

POLYTECHNIQUE MONTRÉAL

affiliée à l'Université de Montréal

**Building and Characterization of Biomedical-Grade Cold Atmospheric Plasma
Jet for Cancer Treatment**

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Département de génie physique

Mémoire présenté en vue de l'obtention du diplôme de *Maîtrise ès sciences appliquées*
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Ce mémoire intitulé :

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Jet for Cancer Treatment**

présenté par **Jean-Baptiste BILLEAU**

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RÉSUMÉ

Le présent mémoire aborde le processus de création et de caractérisation d'une source de plasma pouvant traiter des cellules cancéreuses. L'objectif de cette recherche est d'établir une méthode permettant de lier de manière fiable le processus de traitement plasma et son interaction avec un environnement cellulaire. En supposant qu'une majorité de l'effet biologique du plasma relève des espèces chimiques et de son champ électrique, un jet de plasma à pression atmosphérique a donc été conçu puis caractérisé au niveau thermique, électrique et chimique. Afin de s'assurer d'une utilisation sécuritaire de la source avec un individu, les normes de courant de fuite sur un patient émis par la Commission International Electrotechnique ont été suivies. Avec un courant de fuite limité à moins de $150\ \mu\text{A}$ notamment conditionné par des choix de conceptions tel que la mise en place d'une électrode de mise à terre en forme hélicoïdale, un flux de 2.5 litres par minute et un pourcentage d'opération fixé à 40 %, il est possible d'interagir avec un tissu sans créer des dommages. Similairement, une température biocompatible de $35\ ^\circ\text{C}$ fut caractérisée par comparaison entre trois méthodes différentes: la mesure par thermocouple, la mesure par imagerie Schlieren et par une technique utilisant une fibre ayant un réseau de Bragg exposé directement au jet de plasma. Cette dernière exploite notamment l'effet thermo-optique afin de convertir le décalage de lumière réfléchi dans le réseau en valeurs thermiques. Ces mesures permettent d'obtenir une méthode fiable d'analyse temporelle et spatiale de l'évolution de la température du gaz de sortie du plasma. De manière à comprendre plus en profondeur les principes fondamentaux du processus chimique et électrique au sein du plasma, une technique de mesure de champ électrique appelée Seconde Harmonique Induite par Champ Électrique (E-FISH) fut employée. Cette technique utilise deux faisceaux lasers se croisant et amplifiés par interférométrie par un troisième faisceau communément appelé oscillateur local. Cette technique permet entre autres d'obtenir une précision spatiale et directionnelle de la polarisation du champ électrique encore inégalée, ce qui ouvre la voie vers de nouvelles études de l'effet de la polarisation du champ électrique sur les propriétés physico-chimiques du plasma. Afin de lier l'ensemble de ces expériences avec des effets biologiques, l'aspect chimique de l'interaction entre le plasma et des modèles d'hydrogel servant d'expérience *in vitro* a été effectué. Plus précisément, le pH, étudié par la décroissance d'un fluorophore, soit la fluorescéine, fut examiné spatialement. Cette étude permet de cibler les zones où les espèces réactives sensibles au pH peuvent proliférer tout en observant son effet au niveau cellulaire. Pour des espèces chimiques de longues vies telles que le nitrite et le nitrate, des méthodes de colorimétrie utilisant un réactif chimique, appelé réactif de Griess, fut utilisé. De manière similaire à la mesure de pH, le nitrite fut également

étudié au niveau spatial afin de comprendre le processus de propagation de ce dernier dans les modèles de tissus. L'ensemble de cette recherche permettra d'établir un lien fiable et reproductible entre la physique des plasmas froids et l'étude de la réponse cellulaire des tissus cancéreux.

ABSTRACT

Therapeutic properties of cold plasma have been the subject of many successful studies in cancer research over the last two decades. Innovative designs, such as that of cold atmospheric pressure plasma jets, have enabled local and non-invasive treatment that is able to inhibit cancer growth and trigger cancer cell death. Despite large knowledge of the reactive species production pathways from plasma source to biological material, comprehensive models to translate plasma parameters into biological response are still to be fully developed. Fundamental for this, always, is a well characterized plasma source and biological systems. This thesis addresses the first part of this statement; the design and characterization process of a plasma source able to treat cancer cells. Through the research objectives, I aim to establish a reliable method for linking the plasma treatment process to its interaction with a cellular environment. Assuming that the biological effect of the plasma mainly involves chemical species and electric fields, in this work, an atmospheric pressure plasma jet was designed and the plasma was characterized in its thermal, electrical and chemical properties. To ensure safe use of the source of an individual, leakage current standards on a patient issued by the International Electrotechnical Commission were followed. With a leakage current limited to less than 150 μA , achieved through design choices such as the implementation of a helical ground electrode, a flow rate of 2.5 litres per minute and a duty cycle set at 40 %, plasma jet interaction with tissues without causing damage was assured. The first and basic characterization of the gas temperature plasma jet was performed by comparing three different methods: measurement with a thermocouple, Schlieren imaging and a technique involving a fibre with Bragg grating directly exposed to the plasma jet. The latter exploits the thermo-optic effect to convert the shift in reflected light in the grating into thermal values. These measurements provide a reliable method for the temporal and spatial analysis of the plasma gas temperature evolution and demonstrated the plasma jet's tissue tolerable operation regime of below 35 °C. To gain a deeper understanding of the fundamentals of the chemical and electrical processes within the plasma, an electric field measurement technique called Electric-Field Induced Second Harmonic (E-FISH) light generation was employed. A major achievement of this work is the development of a novel E-FISH technique. This technique uses two intersecting laser beams and is amplified by interferometry of second harmonic light generated from the intersecting beams with a third, external, beam at the second harmonic frequency, referred to as the local oscillator. This technique allows, among other things, for unprecedented spatial and directional precision of the electric field polarization, opening the way for new studies on the effect of electric field polarization on physicochemical

properties of the plasma. Because the amplification process is linear with the electric field – and not quadratic, as is the case for regular E-FISH – the polarisation of the electric field can be determined. In this thesis, for the first time, electric field polarity was measured by second harmonic light generation. To link all these experiments with biological effects, the chemical species produced from the interaction between plasma and simple biophysical model tissues consisting of hydrogel serving as *in vitro* experiments was performed. These experiments will be linked to ongoing experiments in 3D-bioprinted model tissues as part of a parallel ongoing PhD thesis. Specifically, pH was examined spatially through the decay of a fluorophore, called fluorescein. This study allows targeting areas where pH-sensitive reactive species can be catalyzed or inhibited while observing its effect at the cellular level. For long-lived chemical species such as nitrite and nitrate, colorimetric methods using a chemical reagent called Griess reagent was used. Similar to pH measurement, nitrite was also studied spatially to understand its propagation process in tissue models. This research will ultimately help to establish a reliable and reproducible link between the physics of cold plasma and the study of the cellular response of cancerous tissues.

