THE GEORGE WASHINGTON UNIVERSITY
WASHINGTON, DC

Micro-propulsion and Nanotechnology Lab
MPNL
The George Washington University

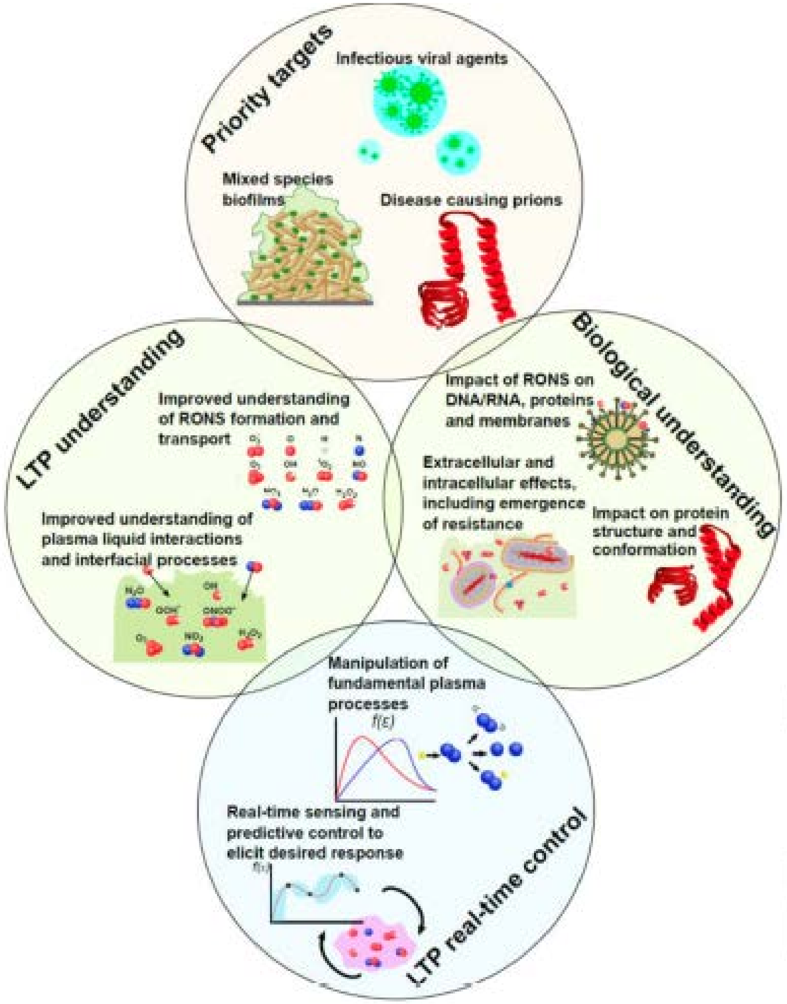
Adaptive Plasmas for Biomedical Applications

Michael Keidar
The George Washington University

Technical Meeting on Emerging Applications of Plasma Science and Technology

Biomedical Applications

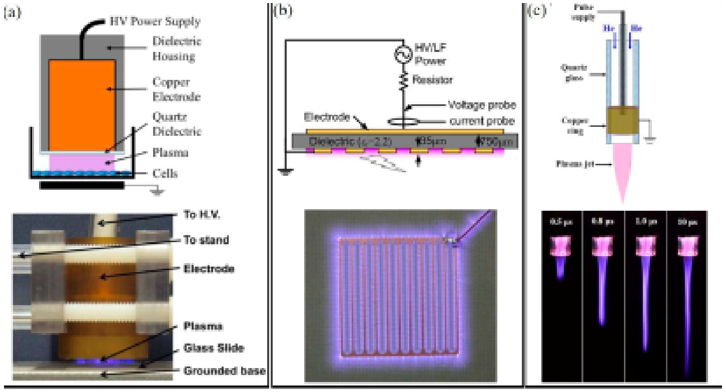
- Bacteria/virus inactivation
- Blood coagulation
- Wound healing
- Cancer therapy

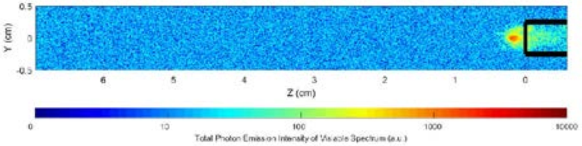


Laroussi et al., IEEE TRRMS, 2022

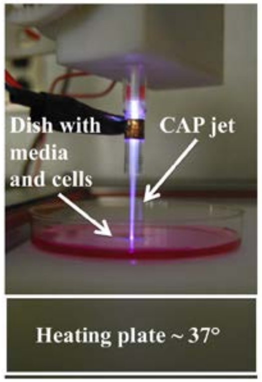
Summary: Plasmas for Medicine

Cold atmospheric plasmas



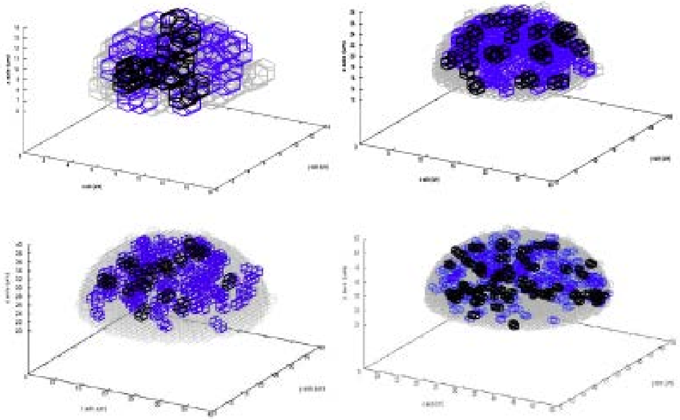


In vitro

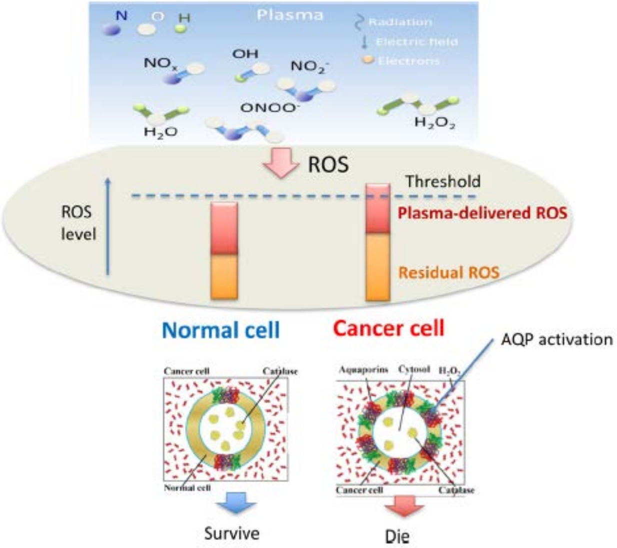


In vivo

Simulation of plasma interaction with solid tumor



Mechanism



Clinical study

Treatment of surgical margins was performed

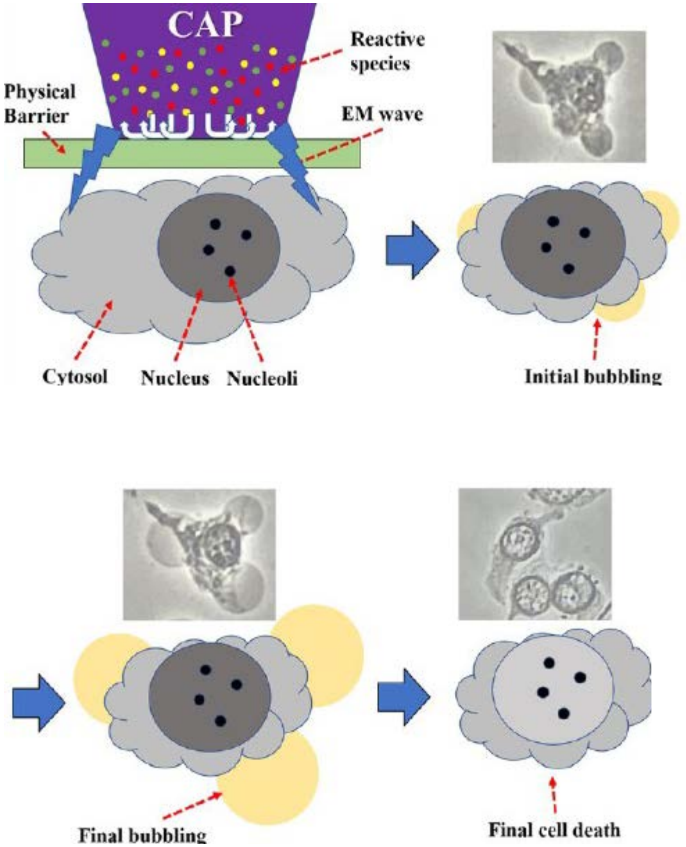
Rush University Medical Center applied USMI/GWU CAP device for treatment for pancreatic cancer, April 2017

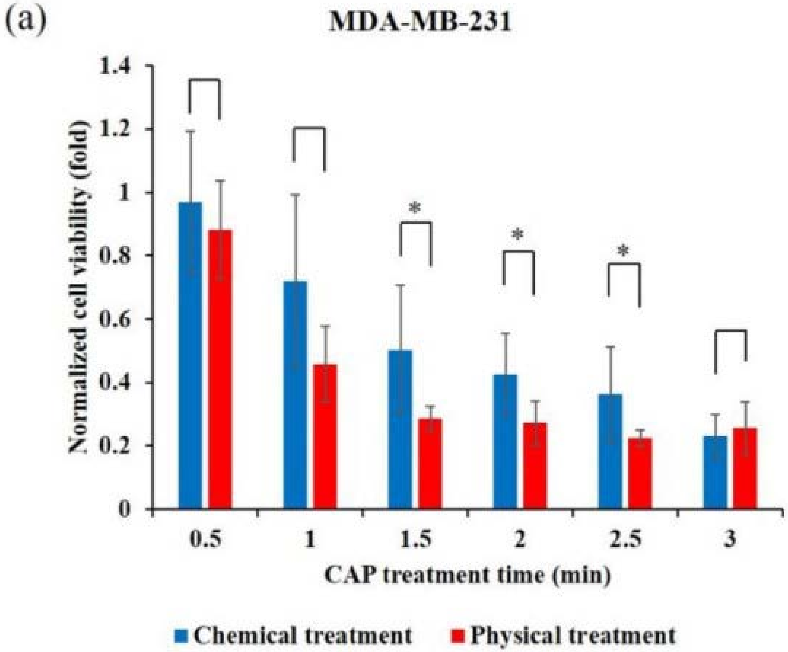
20 new cases starting August 2019

JCRI-ABTS and USMI Successfully Complete Phase 1 Multi-Center Clinical Trial Using Canady Helios™ Cold Plasma for the Treatment of Cancer
April 20, 2021 10:30 AM EST

Baton Rouge General Medical Center,
March 2015

Research Background:
Plasma leads to cell death

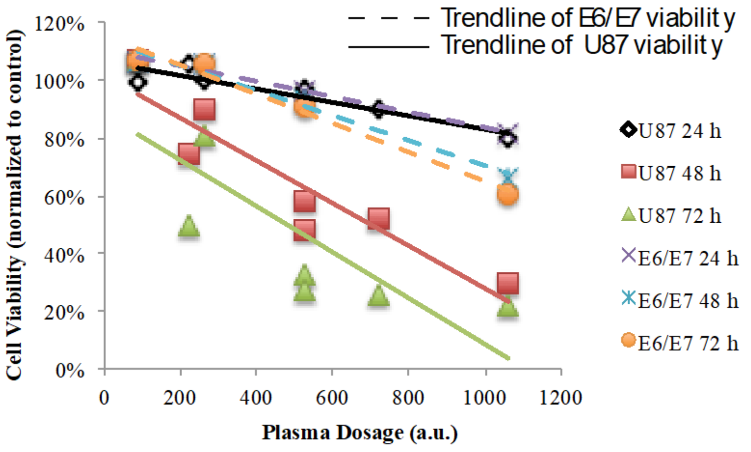




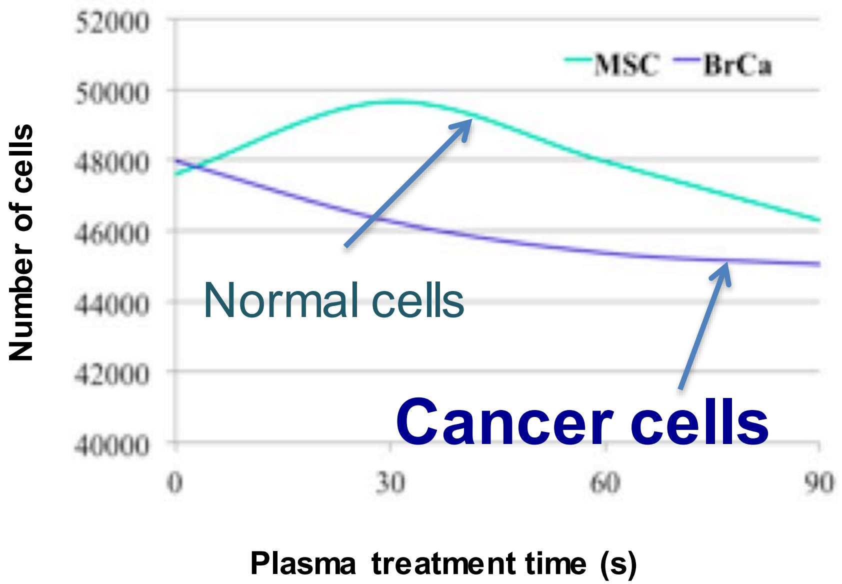
Journal of Physics D: Applied Physics 2021, 54 (9), 095207

ACS Appl. Mater. Interfaces 2020, 12, 31, 34548–34563

Research Background: Selectivity



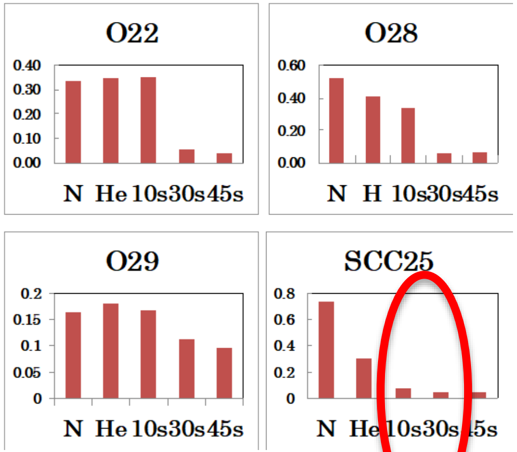
X. Cheng et al., PloS One, 2014



Normal cells

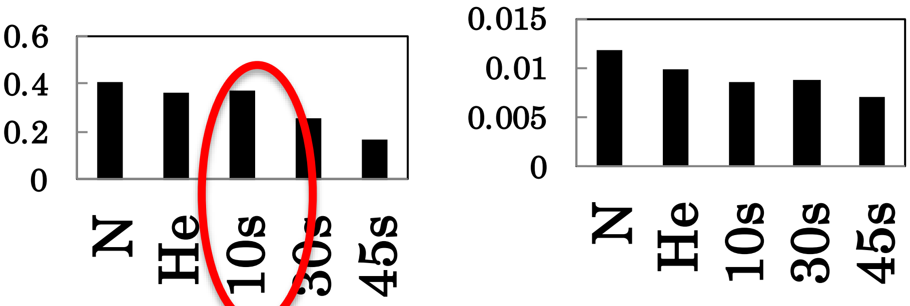
Cancer cells

Wang et al, PloS One, 2013



Head and Neck Squamous Cell Carcinoma (HNSCC)

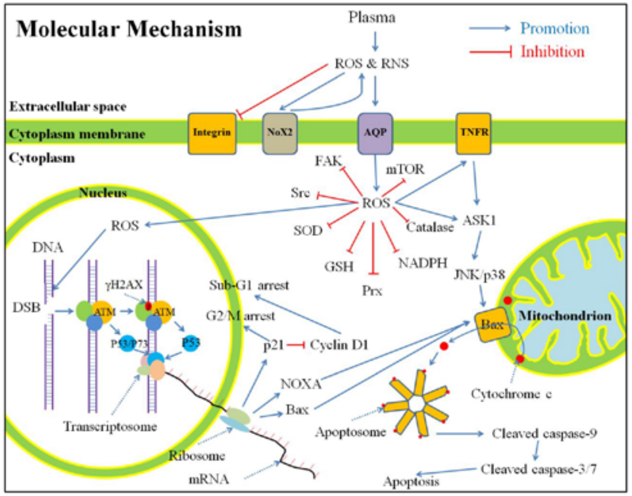
Rafael Guerrero-Preston et al, IJMM 2014



Normal oral cavity epithelium cell lines

Plasma in biomedical application

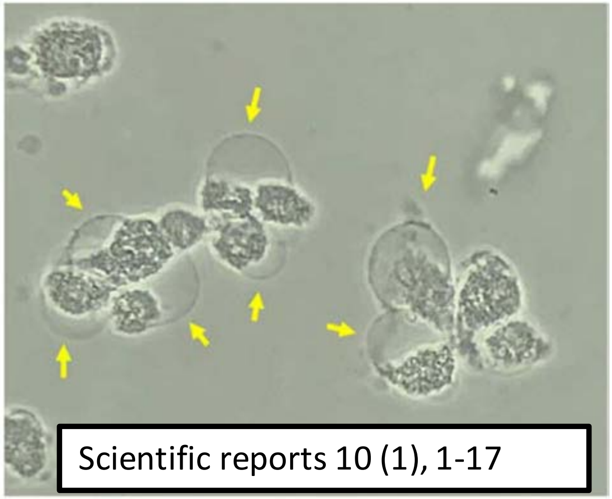
Reactive Oxygen & Nitrogen Species (RONS) leading to apoptosis



Oncotarget, 2017, Vol. 8, (No. 9), 15977

chemical

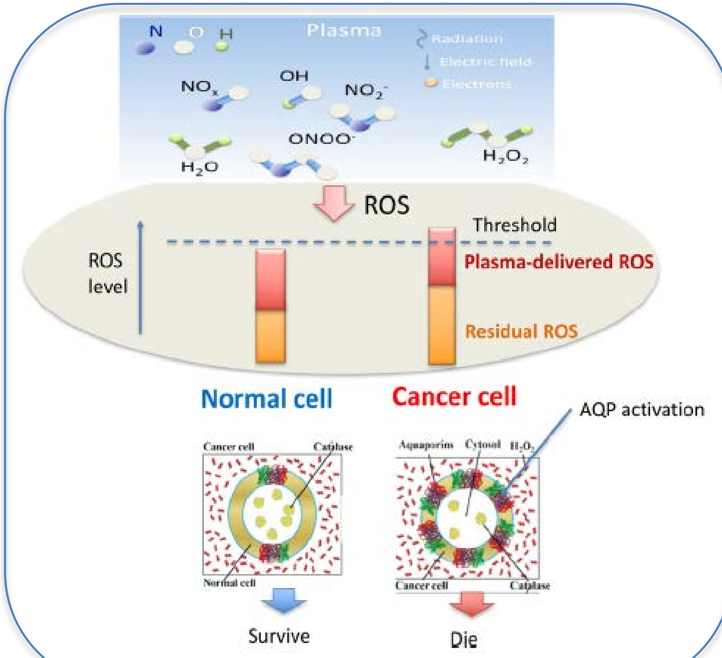
Physical effect: CAP treatment with cover on Petri dish (no RONS)



