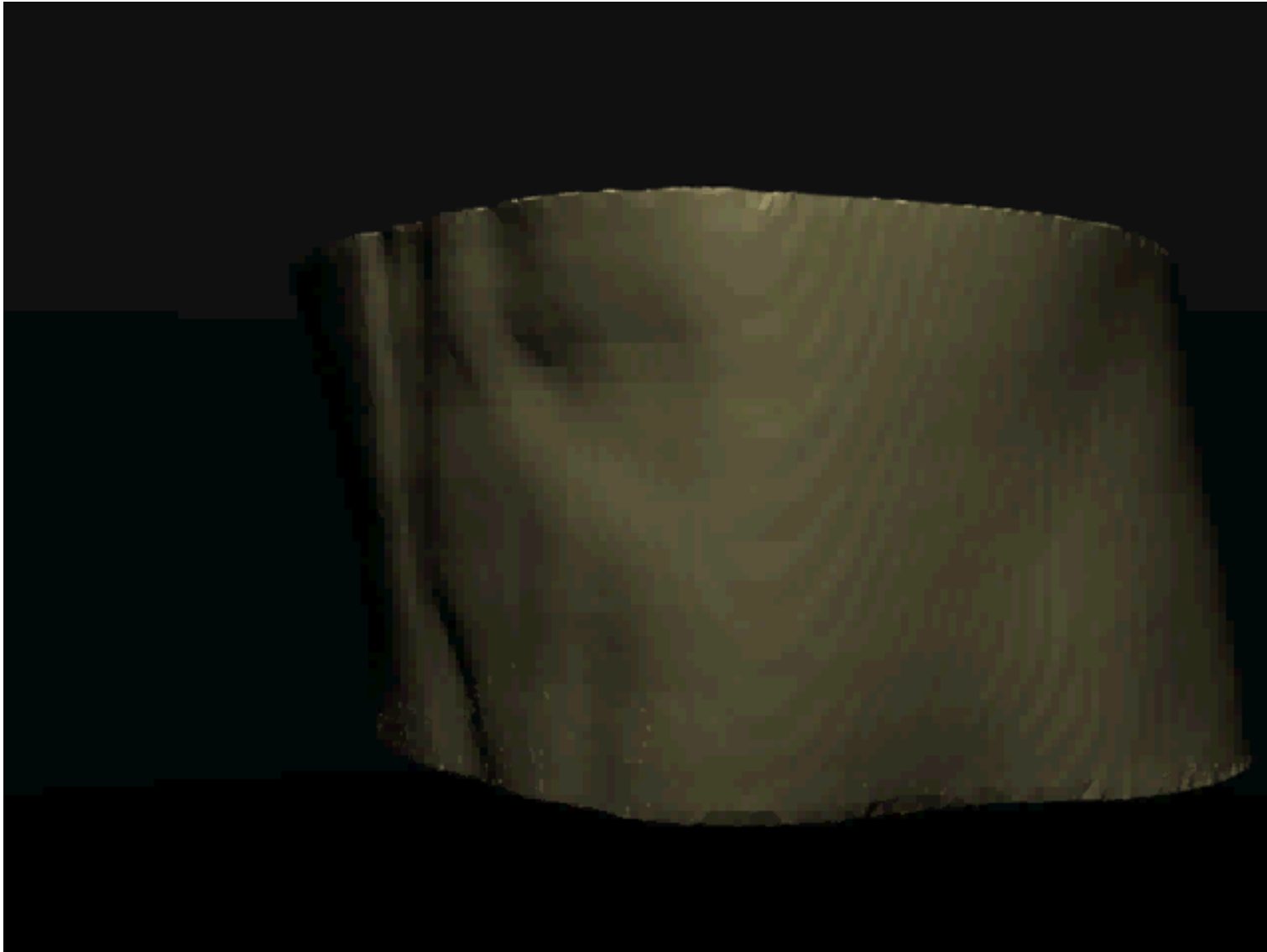
Fat: 0.04 S/m

Muscle: 0.15 S/m (transversal), 0.75 S/m (longitudinal)

3D conductivity data



Building a 3D model



Finite element model - details

- Comsol Multiphysics 3.5 (Comsol AB, Sweden)
- Differential equation: $-\nabla \cdot (\sigma \cdot \nabla \varphi) = 0$
- Conductivity:
 - Tumor: 0.2 S/m
 - Fat: 0.04 S/m
 - Muscle: 0.15 S/m (trans.), 0.75 S/m (longitudinal)
- Boundary conditions:
 - Electric potential (electrodes)
 - Continuity (muscle, tumor)
 - Electric insulation (fat)

Optimization method: genetic algorithm

How does it work?

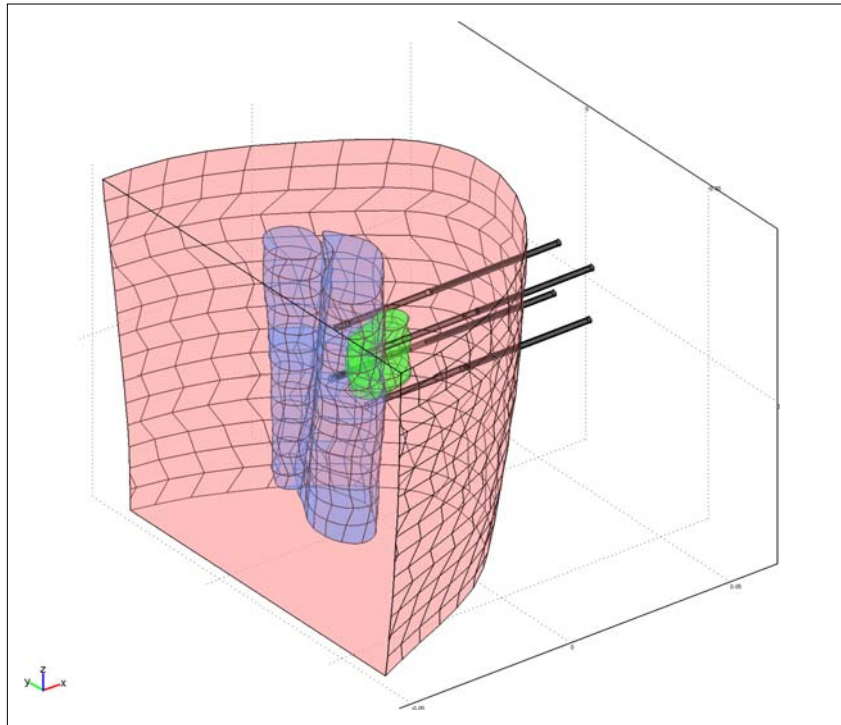
1. Starting with initial population of chromosomes
(optimization variables)
2. Calculating fitness of each chromosome
3. Generating new generation of chromosomes
by mutation or cross-over

$$m_i = x_i + b_i \cdot x_i; \quad b_i \in [-0.3, 0.3] \quad z_i = a_i \cdot x_i + (1 - a_i) \cdot y_i; \quad a_i \in [0, 1]$$

