「2012年度 原子分子データ応用フォーラムセミナー」
（高Zプラズマの原子過程と、酸素分子が関係する原子分子過程とその応用）

2012年12月11日～12月13日　核融合科学研究所

大気圧放電に接する液中に生成される活性酸素・活性窒素

大阪大学工学研究科
浜口智志

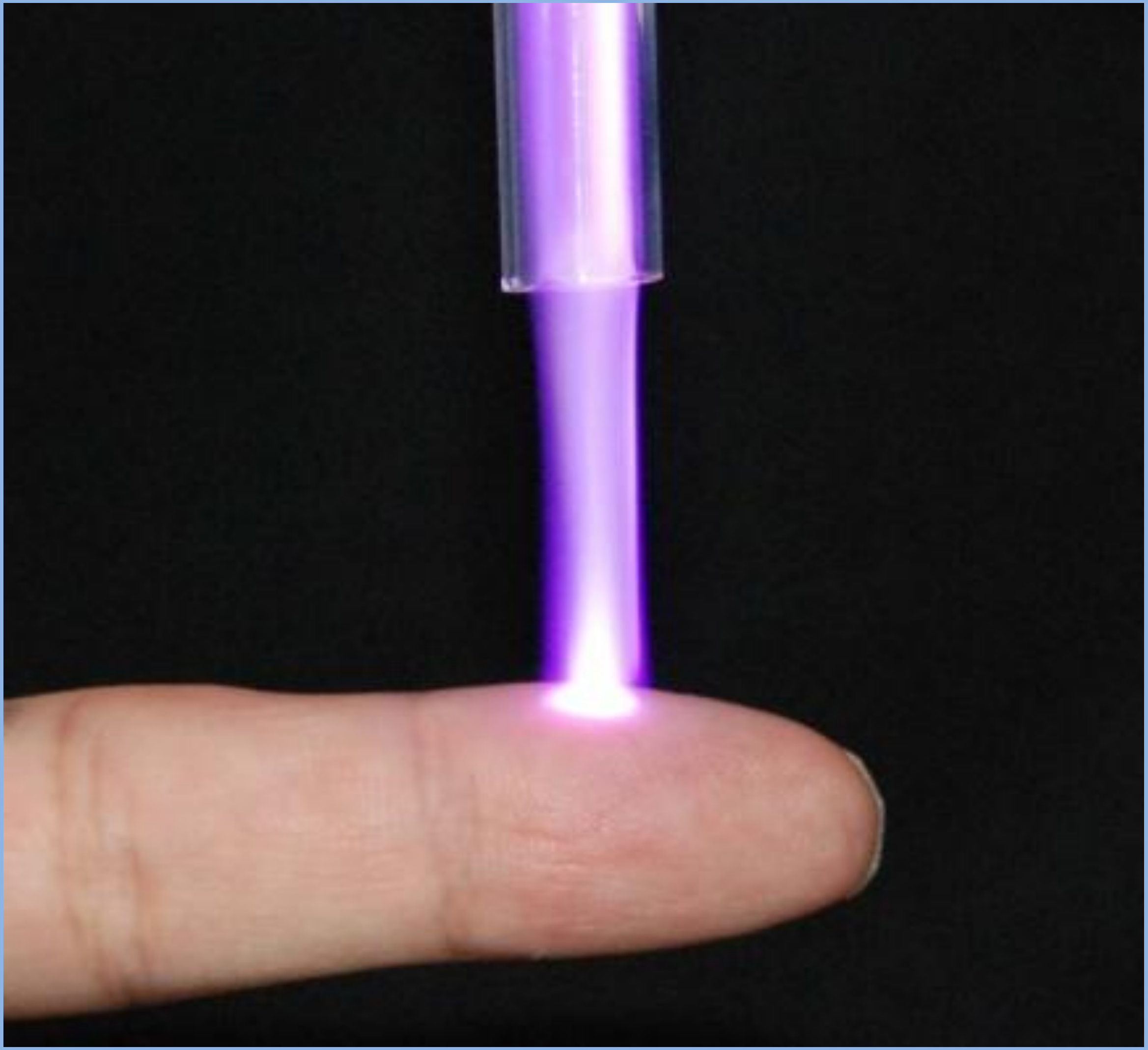
Acknowledgements

1. Numerical Simulation: Tatsuya Kanazawa, Michiro Isobe
2. Atmospheric-pressure plasma experiments: Katsuhisa Kitano
3. Plasma application experiments for biological systems: Hideki Yoshikawa, Akira Myoui, Yu Moriguchi, Keiko Kaneko, Yasuo Kunugiza, Kazuto Masuda, Dae-Sung Lee ,

Outline

1. Motivation and background: plasma technologies for medicine
2. Model system: ROS/RON generation in water exposed to low-temperature atmospheric-pressure plasma
3. Sample simulations

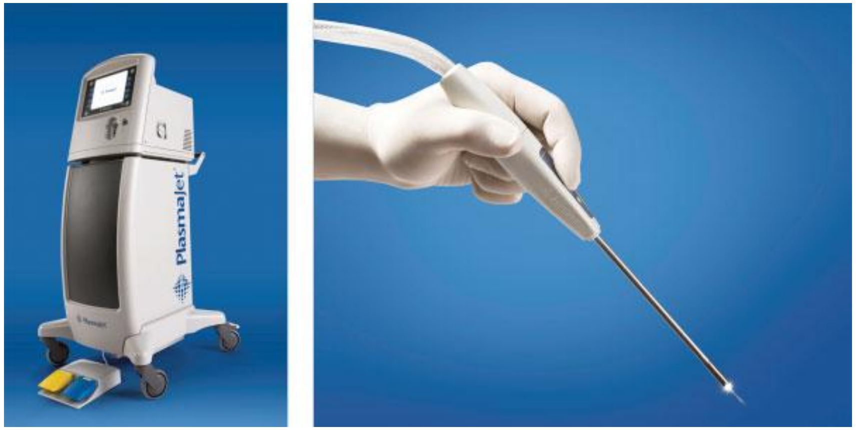
Low-temperature atmospheric-pressure plasmas



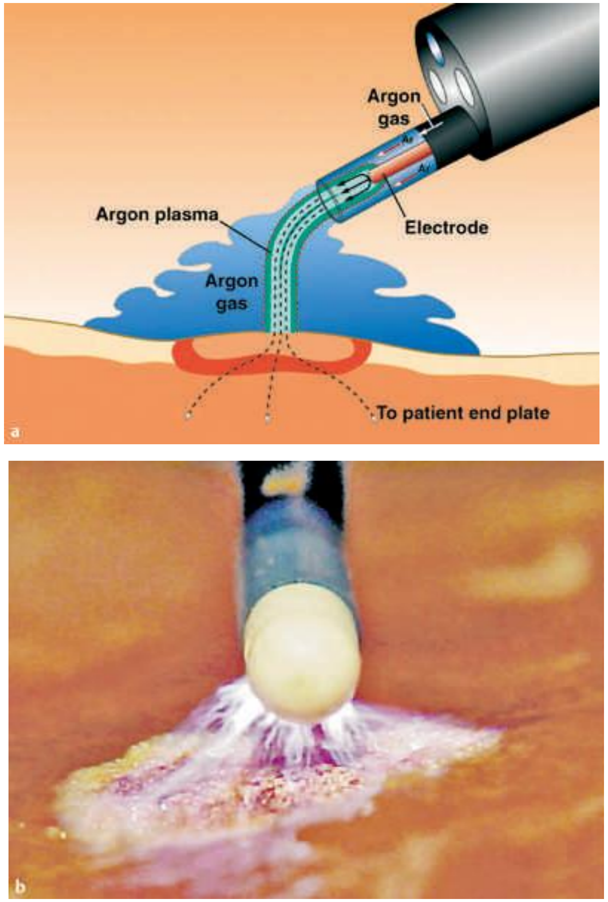
Argon Plasma Coagulator (APC)

Argon Plasma Coagulator (APC)

cauterization by thermal plasma



Plasma Surgical PlasmaJet System
(from Plasma Surgical Limited)
G. Lloyd, et al., Plasma Process. Polym.
7 (2010) 194.

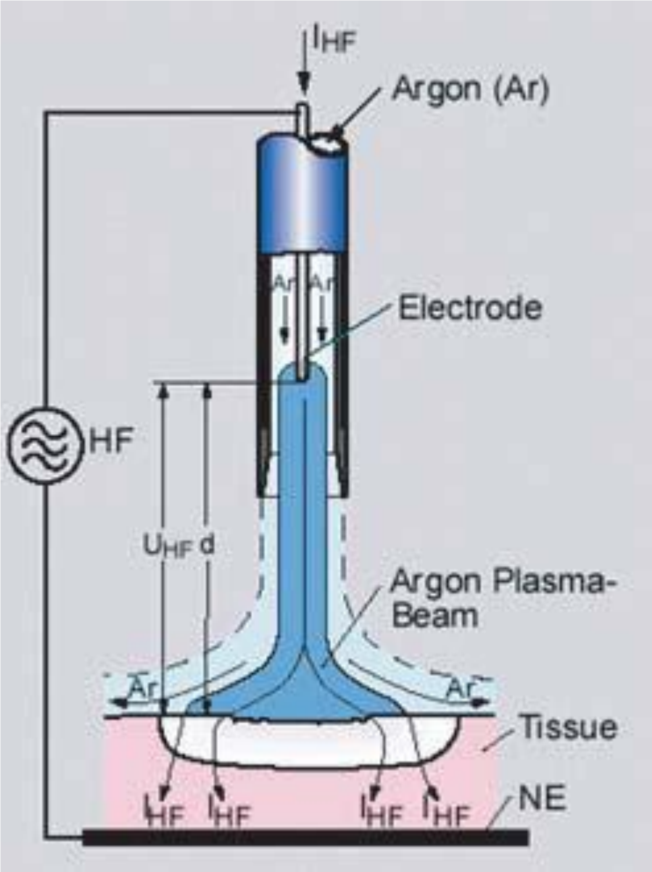


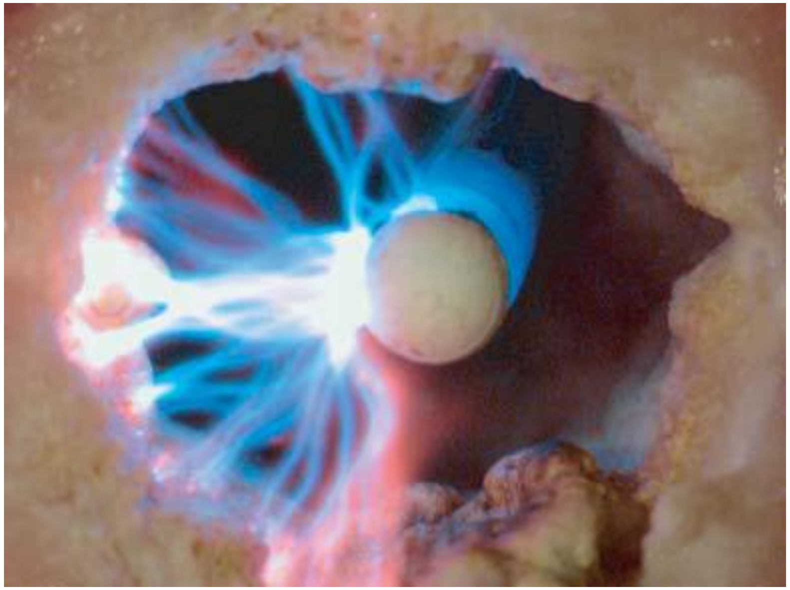
APC basic principles

A. Postgate *et al*., Endoscopy **39** (2007) 361

APC

ERBE (Germany)
power consumption:(50-100 W)
frequency: 350 kHz
plasma temperature : 100°C
(current flows in the tissue)





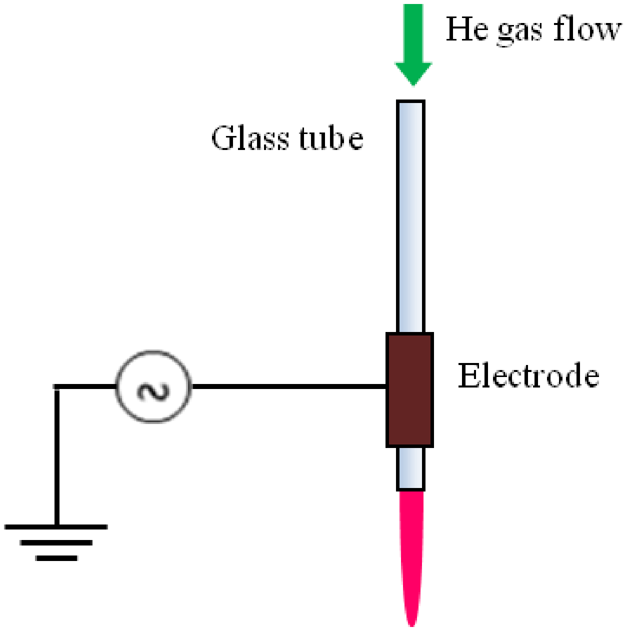
High-frequency Argon Plasma Coagulation unit; left – schematic view, right – interaction with tissue .

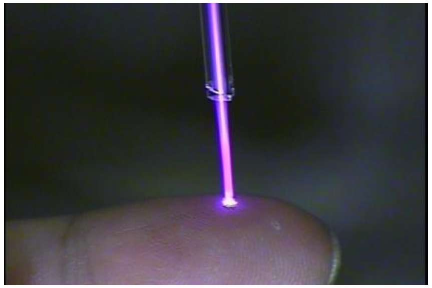
E. Stoffels, Contrib. Plasma Phys. **47**, 40 – 48 (2007)

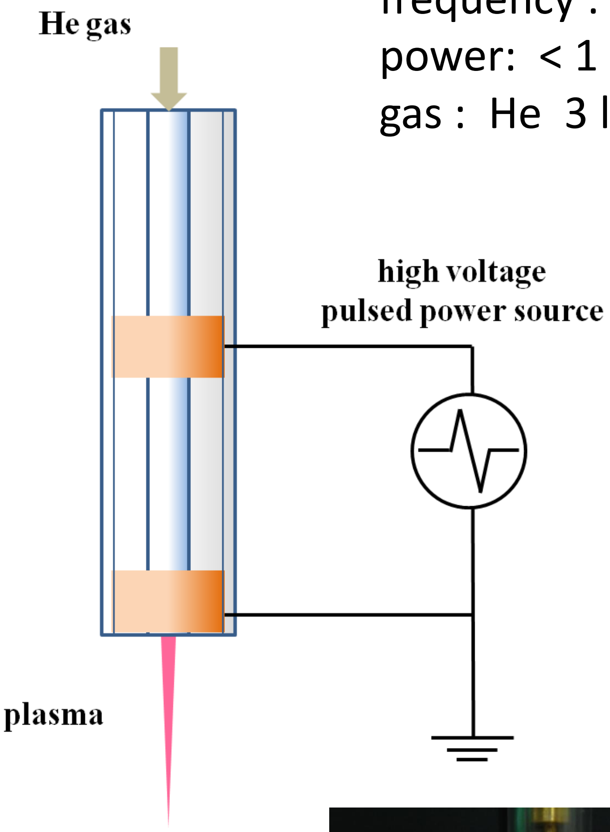
low-temperature atmospheric-pressure plasmas