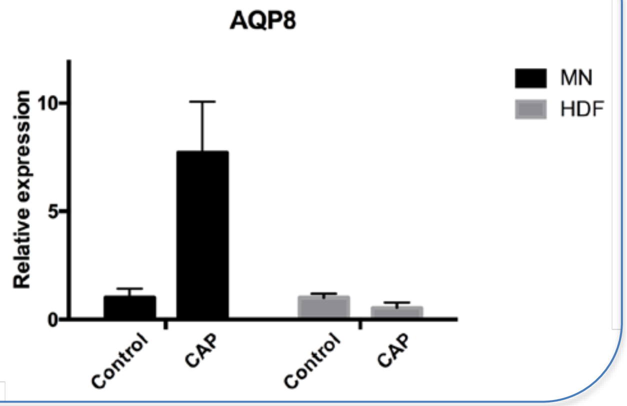
Scientific reports 10 (1), 1-17

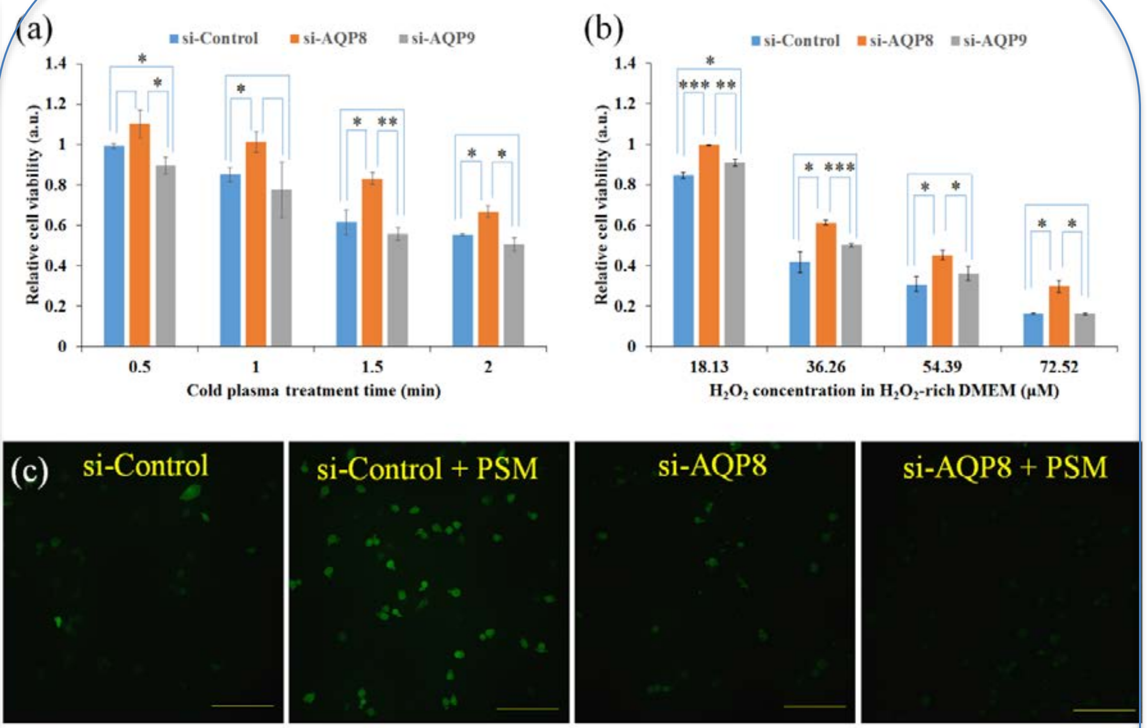
physical

Mechanism of CAP action. Chemical pathway



Plasma turn ON AQP



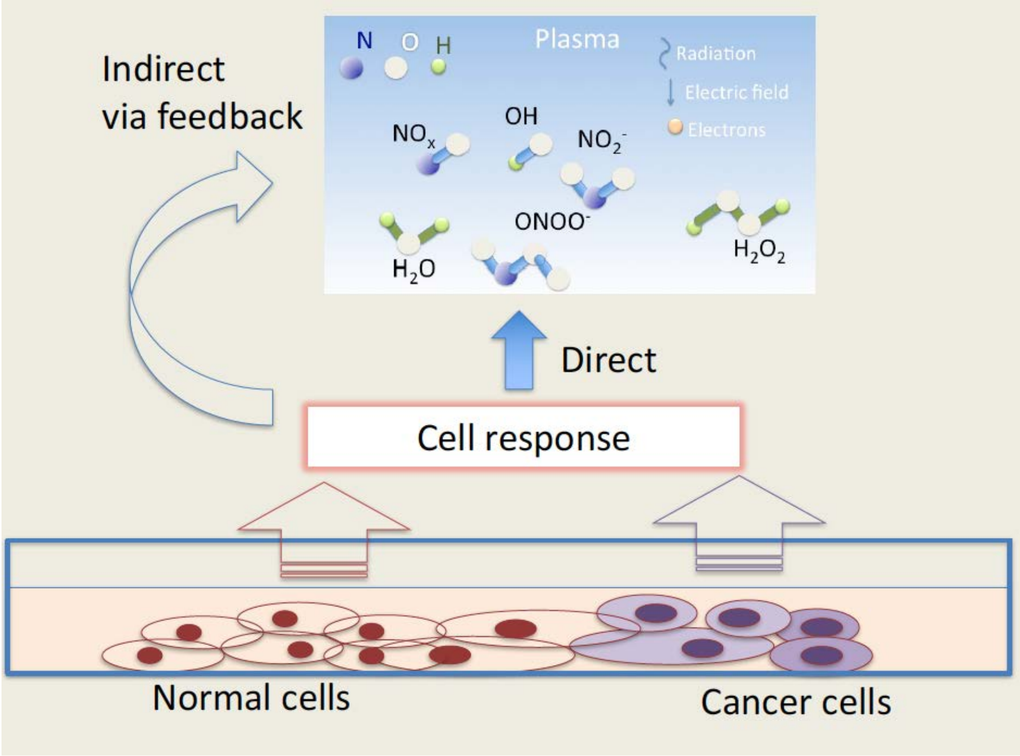


Knocking down AQP8 and AQP9 weaken the CAP action

Keidar, Phys Plasmas, 2018
Keidar, Ed, Plasma Cancer Therapy, 2020, Springer
Yan et al., J. Phys D., 2016

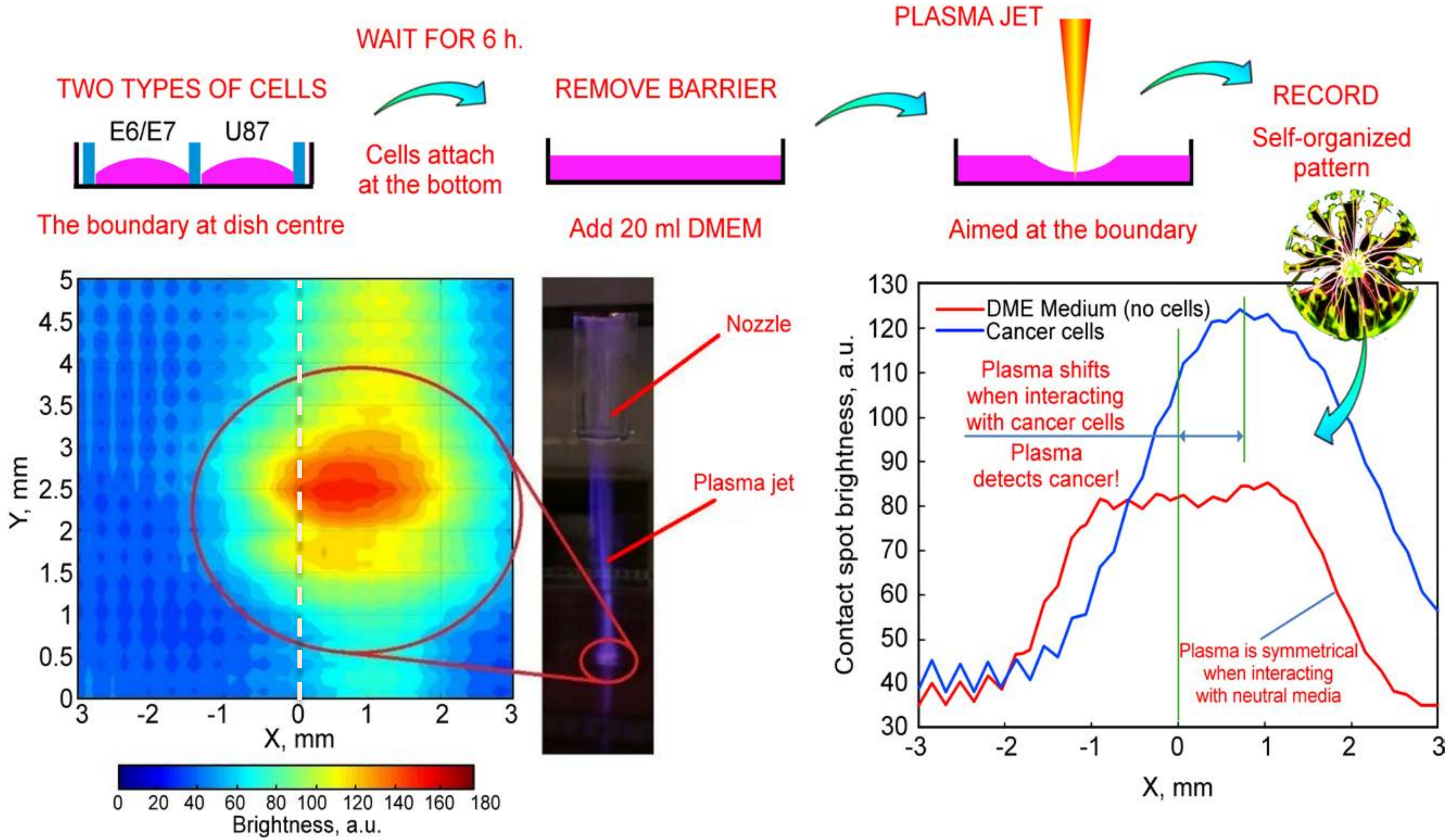
Adaptive plasma platform for cancer therapy

Uniqueness of plasma is its ability to change its composition and key parameters on demand, dependent on specific requirements of diseased cells



Keidar et al, *Trends in Biotechnology*, 2017

Self-adaptive ?



TWO TYPES OF CELLS

The boundary at dish centre

WAIT FOR 6 h.

Cells attach at the bottom

REMOVE BARRIER

Add 20 ml DMEM

PLASMA JET

Aimed at the boundary

RECORD

Self-organized pattern

Plasma shifts when interacting with cancer cells

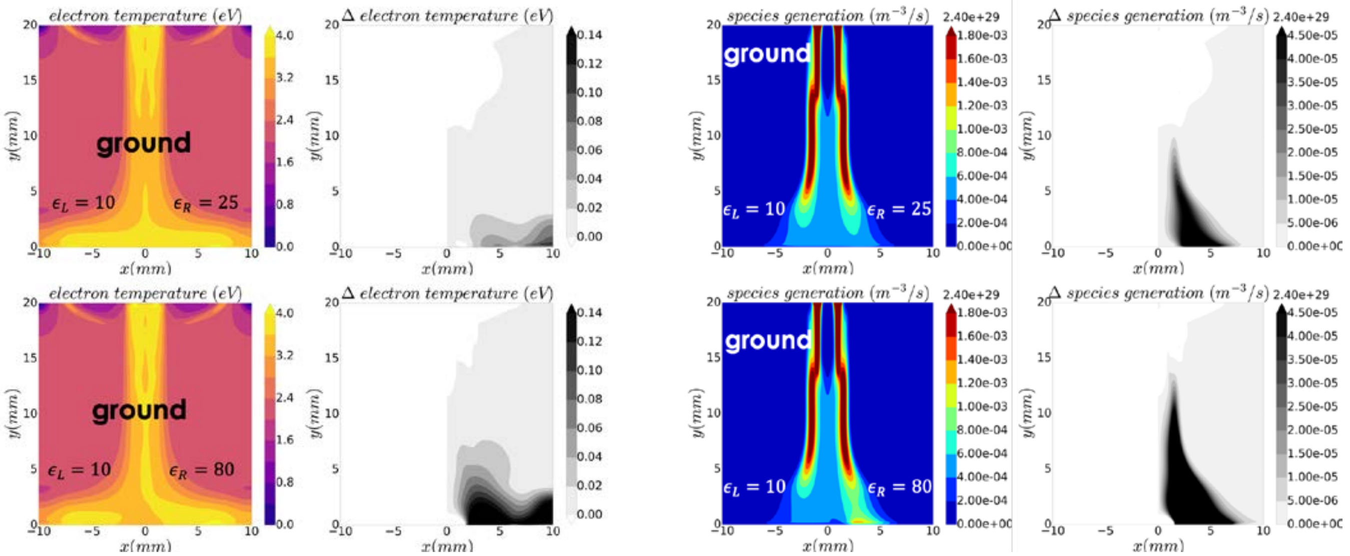
Plasma detects cancer!

Plasma is symmetrical when interacting with neutral media

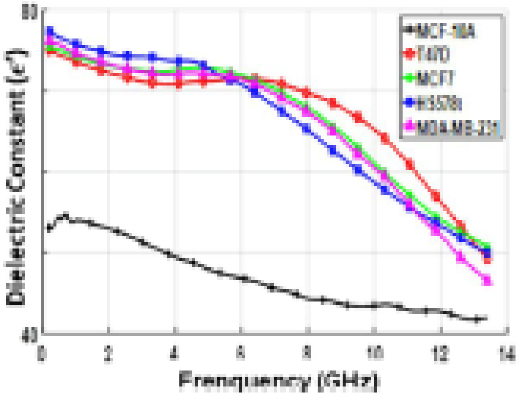
grounded copper plate beneath the dish

Li et al., ACS AMI, 2019

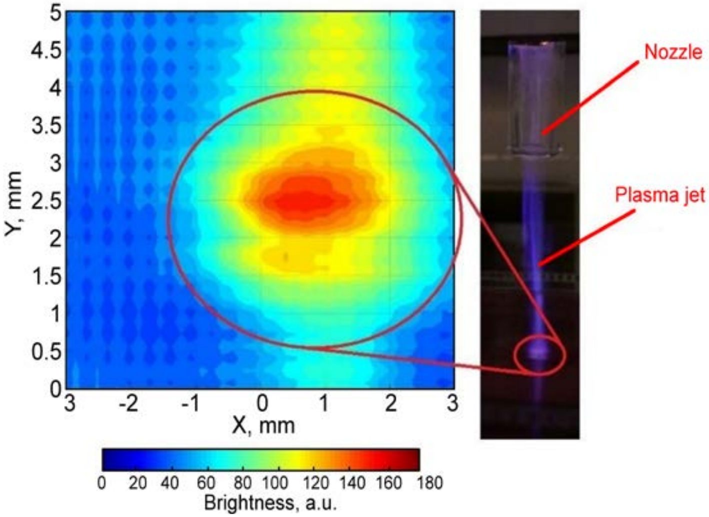
Self-organization simulation

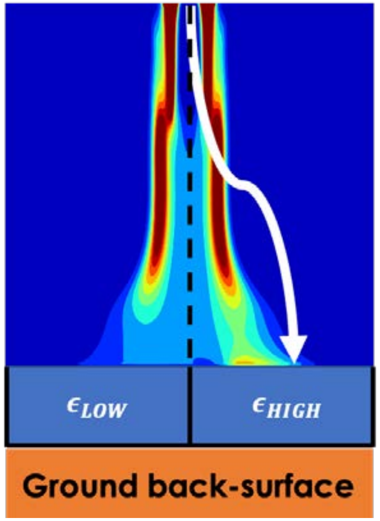


Four breast cancer cell lines exhibit higher dielectric constant when compared to healthy cells