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LIST OF SYMBOLS AND ACRONYMS

a	Cross-section perpendicular to the heat flow
A	First experimental coefficient of the Paschen curve
ACE-FISH	Amplified Crossed beam Electric-Field Induced Second Harmonic
α	First Townsend coefficient
ATP	Adenosine Triphosphate
B	Second experimental coefficient of the Paschen curve
c	Schlieren signal
$\hat{c}$	Abel inverse of the Schlieren signal
CAP-Jet	Cold Atmospheric Pressure Plasma Jet
C_e	Thermal inertia
χ_i	Permeability of species i
$\chi^{(3)}_{i,j,k,l}$	Nonlinear Susceptibility
CMOS	Complementary Metal-Oxide Semiconductor
CNC	Computer Numerical Control
c_p	Heat capacity
D	Electric displacement field
d	Distance between electrodes
DBD	Dielectric Barrier Discharge
DC	Direct Current
Δ_t	Inverse count rate
DNA	Deoxyribonucleic Acid
E	Electric field
E_e	Energy of electron
EEDF	Electron Energy Distribution Function
E-FISH	Electric-Field Induced Second Harmonic
ϵ_0	Permittivity of free space
$\epsilon_{r,i}$	Relative permittivity of species i
f	Pulse repetition rate
F	Factor for change in air mole fractions
$f(E_e)$	Function distribution of electron energy
FBG	Fibre Bragg Grating
γ	Second Townsend coefficient
GUI	Graphic User Interface

I	Patient leakage current
ICD	Immunogenic Cell Death
IEC	International Electrotechnical Commission
ID	Outer Diameter
I_{RMS}	Root mean squared valued of current
k	Heat conductivity
k_x	Reactive rate of species x
λ	Laser wavelength
λ_D	Debye length
L	Length perpendicular to the heat flow
Laser	Light Amplification by Stimulated Emission Radiation
m_e	Mass of electron
n_0	Refractive index of air
n	Refractive index
N	Density of species in a gas
n_A	Density of particle A
n_e	Electron density
N_d	Number density of the gas
N_r	Density of species r
OCT	Optical Coherent Tomography
OD	Outer Diameter
ω	Angular frequency
oxPTMs	Oxidative post-translational modifications
p	Pressure
$P_{average}$	Average electrical power
P_{in}	Input heat power
$P_i^{2\omega}$	Induced polarization at frequency 2ω
PPSL	Plasma Physics and Spectroscopy Lab
q_e	Electron charge
R_2	Resistor 2 in patient leakage current
R_e	Thermal resistance
ρ_v	Space charge density
RONs	Reactive Oxygen and Nitrogen Species
ROS	Reactive Oxygen Species
RF	Radio Frequency
S	Signal of the contrast

$S_{ACE-FISH}$	ACE-FISH signal
$\sigma_x(E_e)$	Electron collision cross-section
SLM	Standard Litres per Minute
SNR	Signal-to-Noise Ratio
T	Temperature
T_0	Temperature of reference
T_c	Cooling temperature
T_e	Electron temperature
T_f	Temperature final
T_h	Heating temperature
θ	Angle of the scalar product
t_{int}	Integration time of a single pulse
τ	Characteristic time
TTL	Transistor-Transistor Logic
V	Applied Voltage
V_b	Voltage Breakdown
$V(t)$	Voltage with a temporal variation
V_{RMS}	Root mean squared valued of voltage
w_0	Minimum beam waist
z_R	Rayleigh length

CHAPTER 1 INTRODUCTION

More than ever, cancer therapy has become a critical field in medicine. The year 2020 alone saw 19.3 million individuals diagnosed with cancer, while an alarming 10 million cancer patients succumbed to this devastating disease, solidifying its rank as the second most lethal global cause of death [28]. While current therapies are mainly based on surgery, chemotherapy, and radiotherapy [29], these treatments are usually characterized by their severe side effect on patients. Finding a cure for cancer as well as putting emphasis on the recovery phase of the treatment is thus imperative. Plasma medicine, the use of cold physical plasma for medical therapy, offers new pathways for adjuvant or complimentary therapies. Yet, many of the processes underlying plasma medicine are still unknown. This is where this project aims to make a difference; opening a new avenue for an adaptive, less invasive, and efficient cancer treatment based on a controlled and well characterized plasma source.

Plasma used in medical therapy, not to be confused with the plasma in blood, can be described as a quasi-neutral ionized gas exhibiting a collective behaviour. To generate plasma, high energy input into a gas induces separation of charges from atoms resulting in a gas mixture of ions, electrons, neutrals, excited species and metastable species. In order to achieve biocompatible conditions, the plasma is put into a state of non-thermodynamic equilibrium. This term refers to the average temperature of ions and other massive particles transporting heat having temperatures averaging room temperature while the electrons still maintain high thermal energy, providing the basis for plasma chemistry. This so-called cold plasma is used to achieve highly reactive chemistry at low thermal conditions, reducing the risks of thermal damages in tissues. More precisely, the key of inducing biological responses have been linked to the production of highly reactive species of oxygen and nitrogen (RONS), electrical field, ultraviolet light and even heat [30]. Use of plasma in medicine improves wound healing performance of the body, inhibits and prevents infection by killing bacteria, and controls tumour spreading [25, 31, 32].

For this thesis, I will focus my interest in the construction and characterization of a biomedical type of plasma sources, called plasma jet, that could be used in clinical application. To be more precise, the ultimate goal is to support the surgical removal of bone metastasis by applying plasma treatment to the surgical site afterwards. With this approach, we would be able to reduce the risk of cancer recurrence and minimize the extent of invasiveness associated with the other aforementioned procedures. This thesis focuses on the plasma side of things, on the design of a biocompatible plasma jet, its characterization in its electric field and

reactive chemistry induced in model tissue systems.

1.1 Problematics

The problematics addressed in this research lies in understanding the complex nature of plasma and the need for precise control over its chemistry. Obtaining the right mixture of reactive species is essential for targeting a specific biological response. The challenge here can be described as followed: **Achieving high chemical output precision in plasma necessitates highly accurate diagnostics.** The issue is threefold.

- 1) The diagnostics must ensure the safe use of a cold atmospheric pressure plasma jet (CAP-Jet) source, preventing inadvertent thermal and electrical damage to biological specimens or surroundings.
- 2) Due to the relation between the propagation of fast ionization waves through a medium and the production of reactive species [33], emphasis on electric field control (see Figure 1.1 [1–4]) is critical. In the context of argon-based plasma jets, plasma typically manifests as small, filamentary discharges propagating at multiple 10s of thousands m/s that can produce intense bursts of RONS. These conditions make high spatial and temporal resolution diagnostic techniques necessary.
- 3) Dosage control of the RONS is imperative. While, inadequate or imprecise diagnostics can jeopardize both the safety and efficacy of the plasma treatment, knowledge over the species distribution is paramount for establishing a reliable treatment method.