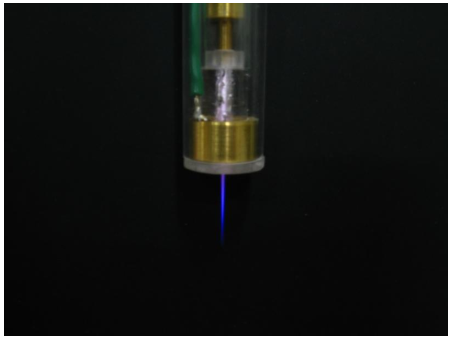
Plasma jet systems

voltage: 1 ~ 10kV
frequency : 10 ~ 30kHz
power: < 1 W
gas : He 3 l/min



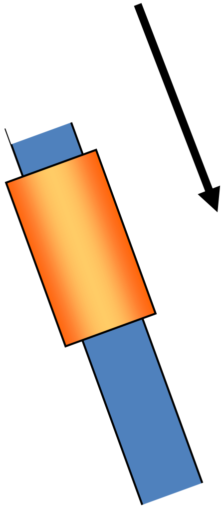






hand-held plasma jet device

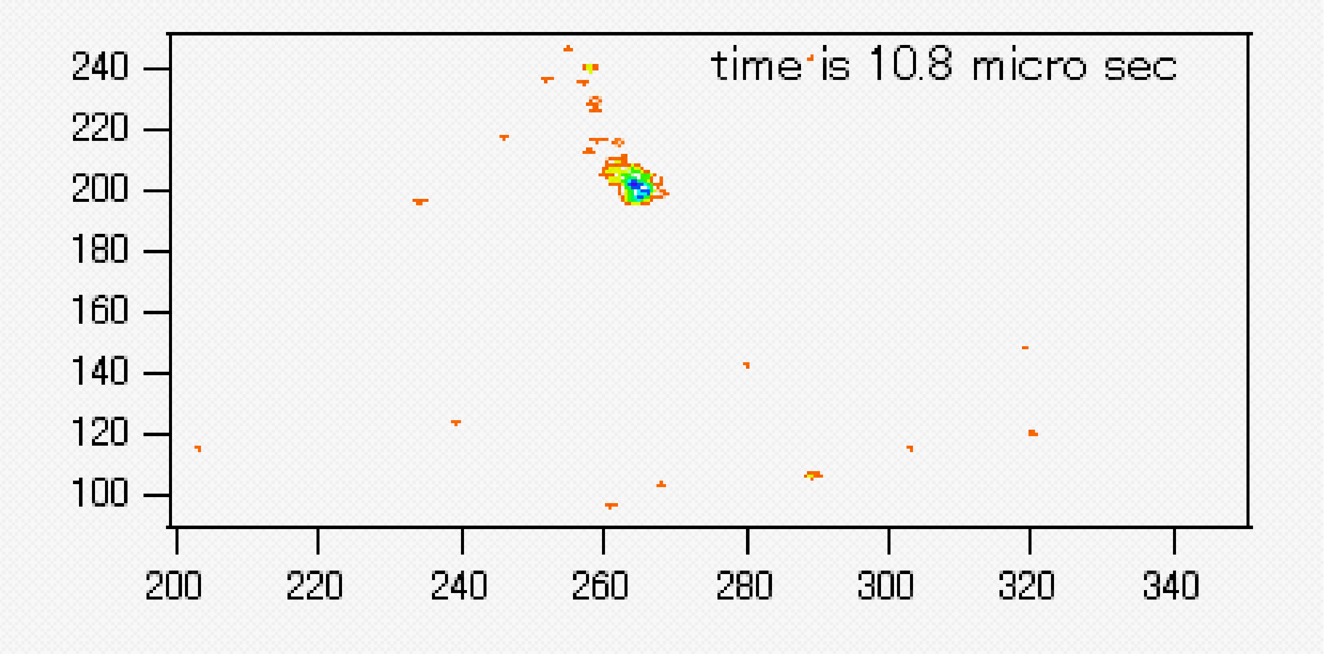
High-speed camera observation

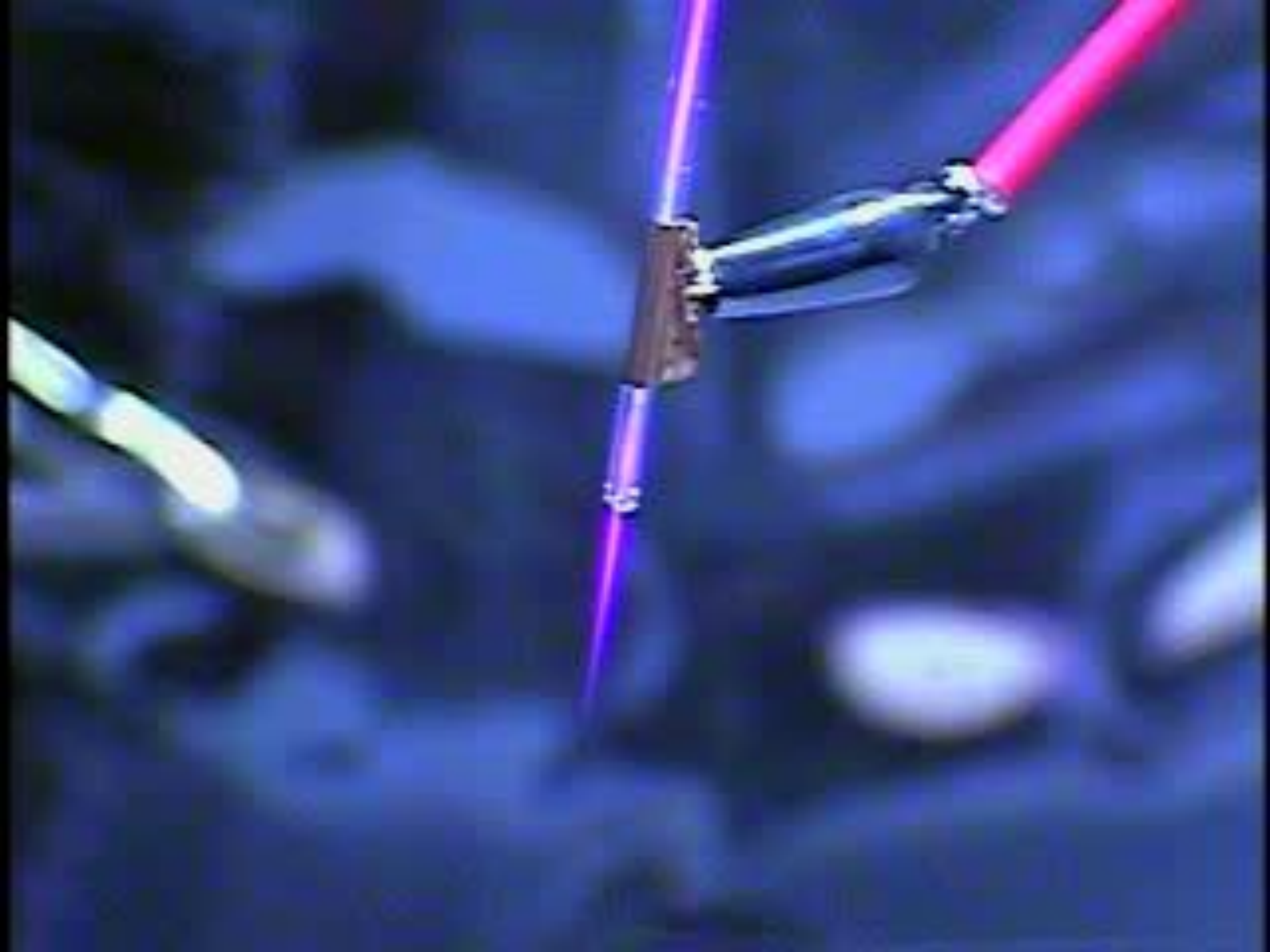


direction of plasma jet injection

plasma bullet : speed（ ≒10km/s）≫ gas velocity

exposure time: 0.1μs
shot at every 0.2μs



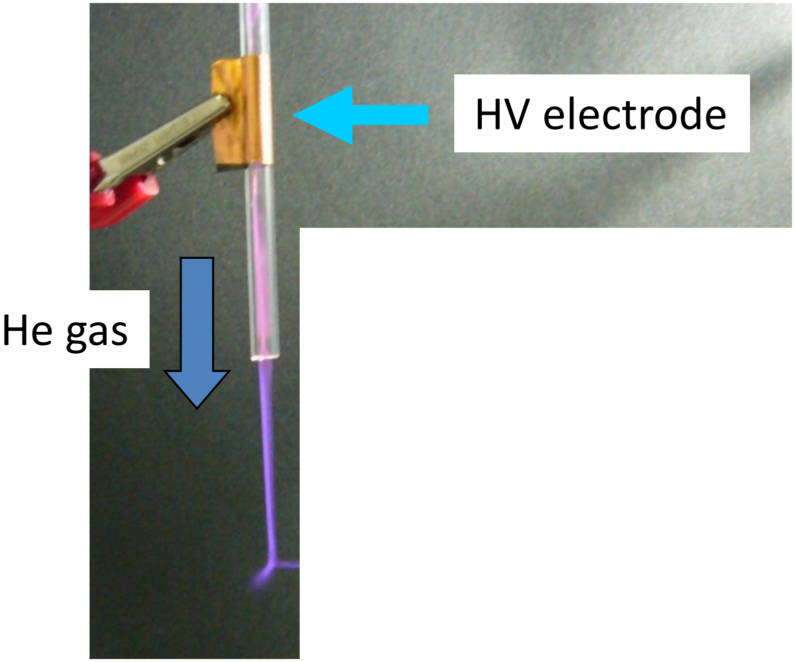


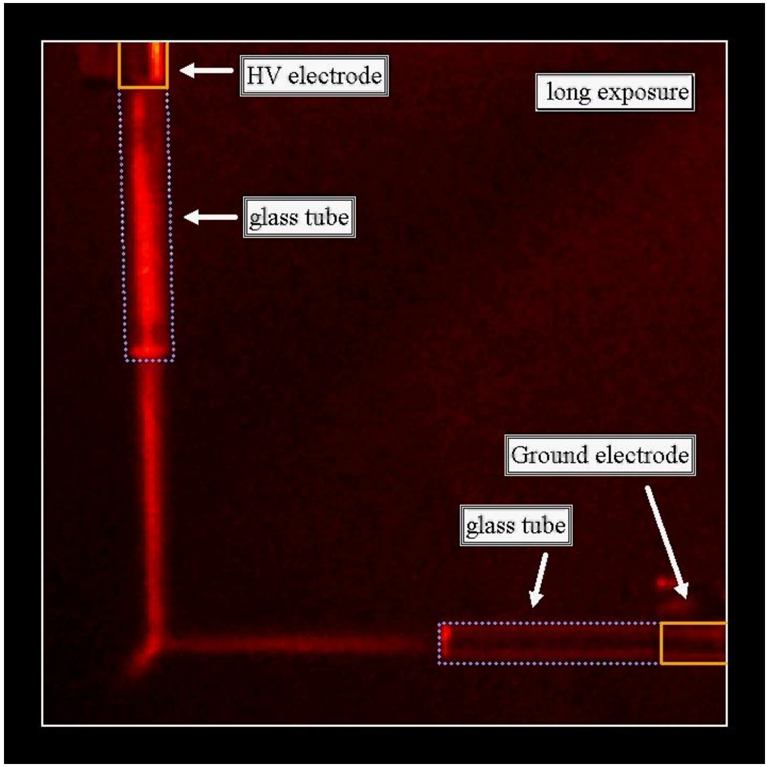
Cross Jet

Cross jet

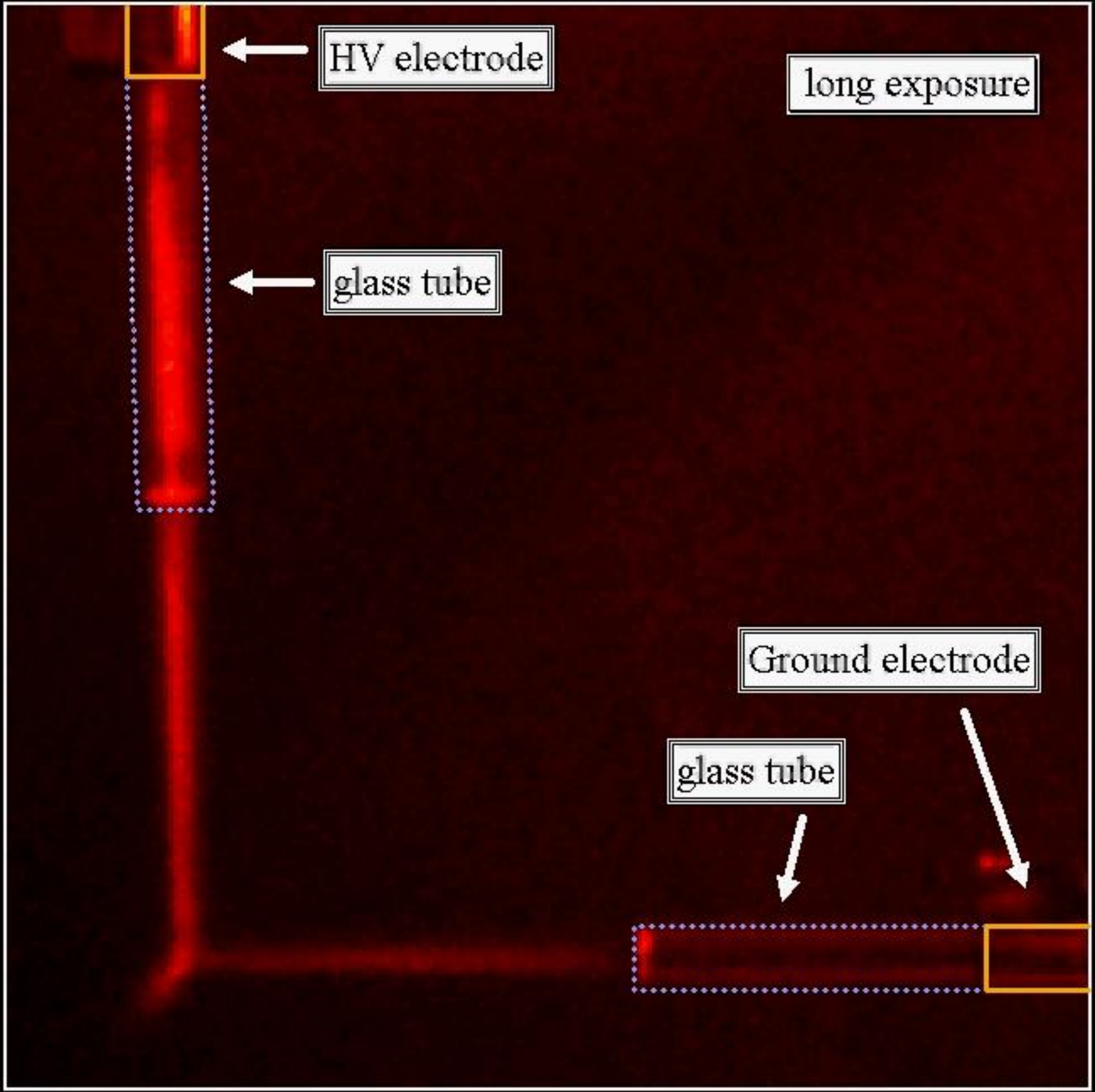
High speed ICCD camera

Exposure time 50ns
Time step 50ns





The ionization front travels along the crossing gas flows


HV electrode
long exposure
glass tube
Ground electrode
glass tube

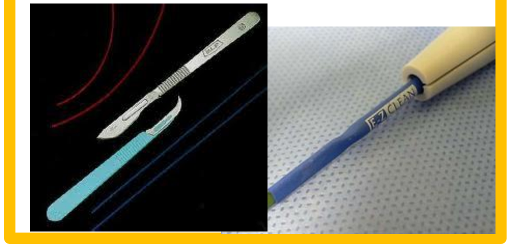
Medical Tools

Plasma medicine

traditional surgical devices

scalpels・electrical scalpels

mechanical force/ heat



radiations

X-ray, heavy ions

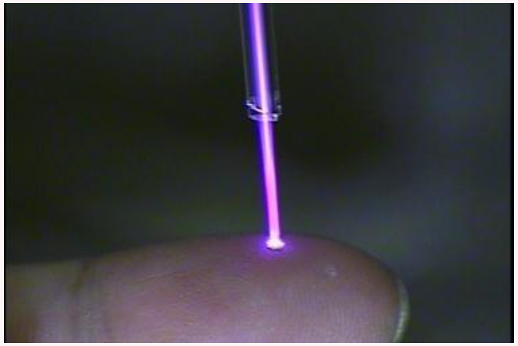
ionization

low-temperature plasma

new generation of plasma device

free radicals, ROS, RON

blood coagulation, would healing, local sterilization, cell proliferation etc.