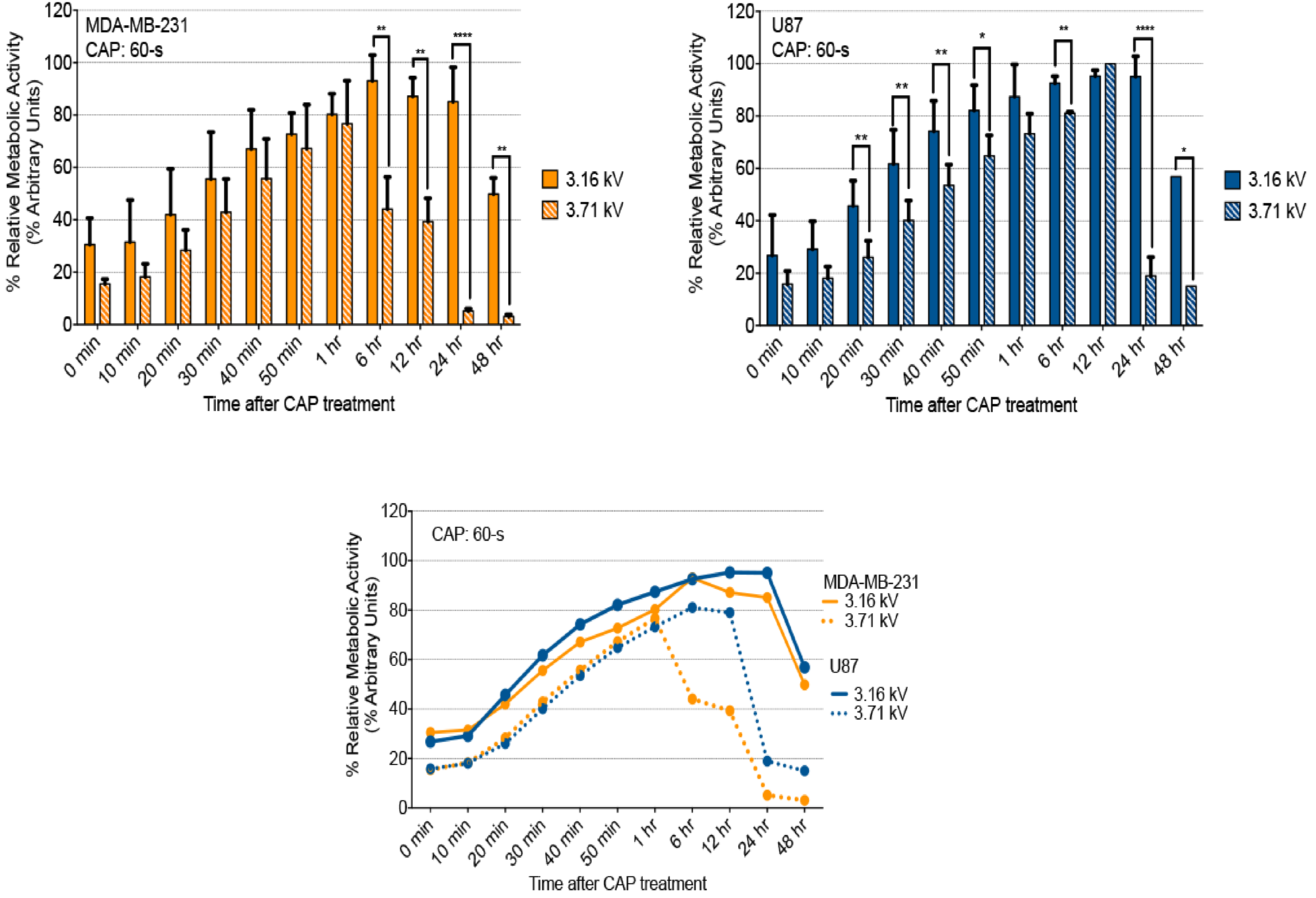


Sci. Rep., (2019) 9:4681



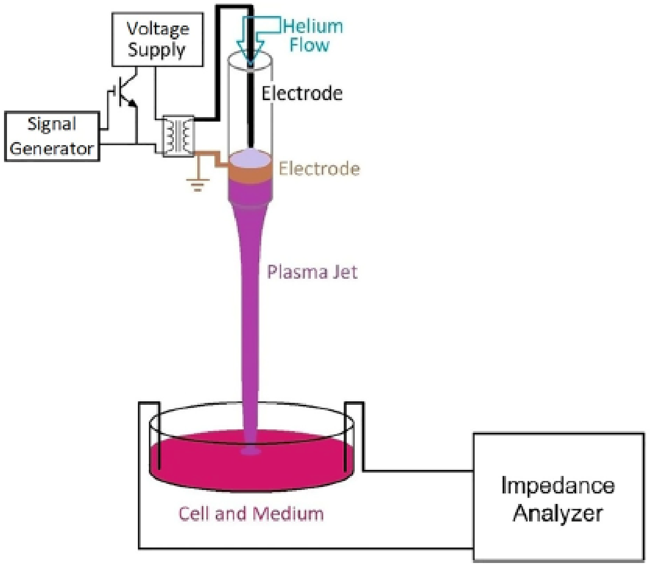


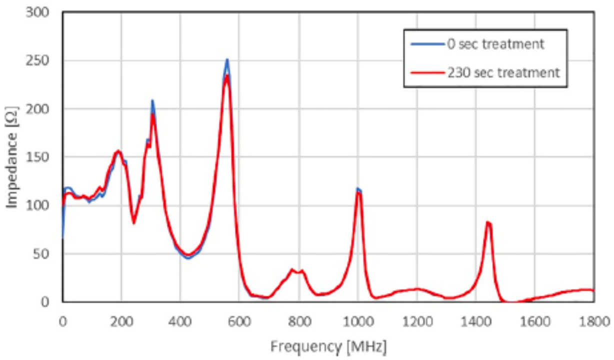
Martinez et al., PSST, 2022



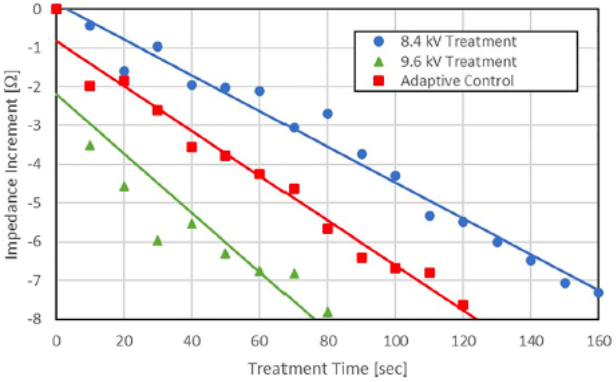
Gjika et al., ACS AMI, 2018

Example of adaptive treatment via electrical impedance spectroscopy

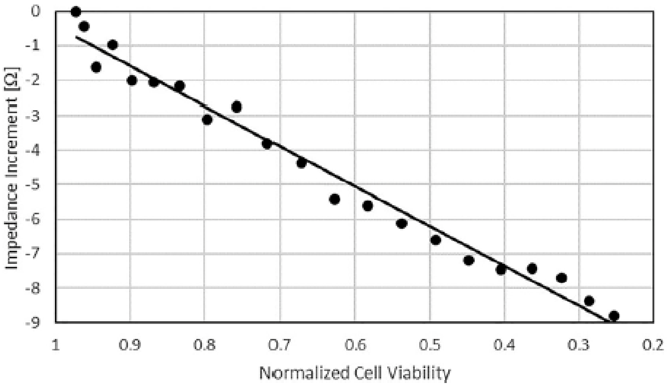


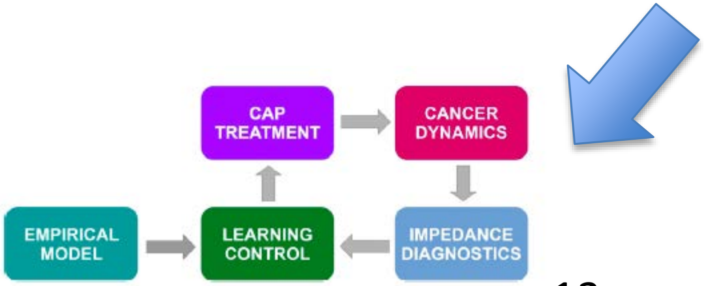


Demonstration of adaptive control



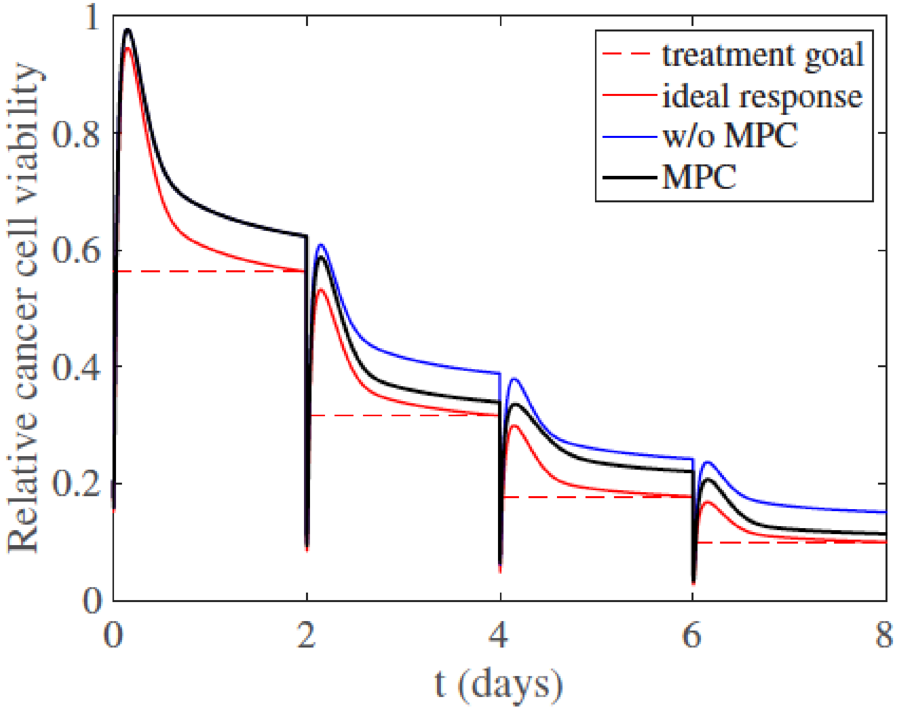
EIS –cell viability correlation

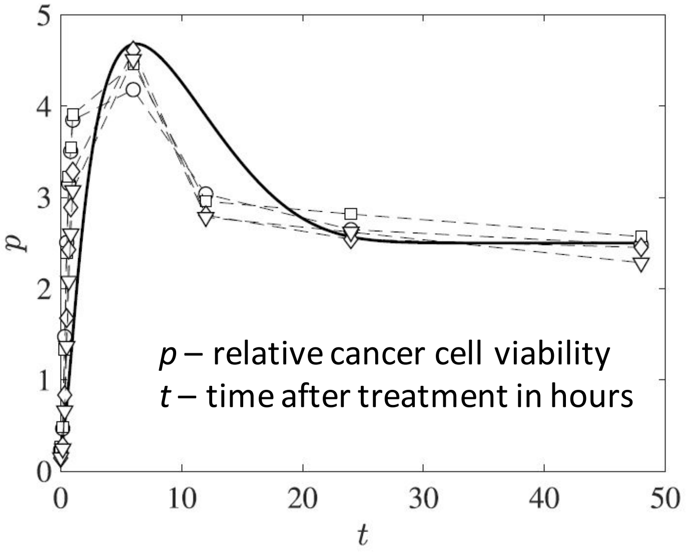




Li et al. Phys. Plasmas 27, 063501 (2020)

Model predictive control (MPC) limitations





p – relative cancer cell viability

t – time after treatment in hours

- Due to the complexity of plasma-target system:
  - The control algorithm should consider multiple status variables and CAP setup parameters.
  - MPC may encounter difficulties during the fitting due to the simplicity of the model.

Lui et al., J. Phys D, 2019

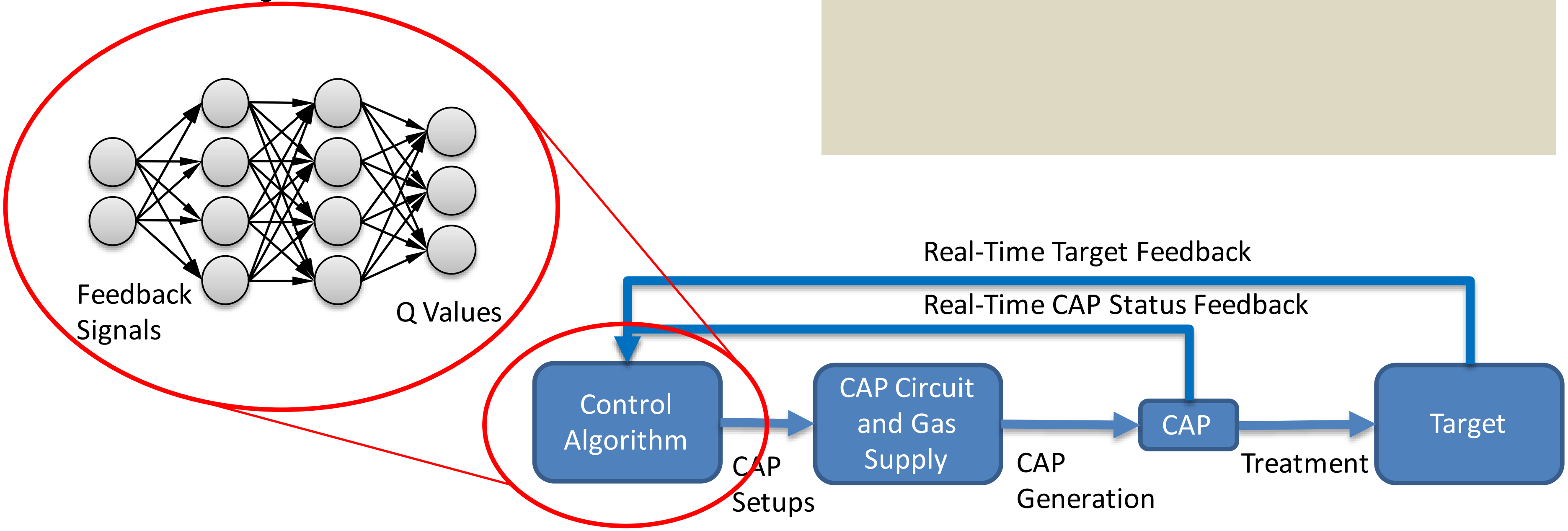
ANN and Deep Q Learning

Artificial neural network (ANN) can be considered to replace the mathematical model.

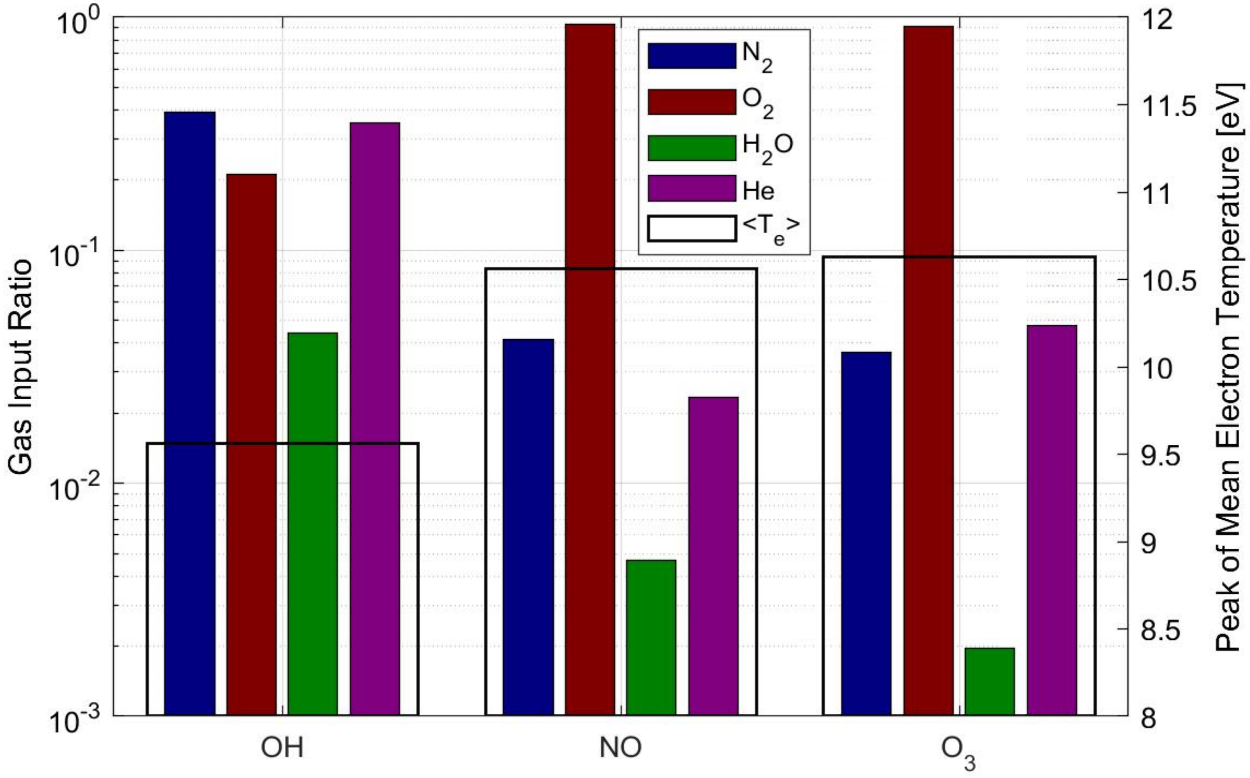
- ANN as a function: computes output vector based on multiple weighted linear combinations of input vector. Thus, it can represent a very complicated function.
- Training ANN: updating weights

= curve fitting an MPC model

- Each neuron at the output layer represents an action such as increasing the discharge voltage, decreasing the gas flow rate, etc.
- The action with the highest Q value will be selected to operate



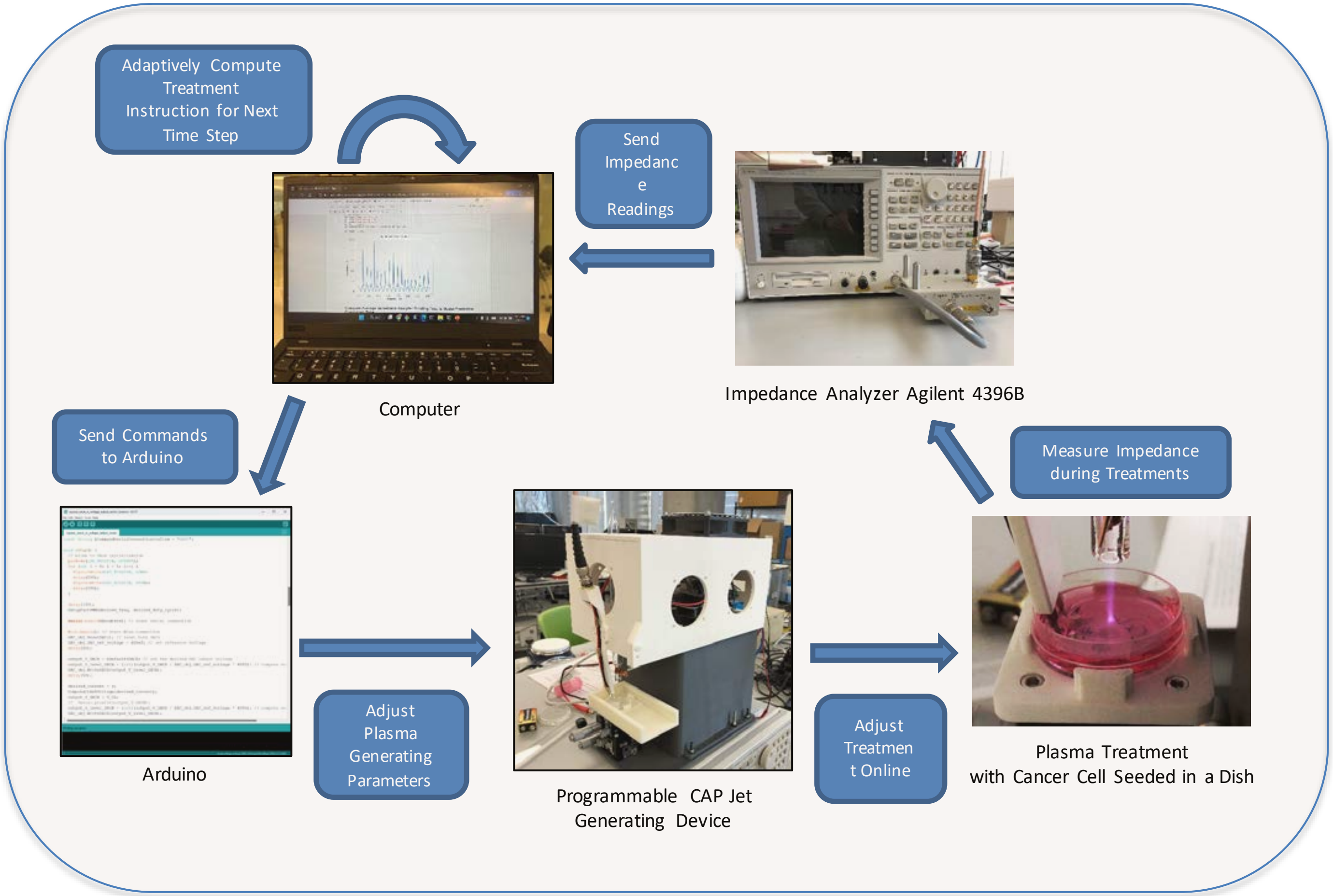
Plasma composition optimization using ANN