Therefore, establishing a plasma source with precise control and characterization of electrical, thermal, and chemical parameters becomes crucial. In summary, all these challenges can be regroup in the following research question: **How can we build a fully tailored plasma source suitable for clinical applications of cancer treatment and ensure its maximum treatment efficiency?**

1.2 Research Objectives

To answer my research questions, three different objectives have been established:

1. Build and certify the safety of a biological-grade plasma source

The first step of this project is to design and build a plasma source that is stable, that can create reproducible results, and that respects thermal and electrical safety requirements for biological interaction. The plasma set-up should be able to be sterilized

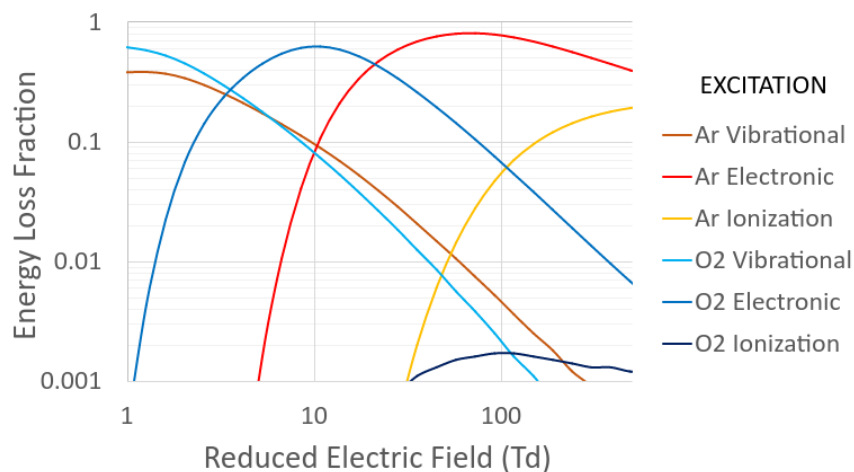


Figure 1.1 Energy loss fraction of different excitation mode in an argon plasma (99%) mixed with oxygen (1 %) as a function of the reduced electric field. This figure highlights the intricate relation between electric field and its ability to inhibit certain reaction pathways [1–4]

and compact enough to work in a laminar flow hood for *in vitro* experiment on cancer cells. Finally, the setup should be simple enough for non-plasma experts to use it safely and efficiently for long treatment times.

2. Design an ultrafast laser experiment that allows to measure accurately amplitude and polarization sensitive measurements of the electric-field of the plasma source

The second objective is aimed toward characterizing the plasma electric-field via improving the E-FISH technique. The setup must be sensitive enough to accurately image spatially and temporally the plasma electrical dynamics. The approach should also be able to detect directionality change of the electric field inside a plasma source.

3. Create reliable tools for reactive species analysis of plasma-hydrogel interaction

This third objective aims to create and use the necessary tools to get information on the concentration of long lived species present in the hydrogel after a plasma treatment. An experiment was designed to be able to perform spatially-resolved measurements of reactive species to investigate the propagation of reactive species and establish reliable method of treatments.

With these objectives, I will be able to build a reliable biomedical plasma source and outline some of its most important inner process for the production of highly reactive species. The

tools developed in this research could then be coupled with a biological study to create a complete picture of the plasma interaction processes.

1.3 Thesis Outline

This thesis is organized in six distinct sections.

Chapter 2 presents a literature review in two parts, the first part provides an overview on the basis of cold plasma medicine and the second part focuses on the many types of biomedical-grade plasma sources used throughout plasma medicine history.

Chapter 3 discusses the details of the experimental procedures and the important steps to assess the safety of plasma medical instruments, ultimately leading to the construction of a plasma jet.

Chapter 4, sets emphasis on temperature characterization, which will directly be linked to the previous chapter in terms of thermal safety.

Chapter 5 shifts the focus towards the diagnostic aspects of the electric field and will elaborate on the applicability and improvement of the diagnostic technique.

Chapter 6 provides a chemical analysis of the designed plasma source when interacting with hydrogel models, completing the basic characterization of the plasma source.

Chapter 7 will address the main outcome of this thesis and its implication in the plasma medicine domain.

The thesis final chapter is comprised of conclusion and discussion that, Chapter 8, summarizes the project and points out its current limitations and the resulting next steps.

CHAPTER 2 LITERATURE REVIEW

Over the years, various types of electrical-based sources have been developed to serve as tools for healing. This section will provide an overview of plasma medicine in general, while also emphasizing the evolution of the technology over time. Understanding the historical development of plasma sources in medicine will help to underscore the engineering challenges and the incredible improvements made over the years leading to the development of some modern plasma sources we know and love.

2.1 Part I: Fundamental Notions of Plasma Medicine

First tests with cold plasma in medical environment were made in 1969 for surface treatment of blood compatible and adhesive materials [34]. From then until the present day, numerous discoveries were made in the domain. In this section, I will highlight some of the most relevant plasma properties in biological applications.

2.1.1 Basic Concepts

The term "plasma," originating from a Greek root meaning "moldable substance" or "jelly," [35] was first coined by the physicist Irvin Langmuir to describe a "region containing balanced charges of ions and electrons" [36]. It is worth noting that while the choice of words could correctly describe this entity, the term was already associated with the liquid component that contains white and red blood cells, where Langmuir was inspired from. This overlap in terminology potentially leads to confusion, particularly when employing "plasma" in the context of biomedical applications¹.

Over the years, the definition was refined as a quasi-neutral gas in which a significant input of energy has led to the dissociation of charges, resulting in its overall behaviour closely resembling that of a fluid [37]. The latter property was an important improvement to the previous definition as it put emphasis on the fact that plasma must be defined by more than just a degree of ionization. For instance, while a fire can be considered highly ionized due to its energetic exothermic reactions, it exhibits little to no collective behaviour. A more fitting example of plasma would be phenomena such as lightning, aurora borealis and even stars.

¹In this thesis the word "plasma" will be kept exclusively to plasma studied in physics; it will be stated when I am referring to blood plasma

Cold vs. Thermal Plasma

Fundamentally, plasma exists in multiple states, either at thermodynamic equilibrium or at thermal non-equilibrium. The difference between the two lies in the energy transmitted by the plasma as a form of heat. Equilibrium plasma, also simply qualified as hot, maintains equally high temperature among its massive particles, such as ions and neutrals, as well as the electron. However, for non-equilibrium plasma or cold plasma, the heavy particles remain at temperatures as low as or lower than room temperature in contrast to the electrons, which can reach temperatures as high as $10^3 \text{ K} - 10^5 \text{ K}$ [38]. This unique property serves as the cornerstone of plasma medicine as it enables direct interaction between plasma and tissue.

Breakdown Mechanism of Electrical Discharge

Electrical discharges usually starts as an electron (created from photoionization or through collision of recombining ions) inducing an ionization avalanche effect on other electrons through acceleration induced by the electric field. The overall shape, luminescence, and power dissipated into a discharge rely on many components: the electric field accelerating the charges, the type of gas where the discharge occurs, the frequency, the shape of the electrodes and the applied pressure [39]. As figure 2.1 can show, for various voltage and current applied, different kinds of discharges become apparent [5].