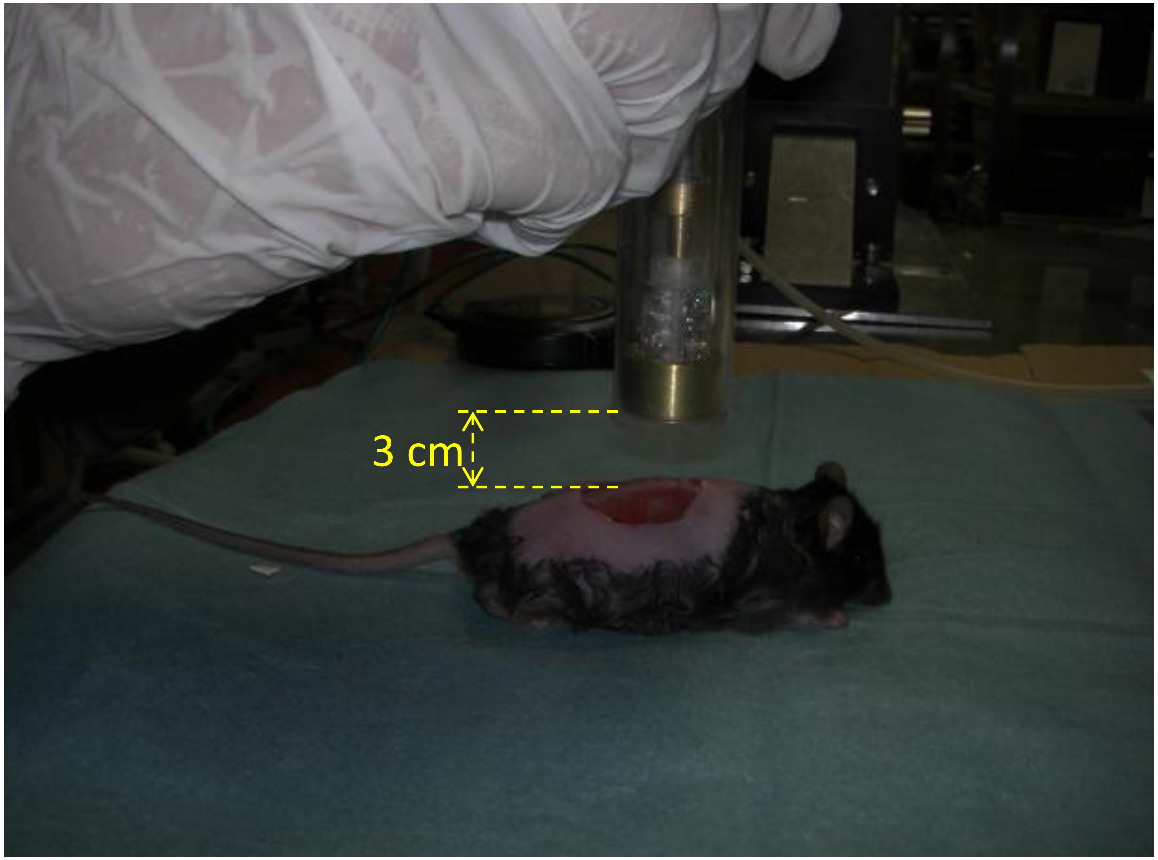


laser

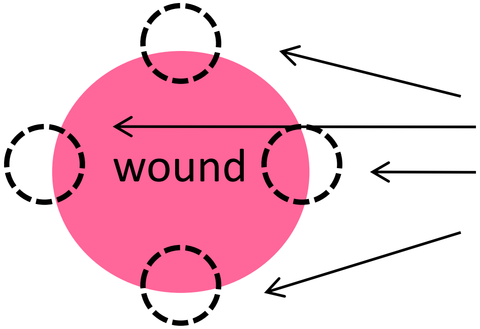
laser scalpels
heat

thermal plasma

argon plasma coagulator
heat



Application Method



1 min plasma application each
in Day 0 and Day 1 (twice)

Day 0 (before plasma application)

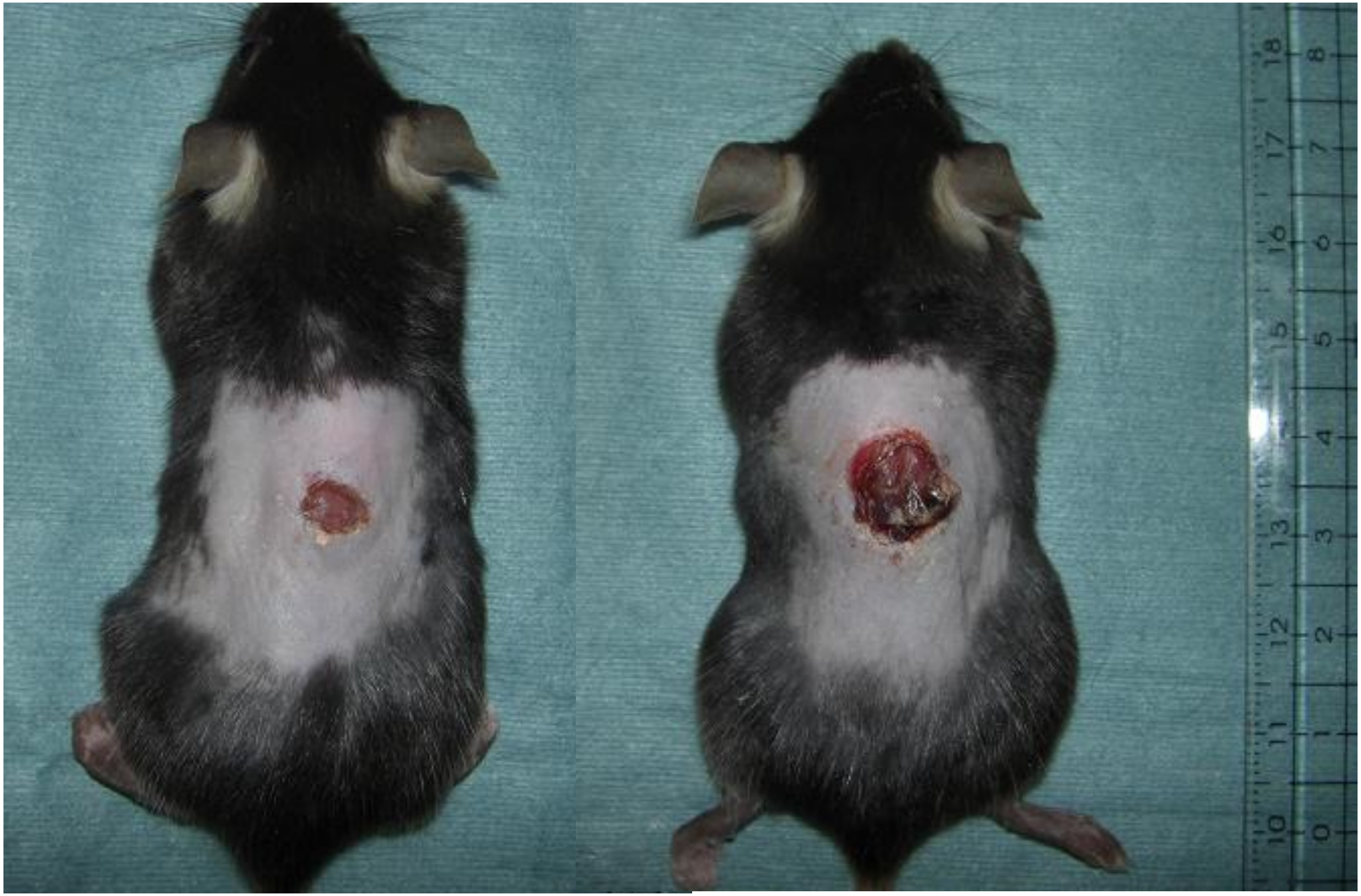
Before Plasma Application

area = 89 units (arb)

Control

area = 111 units

Day 6



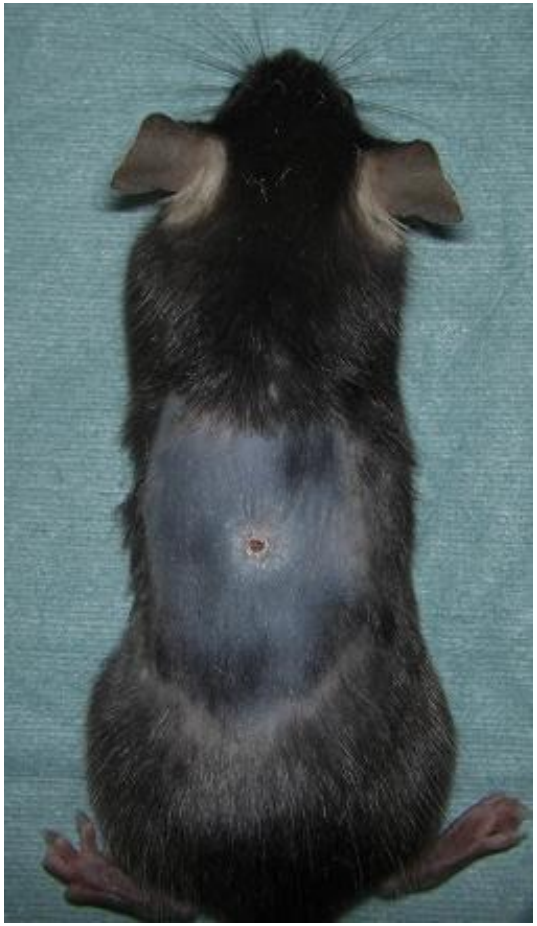
plasma treated: area = 25 units (28%)

untreated: area 87 (78%)

Day 10

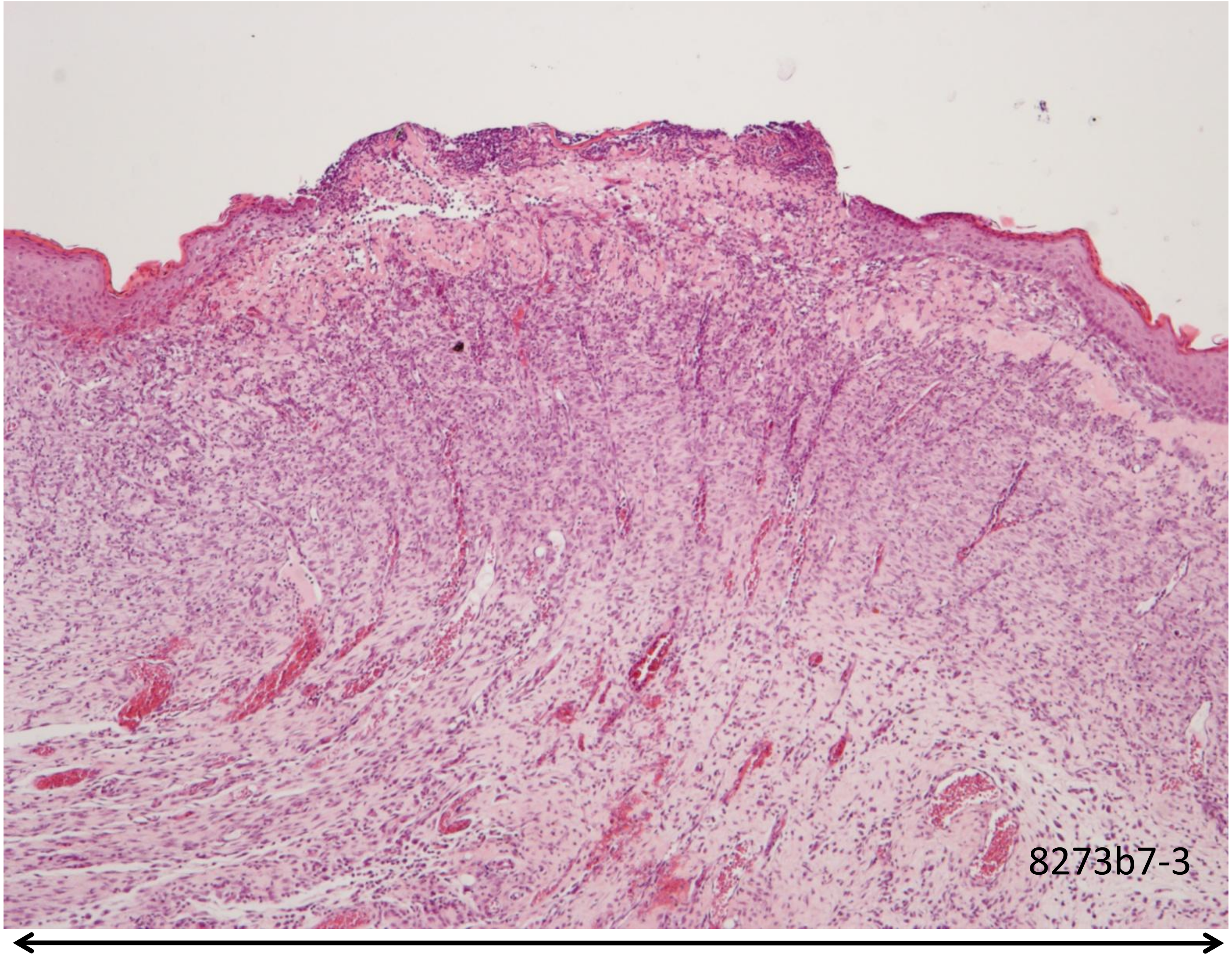
plasma treated: area = 12 units (14%)

Day 12



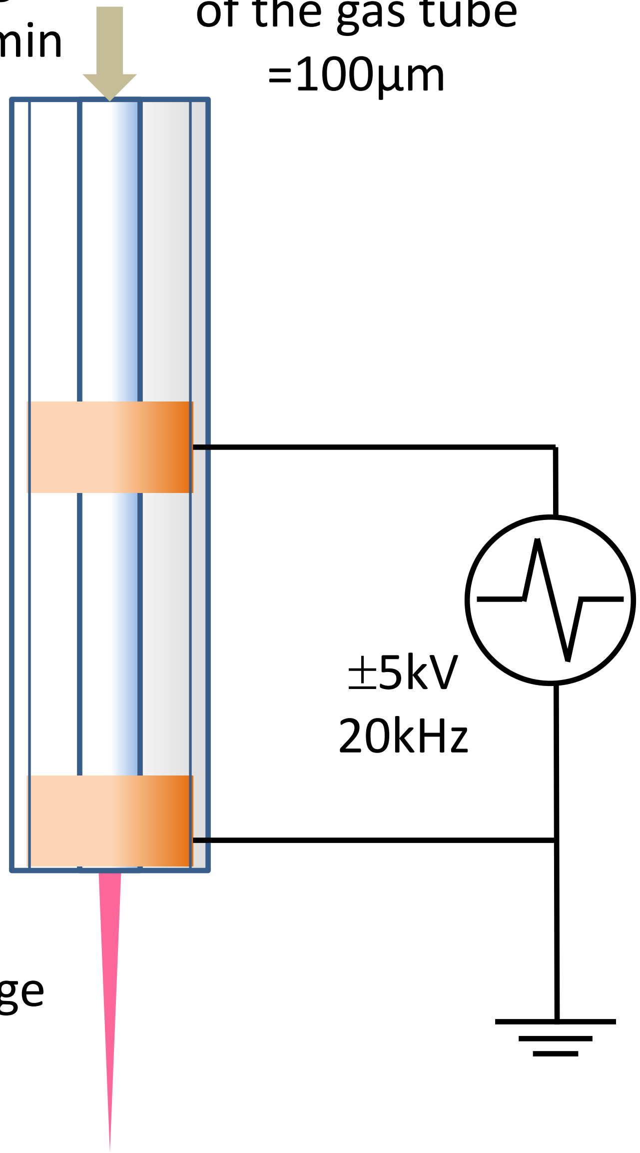
plasma treated

1 minute-single plasma application (7 days after application) => normal skin growth