Cancer therapy **Wound healing** **Decontamination**

Li et al., Adv. Intel. Mater. 2021

State of the art: adaptive CAP jet device



Adaptively Compute Treatment Instruction for Next Time Step

Computer

Send Commands to Arduino

Arduino

Adjust Plasma Generating Parameters

Programmable CAP Jet Generating Device

Adjust Treatment Online

Plasma Treatment with Cancer Cell Seeded in a Dish

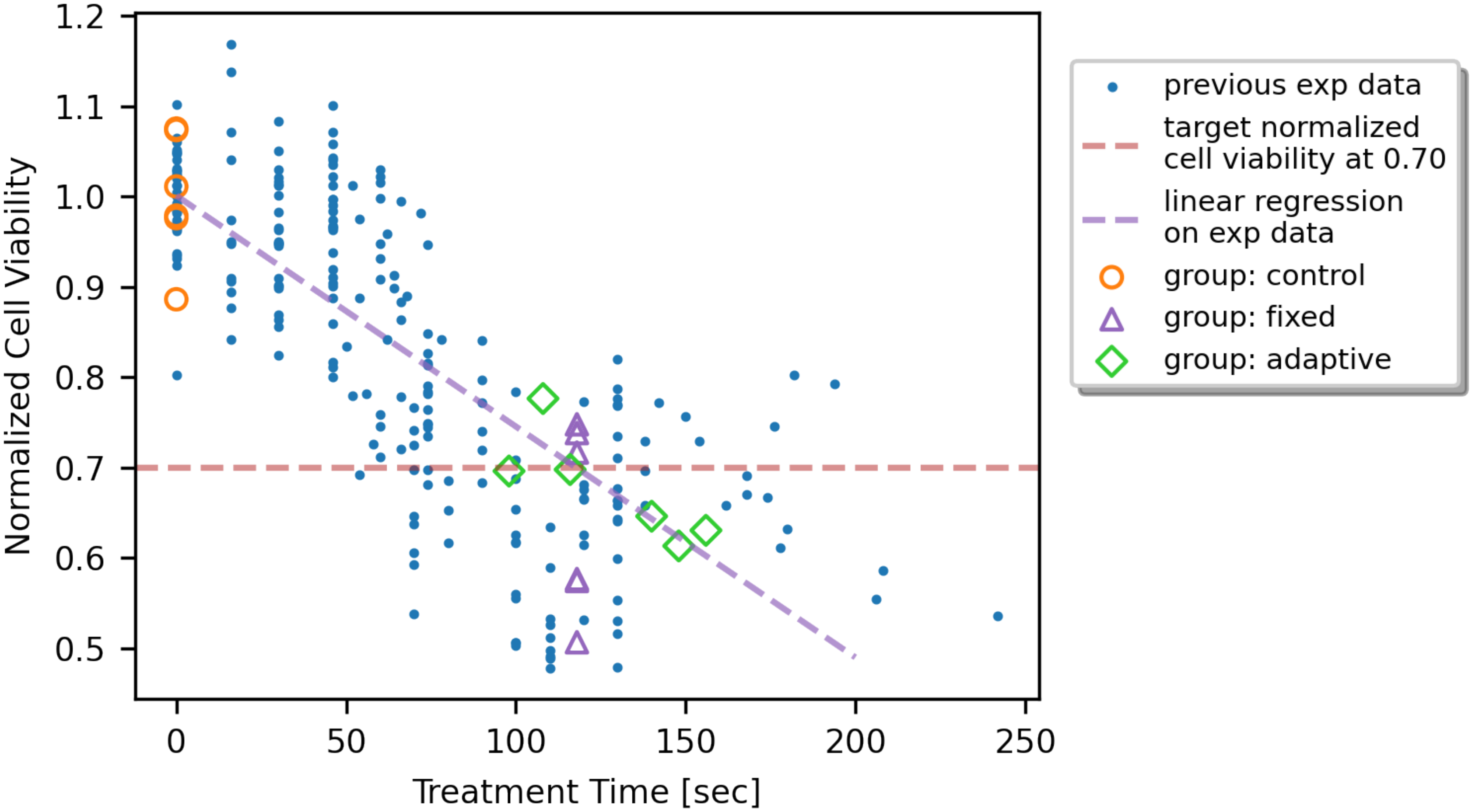
Measure Impedance during Treatments

Impedance Analyzer Agilent 4396B

Send Impedance Readings

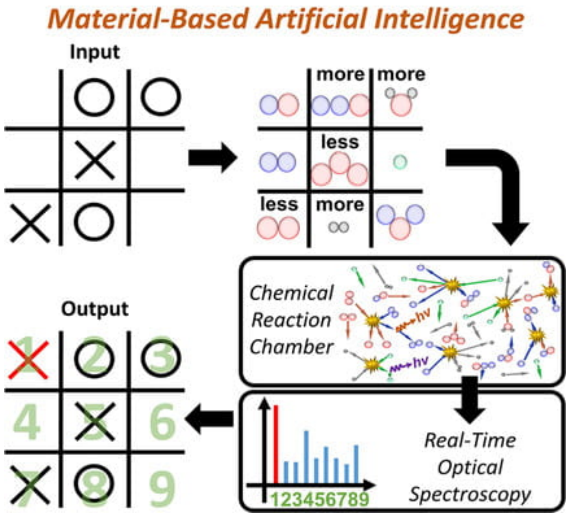
Hou et al., IEEE TRPMS 2021

Adaptive CAP jet device



Intelligent Plasma

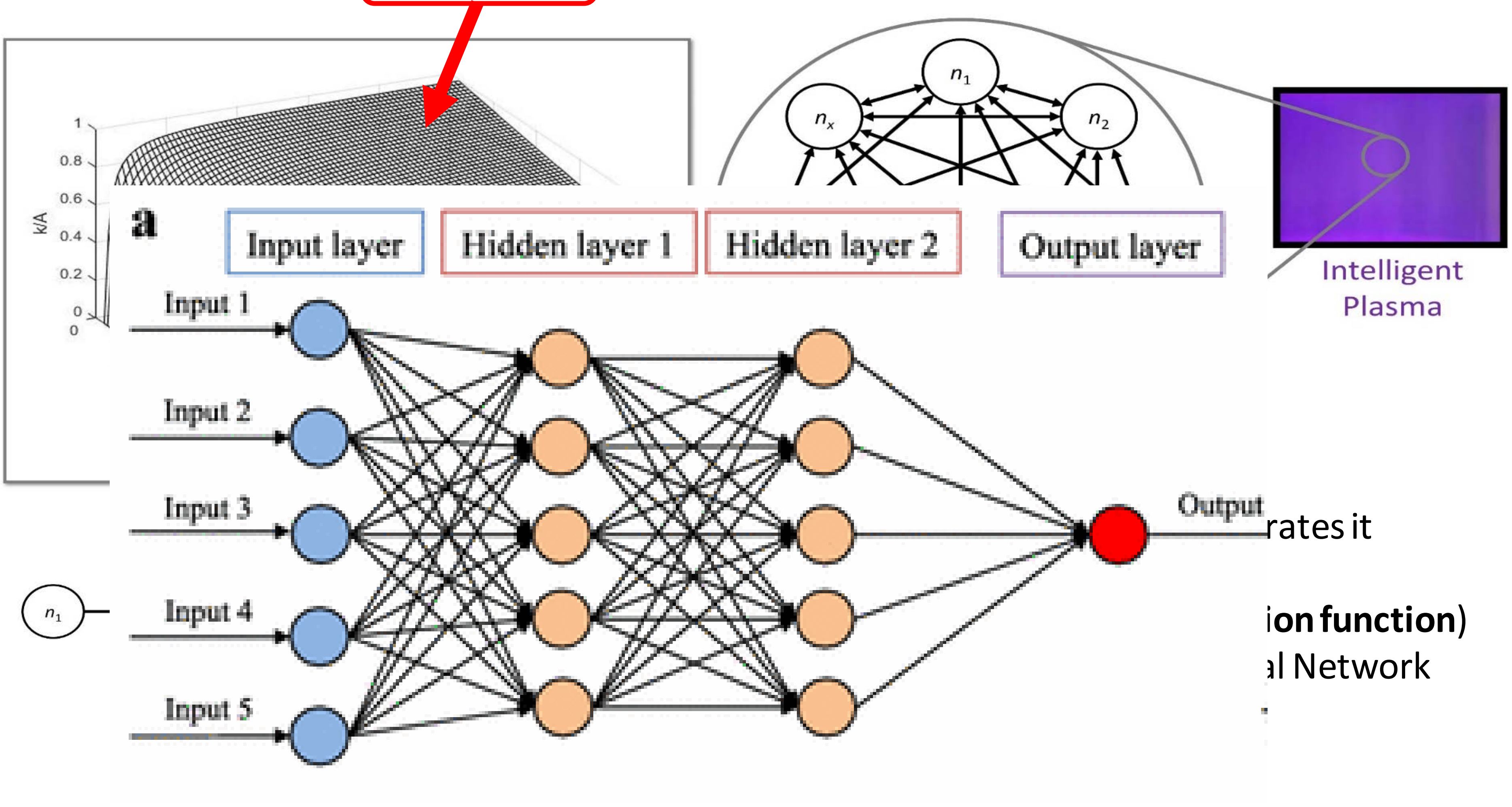
- The plasma itself can be a programmable intelligent material
- Train a low-temperature plasma system to play a board game Tic-Tac-Toe



Li & Keidar, Adv. Intell. Syst. 2022, 2200157

Chemical Pathway Network (CPN)

$$\boldsymbol{\Psi}_{output} = \boldsymbol{\Psi}_{input} + \boxed{\mathcal{F}\left(\boldsymbol{T}, \boldsymbol{\Psi}_{input}\right)} dt$$



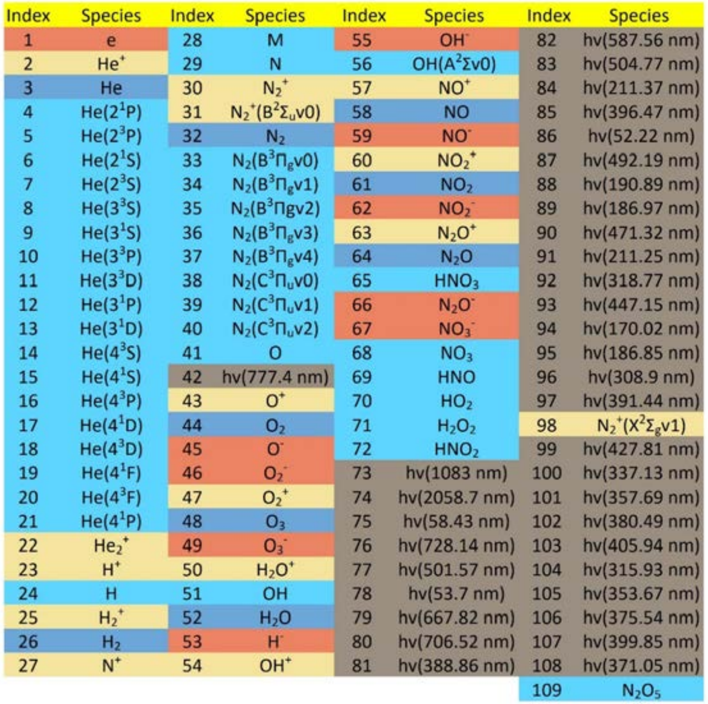
rates it

ion function)

al Network

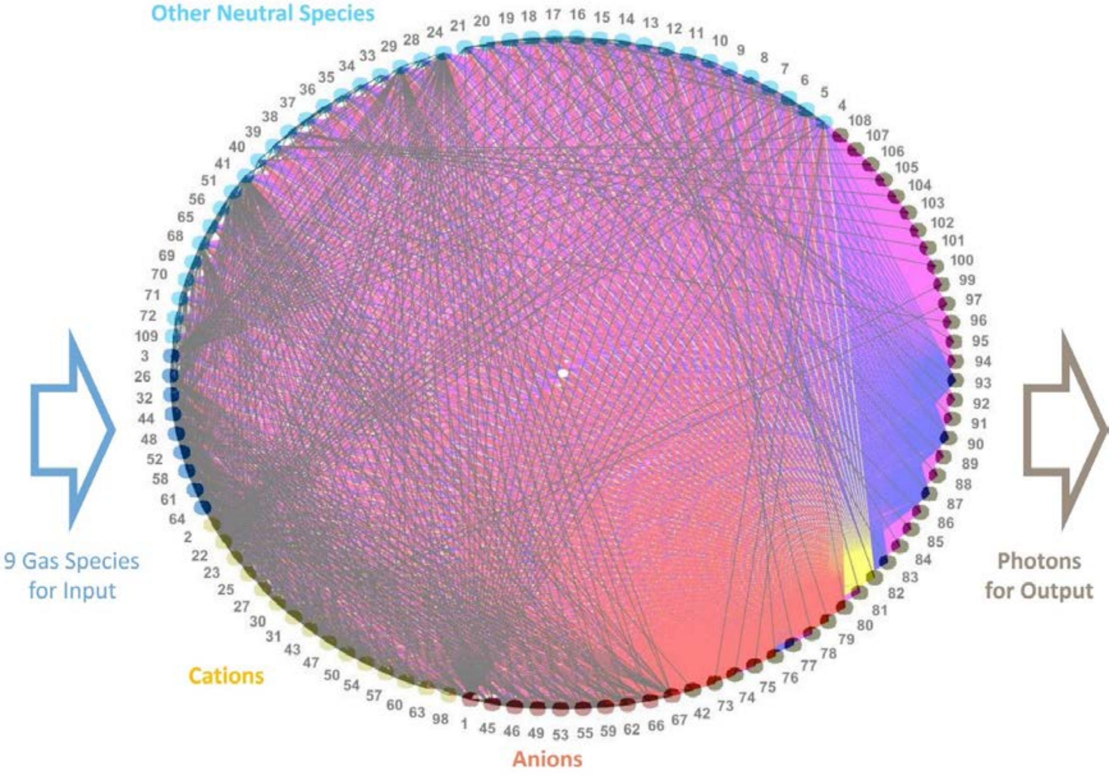
Similar to Artificial Neural Networks

Main Species in a helium-air plasma

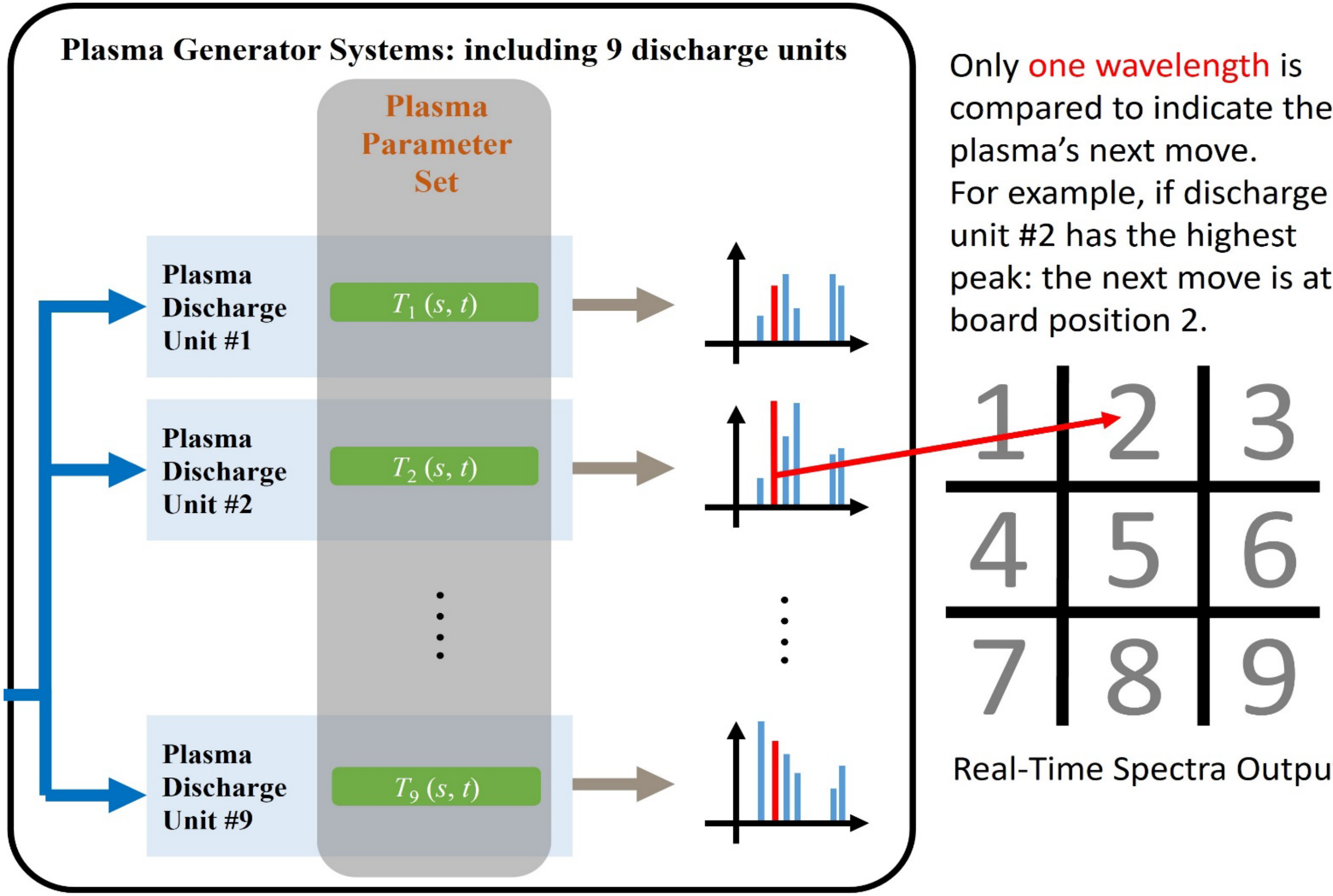


Index	Species	Index	Species	Index	Species	Index	Species
1	e	28	M	55	OH^-	82	hv(587.56 nm)
2	He^+	29	N	56	$OH(A^2\Sigma v0)$	83	hv(504.77 nm)
3	He	30	N_2^+	57	NO^+	84	hv(211.37 nm)
4	$He(2^1P)$	31	$N_2^+(B^2\Sigma_u v0)$	58	NO	85	hv(396.47 nm)
5	$He(2^3P)$	32	N_2	59	NO^-	86	hv(52.22 nm)
6	$He(2^1S)$	33	$N_2(B^3\Pi_g v0)$	60	NO_2^+	87	hv(492.19 nm)
7	$He(2^3S)$	34	$N_2(B^3\Pi_g v1)$	61	NO_2	88	hv(190.89 nm)
8	$He(3^3S)$	35	$N_2(B^3\Pi gv2)$	62	NO_2^-	89	hv(186.97 nm)
9	$He(3^1S)$	36	$N_2(B^3\Pi_g v3)$	63	N_2O^+	90	hv(471.32 nm)
10	$He(3^3P)$	37	$N_2(B^3\Pi_g v4)$	64	N_2O	91	hv(211.25 nm)
11	$He(3^3D)$	38	$N_2(C^3\Pi_u v0)$	65	HNO_3	92	hv(318.77 nm)
12	$He(3^1P)$	39	$N_2(C^3\Pi_u v1)$	66	N_2O^-	93	hv(447.15 nm)
13	$He(3^1D)$	40	$N_2(C^3\Pi_u v2)$	67	NO_3^-	94	hv(170.02 nm)
14	$He(4^3S)$	41	O	68	NO_3	95	hv(186.85 nm)
15	$He(4^1S)$	42	hv(777.4 nm)	69	HNO	96	hv(308.9 nm)
16	$He(4^3P)$	43	O^+	70	HO_2	97	hv(391.44 nm)
17	$He(4^1D)$	44	O_2	71	H_2O_2	98	$N_2^+(X^2\Sigma_g v1)$
18	$He(4^3D)$	45	O^-	72	HNO_2	99	hv(427.81 nm)
19	$He(4^1F)$	46	O_2^-	73	hv(1083 nm)	100	hv(337.13 nm)
20	$He(4^3F)$	47	O_2^+	74	hv(2058.7 nm)	101	hv(357.69 nm)
21	$He(4^1P)$	48	O_3	75	hv(58.43 nm)	102	hv(380.49 nm)
22	He_2^+	49	O_3^-	76	hv(728.14 nm)	103	hv(405.94 nm)
23	H^+	50	H_2O^+	77	hv(501.57 nm)	104	hv(315.93 nm)
24	H	51	OH	78	hv(53.7 nm)	105	hv(353.67 nm)
25	H_2^+	52	H_2O	79	hv(667.82 nm)	106	hv(375.54 nm)
26	H_2	53	H^-	80	hv(706.52 nm)	107	hv(399.85 nm)
27	N^+	54	OH^+	81	hv(388.86 nm)	108	hv(371.05 nm)
						109	N_2O_5

Chemical Pathway Network (CPN) of a helium-air plasma



Train the plasma to play a boardgame



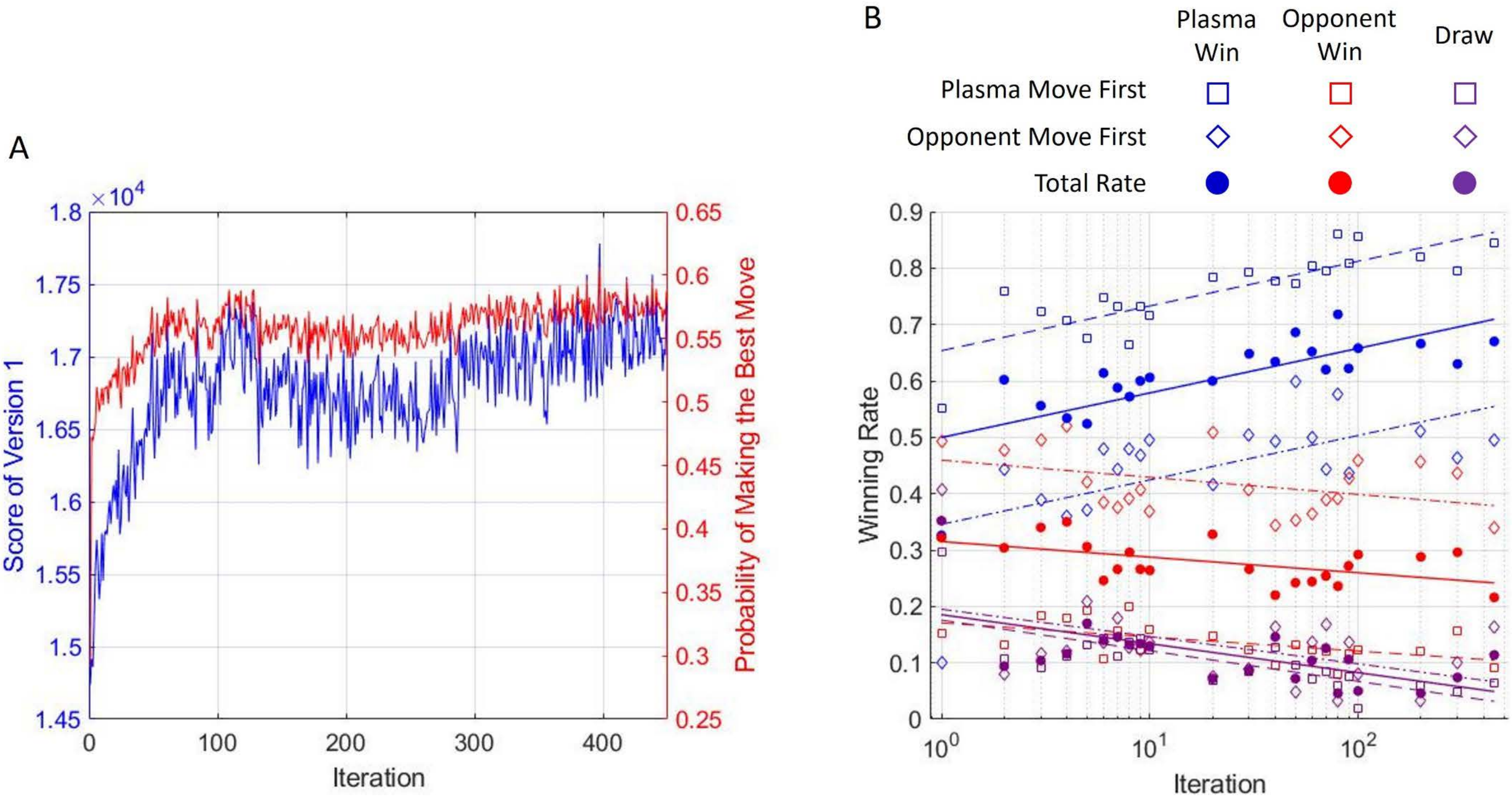
Only one wavelength is compared to indicate the plasma’s next move.
For example, if discharge unit #2 has the highest peak: the next move is at board position 2.