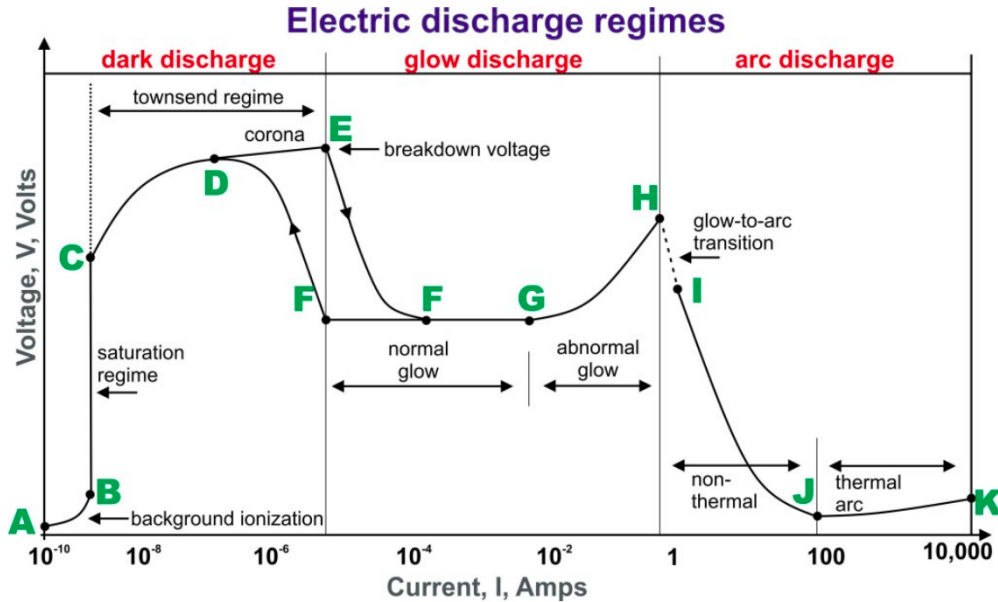


Figure 2.1 Multiple types or discharge regime as a function of applied voltage and current taken from [5].

Usually, to create an ionization wave for the plasma to propagate, an electron avalanche, or also called Townsend's discharge, is produced before transitioning to other states such as glow discharge [40]. For a discharge to be self-sustained, one criterion should be respected [41]:

$$1 - \gamma(e^{\alpha d} - 1) = 0 \quad (2.1)$$

where α is Townsend's first ionization coefficient, γ Townsend's second ionization coefficient and d the distance between the electrodes. This criterion essentially describes the minimum rate at which: 1) the electron should ionize neutrals and ions to create an avalanche effect and 2) where the ionized particle should ionize other neutrals and ions to sustain the avalanche effect.

For plasma using direct current DC or radio frequency RF, the information on a discharge is crucial. A more continuous current will enable high density of electrons, which will be able to claim a high current leading to possible arcing effects. To prevent these effects, especially in medical applications, RF is used. The transition to the thermal and high current regime are then limited by their transition time due to a fast alternative or short pulsed variation of the current [42]. For medical purpose, we aim to minimize the current while maximizing the chemical efficiency as an output. The Townsend or glow discharge regime is thus the regimes usually preferred.

Under Pressure

Another important parameter in electrical discharge comes at the relation between the breakdown voltage, the pressure and the interelectrode distance. While Townsend discharges predict the transition from gas to discharge, Paschen's law generalizes the conditions when the breakdown can happen. In general, the law is able to get a rough image on the collisional behaviour of electrons. It describes the competing aspect between ionizing and non-ionizing collision [43] approximated by [44]:

$$V_b = \frac{Bpd}{\ln(Apd) - \ln(\ln(1 + \frac{1}{\gamma}))} \quad (2.2)$$

where the breakdown voltage V_b is linked to the pressure p , the distance between the electrode d and A and B are experimental values approximated to be constant for a specific gas.

As seen in Figure 2.2, for high pressure or long electrode distance, the distance of interaction, called Debye length, is too short for the electrons to gain enough energy to ionize. Similarly, in low pressure conditions where the Debye length is much longer, the amount of ionization

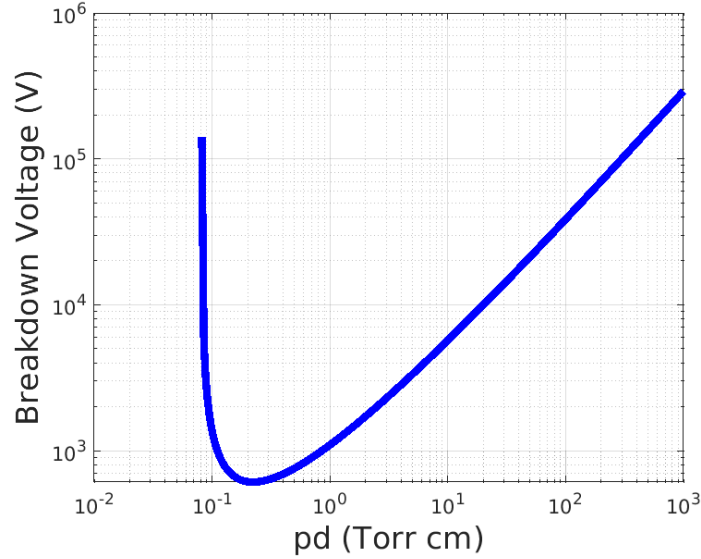


Figure 2.2 Example of a Pachen curve in Air. The data has been taken from [6]

collision is too small to sustain a chain reaction for an electrical discharge to occur. In both situations, to cope with the inability to ionize, the breakdown voltage must be higher. To estimate the Debye length, one can use the following relation:

$$\lambda_D = \sqrt{\frac{\epsilon_0 T_e}{n_e q_e^2}} \quad (2.3)$$

where the Debye length λ_D is related to the permittivity of free space ϵ_0 , the electron temperature T_e , the electron density n_e and the electron charge q_e .

Another particularity of the Debye length is the Debye shielding. In essence, the Debye shielding is the core property that enables the inherent collective behaviour of plasma; it is a direct consequence of the Debye length effect. By definition, the quasi-neutrality of plasma stipulates that plasma must have similar amount of ions and electrons. However, the term "quasi" means that locally, the charge distribution might not be uniform. When interacting with an external ion for example, the overall distribution of the charges inside the plasma will change due to the electrons attracted to it and the ions repulsed by it. The phenomenon will thus create a screening effect also qualified as Debye shielding protecting from electrostatic fields within the range of the Debye length [45]. For medical devices using atmospheric pressure, the Debye length is usually in the range of μm to tens of nm [46], meaning that the atmospheric pressure plasma will take shapes much smaller than their counterparts in low pressure. For argon CAP-Jet, the shape of the plasma can be characterized as filamentary

due to its small elongated form.

Plasma Sheath

First observed by Irvin Langmuir in 1923, the plasma sheath was characterized as a thin region forming around the plasma when interacting with a solid interface [47]. More precisely, a plasma sheath can be described as a depletion zone of charges where the electrons are absorbed quicker than the ions due to their lower mass. This behaviour creates a potential difference slowing down electrons and accelerating ions [48, 49]. For applications such as biological treatment where the interaction between a plasma and a liquid layer is almost ubiquitous, the notion of a plasma sheath is very important. Knowing that the surface interacting with the plasma and the sheath itself has its own set of chemical agents than the plasma or liquid itself, modification of surface charge will directly interact with its properties. This region also becomes highly important in direct treatment through mechanisms such as electroporation or iontophoresis [50].

2.1.2 Reactive Species and Where to Find Them

In plasma cancer treatments applications, one of the main mechanisms inducing selective programmed cell death, or apoptosis, is directly linked to the production of RONS [30]. As seen in 2.3 and as for the majority of plasma medical devices, the use of plasma in open-air environment enables production of various RONS, from short-lived to long-lived species.