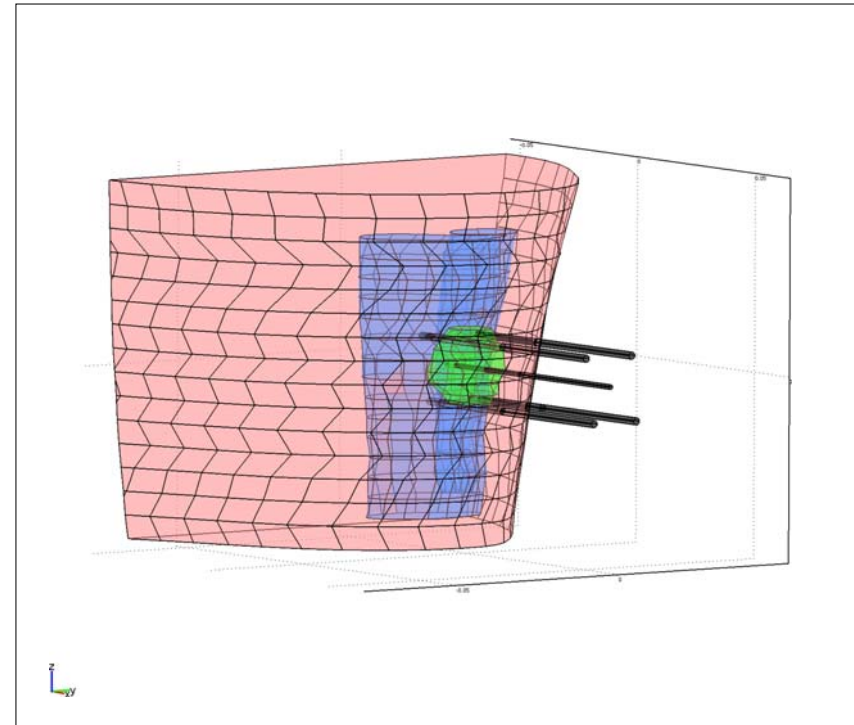
4. Calculating fitness for new generation
5. Repeat step 3 and 4 until Stop condition

Mutation probability: 0.2
Cross_over probability: 0.8

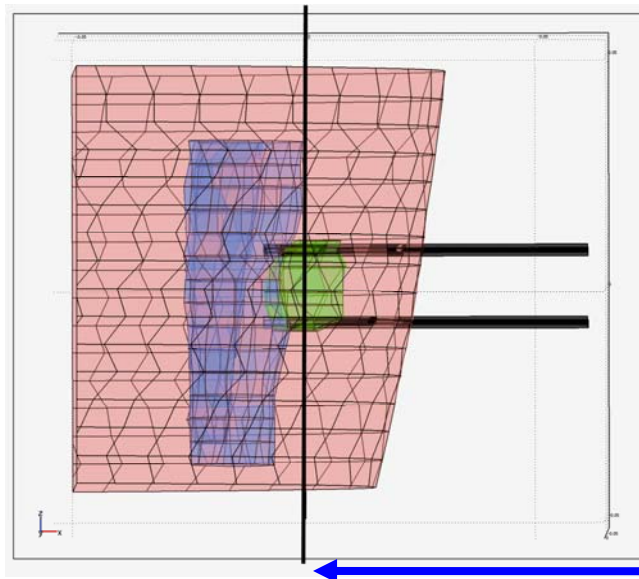
Čorović S, Županič A, Miklavčič D., *IEEE Trans. Plasma Sci.* 36: 1665-1672, 2008
Županič A, Čorović S, Miklavčič D., *Radiol. Oncol.* 42: 93-101, 2008



Four electrodes



Five electrodes



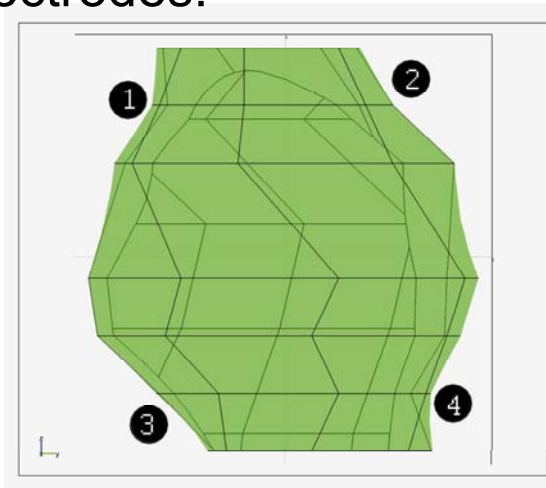
- Tumor
- Muscle
- Fat
- Electrodes

Point of view

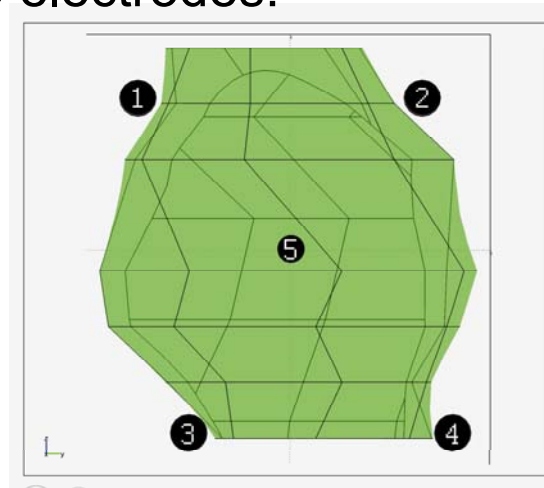
Optimization method: genetic algorithm

- Random initial population (40)
- Objective function: $I = 1000 \cdot \text{electroporated tumor} - \text{electroporated muscle}$

Four electrodes:



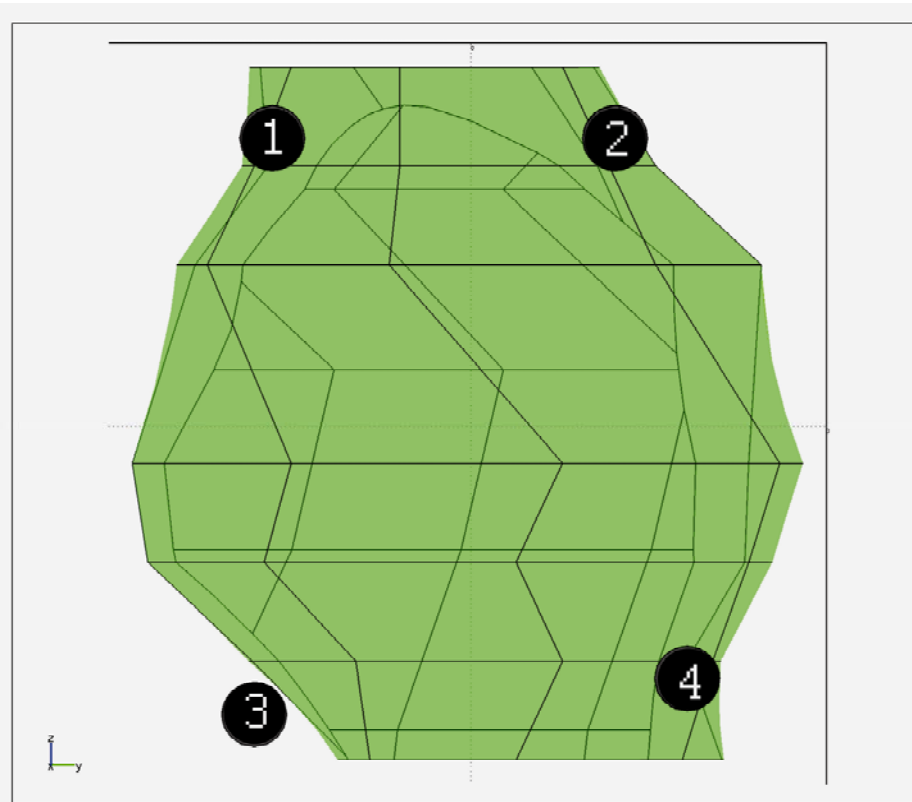
Five electrodes:



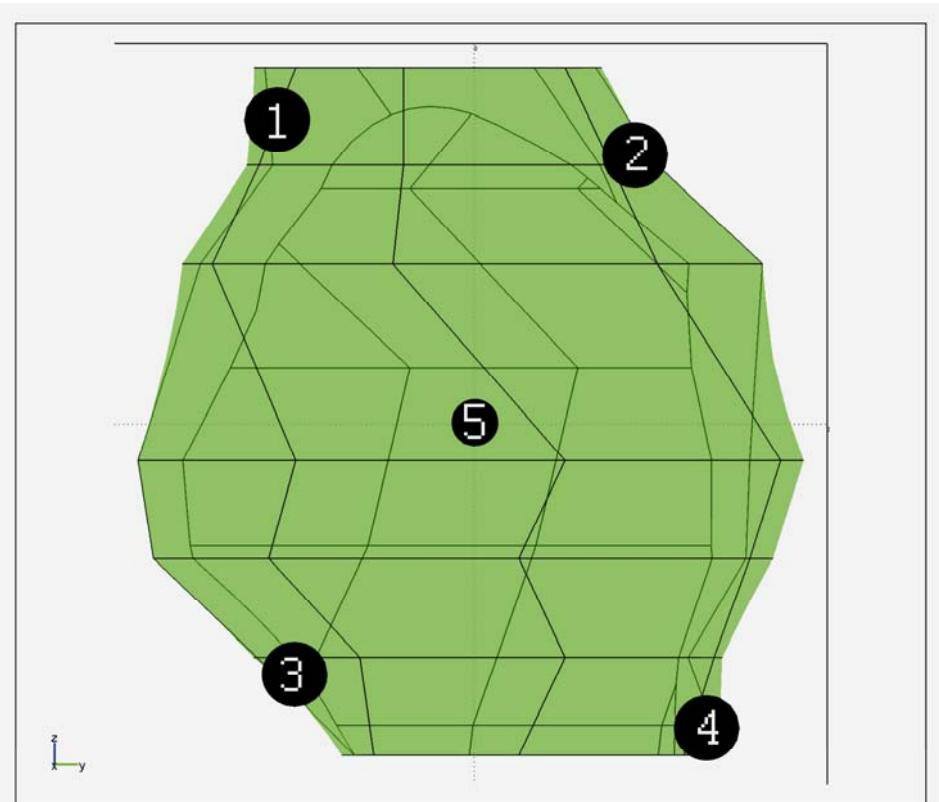
- Optimization variables (18)
 - Electrode positions: $x_{1-4}, y_{1-4}, z_{1-4}$
 - Voltages: $U_{1,2}, U_{1,3}, U_{1,4}, U_{2,3}, U_{2,4}, U_{3,4}$
 - Voltage constraint: 3000 V
 - Stop condition: 100 iterations
- Optimization variables (21)
 - Electrode positions: $x_{1-4}, y_{1-4}, z_{1-4}, x_5$
 - Voltages: $U_{1,2}, U_{1,3}, U_{2,3}, U_{2,4}, U_{1,5}, U_{2,5}, U_{3,5}, U_{4,5}$
 - Electrode 5 – only depth of insertion (x_5) was optimized

Optimal treatment plan according to the genetic algorithm

Four electrodes



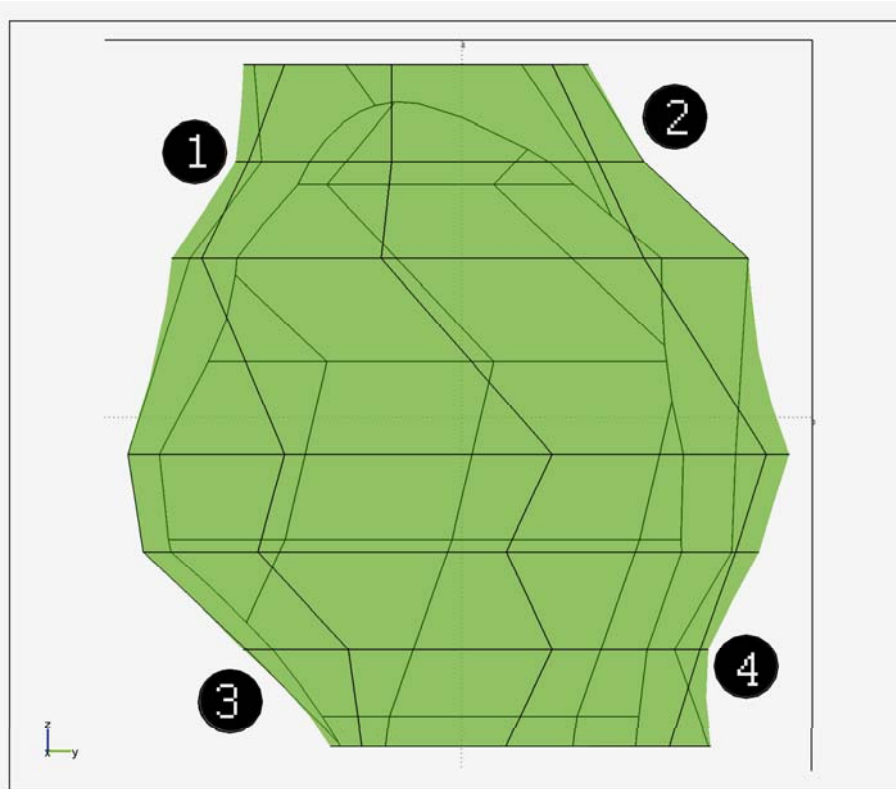
Five electrodes



Total computation time: 4h; Desktop PC (Core2 Duo, 2.66 GHz, 4 GB RAM)