Real-Time Spectra Output

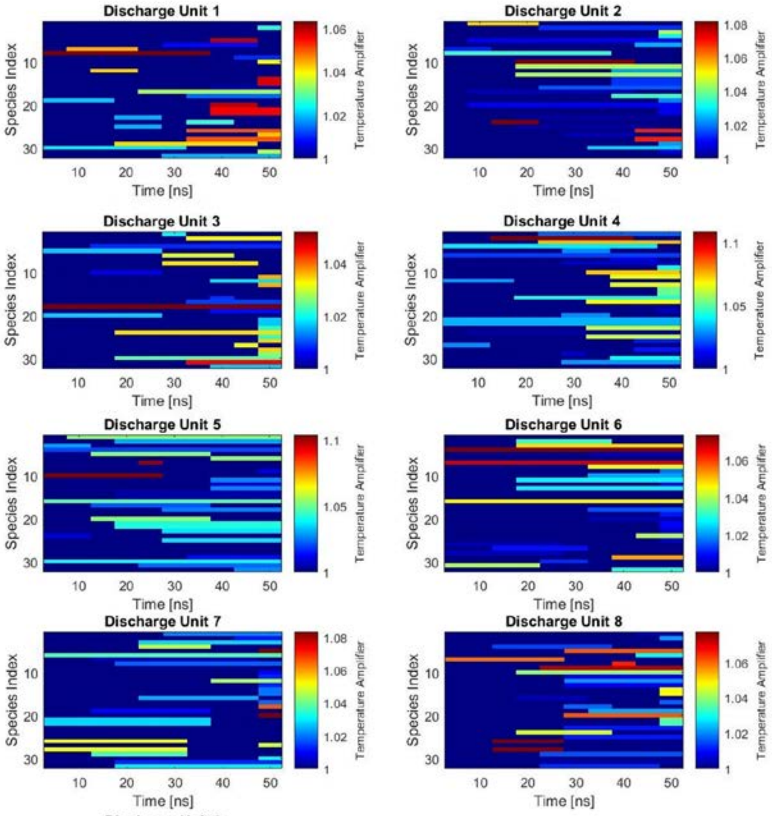
Intelligent Plasma Processor

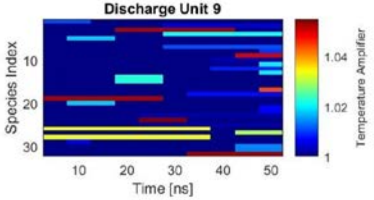
Lin & Keidar, Adv. Intell. Syst. 2022, 2200157

Training Progress



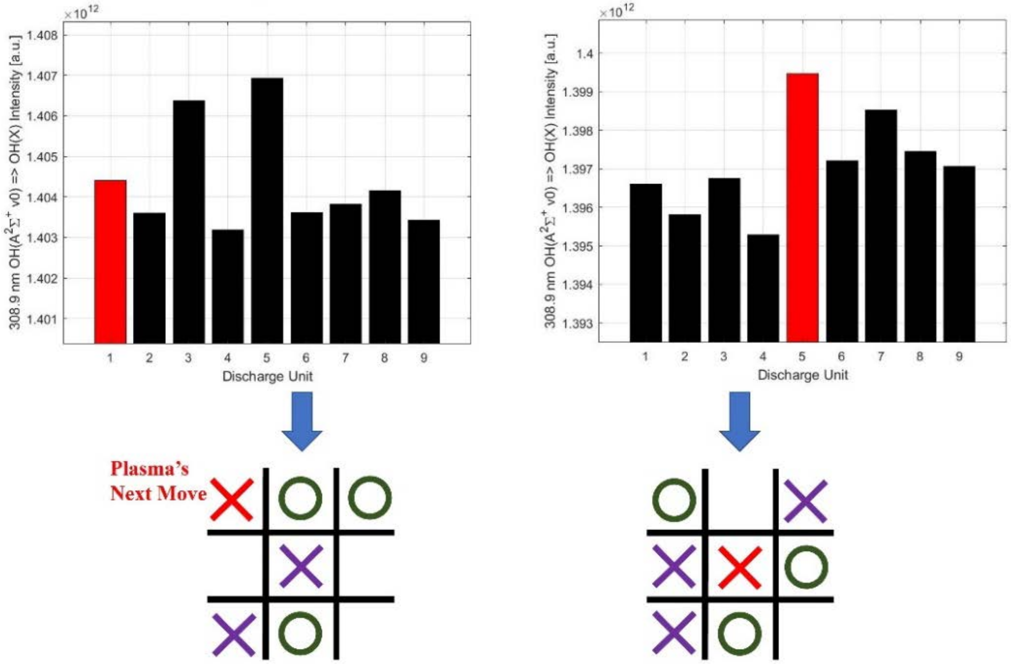
Examples

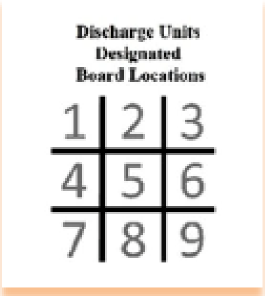




Species Index

1	2	3	4	5	6	7	8	9	10	11
e	He^+	He	He_2^+	H^+	H_2^+	H_2	N^+	N_2^+	N_2	O^+
12	13	14	15	16	17	18	19	20	21	22
O_2	O^-	O_2^-	O_2^+	O_3	O_3^-	H_2O^+	H_2O	H^-	OH^+	OH^-
23	24	25	26	27	28	29	30	31	32	
NO^+	NO	NO^-	NO_2^+	NO_2	NO_2^-	N_2O^+	N_2O	N_2O^-	NO_3^-	





Discharge Units Designated Board Locations

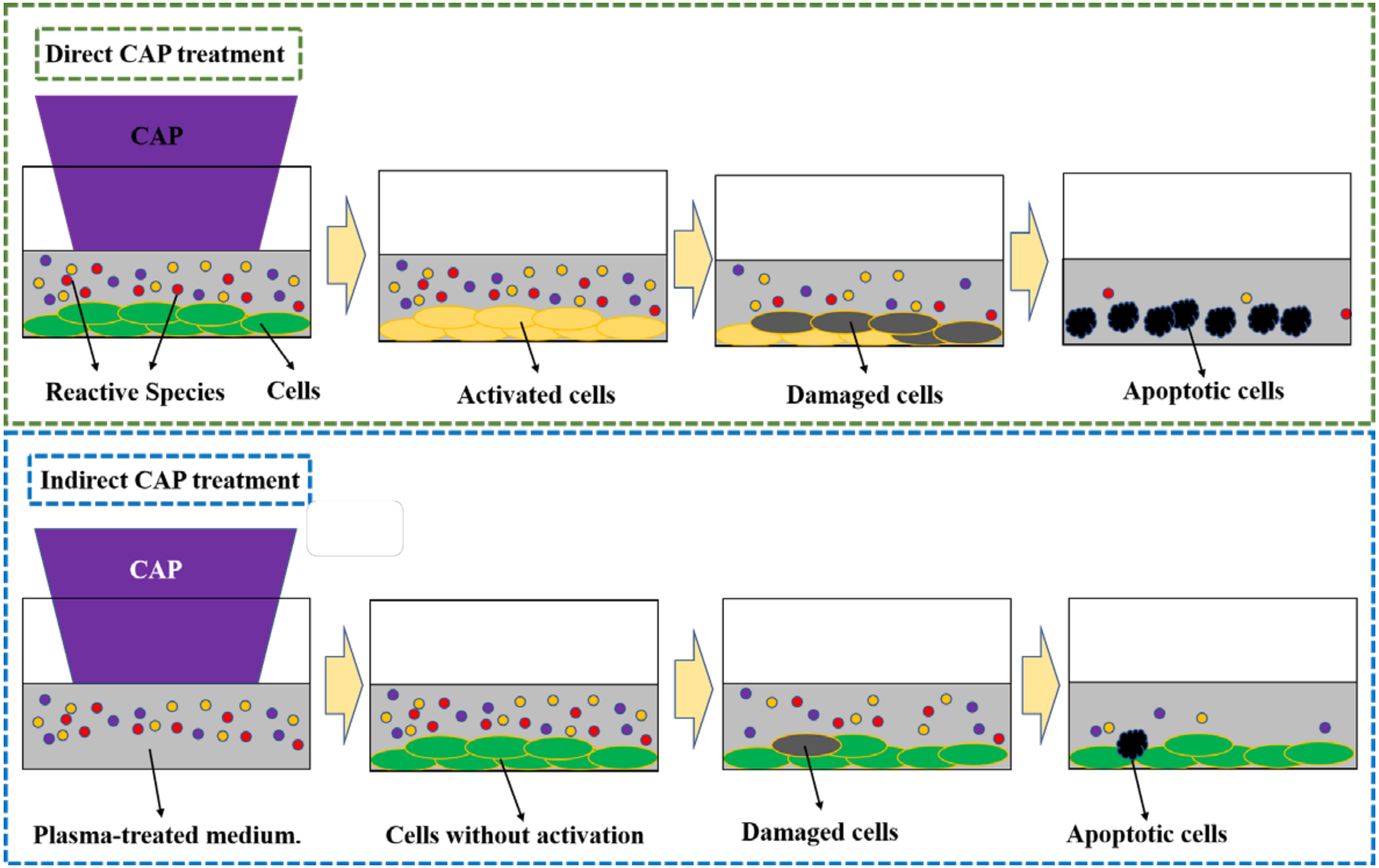
1	2	3
4	5	6
7	8	9

Lin & Keidar, Adv. Intell. Syst. 2022, 2200157

New plasma modality

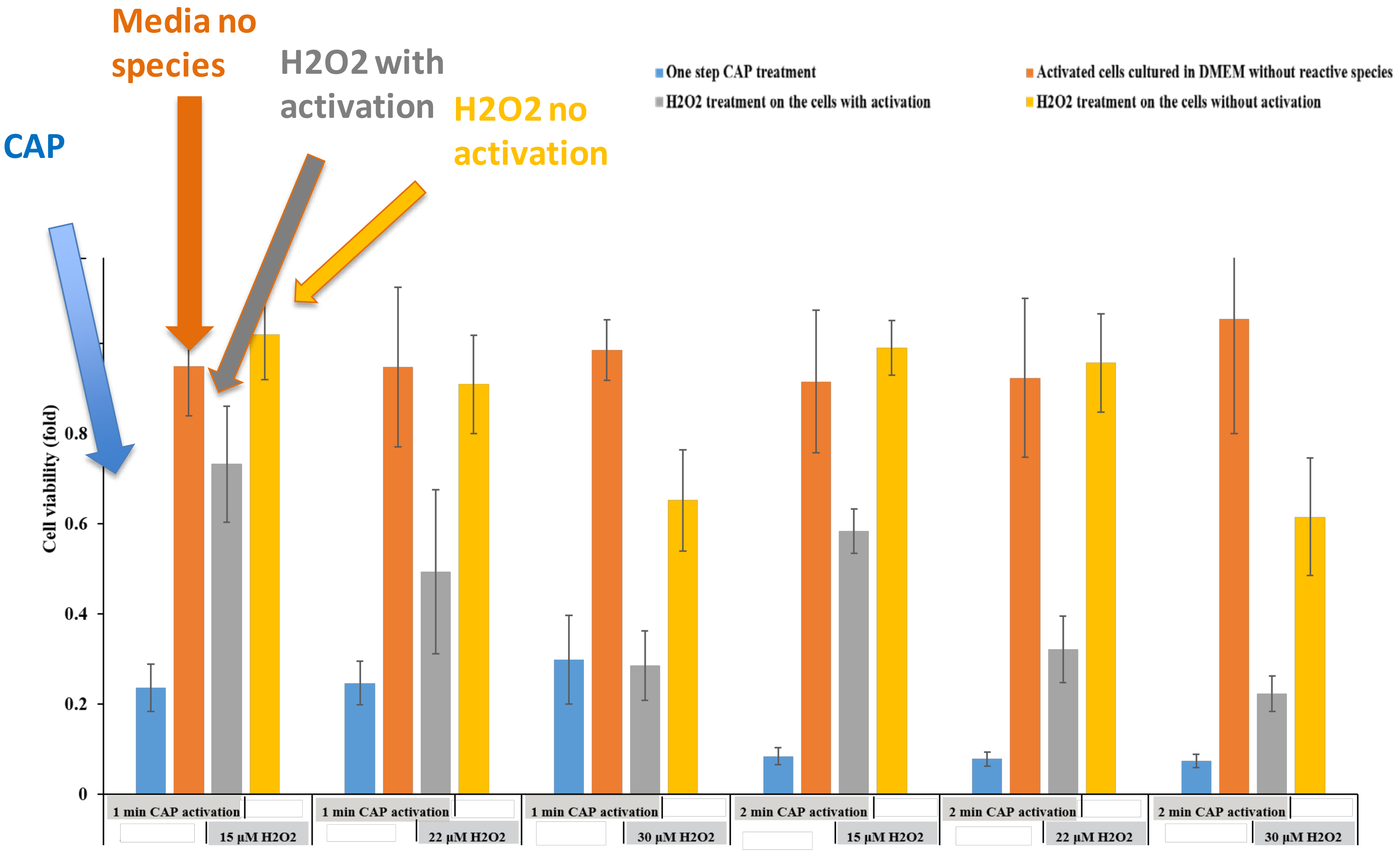
Treatment via physical barrier.

The activation state of the CAP-treated cancer cells



Yan, et al, Sci. Rep., 2018.

Activation state of the CAP-treated cells


Media no species

H2O2 with activation

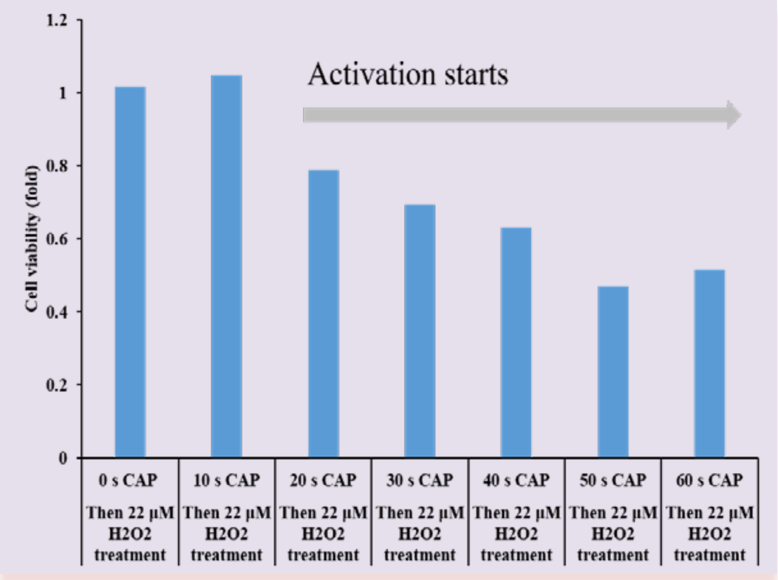
H2O2 no activation

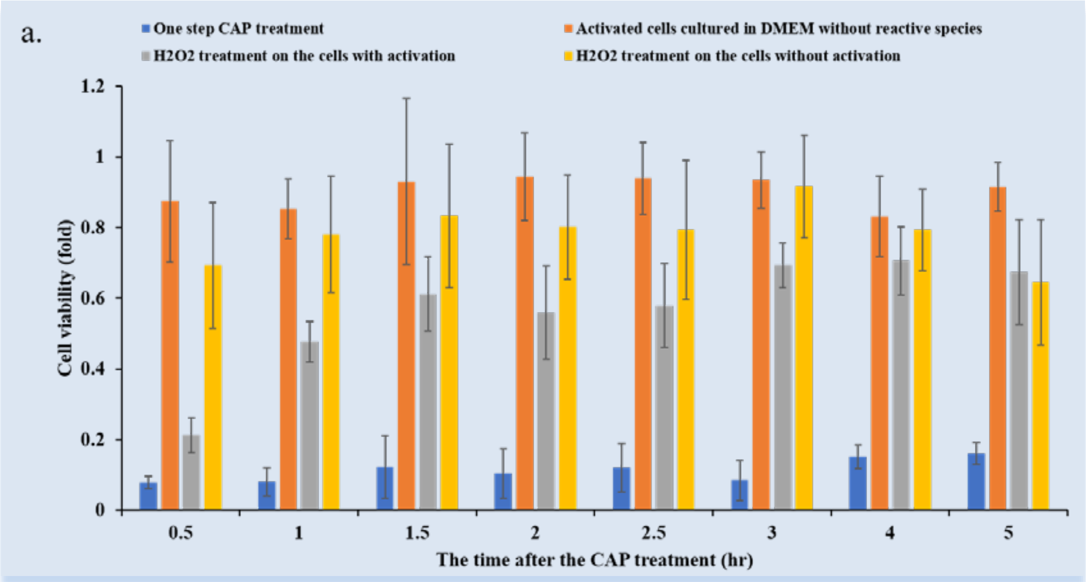
CAP

Dayun Yan, et al, Sci. Rep., 2018.