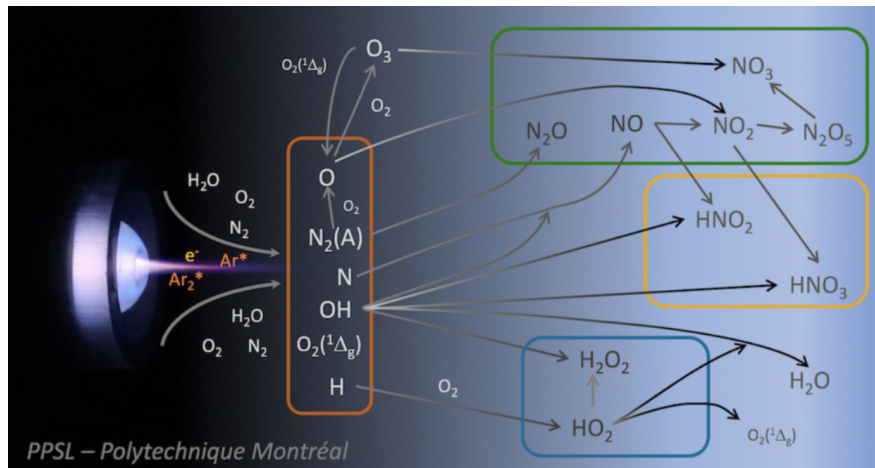


Figure 2.3 Chemical pathway production of an argon plasma jet in air [7]

In essence, the plasma creates its chemistry by first exciting the electrons through an electrical field. Depending on the intensity of the electric field, the electrons will then proceed to share

it with atoms and molecules. The plasma reactivity can thus be described by the following equation [51]:

$$k_x = n_A \int_0^\infty \sigma_x(E_e) \sqrt{\frac{2m_e}{E_e}} f(E_e) dE_e \quad (2.4)$$

where n_A is the density of the particle A, $\sigma_x(E_e)$ is the electron collision cross section, $f(E_e)$ the electron energy distribution function (EEDF), m_e the mass of the electron and E_e the electron energy. Through collision giving rise to vibration, excitation, dissociation and ionization, the high energy transmitted from electrons to ions can contribute to important plasma-kinetic phenomena such as suppressing activation barriers of chemical reactions and act as a catalyst for multiple reactions [52]. In terms of excitation, many excited and metastable species created can contribute directly into bioreactive pathways.

Nitrogen Species

From the combination of oxygen and nitrogen in air through plasma excitation and ionization, the **nitric oxide radical** $\bullet\text{NO}$ is one of the most important short-lived species for the reactive nitrogen species. It reacts with oxygen species to form two key molecules: **peroxynitrite** ONOO^- and **nitrogen dioxide** NO_2^* **radical**. First, peroxynitrite is a short-lived oxidant species issue from the reaction of superoxide $\text{O}_2^{\bullet-}$ and $\bullet\text{NO}$. In nature, peroxynitrite is usually found in plasma membrane NAD(P)H oxidases or mitochondrial respiratory complexes [9]. In low pH environment, peroxynitrite will tend to recombine with protons to form peroxynitrous acid, but can also recombine in more stable species such as nitrite and nitrate. These species are highly relevant for biochemical interaction processes. Secondly, nitrogen dioxide radicals can also be created when nitric oxide radical is oxidized by oxygen in air. It can contribute to the formation of reactive nitrogen species that can participate in processes related to inflammation and the immune response [12].

The interplay between nitric oxide radical, peroxynitrite, and nitrogen dioxide radical in biological systems is complex and has diverse implications for human health. These molecules can influence various cellular signalling pathways and impact physiological processes. Detailed biological responses involving these species can be described in Figure 2.4, which provides a summary of these interactions. While further research is needed in this domain to fully uncover the intricacies of the effects caused by nitrogen-related species, existing studies have already shed light on several key areas where these molecules play crucial roles in biological processes. For the moment, these species have been shown to be linked to blood pressure regulation, immune response modification and cell-to-cell communication [53].

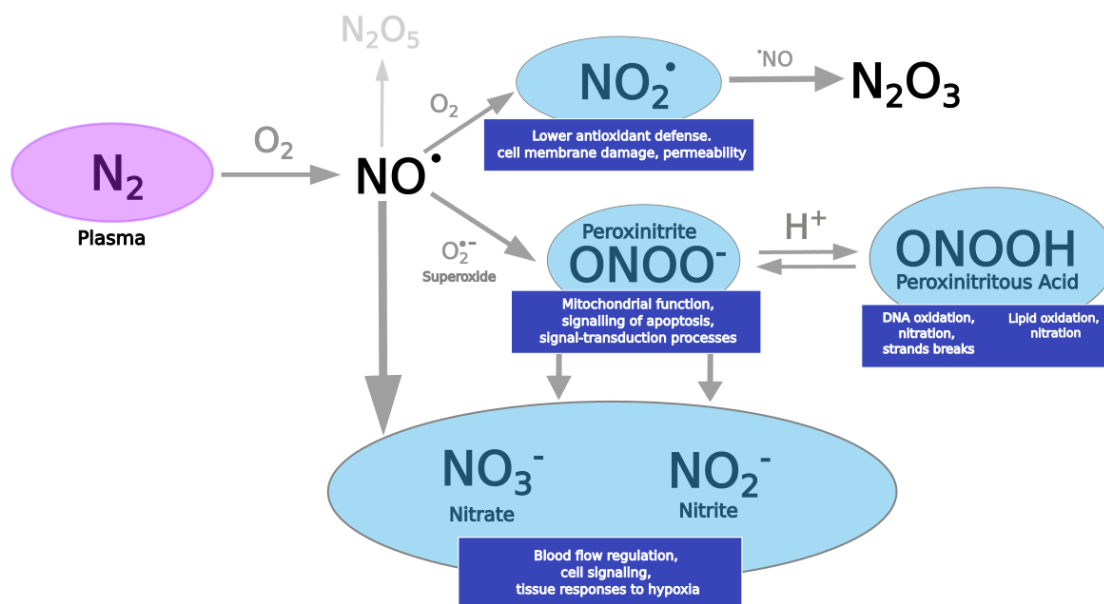


Figure 2.4 Summary of general nitrogen chemical pathway relevant in plasma medicine [8–12]

Oxygen Species

Numerous biological processes produce reactive oxygen species (ROS), from oxidative ATP production in mitochondria to its generation on the extracellular face of the cell plasma membrane [54]. At Figure 2.5 is represented the main production pathways for relevant ROS in plasma medicine.

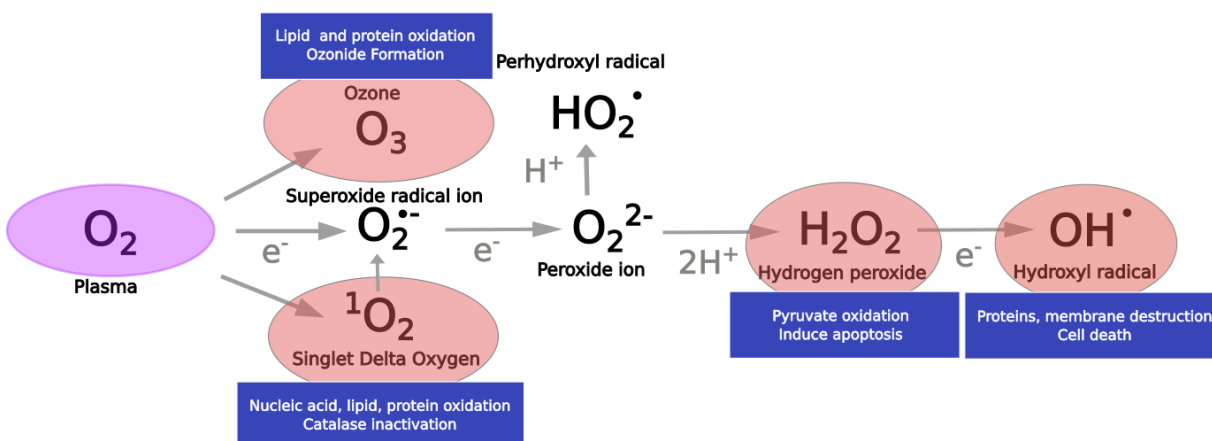


Figure 2.5 Simple oxidative pathway relevant in plasma medicine [13–15]