Cell Proliferation

Plasma System

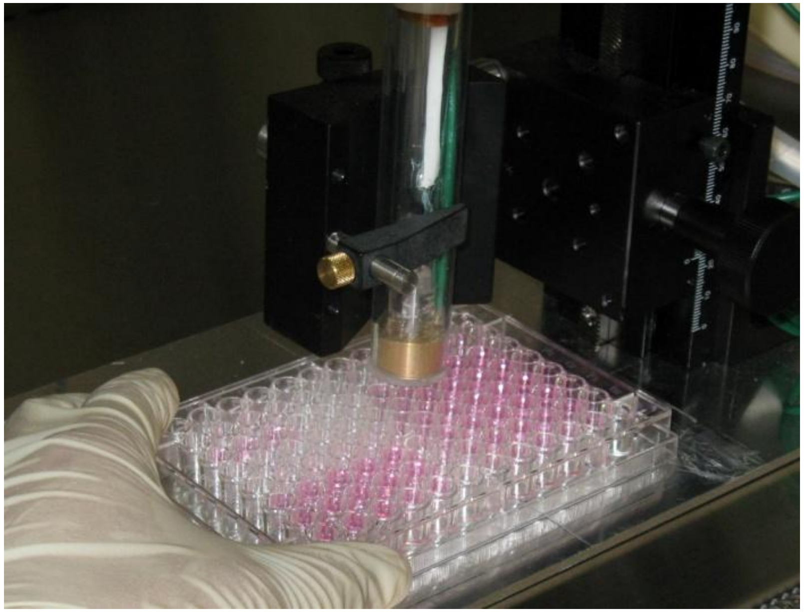


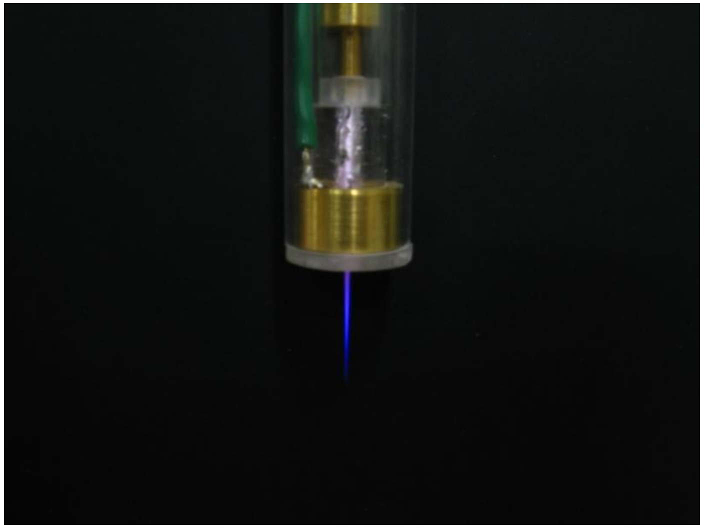
He gas
3L/min

inner diameter
of the gas tube
=100µm

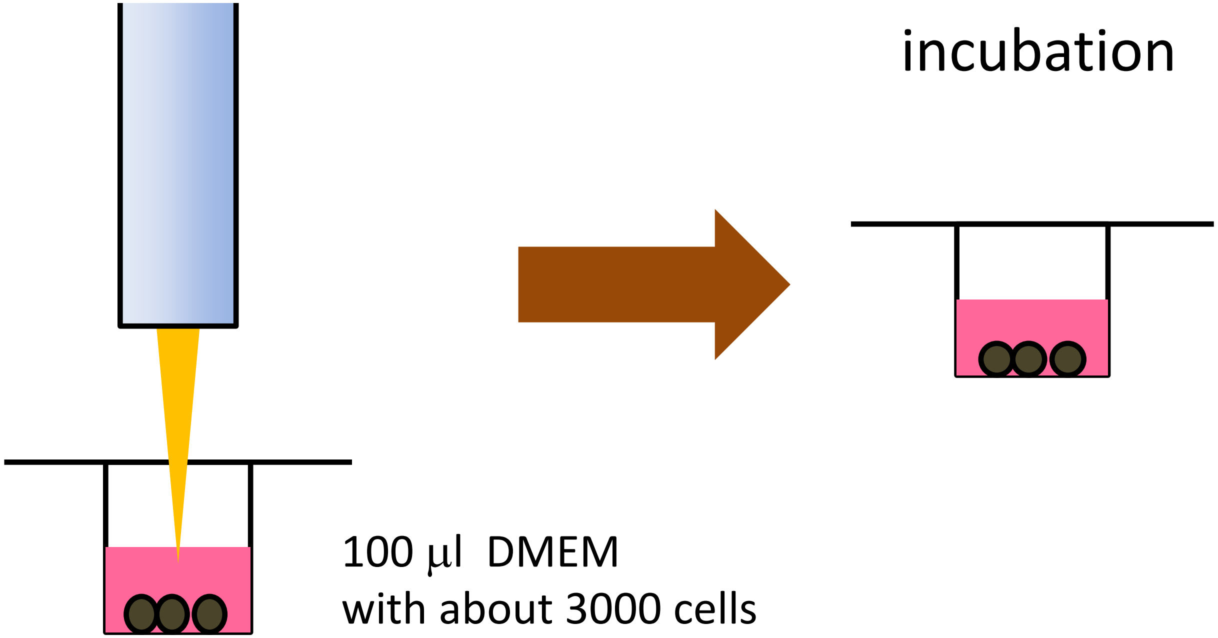
±5kV
20kHz

discharge
in air





procedure



incubation

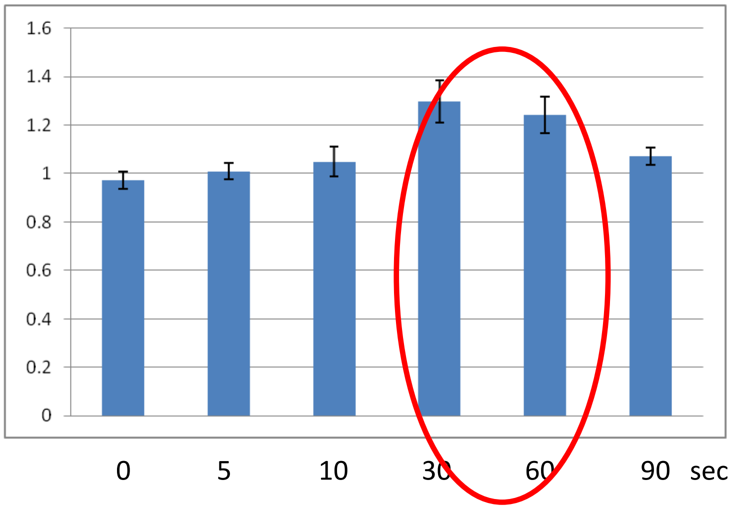
100 μl DMEM
with about 3000 cells
(for a 96 well process)

Cell count : WST kit CCK-8
or typan blue & Beckman Coalter Counter

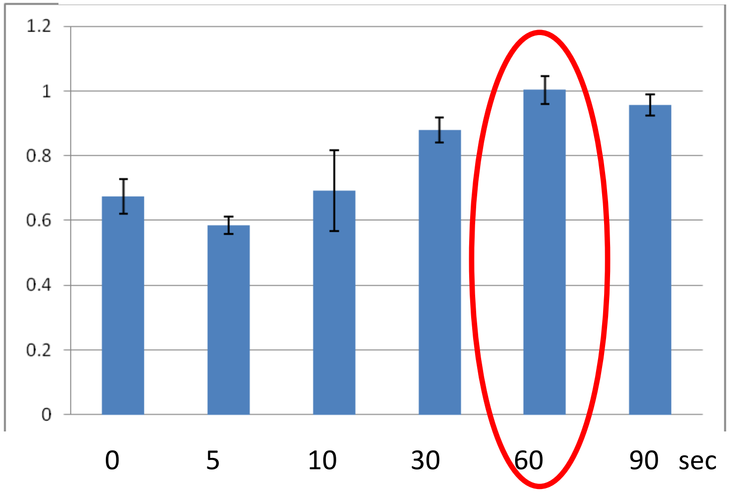
N=3 with a Bonferroni-Dunn test

Human primary culture

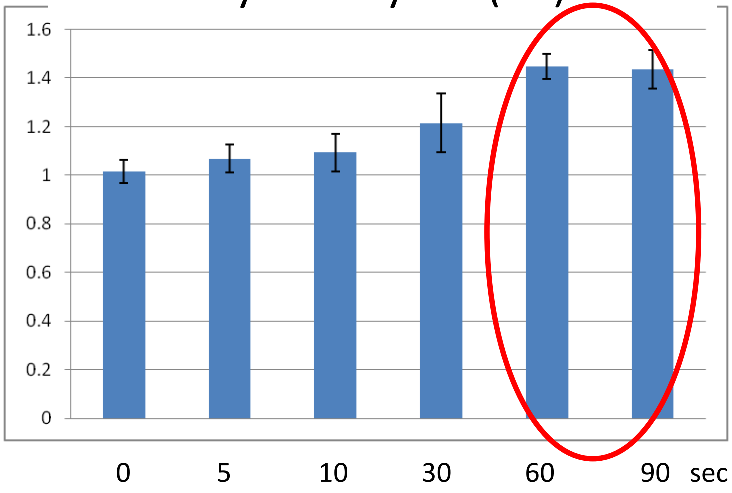
human synoviocytes (HS) 6hr



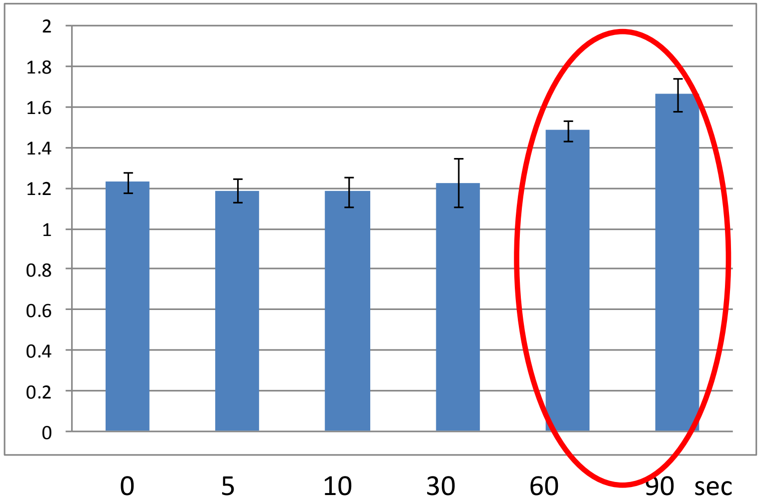
human synoviocytes (HS) 12hr



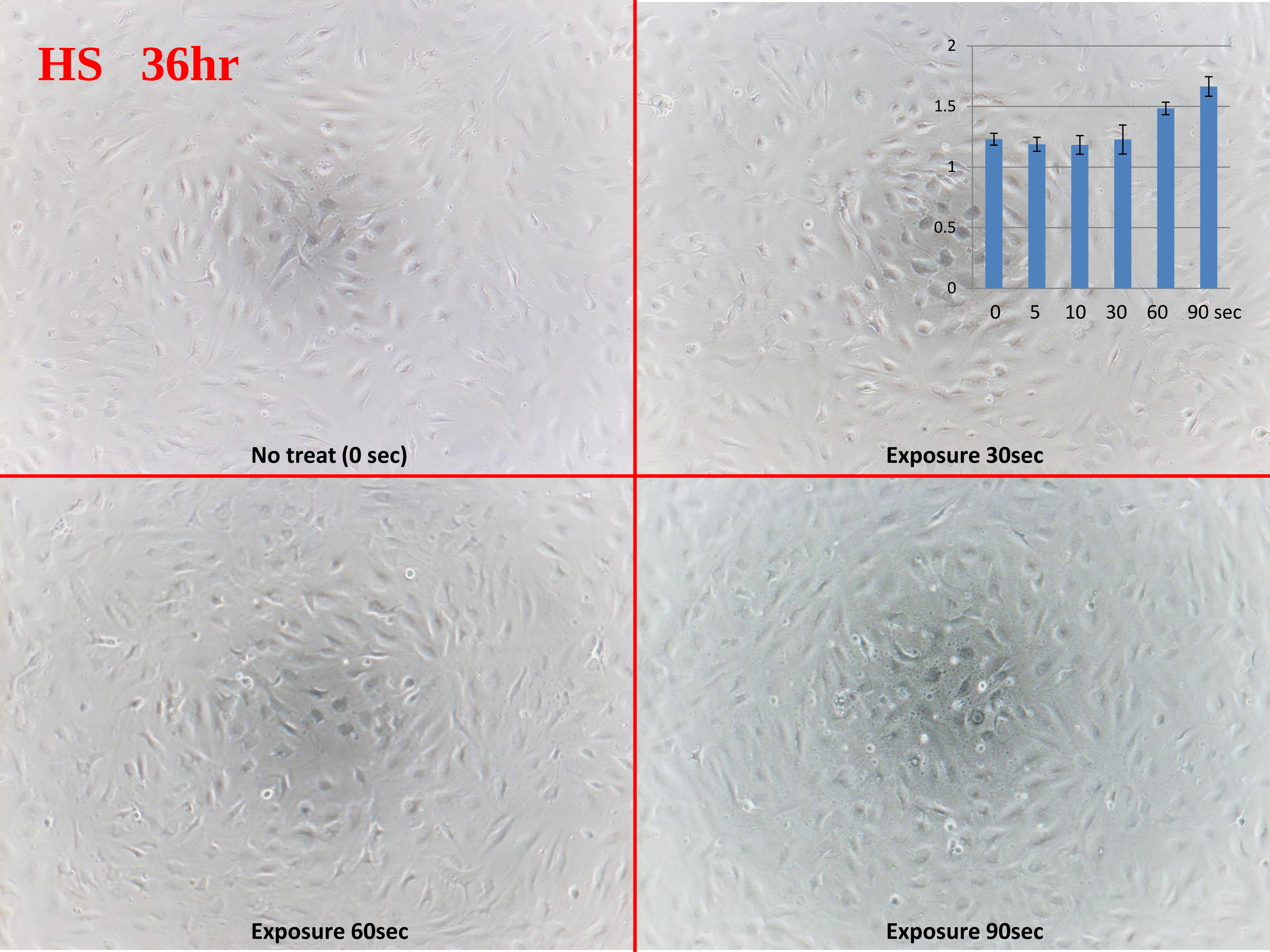
human synoviocytes (HS) 24hr



human synoviocytes (HS) 36hr



HS 36hr



No treat (0 sec)

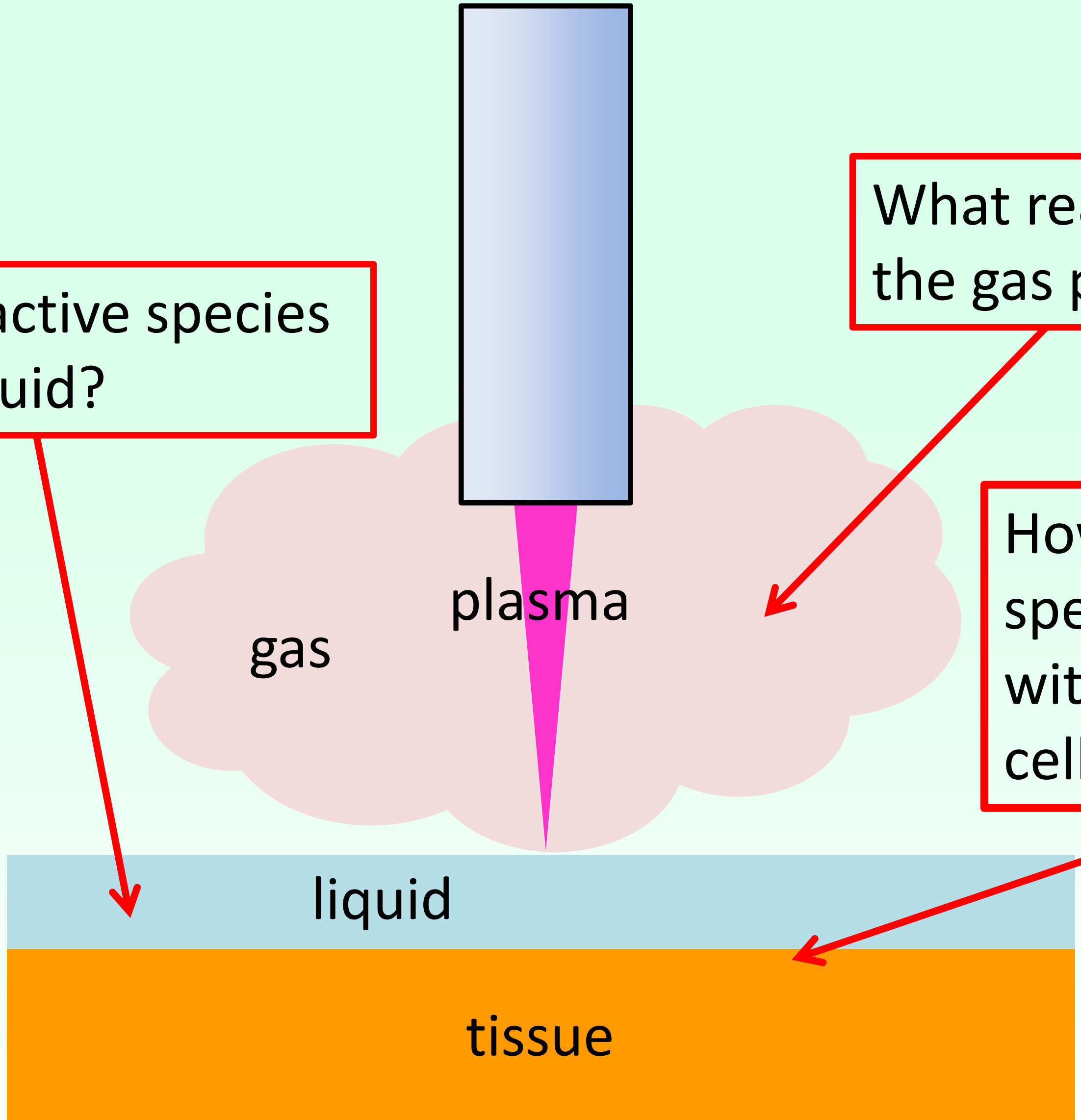
Exposure 30sec

Exposure 60sec

Exposure 90sec

model system to study

physics questions



What reactive species in the gas phase?