Sensitization

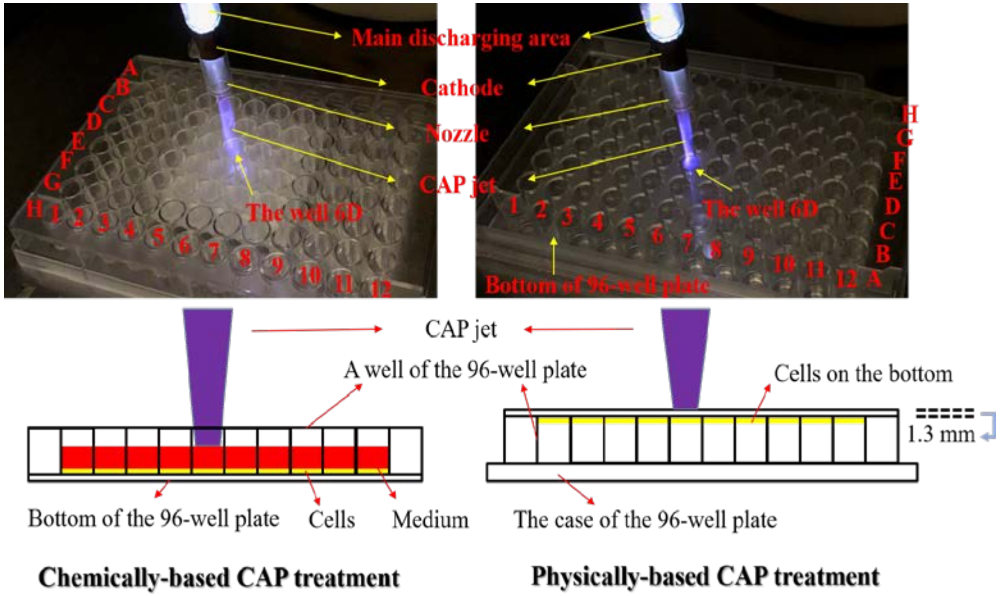
The slow de-sensitization

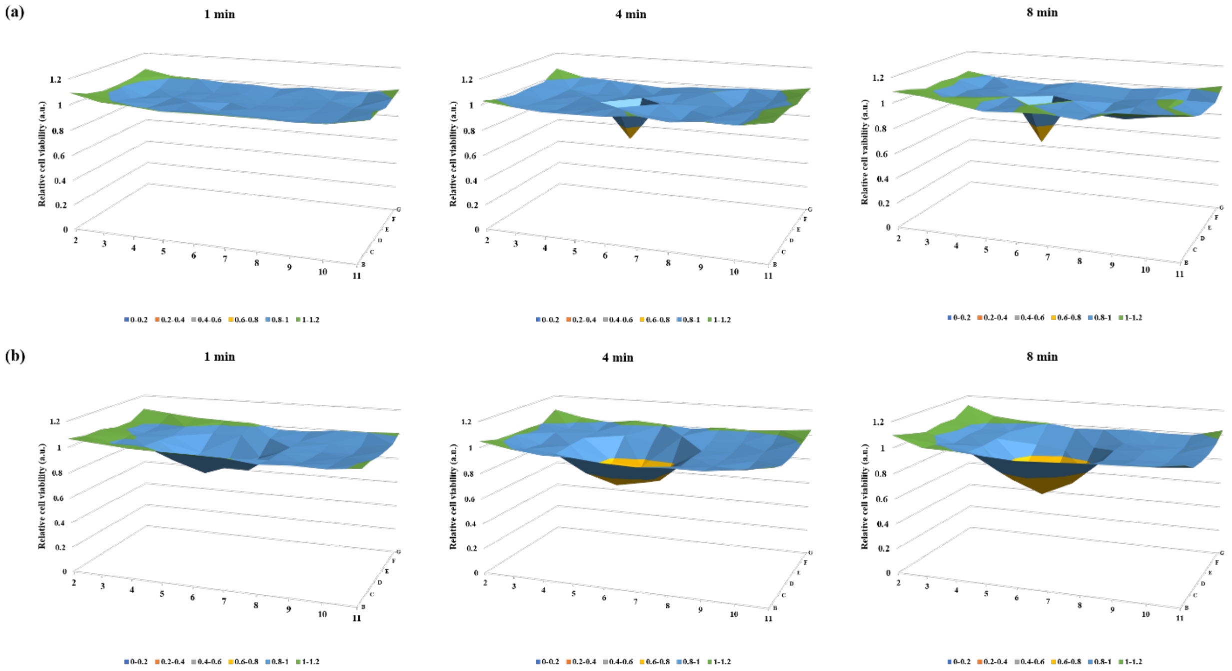




Dayun Yan, et al, Sci. Rep., 2018.