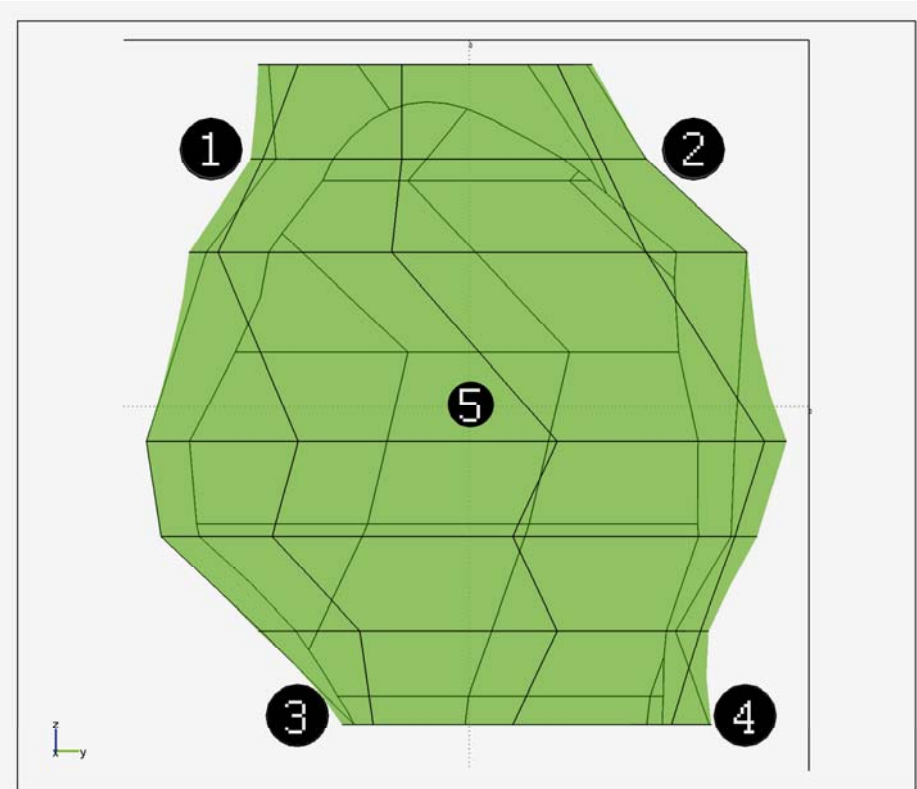
Adjustment of treatment plan by forward modeling

Four electrodes



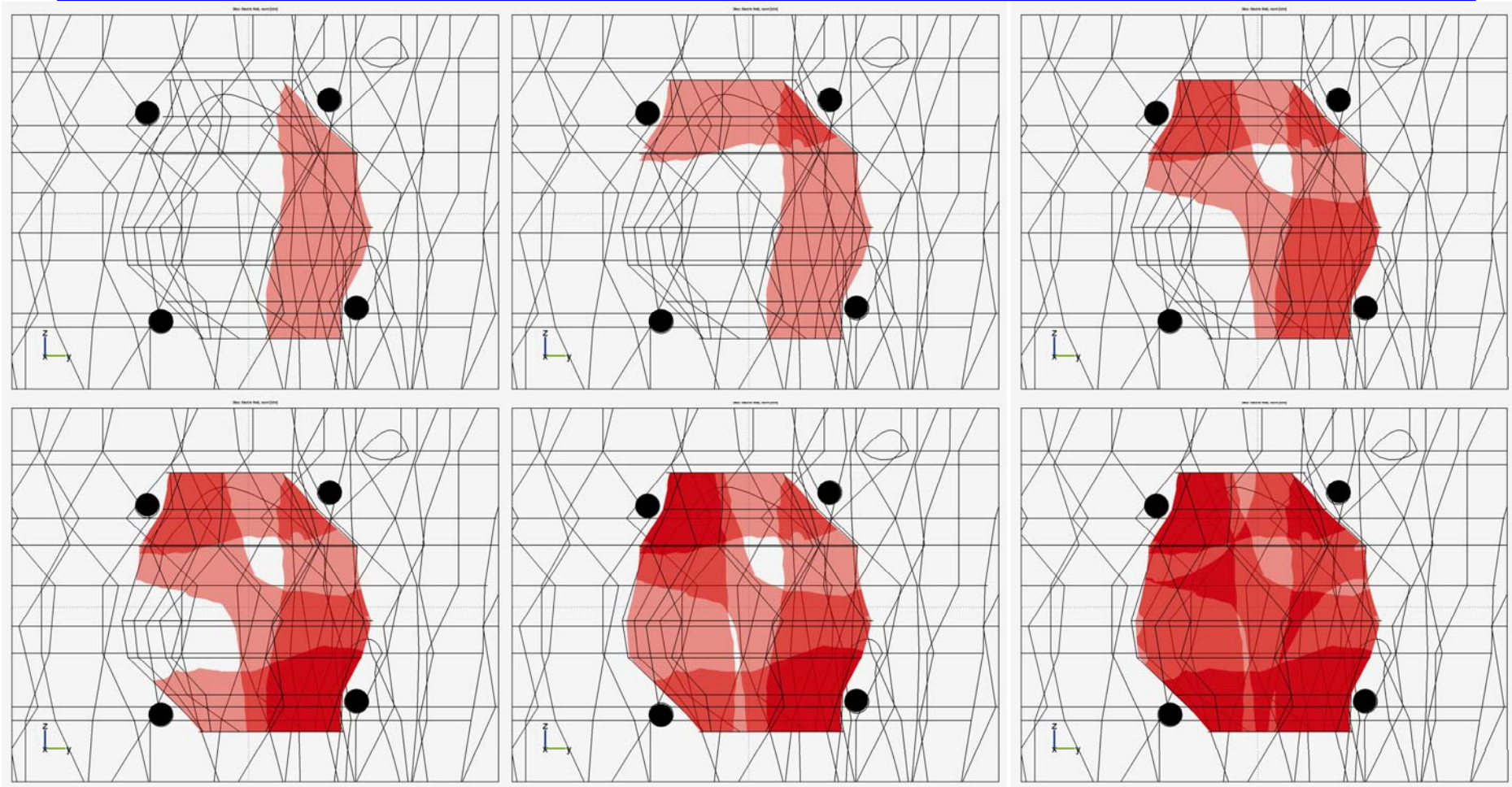
$$\begin{aligned}U_{1,2} &= 2000 \text{ V}, U_{1,3} = 2700 \text{ V} \\U_{2,4} &= 2700 \text{ V}, U_{3,4} = 2000 \text{ V} \\U_{1,4} &= 3000 \text{ V}, U_{2,3} = 2900 \text{ V}\end{aligned}$$