Due to the high energy of the plasma, the dioxygen molecule contained in air tend to either recombine with an atomic oxygen molecule to form **ozone** O_3 , or excite to higher energies, thus producing **singlet delta oxygen** $^1\text{O}_2$ and **superoxide radical ion** $\text{O}_2^{\bullet-}$. From their unstable form, these species usually react to create more long-lived species such as **hydrogen peroxide** H_2O_2 . Since the chemical mechanism is not as linear as depicted in Figure 2.5, it is still possible to observe highly reactive species formation of long-lived species, such as **hydroxyl radicals** $\text{OH}^\bullet$. In essence, oxygen-based redox chemistry plays a fundamental role in biological systems as it exhibits a high reactivity with lipid membranes and proteins. This reactivity can have profound implications for various aspects of cellular function and health.

All about equilibrium

As for many mechanisms in biology, the amount of chemical species present in an organism can affect its response toward it. The ability of a body to maintain homeostasis by modifying its internal behaviour to adapt is a key component in the concept of hormesis and hormetic response. As depicted in Figure 2.6, a biological system tends to restore and even overcompensate when exposed to an external agent. In the case of ROS species, this overcompensation increase cell proliferation, which is a process used for wound healing. However, longer treatment time might create unwanted effect and kill healthy cells [55,56].

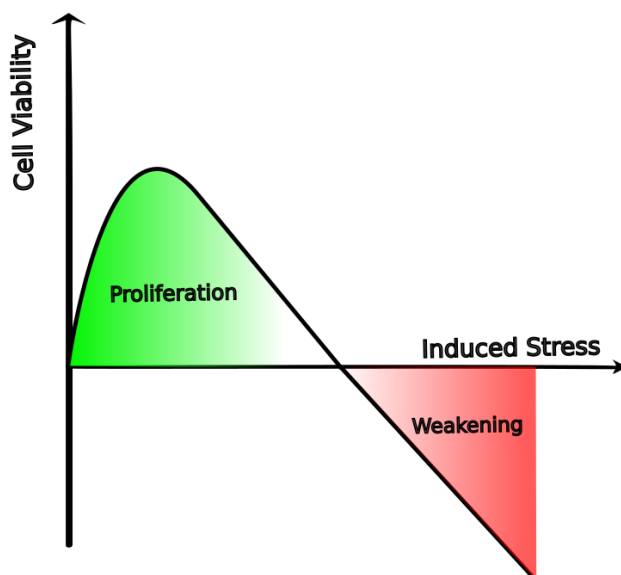


Figure 2.6 Hormesis conceptual graph

In the context of cancer treatment, this concept is very important since the hormetic response

varies from cell to cell [57]. The differentiation of response between healthy cells and cancer cells is the reason why plasma treatment and other types of cancer treatments are able to selectively target tumorous material and why proper dosage characterization is crucial.

Biological Mechanisms of Plasma Cancer Treatment

The competitiveness of plasma therapy to conventional therapy lies in its possibility to induce selectively cell death through multiple mechanisms. More precisely, plasma treatment can act on a biological system by directly triggering cell death mechanisms or immunological types of responses [16].

When referring to cell death mechanism, it is implied that necrosis (damaged-induced cell death), apoptosis (programmed cell death), necroptosis (regulated cell death), ferroptosis (iron-dependent apoptosis), autophagy (self-destruction), and pyroptosis (inflammatory lytic cell death) are the subjects of interest [58–60]. These processes are usually triggered by the plasma via plasma-induced ROS. By increasing the intracellular concentration of ROS, plasma treatment can effectively induce stimulation of tumour suppression proteins by reacting with enzymes such as MAP-kinase. In the case of ferroptosis, the triggering mechanism lies into lipid peroxidation and accumulation of iron [61]. These pathways are described more thoroughly in Figure 2.7.

For immunological triggering, plasma treatment have been to this date linked to four different mechanisms: induction of immunogenic cell death (ICD), introduction of oxidative post-translational modifications (oxPTMs) to proteins and peptides, epigenetic modification of aberrant gene expression, and enhancement of the anti-tumour functions of immune cells [62]. In cancer treatment, ICD reveal to be especially relevant as it provides an innate immune response specifically targeting the type of tumour inside the system [63]. Similar to cell death mechanisms, the activation of this mechanism is related to ROS and charged species concentration [64]. Through oxPTMs, plasma can upregulate antigenicity to the tumour ligands and receptors, and cell activity, or downregulate binding affinity and structural integrity [62]. Cold plasma has also shown some effect on the methylation process of DNA and histones as well as acetylation [65].

While cold plasma can target anti-tumorous processes, it has the ability to selectively target tumorous material. Due to the high level of intracellular ROS in tumours [66], the lethal dose is much lower than the one of a healthy cell, which provide a sweet region for proper dosage. The fact that cancer cells are reproducing faster is also thought to be a cause of selectivity. During the replication phase, the DNA is more vulnerable to ROS [67], which means that an extended exposure period of replication, i.e. a cell replicating more often, will increase the

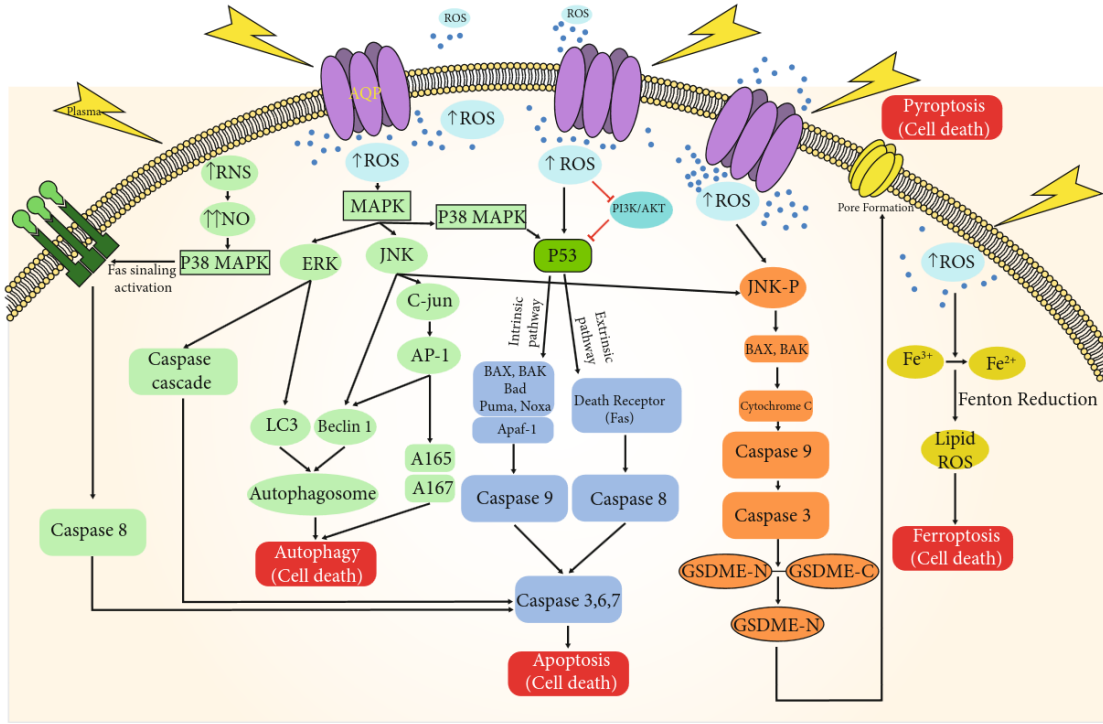


Figure 2.7 Cell death triggering pathways during plasma treatment. Reproduced from [16]

likelihood of triggering cell death processes.