Physical treatment

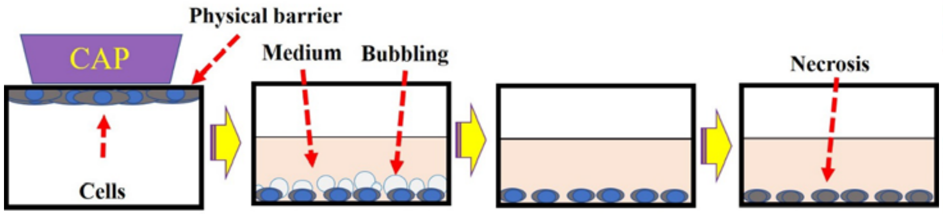


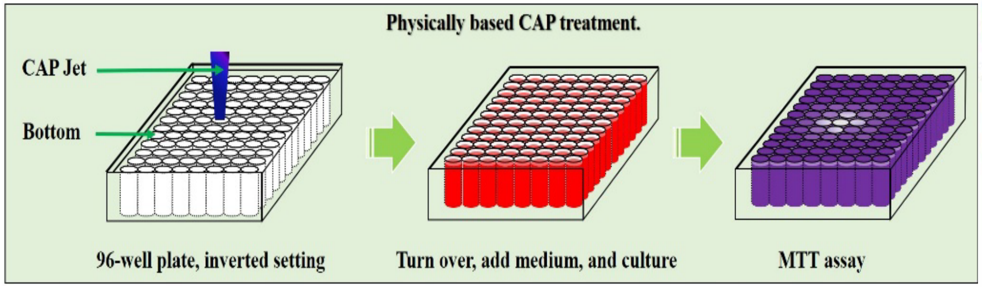


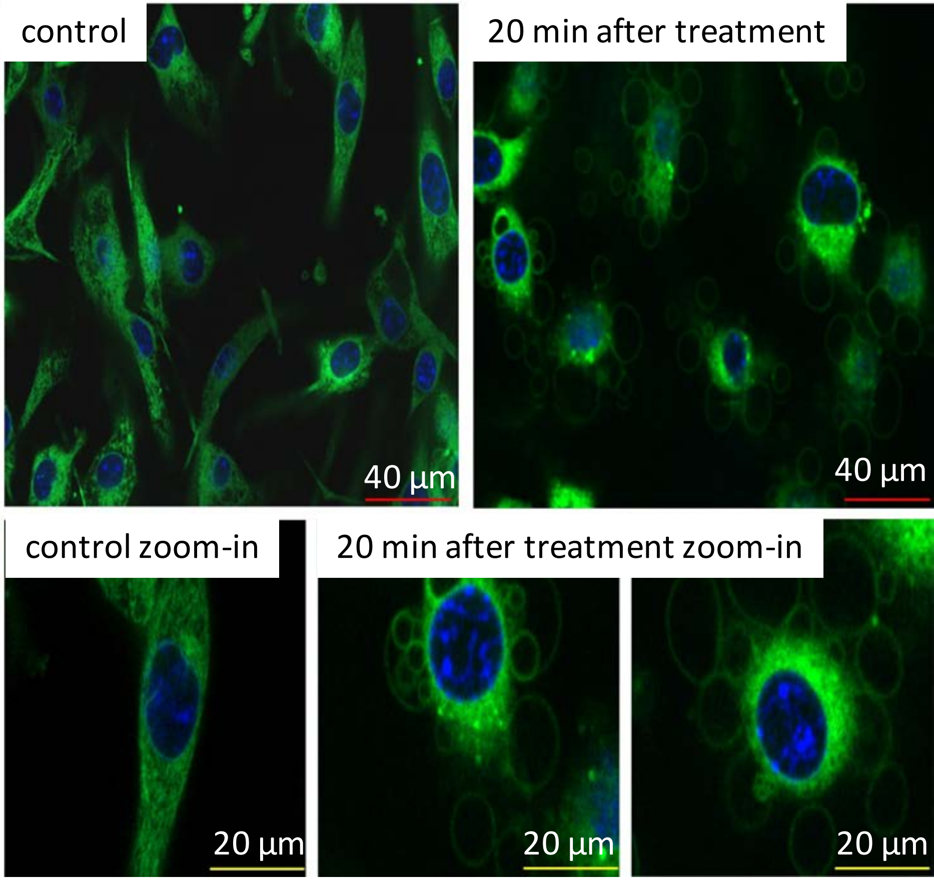
Treatment of diffuse tumors

D. Yan, et al, ACS AMI 2020

Physical Effects of CAP

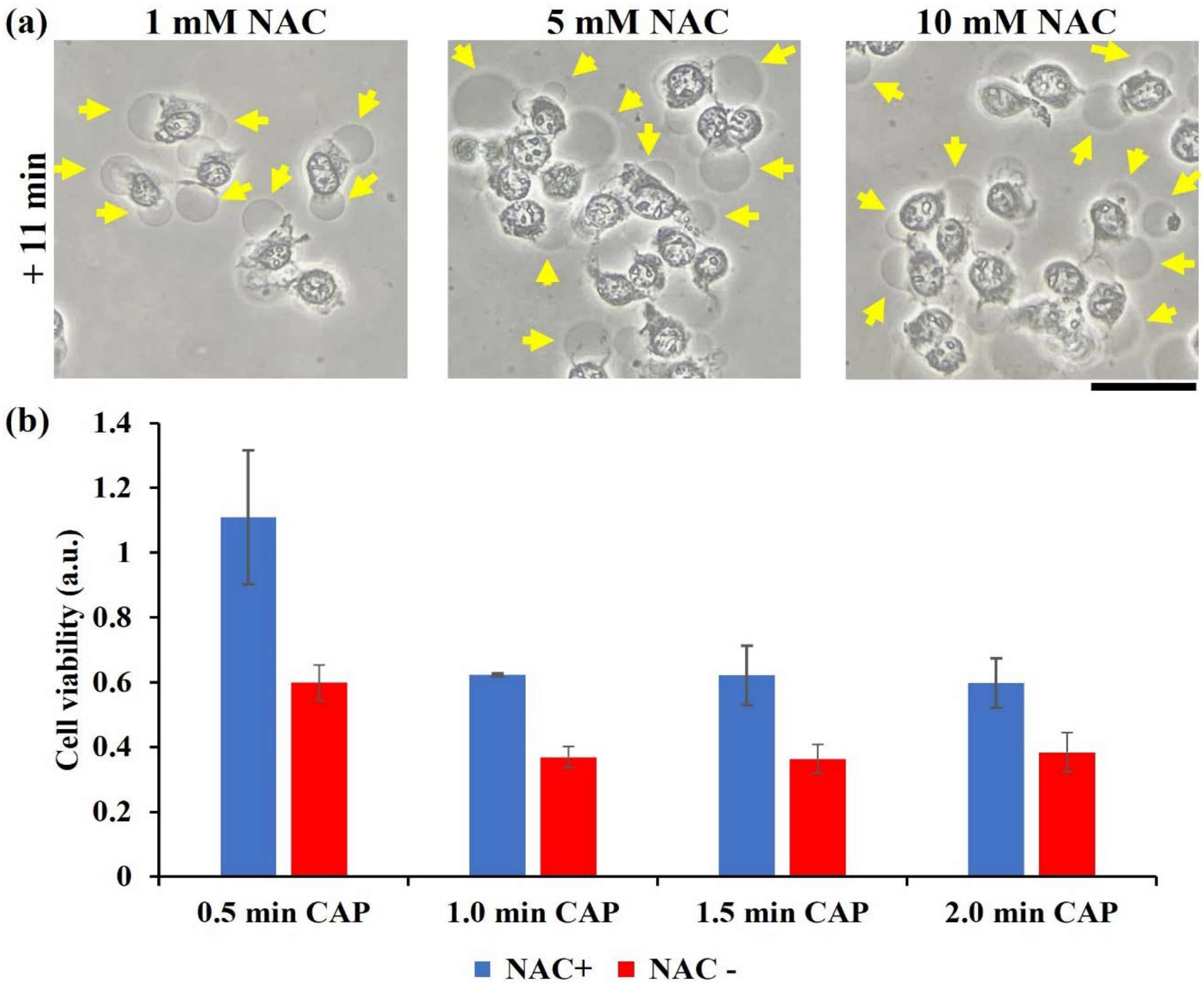




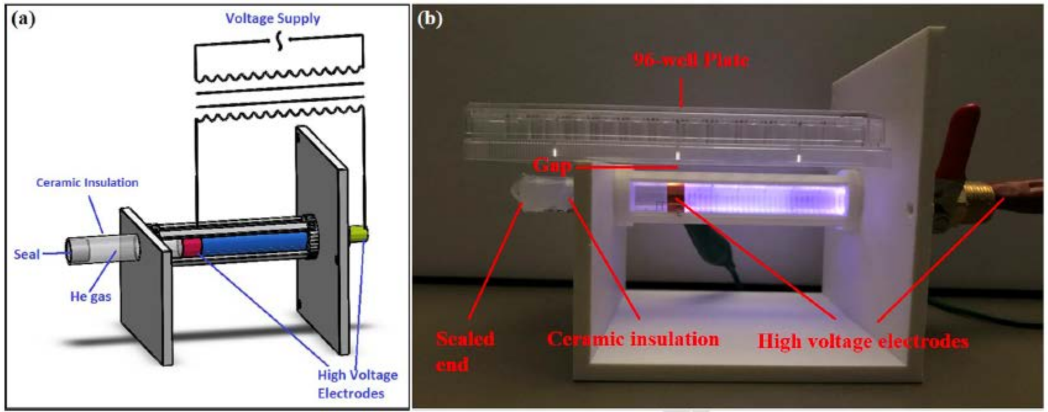


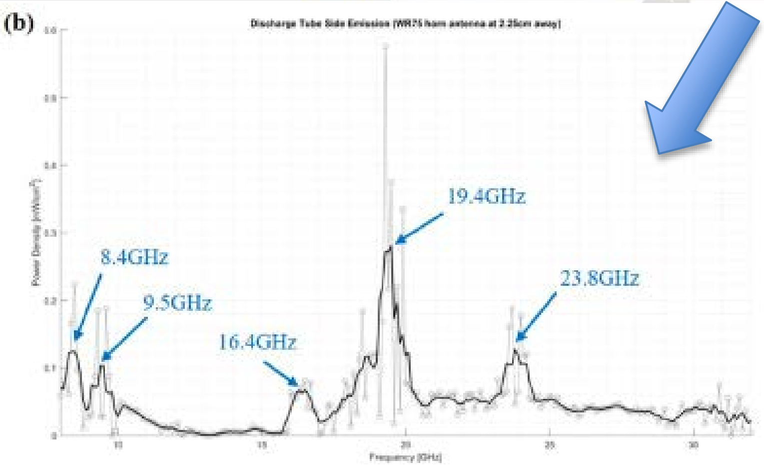
D. Yan, et al, ACS AMI 2020

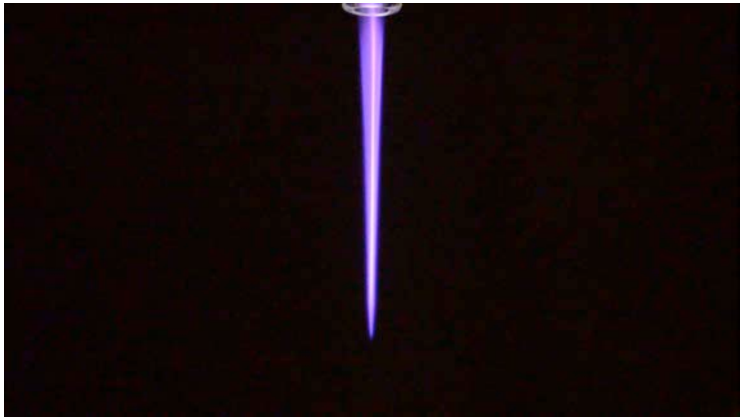
ROS scavenger cannot inhibit necrosis

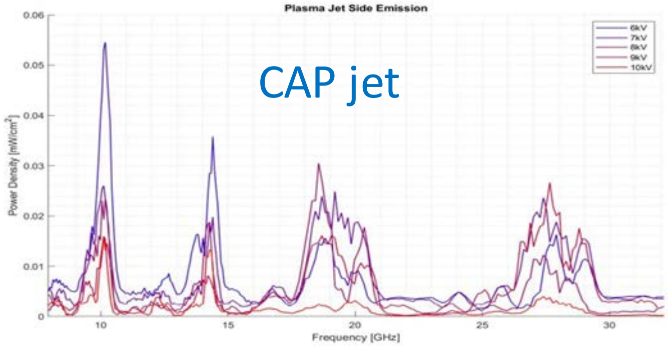


Discharge Tube





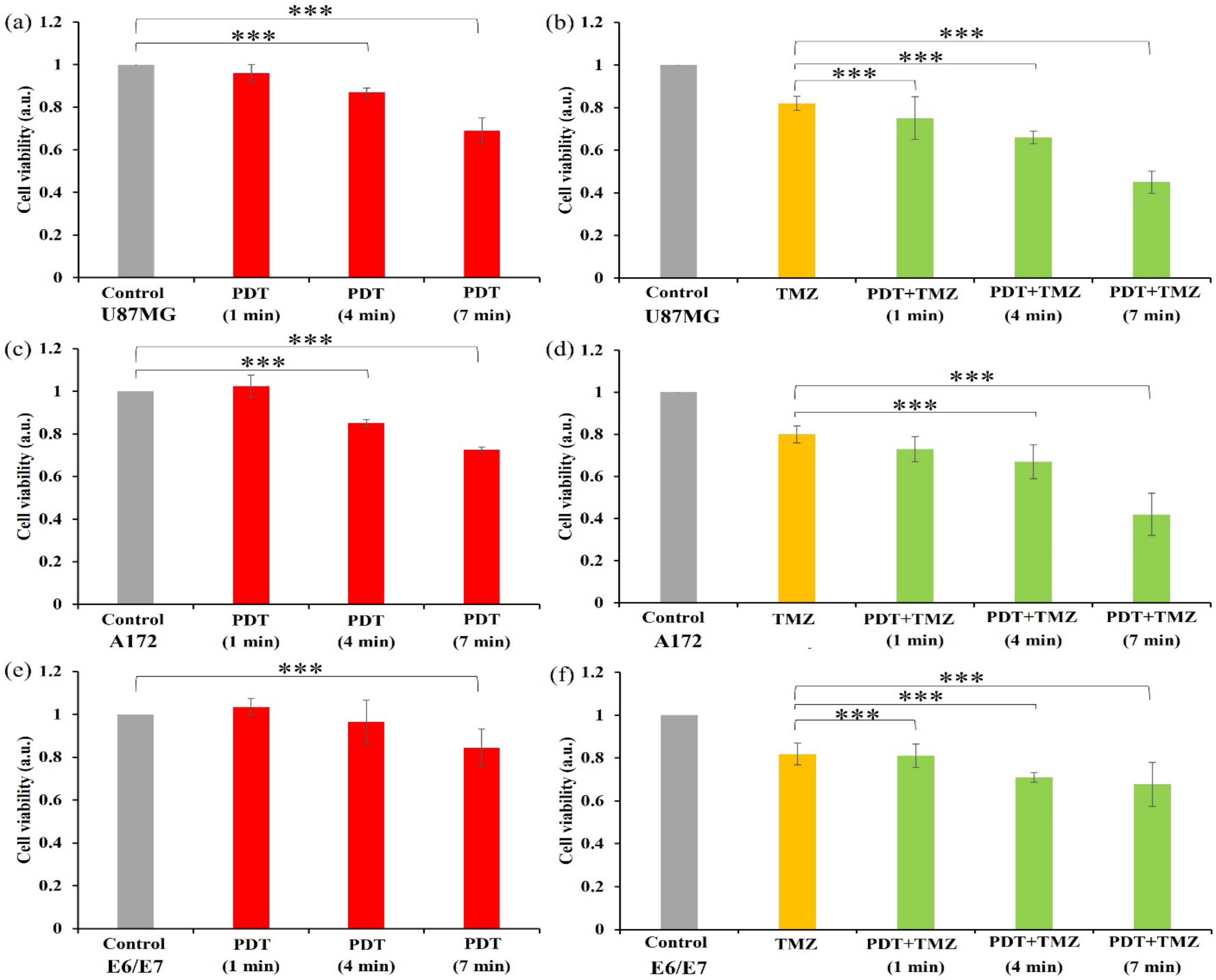


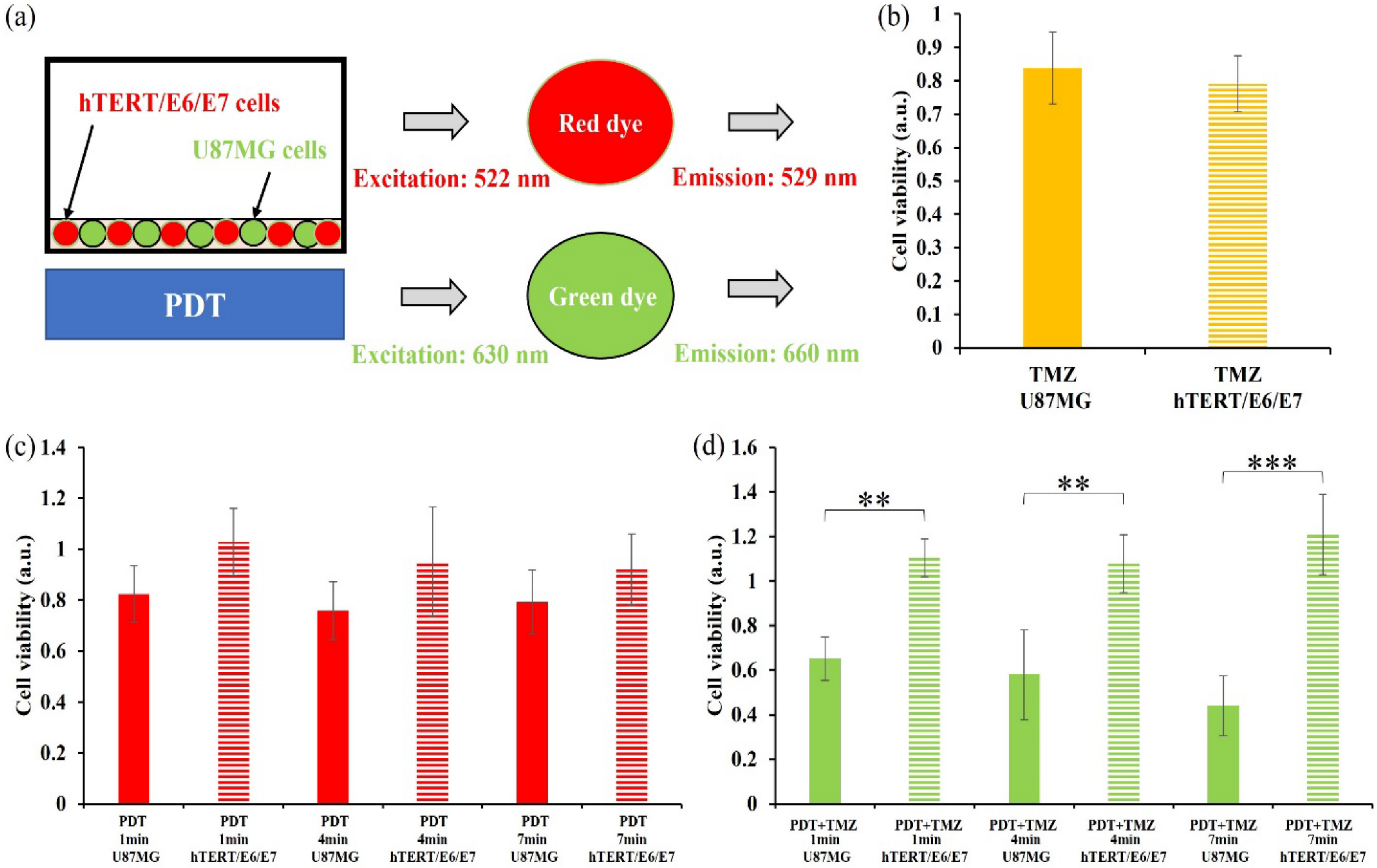


CAP jet

Yao et al., Phys. Plasmas (2020)

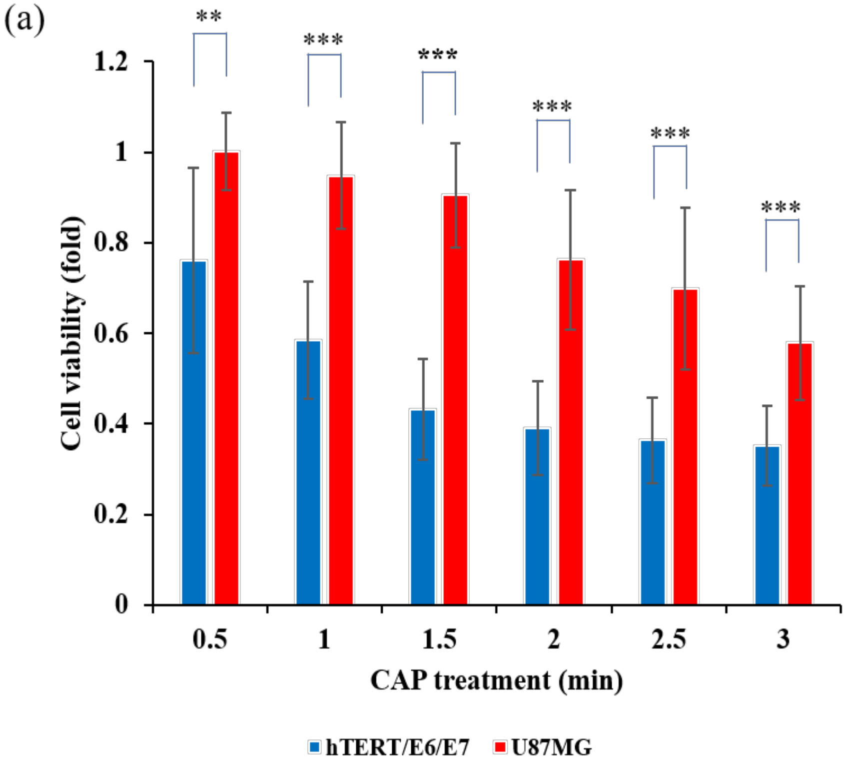
The cytotoxicity of TMZ on the cancer/normal cells




(a)
hTERT/E6/E7 cells
U87MG cells
PDT
Red dye
Excitation: 522 nm
Emission: 529 nm
Green dye
Excitation: 630 nm
Emission: 660 nm
(b)
Cell viability (a.u.)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
TMZ U87MG
TMZ hTERT/E6/E7
(c)
Cell viability (a.u.)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
PDT 1min U87MG
PDT 1min hTERT/E6/E7
PDT 4min U87MG
PDT 4min hTERT/E6/E7
PDT 7min U87MG
PDT 7min hTERT/E6/E7
(d)
Cell viability (a.u.)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
**
**
***
PDT+TMZ 1min U87MG
PDT+TMZ 1min hTERT/E6/E7
PDT+TMZ 4min U87MG
PDT+TMZ 4min hTERT/E6/E7
PDT+TMZ 7min U87MG
PDT+TMZ 7min hTERT/E6/E7

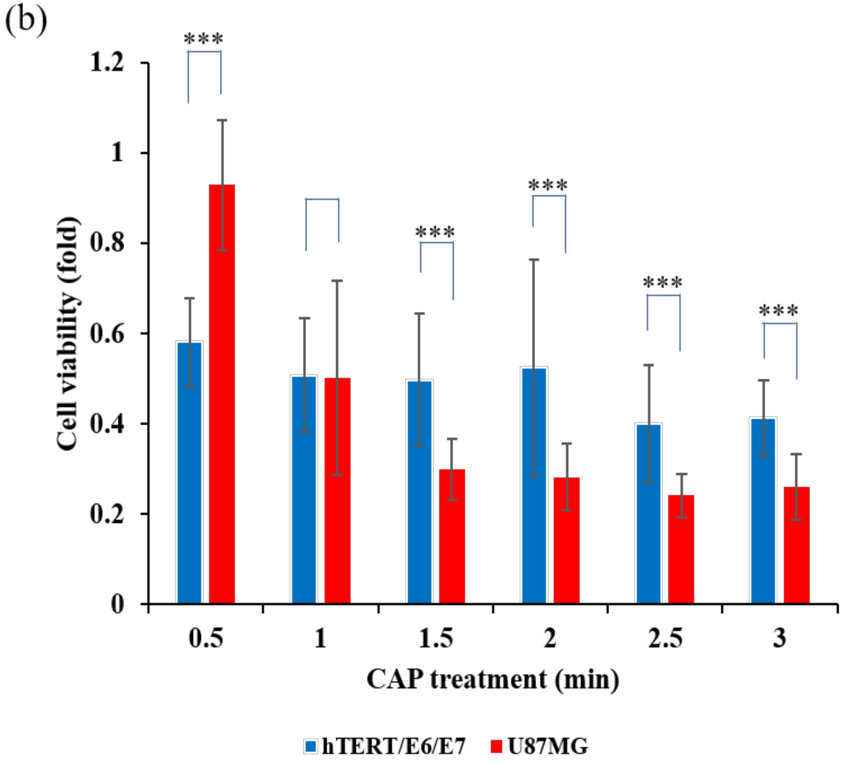
Chemical vs Physical

Chemical not selective



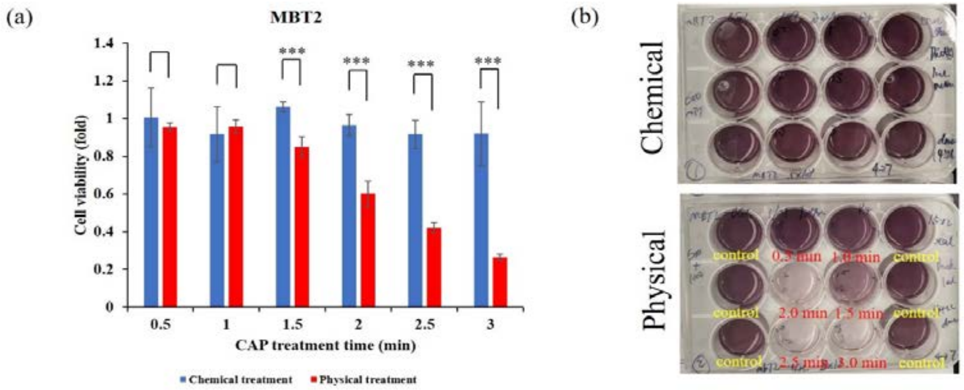
Normal Cancer cells

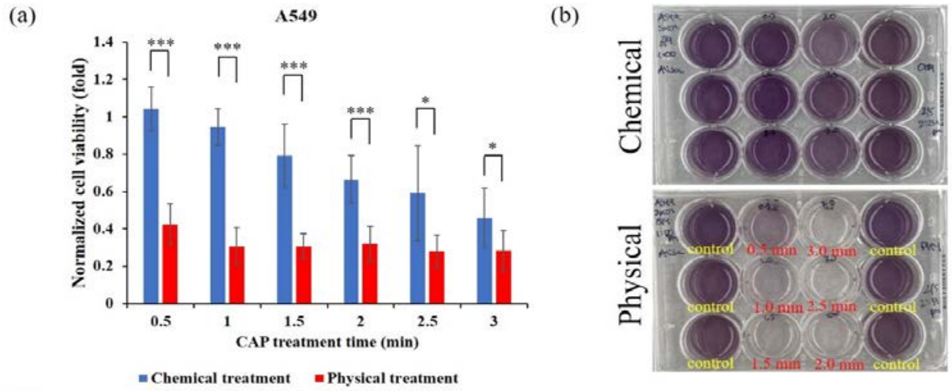
Physical is selective

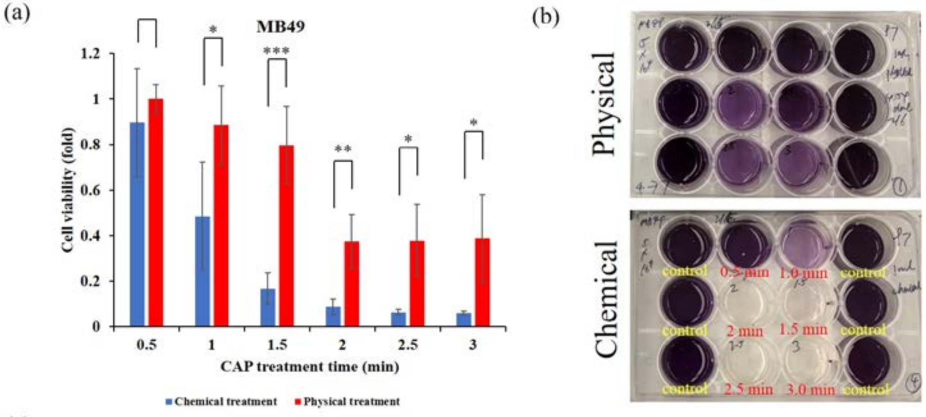


Yan, et al, *Sci. Rep.*, 2020.