Five electrodes



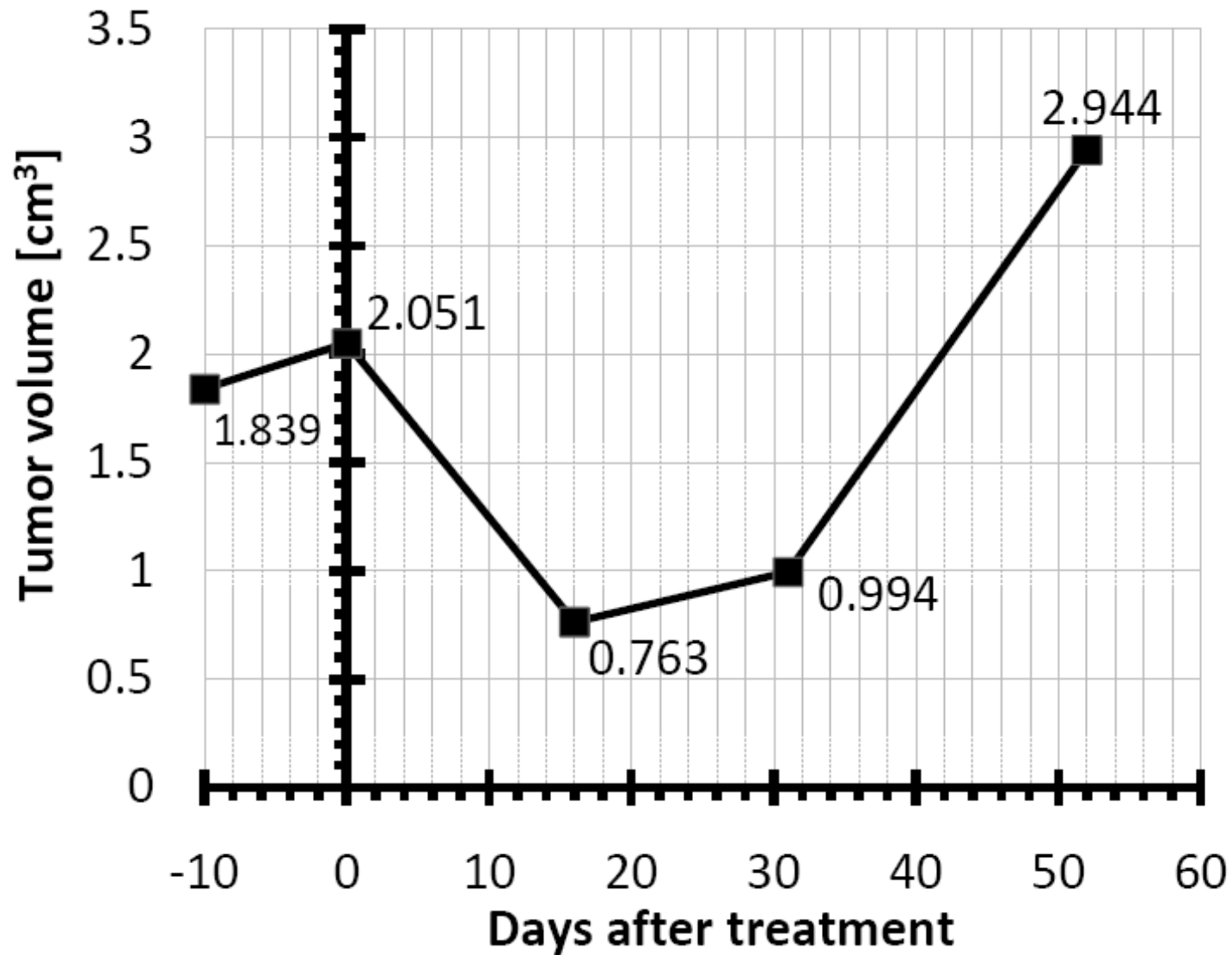
$$\begin{aligned}U_{1,2} &= 2000 \text{ V}, U_{1,3} = 2500 \text{ V} \\U_{2,4} &= 2500 \text{ V}, U_{3,4} = 2000 \text{ V} \\U_{1,5} &= 1700 \text{ V}, U_{2,5} = 1700 \text{ V} \\U_{3,5} &= 1700 \text{ V}, U_{4,5} = 1700 \text{ V}\end{aligned}$$

Sequence of pulses applied – tumor covered with $E > E_{rev}$ (550^* V/cm)