What reactive species in the liquid?

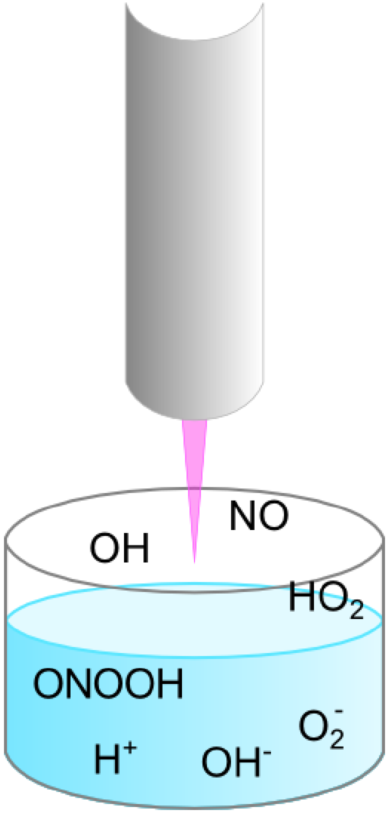
How the reactive species interact with the tissue or cell membranes?

Goal of this study

Chemically reactive species generated in liquid have some strong biological effects

ROS (Reactive Oxygen Species)
OH (hydroxyl radical), O_2^-(superoxide anion radical),
HO_2 (hydroperoxyl radical) etc

RNS (Reactive Nitrogen Species)
NO (nitric oxide), NO_2 (nitrogen dioxide),
ONOOH (peroxynitrous acid), $ONOO^-$ (peroxynitrite) ,etc

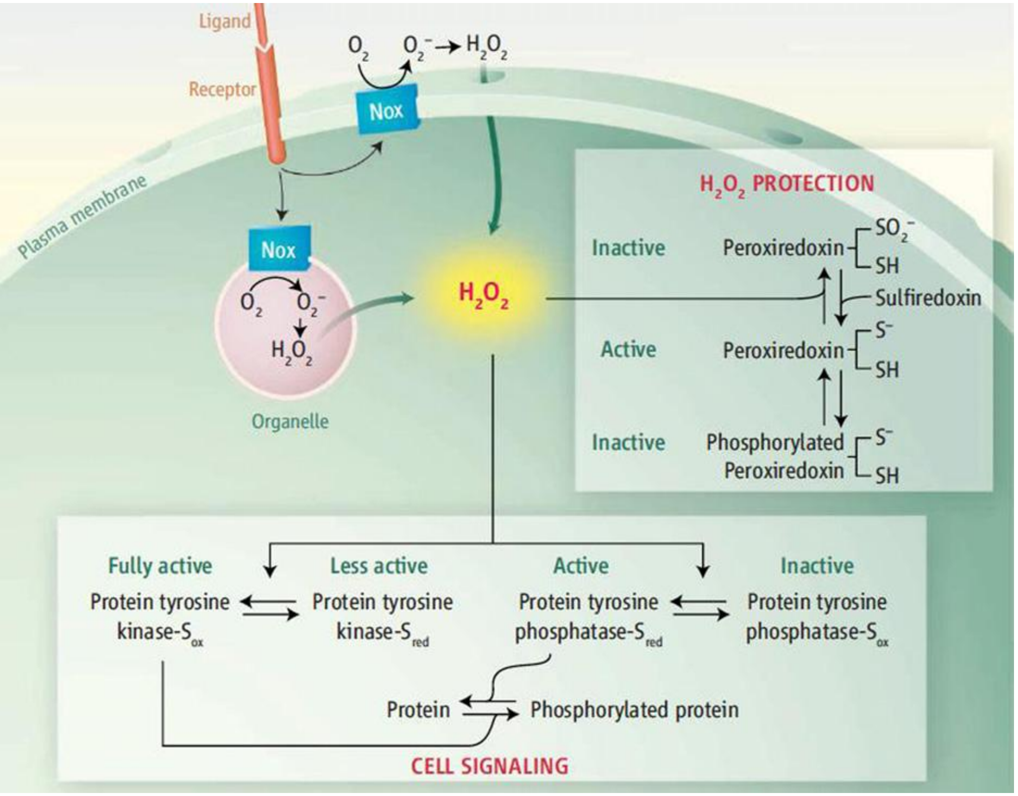


Goal

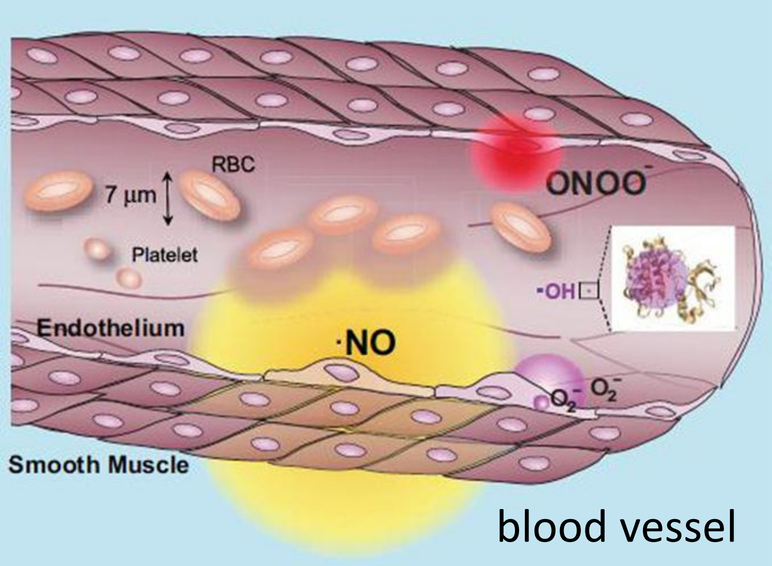
To understand their generation and reaction processes in liquids by numerical simulations

ROS/RNS

activation of the Nox (i.e. NADPH oxidase) enzyme



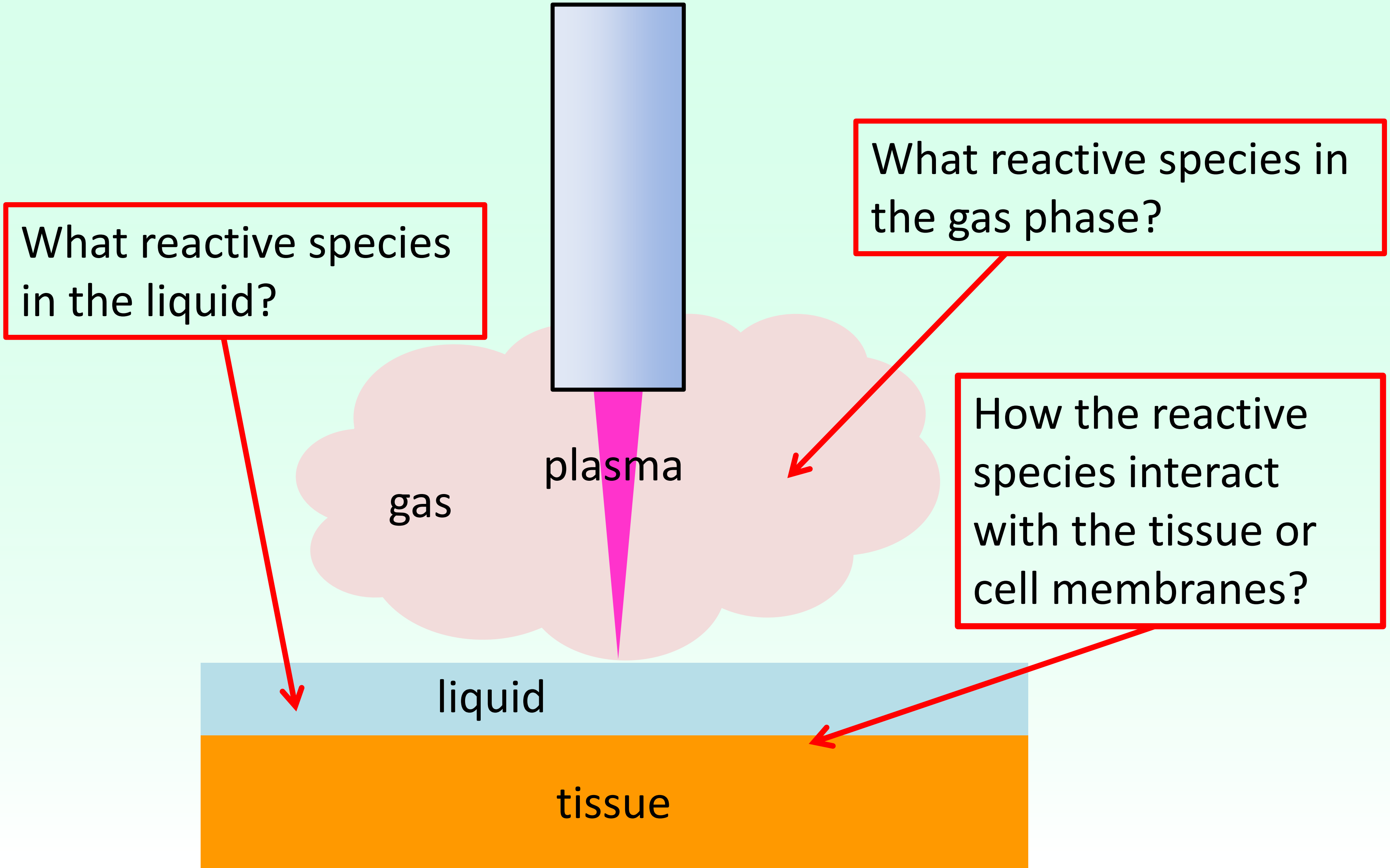
cell signaling in vascular relaxation,



blood vessel

D. B. Graves, J. Phys. D: **48** (2012) 263001

physics questions



What reactive species in the gas phase?

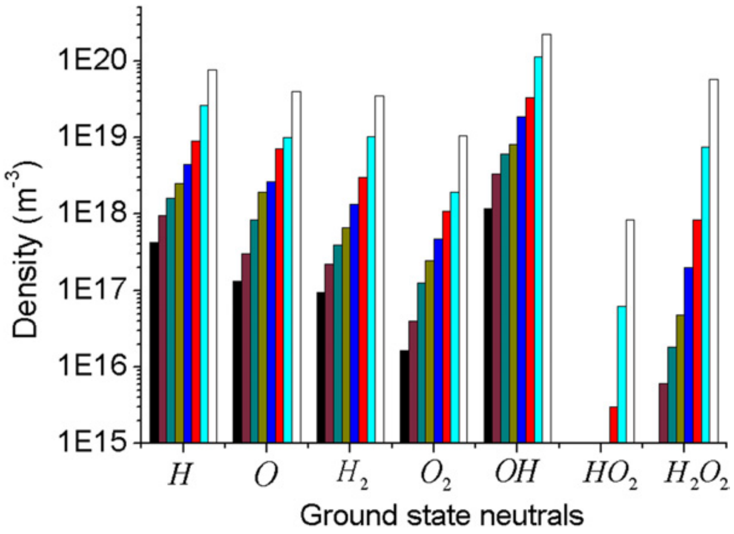
What reactive species in the liquid?

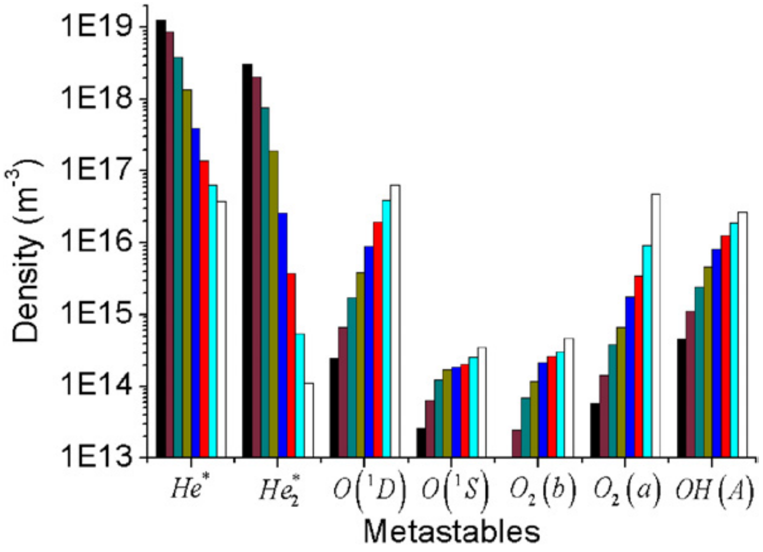
How the reactive species interact with the tissue or cell membranes?

gas-phase simulation

with rate equations

0 D (i.e., global) simulation for He & H_2O

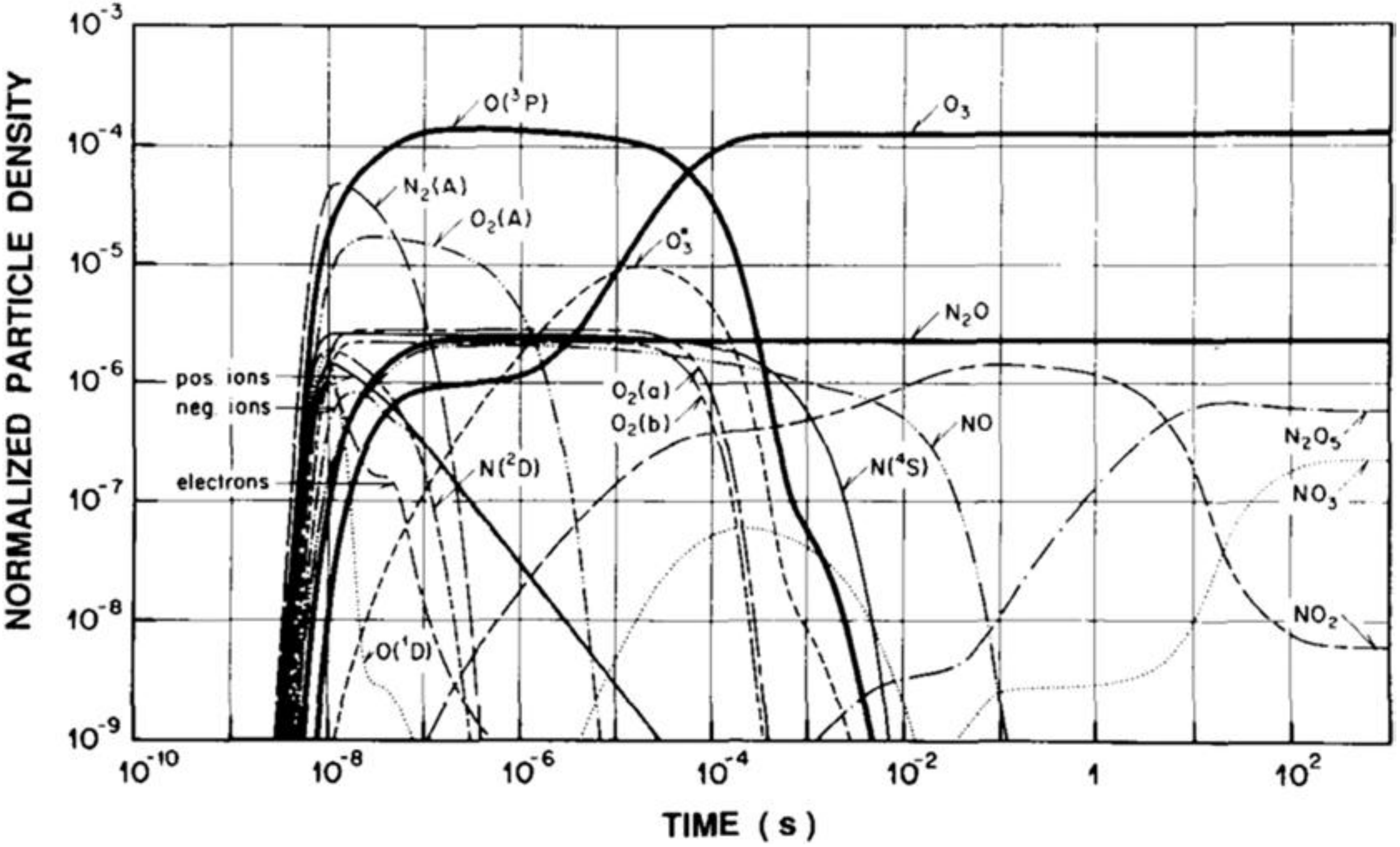




D. X. Liu, P. Bruggeman, F. Iza, M. Z. Rong and M.G. Kong,
Plasma Sources Sci. Technol. 19 (2010) 025018

Reactive species generated in atmospheric-pressure plasmas (simulation : in gas phase)