Chemical vs Physical





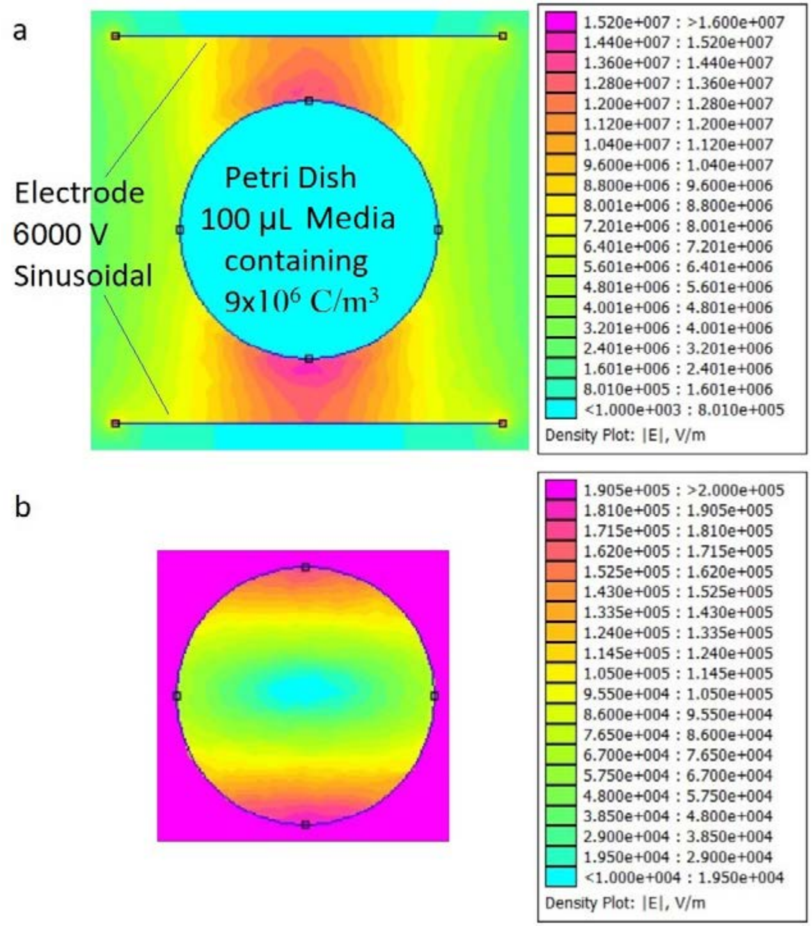


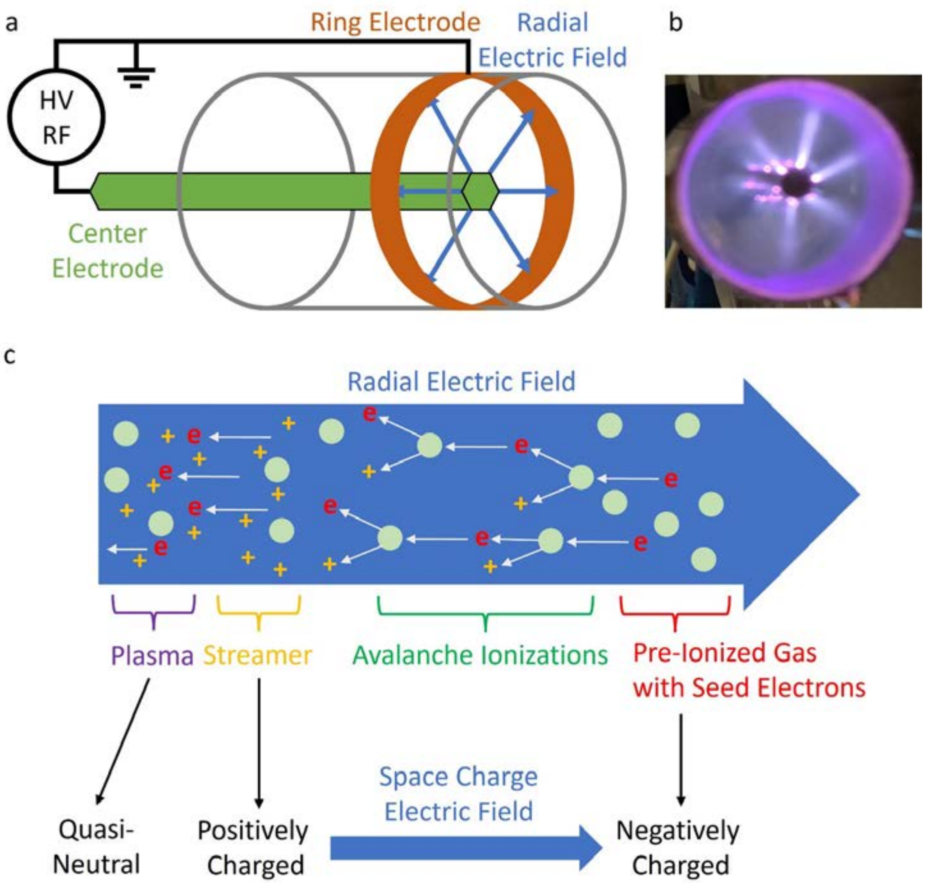
Yan et al., J Phys D, 2020

Cell interaction with EM fields

Methods: Laser Interferometer

Two External Electric Field Sources

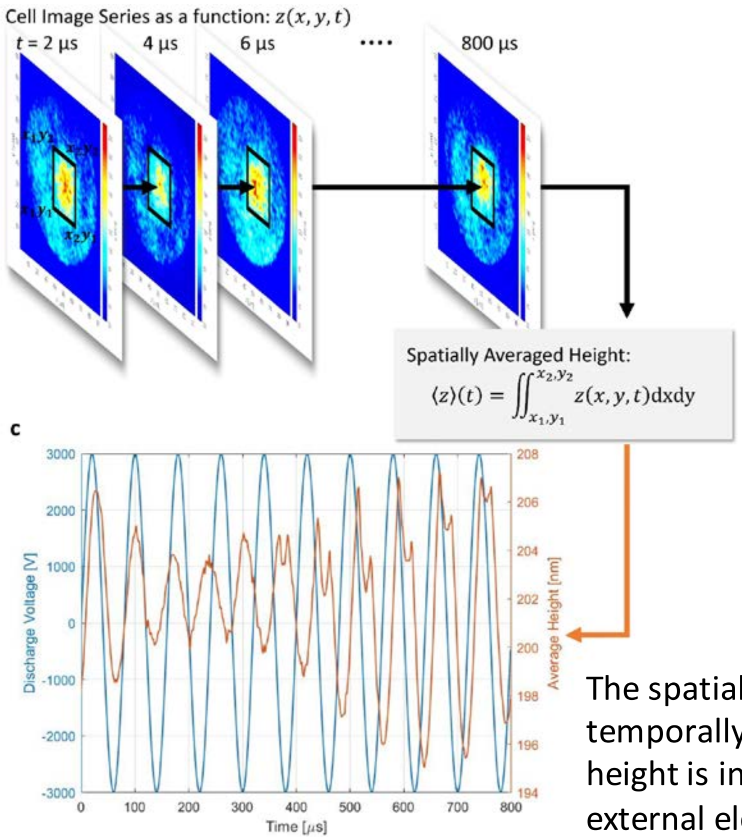




Li et al, *Langmuir*., 2023

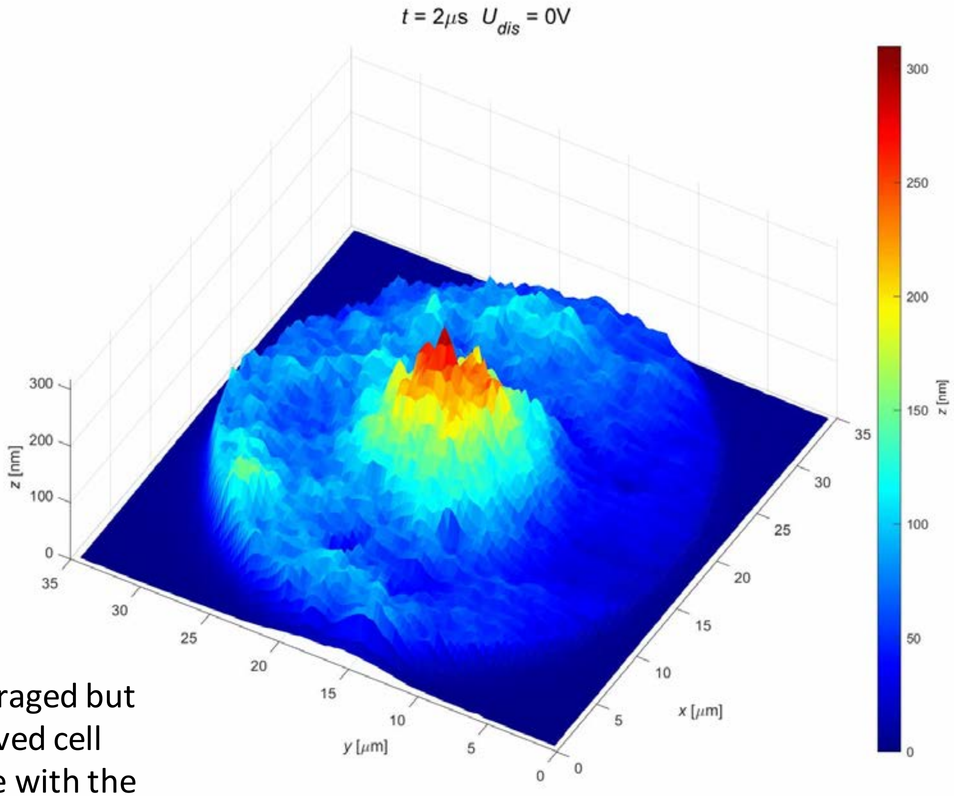
Cell Images of Oscillations

An example of real-time 3D single-cell imaging of A549 Exposed in the electric field of a pair of plate electrodes (6000 V pk-pk at 12.5 kHz)



$$\langle z\rangle(t) = \iint_{x_1,y_1}^{x_2,y_2} z(x, y, t)\mathrm{d}x\mathrm{d}y$$

The spatially averaged but temporally resolved cell height is in phase with the external electric field.



Li et al, *Langmuir*., 2023

The Natural Frequency of Membrane

Wave equation of an arbitrary 2D membrane:

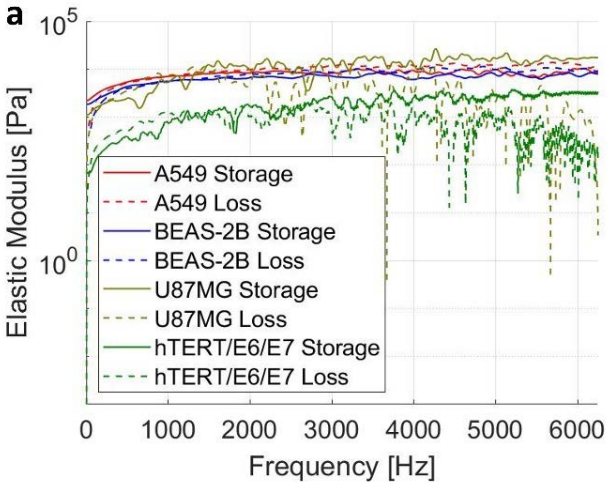
$$D\nabla^4 z + \rho_m \frac{\partial^2 z}{\partial t^2} = 0$$

For specific mode

$$\nabla^2 z_{0\eta} + \Lambda_\eta^2 z_{0\eta} = 0$$

where $f_\eta = \frac{\Lambda_\eta^2}{2\pi}\sqrt{\frac{D}{\rho_m}}$ and $D = \frac{E_m d_{th}^2}{12(1-v^2)}$

Atomic-Force Microscopy



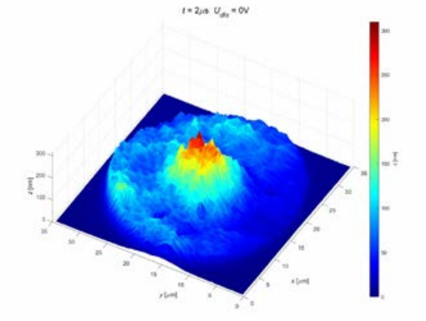
E_m – elastic modulus (measured using AFM)

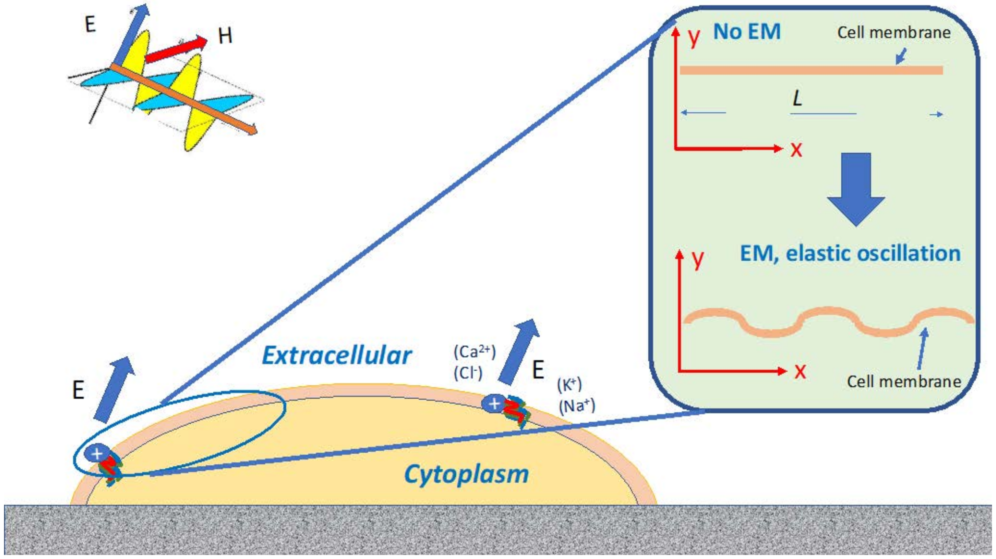
d_{th} – membrane thickness

v – Poisson ratio

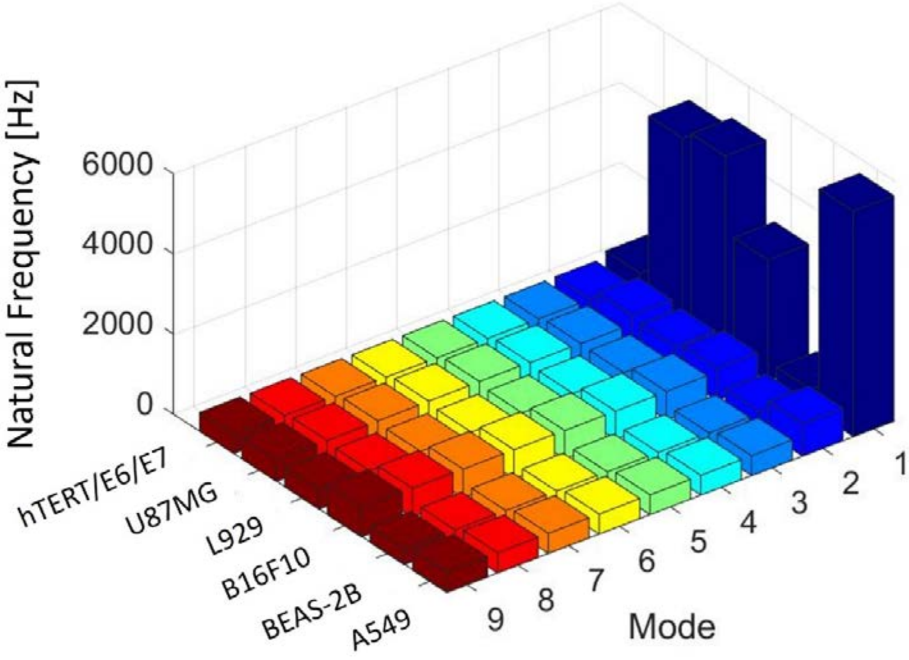
$z_{0\eta}$ – the amplitude of the η^{th} mode

f_η – the natural frequency of the η^{th} mode





The Average Natural Frequencies of the Membranes



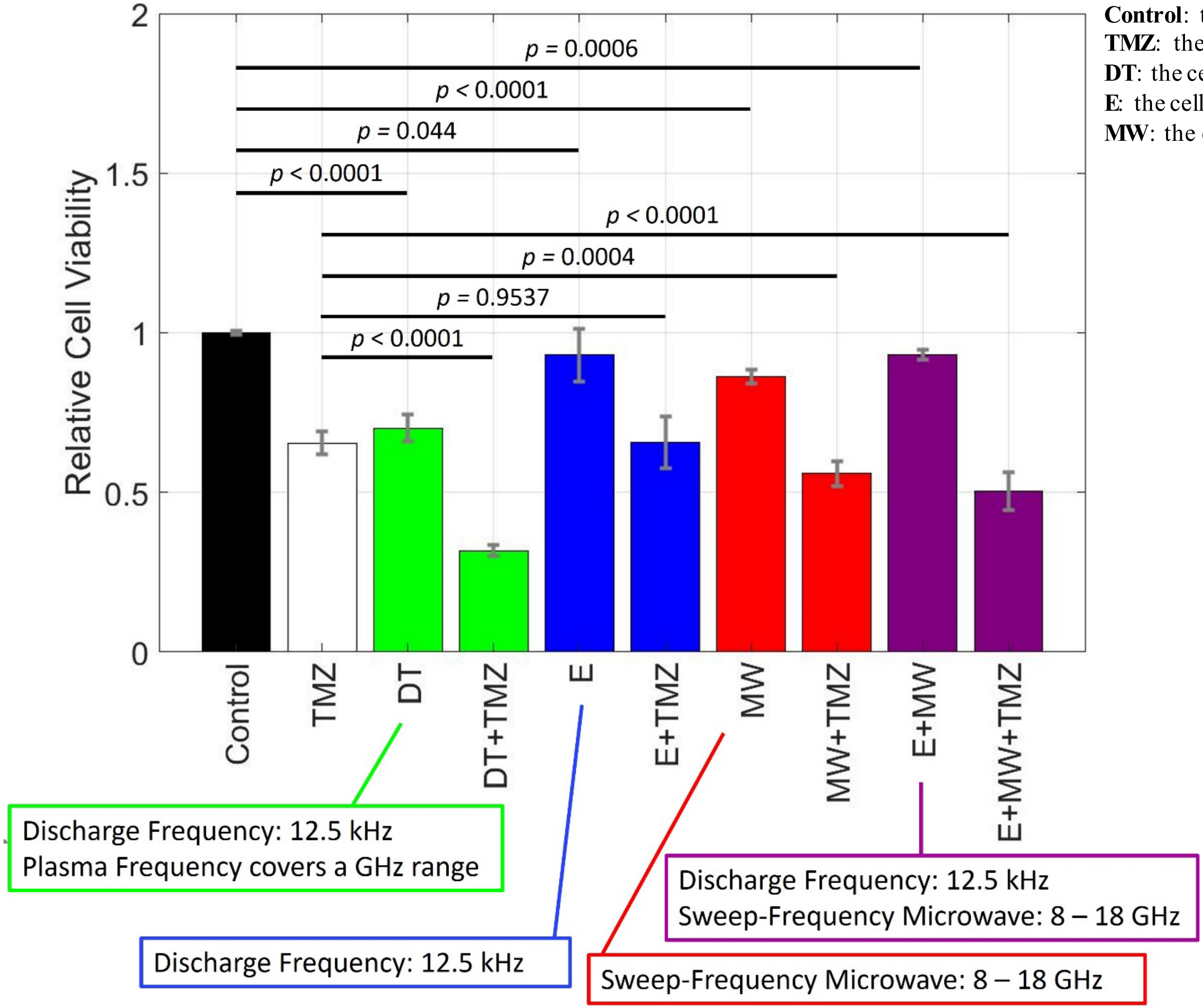
The natural frequencies of Mode 1 (the most contribution mode) agree with the selectivity relations:

U87MG easier to kill than hTERT/E6/E7
L929 easier to kill than B16F10
A549 easier to kill than BEAS-2B

The discharge frequency of plasma is 12.5 kHz. A higher frequency in this figure is closer to the discharge frequency. The plasma has a bandwidth (peak width) of the 12.5 kHz, therefore, covers the natural frequencies.

Li et al, *Langmuir*., 2023

U87MG Sensitization Test



Discharge Frequency: 12.5 kHz
Plasma Frequency covers a GHz range

Discharge Frequency: 12.5 kHz

Discharge Frequency: 12.5 kHz
Sweep-Frequency Microwave: 8 – 18 GHz

Sweep-Frequency Microwave: 8 – 18 GHz

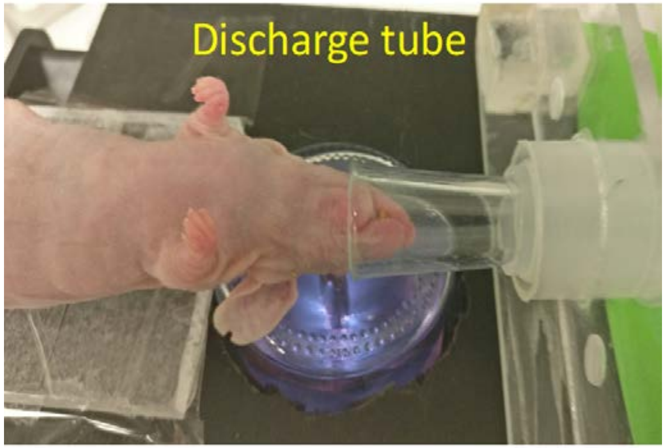
Control: the cells with **no treatment**.
TMZ: the cells were treated with **temozolomide**.
DT: the cells were treated with the **discharge tube**.
E: the cells were treated with the **electrodes**.
MW: the cells were treated with the **microwave**.

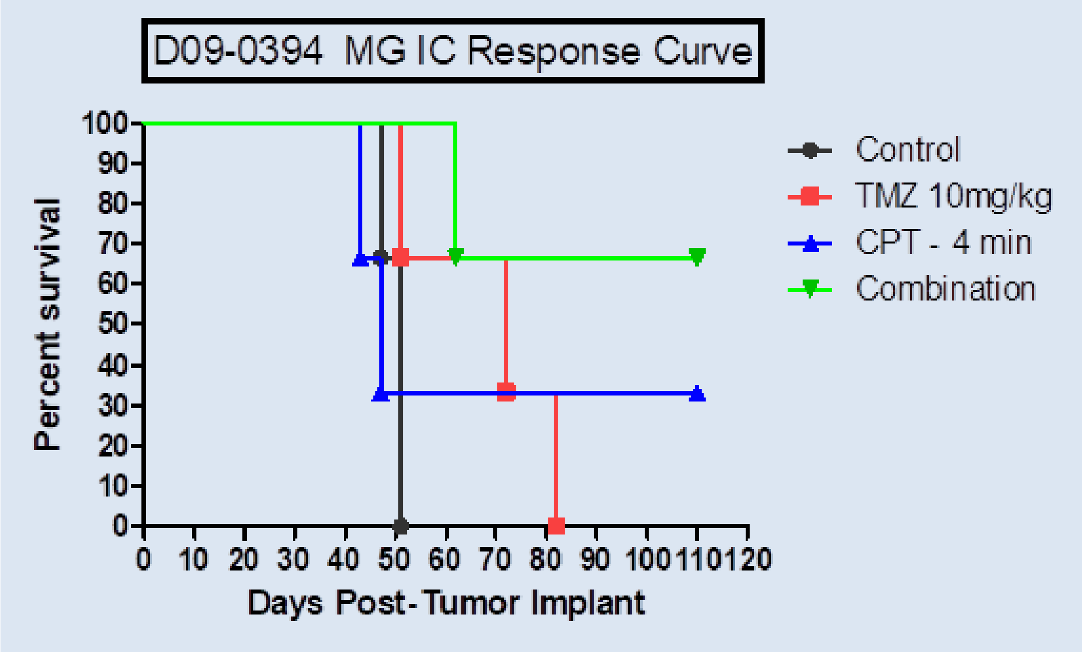
The microwave emissions from the plasma oscillation can resonate smaller structures such as protein molecules, a part of DNA molecules, TMZ drugs, etc.

Li et al, *Langmuir*., 2023

Discharge Tube *in vivo*

- Groups of 3 mice were randomized into groups 10 days post tumor implantation:
  - control group
  - 'TMZ alone' group
  - 'PDT alone' group
  - 'combination' (PDT+TMZ) group
- TMZ was administered IP using a dosing strategy below its maximum tolerated dose at 10 mg/kg IP x 3 days
- PDT treatment was performed for 4 min





Based on the patient-derived xenograft model, PDT treatment drastically improved mean survival days of the tumor-barrier mice by more than 100% compared to control

Yao et al., Phys. Plasmas, 2020
Yao et al., ACS Appl. Biomat., 2022

Conclusions

Adaptive plasma for biomedical application: Uniqueness of plasma is its ability to change its composition and key parameters on demand, dependent on specific requirements of diseased cells. **Intelligent plasma**. Key: **in situ diagnostics**

Chemical vs Physical Treatment is introduced

In chemical model, plasma adaptation might be important way to optimize treatment by plasma. It uniquely utilizes potential of CAP to produce RONS in real time

Plasma-based activation (sensitization) has been discovered. Fast activation and slow de-activation. **EM causes cell membrane oscillation**

Physical treatment might lead to translational pathway

Acknowledgement

National Science Foundation
WHERE DISCOVERIES BEGIN

U.S. DEPARTMENT OF ENERGY | Office of Science

CHARLIE TEO FOUNDATION
• funding brain cancer research •

MPNL
Micro-propulsion and Nanotechnology Lab
The George Washington University

Thank you !