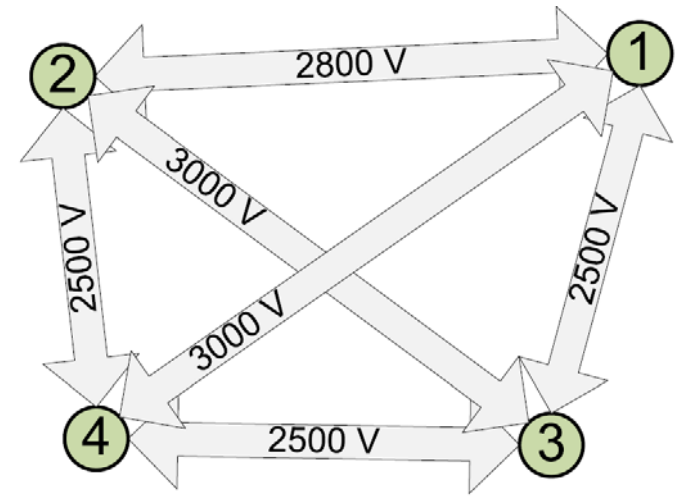
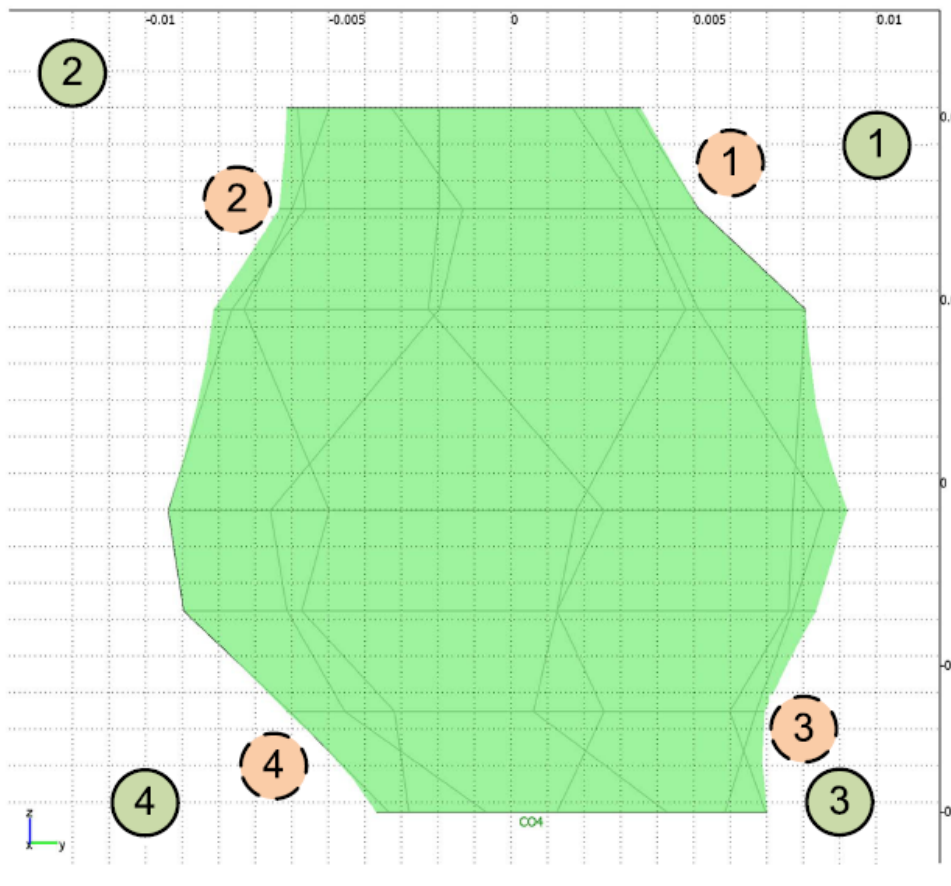


*Pavšelj N et al, *IEEE Trans. Biomed. Eng.* **52**: 1373-1381, 2005

Tumor growth

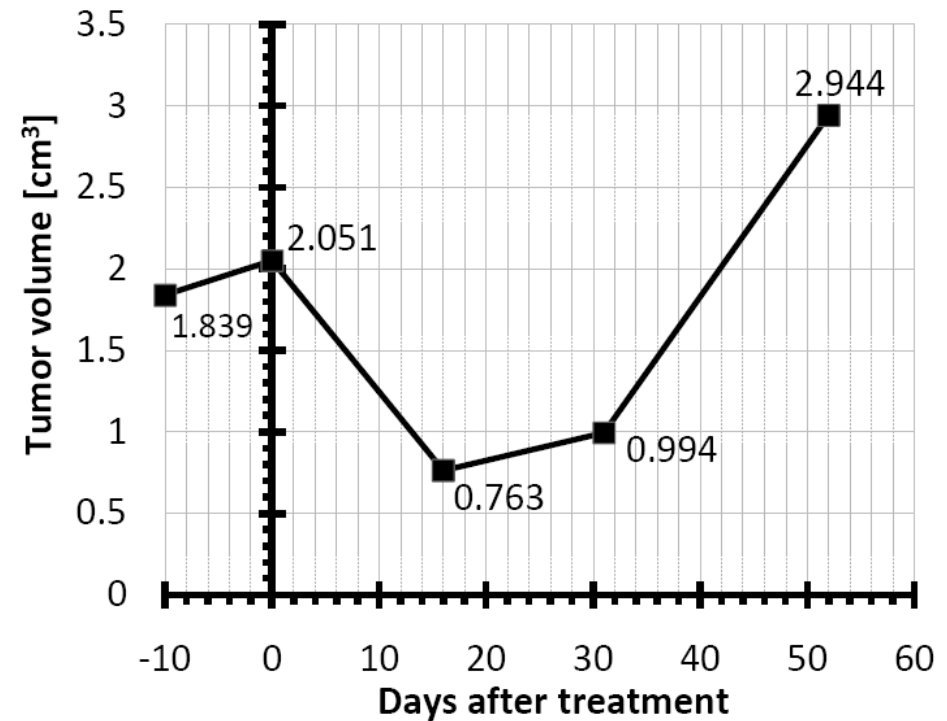
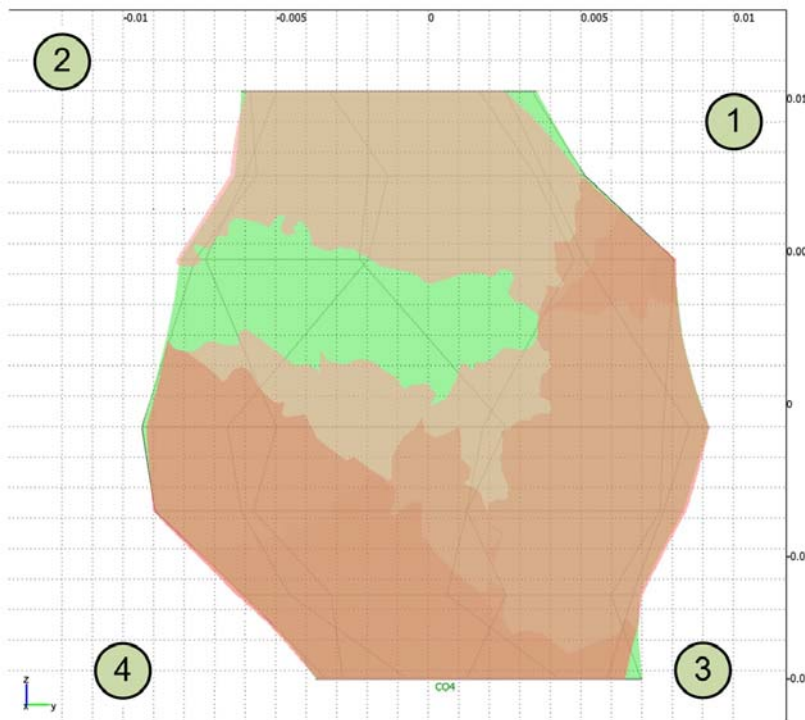


After the treatment:



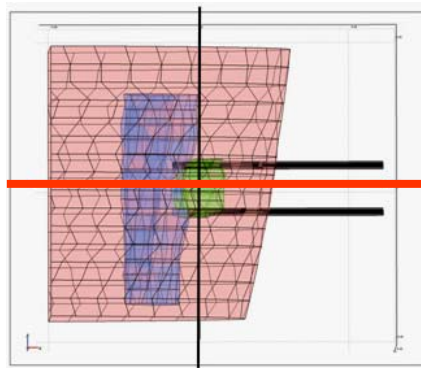
Electrode pair	Measured current [A]	Calculated current [A]	Error [%]
1-2	16	16.2	1
1-3	18	17.3	-4
1-4	10	9.5	-5
2-3	19	17.2	-9
2-4	9	8.4	-7
3-4	9	8.0	-11