Eliasson B and Kogelschatz U 1991 IEEE Trans. Plasma Sci.**19** 309



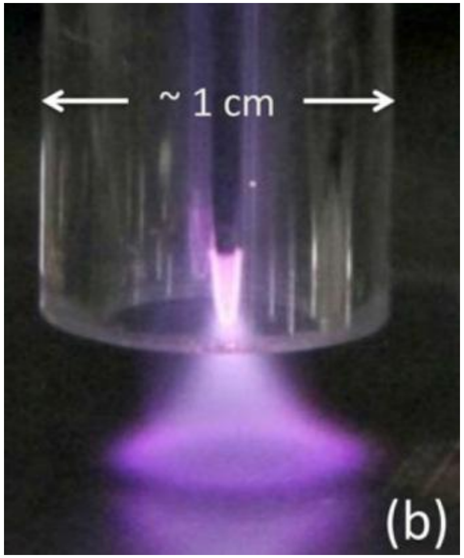
Zero-dimensional numerical simulation of chemical species generated by a microdischarge in a dielectric barrier discharge in air

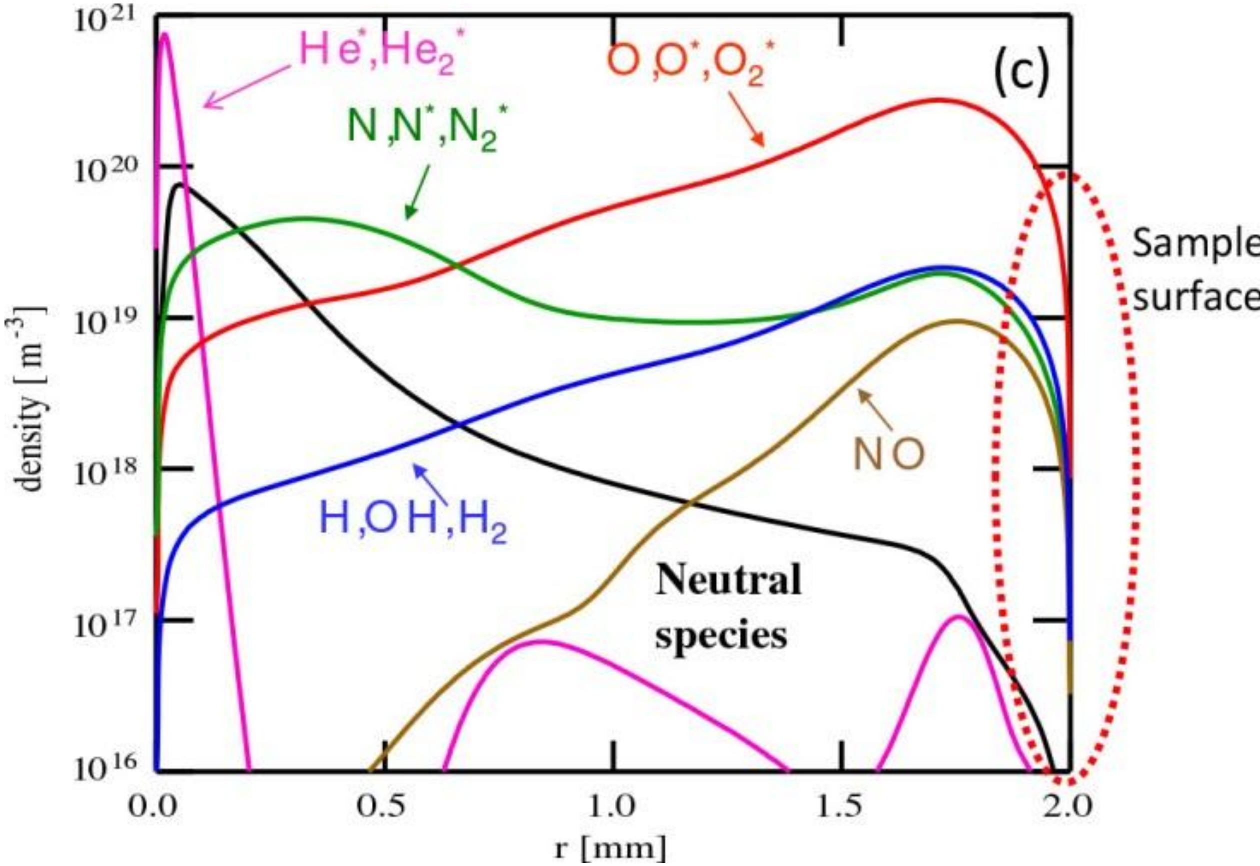
(80% of N2 + 20% of O2, p = 1 atm, T = 300 K).

gas-phase simulation

Rate equations with transport

1D simulation





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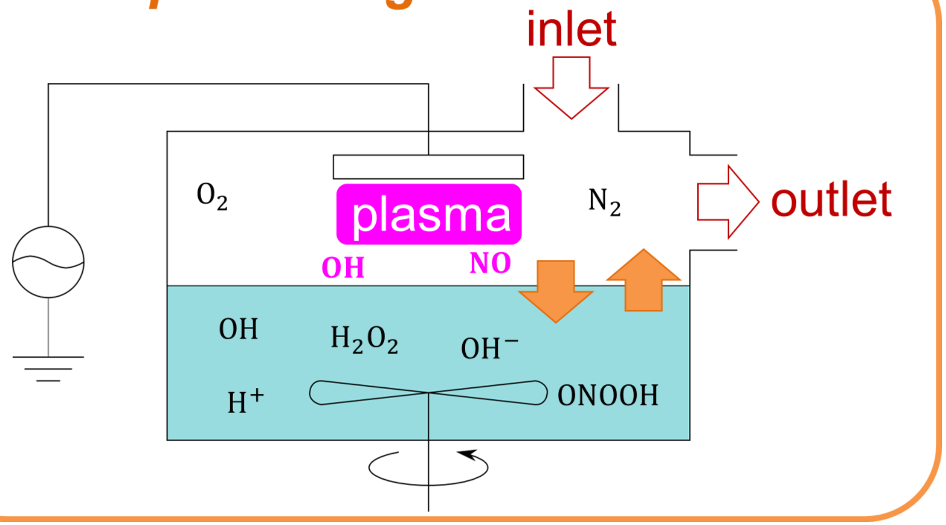
If all gas-phase species – electrons, ions, and neutral (reactive) species – are known, can we predict what species are generated in liquid exposed to the plasma?

Model System

System

- Low temp. Atmospheric Pressure Plasma (APP)
  →provide reactive species
- Pure water (pH=7)

Conceptual Diagram



Computation

- Rate equations／0 D simulation
- No transport (no flow or diffusion) in each phase

(transport of species between the gas and liquid phases)

Henry's law: transport of matters through the gas-liquid interface in equilibrium

Henry's law

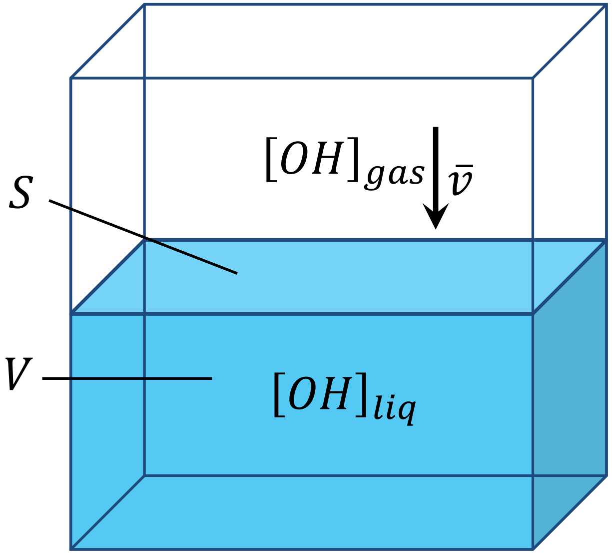
$$[OH]_{liq} = k_H P_{OH} = k_H [OH]_{gas} RT_g$$

Transport of matters through the gas-liquid interface

Henry's needs to be satisfied in equilibrium

$$\frac{d[OH]}{dt} = \underbrace{\frac{S}{V} \cdot \bar{v} \left([OH]_{gas} - \frac{1}{k_H R T_g} [OH]_{liq} \right)}_{\text{transport term}} + Chemical\ Reactions$$

k_H : Henry's constant R : gas constant T_g : gas temp (300K)



Assumptions

- Every species enters the interface at the thermal velocity $\bar{v}$ with no reflection.
- Desorption from the liquid is determined to satisfy the Henry's law in equilibrium

Henry's law

$$[OH]_{liq} = k_H P_{OH}$$
$$= k_H [OH]_{gas} R T_g$$

Rate equations

Chemical Reactions (Global Model)
35 chemical species & 98 rate equations

Rate eqn.:
$$\frac{d[H_2]}{dt} = \cdots + \boxed{k_1}\boxed{[e_{aq}{}^-][e_{aq}{}^-]} + \boxed{k_3}\boxed{[e_{aq}{}^-][H]} + \cdots$$

change of density in time = rate const. × **product of densities** + ···

Reaction Scheme	Rate Constant(at 298K) $[\mathrm{M^{-1}s^{-1}}]$
$e_{aq}{}^- + e_{aq}{}^- \rightarrow H_2 + 2OH^-$	$k_1 = 5.1 \times 10^9$
$e_{aq}{}^- + H^+ \rightarrow H$	$k_2 = 2.4 \times 10^{10}$
$e_{aq}{}^- + H \rightarrow H_2 + OH^-$	$k_3 = 2.5 \times 10^{10}$
$e_{aq}{}^- + OH \rightarrow OH^-$	$k_4 = 3.0 \times 10^{10}$

**NDRL/NIST Solution Kinetics Database on the Web*

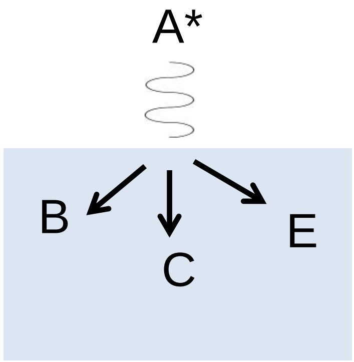
Sample Simulations

To understand what reactive species are generated in liquid by *each* gaseous species

Cases

1. Only OH (hydroxyl) radicals are provided [from the plasma].
2. Only NO (nitric oxide) is provided.
3. Both OH and NO are provides (with nothing else).
4. After OH and NO are provided for 10 sec. and the plasma is turned off.

   → How the reactive species get lost in liquid



Fluxes of OH and NO (typical values from a plasma)

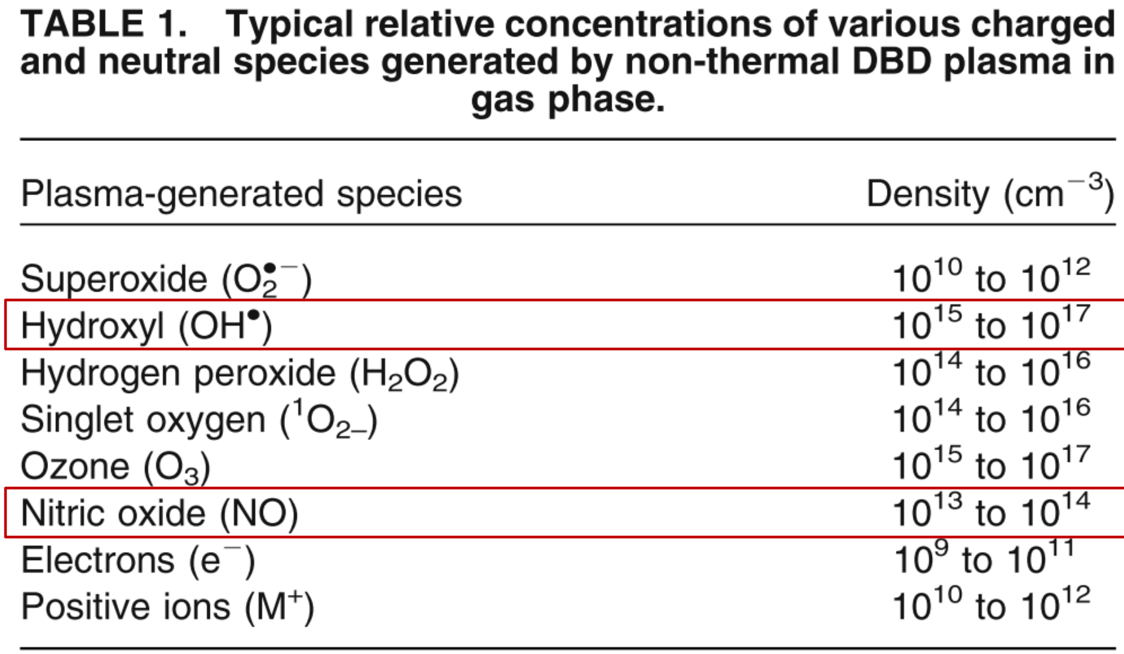
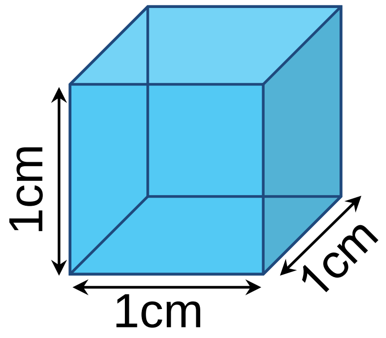


TABLE 1. Typical relative concentrations of various charged and neutral species generated by non-thermal DBD plasma in gas phase.