2.2 Part II: A Brief History of Plasma Medical Devices Design

History of plasma jets have passed through a long series of design iterations before developing bio-suitable plasma sources. In this section, the main design steps of design of biomedical plasma sources will be presented as well as the current sources used in clinical environment.

2.2.1 First Steps Toward Plasma Medicine

For most experts history of plasma in medicine has begun with the mastery of electricity. One of the brightest minds of the 19th century, Nikola Tesla, was the first person to demonstrate the ability of high frequency currents to pass through a human body without causing any harm [17, 68] (see Figure 2.8A).

While Tesla did not try to pursue directly the medical possibilities of his discoveries, he was one of the first to propose the idea to use this technology for medical purpose. The strong impression made during his demonstrations led Jacques-Arsène d'Arsonval to use this technology for a novel type of electrotherapy, thus starting the darsonvalisation [17]. The

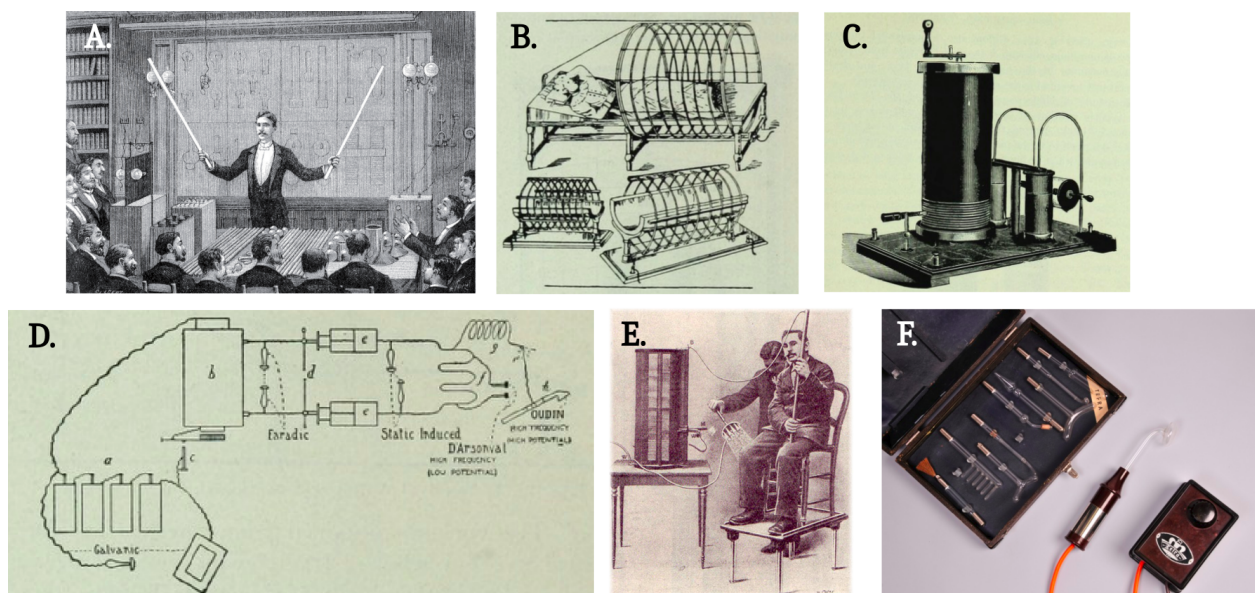


Figure 2.8 Historical development in plasma medicine during the 19th and beginning of the 20th century. A) Nikola Tesla demonstrating the effect high frequency current passing through his body using thousands of volts [17], B) Model of Oudin resonator [18], C) Inner electrical system of the Oudin Resonator. The components are a. Battery in Series, b. Induction Coil, c. Vibrator of Interrupter, d. Spark-gap, e. Condensers for Leyden Jars, f. D'Arsonval Solenoid, g. Oudin Resonator, h. Vacuum Electrode [18], D) Small cages for treatments of arms and legs using the darsonvalisation technique [18], E) Image of a knee treatment using an Oudin resonator [19] and F) Home-care medical hand-held plasma wand assortment. The different shapes of electrodes reflect the many possible use of this technology, from dental to hair treatment [20]

darsonvalisation is a type of therapy using high frequency currents to treat affection notably linked to skin such as scars, acne, dermatitis and other health problems (see Figure 2.8B). These direct treatments relied on electrodes covered in sponges wet with salt solution applied in the areas of interest where a current estimated to be more than 500 mA was applied [18]. It is through the following of this work that a physician called Paul Oudin created what we can only compare to be the first plasma medical device. By creating the Oudin resonator (see Figure 2.8C-D), Oudin used the previous design of the Small Solenoid created by d'Arsonval and connected it to another solenoid at resonance frequency of the circuit [18].

This improvement on the adding of the solenoid to a metal electrode that has a moistened leather surface helped locate the treatment established by d'Arsonval. This new device enabled the creation of visible "rain of little, violet-coloured sparks" [18]. The device was not only the first plasma-based biomedical device, but also one of the ancestor of what we call today the dielectric barrier discharge (DBD), where we can deduce that the small violet spark

is now-called corona discharges in air. However, it was also revealed that during treatments, involuntary movements of the operator or the patient could create sharp spark or arc when approaching the device too close, which resulted in a sharp pain at the treated region [18]. This phenomenon also notes the first steps toward electrical safety for plasma-based medical devices. An example of treatments with the Oudin resonator is shown in Figure 2.8E [19].

One innovative way to prevent the sparks from hurting the patient in the previous model was to use an insulated electrode inside a vacuum chamber (see Figure 2.8 F). This created not only the first floating electrode DBD-type discharge, but it also marks the first use of a second type of state in plasma medicine, the glow discharge [20].

For the majority of the 20th century, the technology has stayed quite similar after the vacuum electrode. While some interesting studies were made on electrical properties of the human body, the biomedical advantages of the device were oversold and unrealistic expectation were created by companies, leading to a loss of interest on the medical value of the device until the 1990s [69]. The resurgence of interest in the device has been linked directly to the great improvement in the human anatomy model leading to better scientific approach in the medical domain [70].

2.2.2 Into Modern Biomedical Plasma Sources

With many improvements over the years, modern plasma sources have been able to show serious and promising results in medicine in terms of wound healing and cancer treatment. Here I will present two of the main designs that are currently used and even certified in clinical environment: the Dielectric Barrier Discharge (DBD) and the Cold Atmospheric Pressure Plasma Jets (CAP-Jet).