Tumor covered by sufficiently high electric field

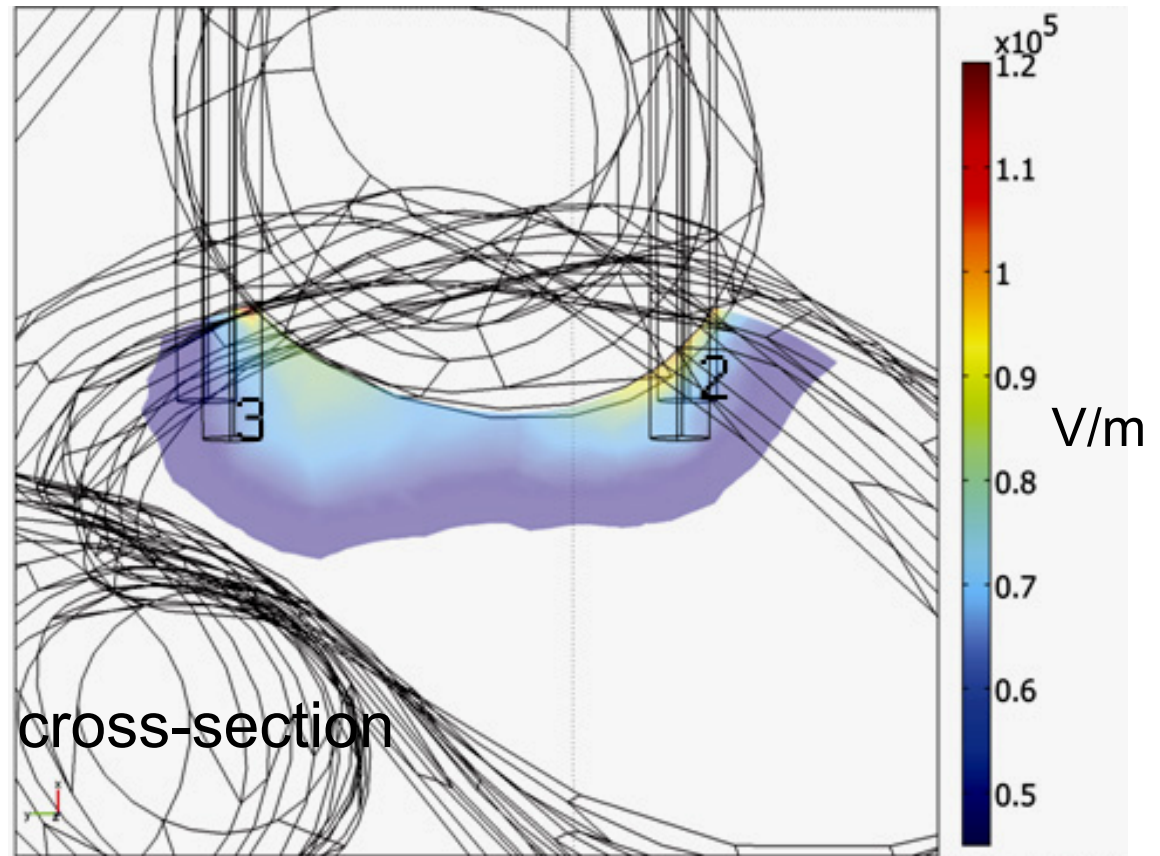


Electrical pulses applied – possible damage to healthy tissue - muscle

$E > 450 \text{ V/cm}$
(irreversible threshold)
Four electrodes used
 $U_{2,3}$ (diagonal) = 2900 V