Plasma-generated species	Density (cm^{-3})	Density (mol L^{-1})
Superoxide ($O_2^{\bullet -}$)	10^{10} to 10^{12}	
Hydroxyl ($OH^{\bullet}$)	10^{15} to 10^{17}	1.66×10^{-6} to 1.66×10^{-4}
Hydrogen peroxide (H_2O_2)	10^{14} to 10^{16}	
Singlet oxygen ($^1O_{2-}$)	10^{14} to 10^{16}	
Ozone (O_3)	10^{15} to 10^{17}	
Nitric oxide (NO)	10^{13} to 10^{14}	1.66×10^{-8} to 1.66×10^{-7}
Electrons (e^-)	10^{9} to 10^{11}	
Positive ions (M^+)	10^{10} to 10^{12}	

**R.Sensenig et al. Annals of Biomedical Engineering 39 (2011) 674-687*

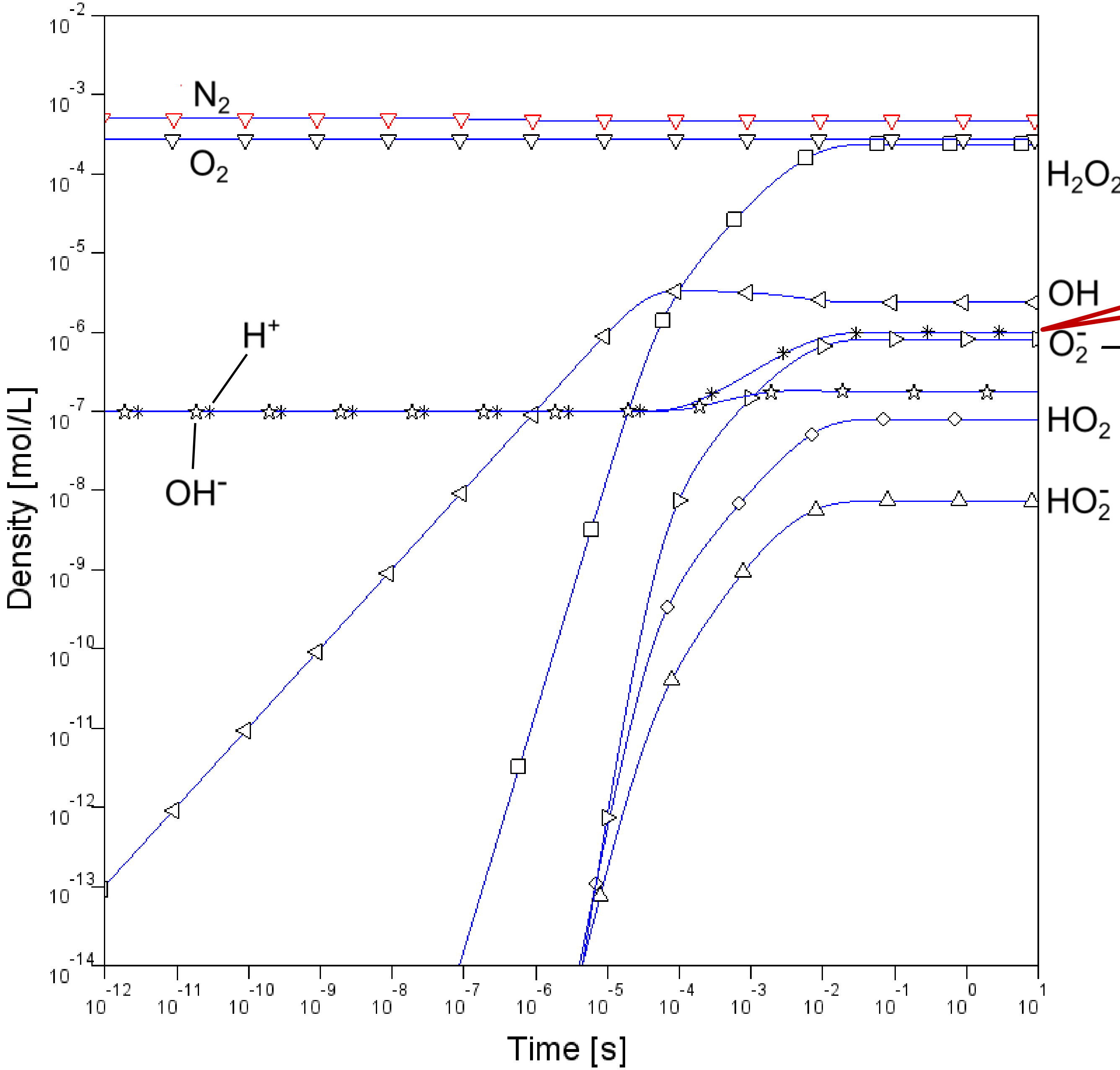


fluxes

$$\frac{S}{V}\cdot\bar{v}_{OH}\cdot[OH]_{gas} = 1.0\times10^{-1}\ mol\cdot L^{-1}\cdot s^{-1}$$

$$\frac{S}{V}\cdot\bar{v}_{NO}\cdot[NO]_{gas} = 7.6\times10^{-4}\ mol\cdot L^{-1}\cdot s^{-1}$$

Case1 : OH only from the gas phase



H_2O_2—*hydrogen peroxide*

OH

O_2^- —*superoxide anion radical*

HO_2 —*hydroperoxyl radical*

HO_2^-

pH=6

OH flux

$$OH$$

$$1.0 \times 10^{-1}\ mol \cdot L^{-1} \cdot s^{-1}$$

dominant reaction

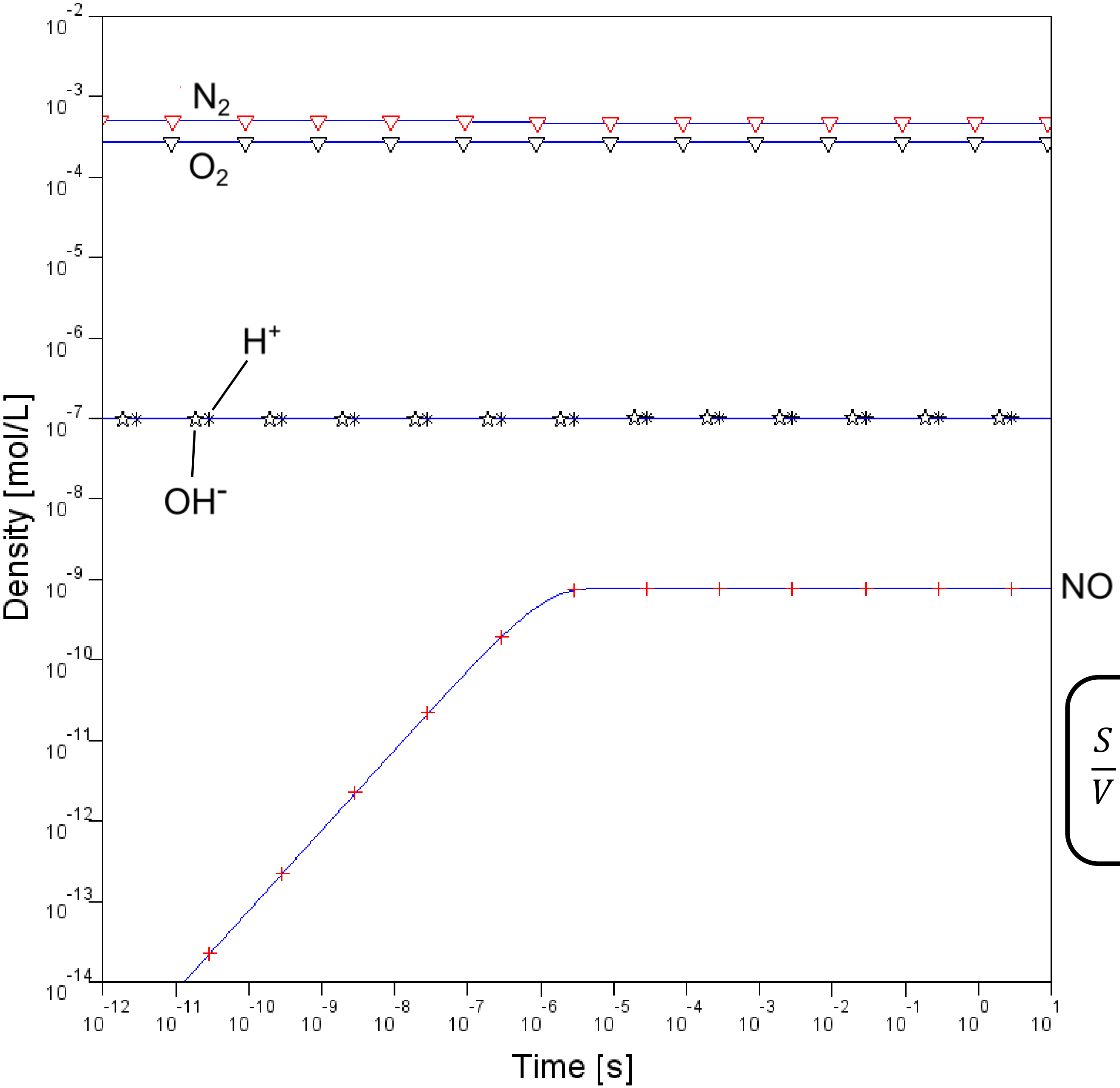
$$OH + OH \rightarrow H_2O_2$$

acid dissociation constant

$$pKa = 4.8$$

$$HO_2 \leftrightarrow O_2^- + H^+$$

NO only from the gas phase



NO flux

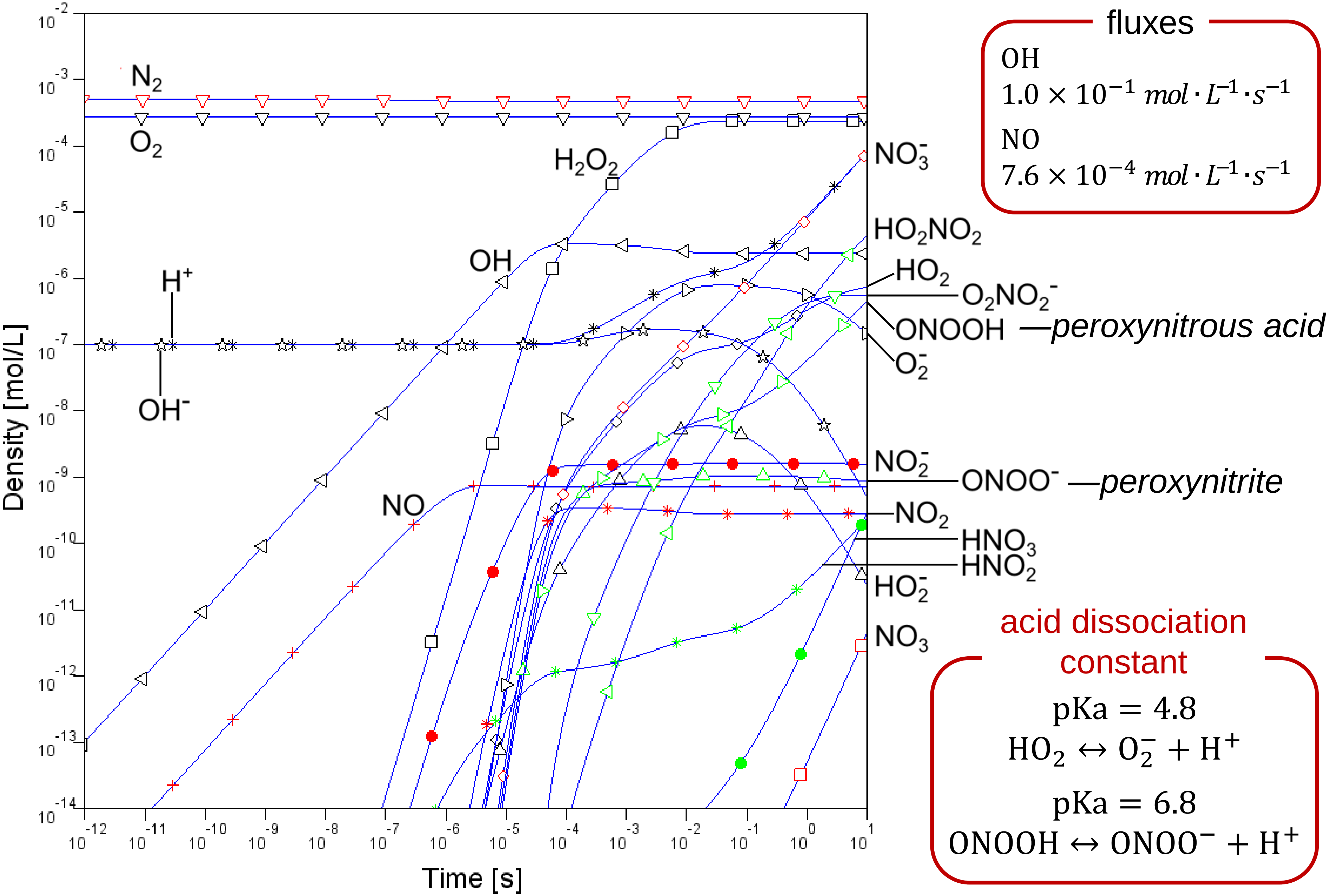
NO

$7.6 \times 10^{-4}\ mol \cdot L^{-1} \cdot s^{-1}$

NO flux from the gas

$$\frac{S}{V} \cdot \bar{v} \left([NO]_{gas} - \frac{1}{k_H R T_g} [NO]_{liq} \right)$$

OH & NO supplied simultaneously for 10s



fluxes

OH

$1.0 \times 10^{-1}\ mol \cdot L^{-1} \cdot s^{-1}$

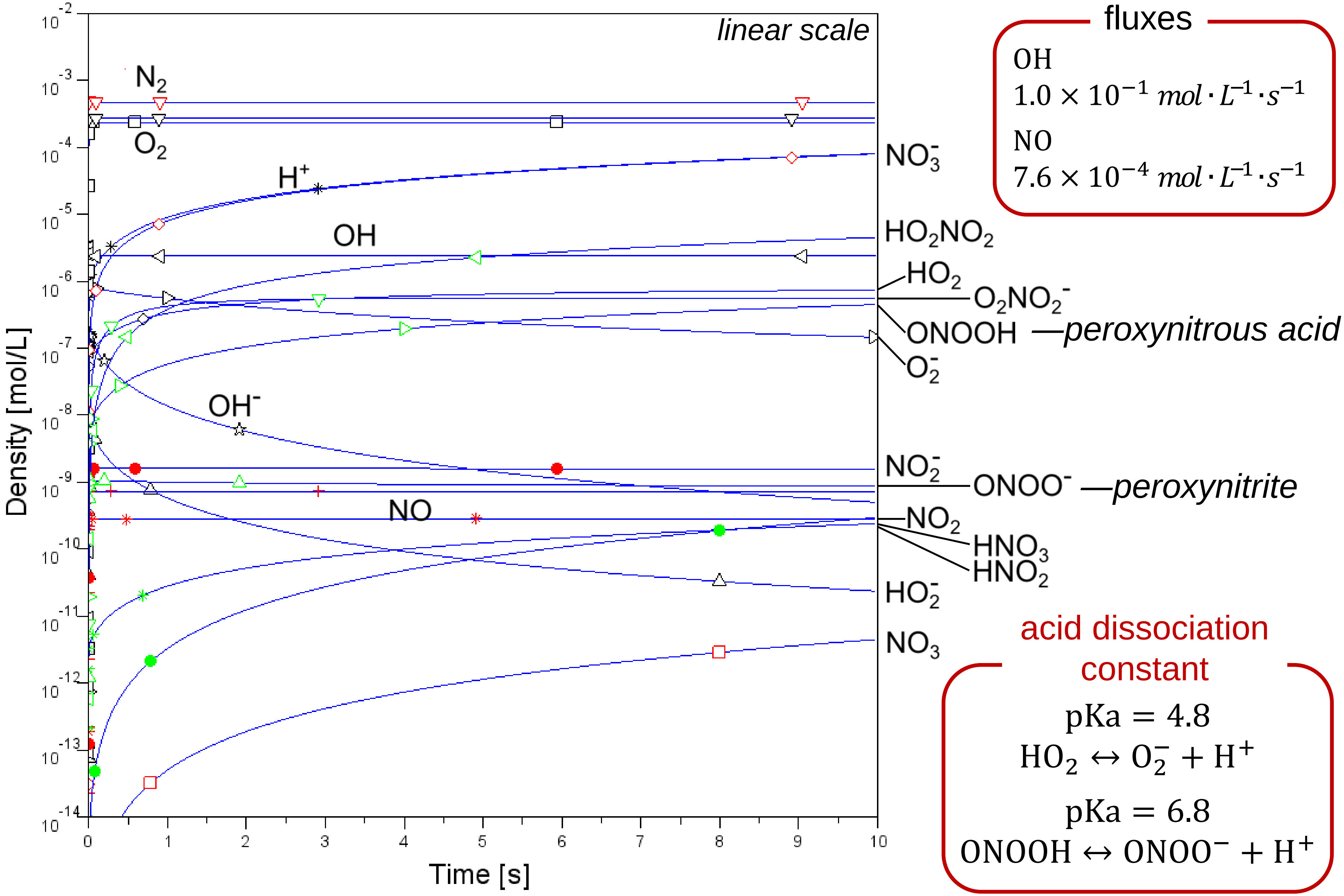
NO

$7.6 \times 10^{-4}\ mol \cdot L^{-1} \cdot s^{-1}$

acid dissociation constant

$$\mathrm{pKa} = 4.8$$
$$\mathrm{HO_2} \leftrightarrow \mathrm{O_2^-} + \mathrm{H^+}$$
$$\mathrm{pKa} = 6.8$$
$$\mathrm{ONOOH} \leftrightarrow \mathrm{ONOO^-} + \mathrm{H^+}$$