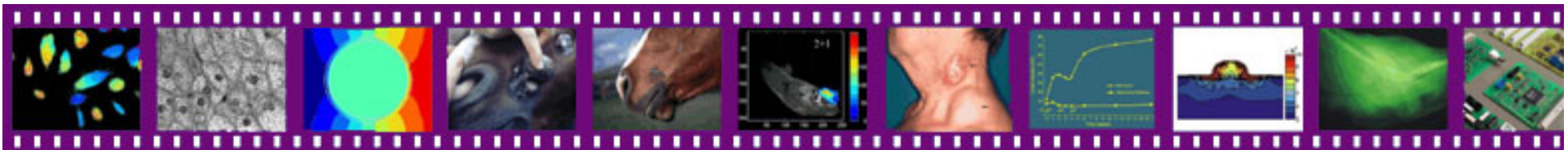
Muscle cross-section



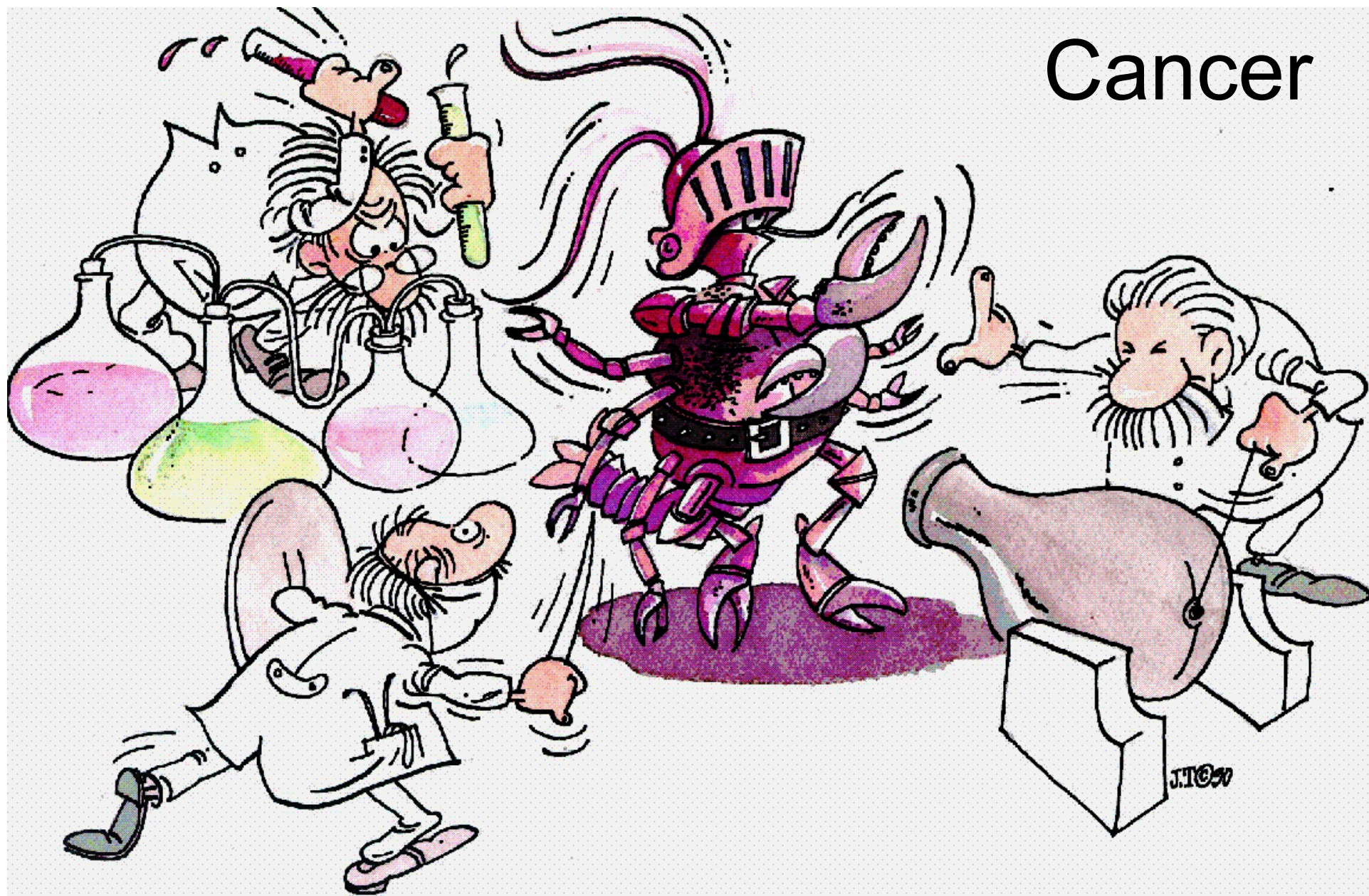
Min $E = 450 \text{ V/cm}$

Aims and scopes

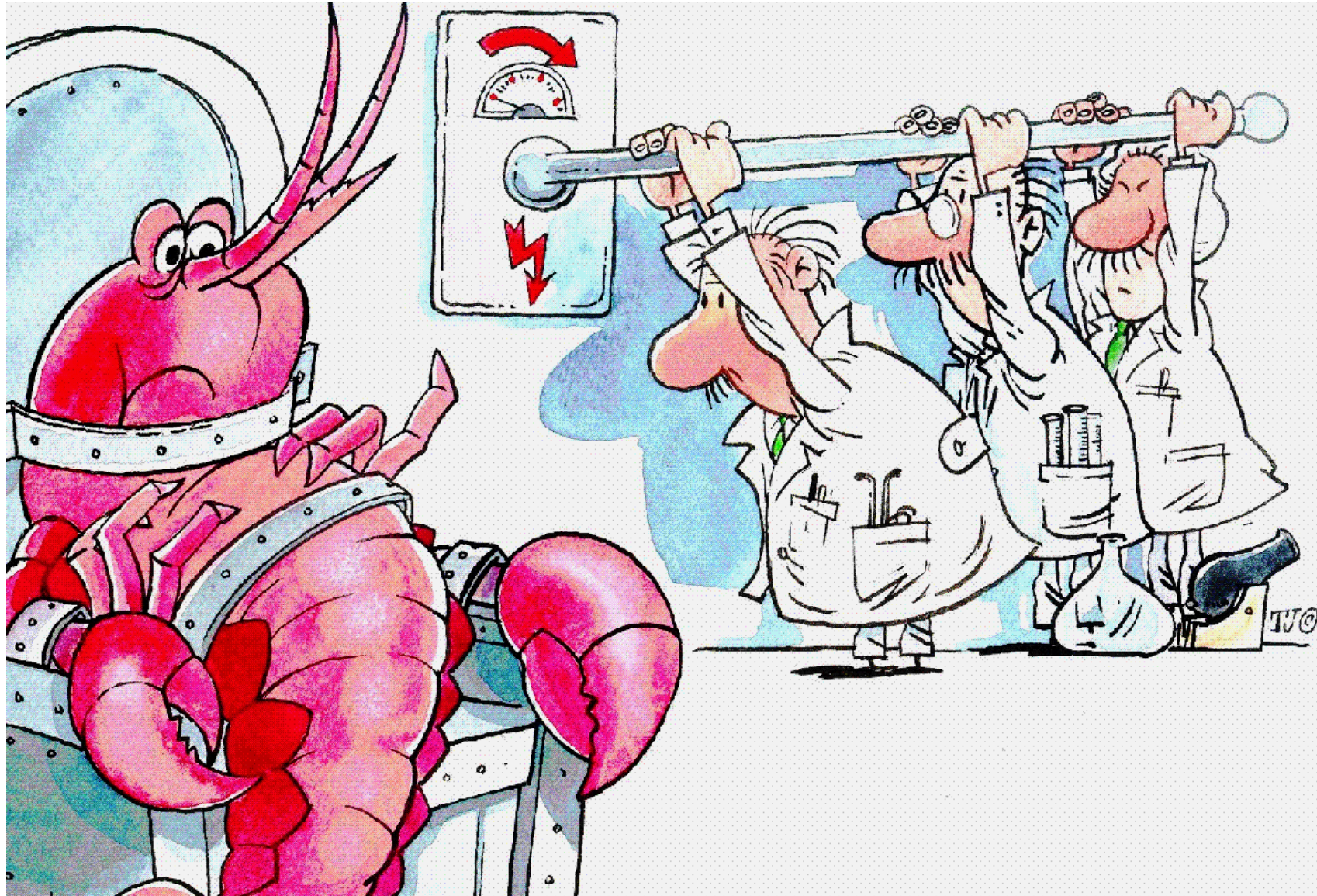
- What is electroporation?
- Use of electroporation in biology, biotechnology and medicine
- Electrochemotherapy
- Electrochemotherapy treatment planning



Cancer



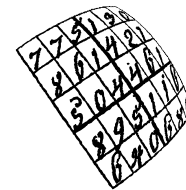
Electrochemotherapy



Acknowledgements

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JAVNA AGENCIJA ZA RAZISKOVALNO DEJAVNOST
REPUBLIKE SLOVENIJE

Laboratory of *bio*cybernetics



<http://lbk.fe.uni-lj.si>

Peter, Katja, Marko, Jakob, Tadej, David, Matej K., Tomaž, Matej R., Denis,
Blaž, Damijan, Ivan, Janez, Mojca, Stanislav, Gorazd, Vilko, Selma, Čarli,
Nataša, Maša, Alenka, Barbara
(missing from the photo: Anže, Tina, Jerneja, Saša, Duša, Bor, Jaka)

Hvala na pažnji !

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