OH & NO supply for 10 s (linear time scale)



fluxes

OH

$1.0 \times 10^{-1}\ mol \cdot L^{-1} \cdot s^{-1}$

NO

$7.6 \times 10^{-4}\ mol \cdot L^{-1} \cdot s^{-1}$

acid dissociation constant

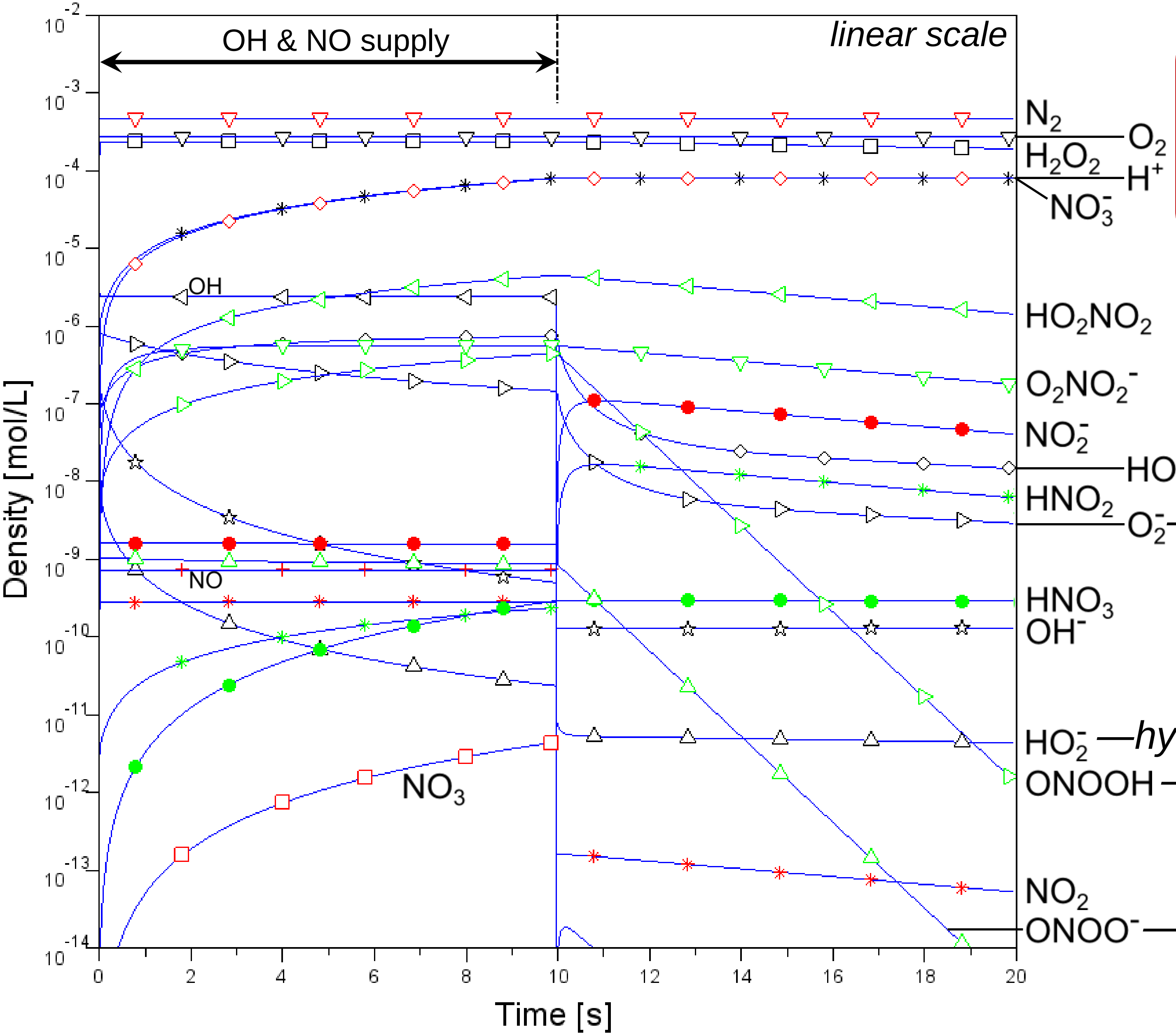
$\mathrm{pKa} = 4.8$

$HO_2 \leftrightarrow O_2^- + H^+$

$\mathrm{pKa} = 6.8$

$ONOOH \leftrightarrow ONOO^- + H^+$

OH&NO supplied for 10s and stopped



fluxes

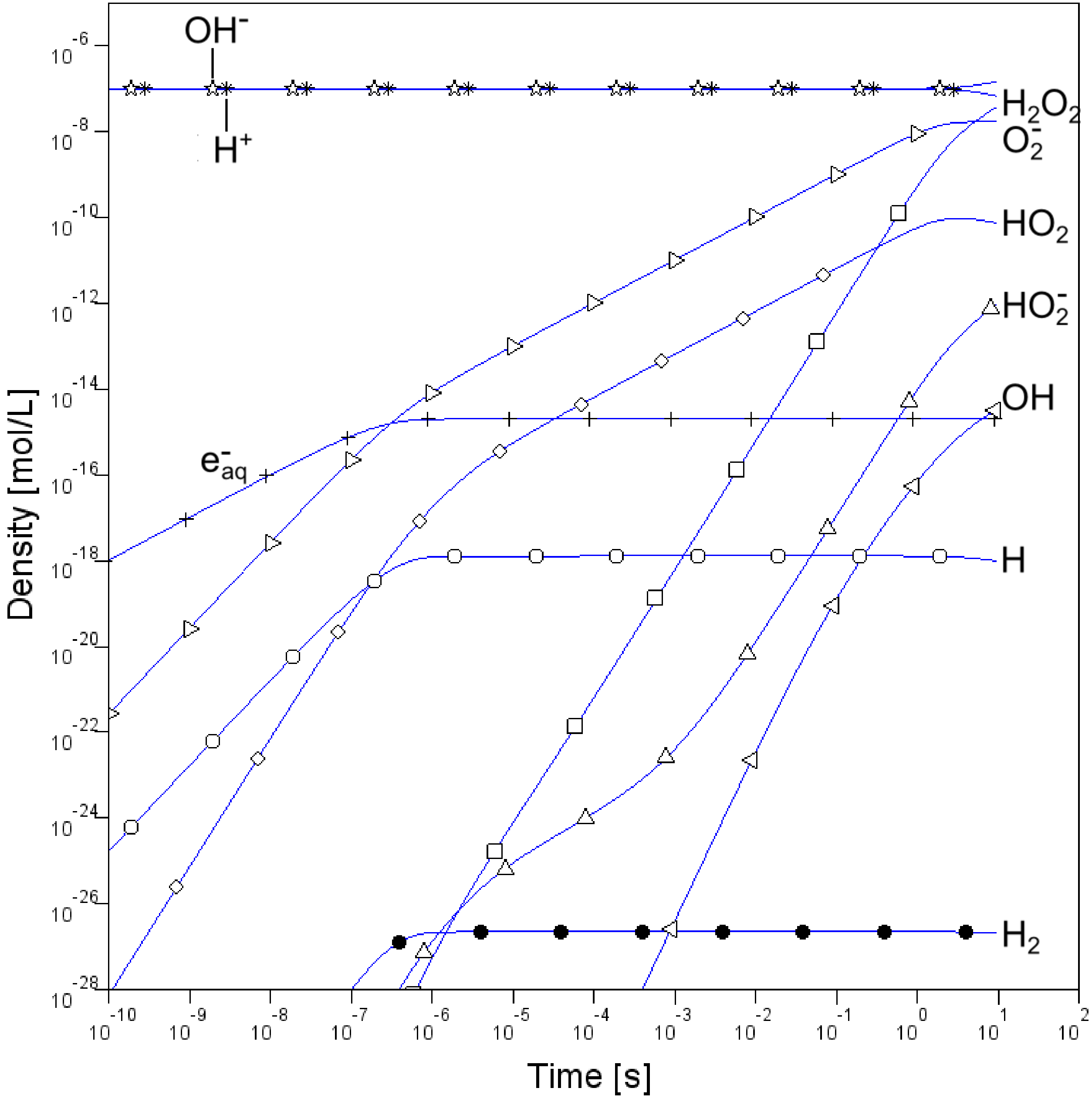
OH

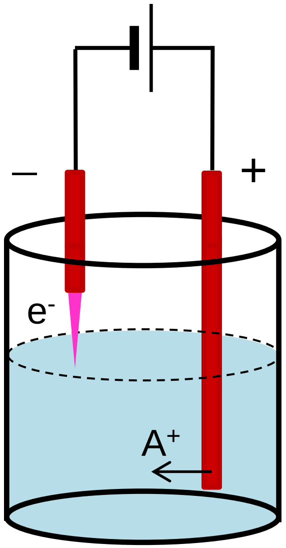
$1.0 \times 10^{-1}\ mol \cdot L^{-1} \cdot s^{-1}$

NO

$7.6 \times 10^{-4}\ mol \cdot L^{-1} \cdot s^{-1}$

1A current only for 100s



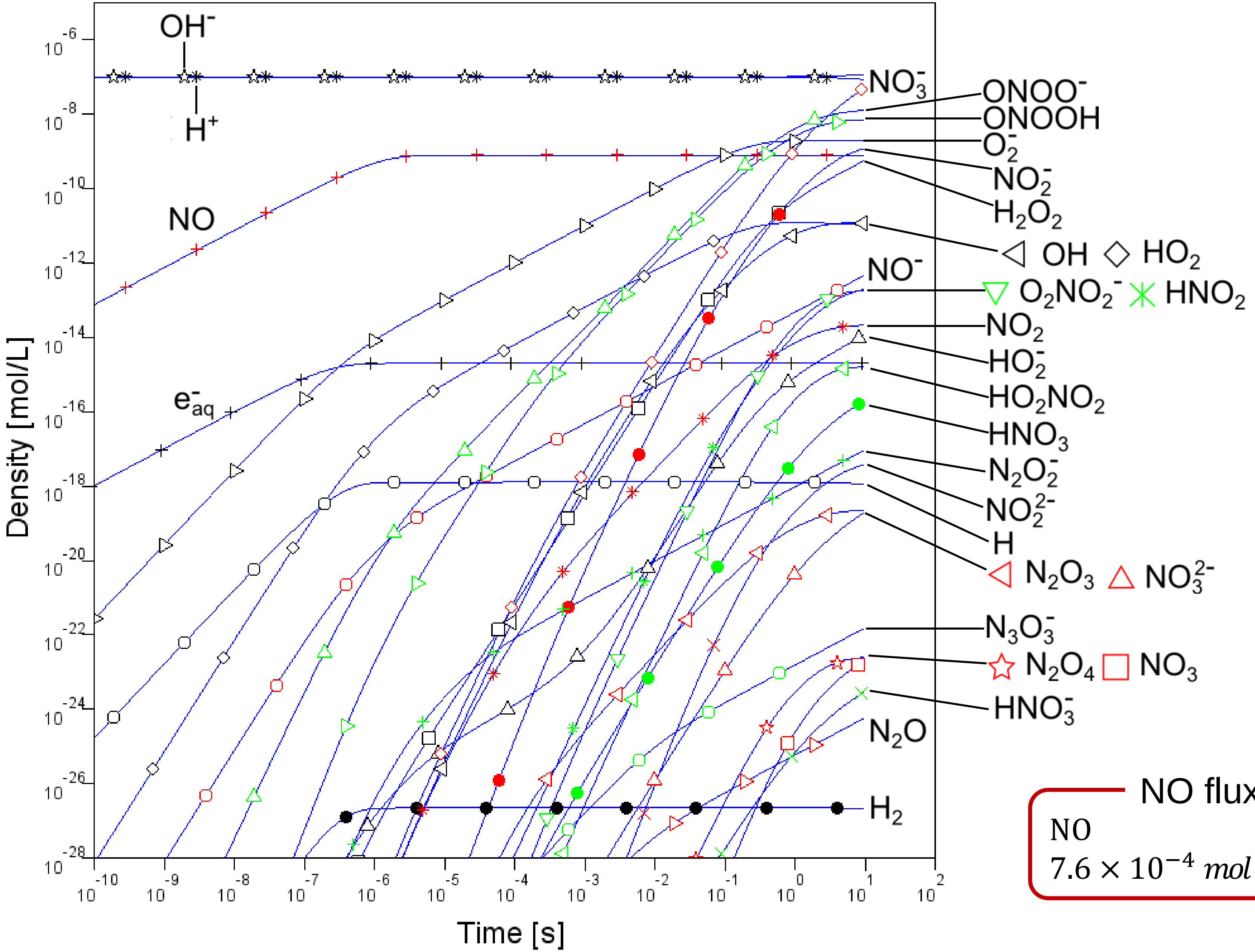


Assume both

$e^- + A^+$

are provided (with A^+ bine non-reactive), so that the charge neutrality of liquid is maintained.

1A current and NO supplied for 100s

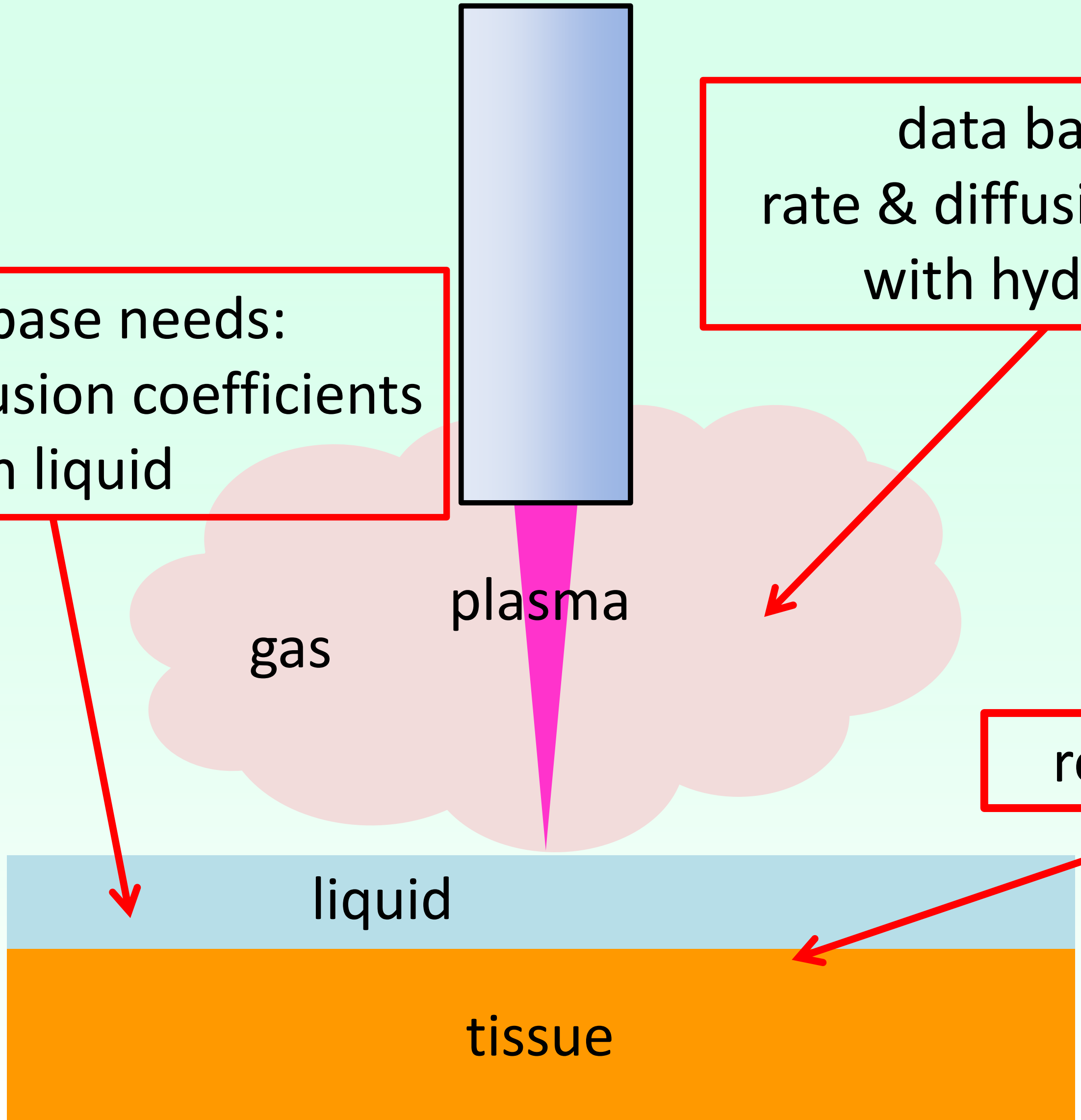


NO flux

NO

$7.6 \times 10^{-4}\ mol \cdot L^{-1} \cdot s^{-1}$

Summary



data base needs:
rate & diffusion coefficients
with hydrogenation

data base needs:
rate & diffusion coefficients
in liquid

realm of biology