Dielectric Barrier Discharge

As its name might suggest, DBD plasma sources usually take the form of two electrodes separated by a dielectric to which a high voltage is applied. As long as a dielectric is placed between two or more sets of electrodes, any type of configuration is possible (see Figure 2.9). With either one or many dielectrics are covering an electrode, it will be able to act as a current limiter which prevents any strong disturbance of the plasma state. When considering electrode placement, there are several options available. Electrodes can be positioned inside a dielectric material, located outside of the dielectric, placed adjacent to each other, separated completely on both sides or use a single covered electrode with the treated surface serving as the ground potential. DBDs typically can create filamentary discharges, which quickly

accumulates on the surface to self-terminate. These discharges usually tend to last from 10^{-7} to 10^{-8} seconds [71].

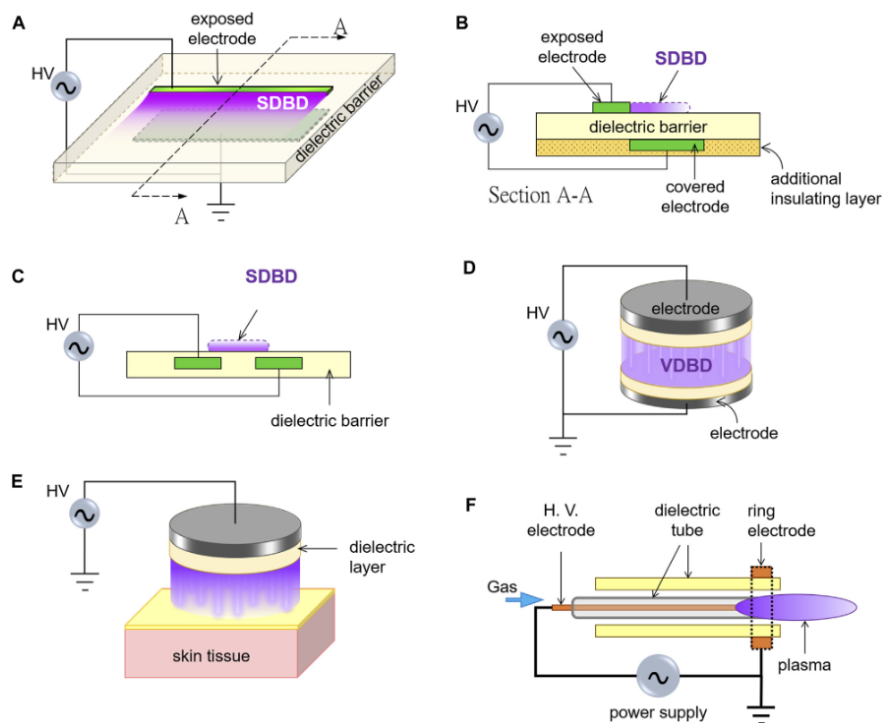


Figure 2.9 Various types of DBD configurations. A. and B. Different views of a surface DBD, C. Surface DBD with electrodes placed on the same side, D. Volume DBD, E. Floating-Electrode DBD, F. Plasma Jet [21]

While DBDs were first developed for a large range of applications such as a tool for ozone generation, material surface modification, surface disinfection, chemistry, aeronautics and much more [72–80], they also found a deep interest in the biomedical domain mainly under the form of a Floating-Electrode DBD and a CAP-Jet (the latter will be discussed in the next section) [81–84]. Official clinical instruments using this method, such as the PlasmaDerm[®] VU-2010 (CINOGY GmbH, Duderstadt, Germany), uses the ability of DBDs to treat large areas of tissue externally. For the PlasmaDerm[®], the skin of the patient acts as a grounded electrode for the current to pass through, meaning that no plasma is produced if not interacting directly with a grounded surface. For the moment, clinical studies on chronic leg ulcers have shown promising results [85].

Cold Atmospheric Pressure Plasma Jets

Similar to the DBDs, plasma jets exist in various configurations (see Figure 2.11). A plasma jet can be typically described as a set of one or multiple electrodes using a cylindrical dielectric barrier and a noble gas to produce plasma in high voltage conditions. The use of this disposition of electrodes to produce plasma is classified as a capacitively coupled system. Inductively coupled plasma sources also exist, but its broader effluent size and overall function differ from what is characterized as a jet. Its main specificity compared to the DBDs and plasma torches lies in its small effluent shape, called plasma plume, directed by a flow of a noble gas or a mixture of gas [86].

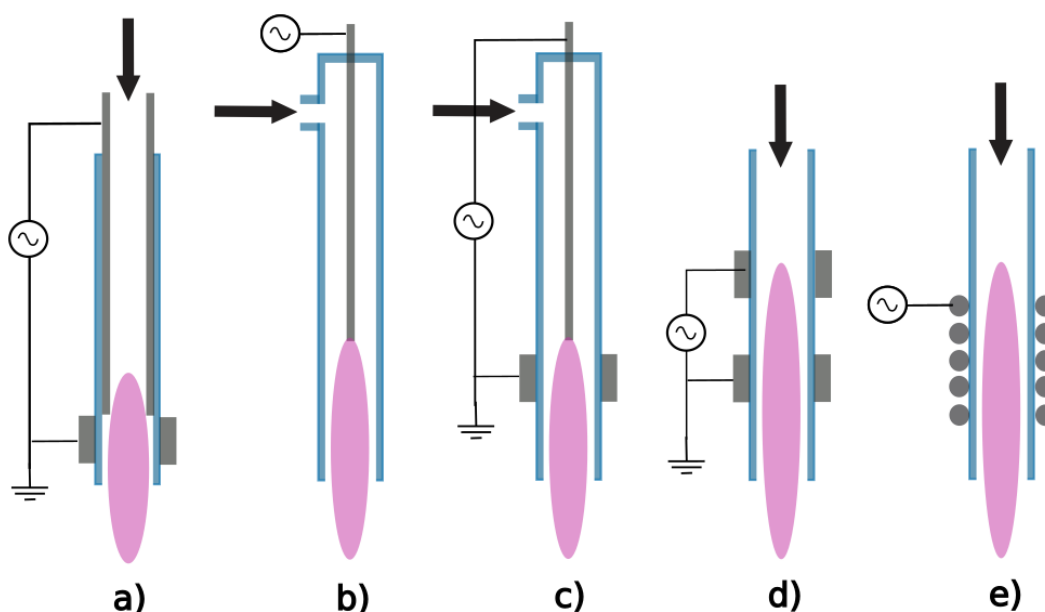


Figure 2.10 Various configurations of plasma jets. A) Coaxial DBD plasma jet, B) Floating needle electrode jet, C) Needle electrode jet, D) Surface coaxial DBD plasma jet and E) Inductively coupled plasma torch [22–25]

In clinical environment, one of the most famous plasma jet is the kINPen® MED (neoplas tools GmbH, Greifswald, Germany). It uses a RF system similar the design C) presented at Figure 2.11 and has a second source of gas around the plasma, called gas curtain. This characteristic enables high control over plasma chemistry through control of its surrounding conditions. For *in vitro*, *in vivo*, and *in ovo* treatments, the kINPen® MED has shown great success in inducing apoptosis in cancer [87–91].

Although the previously mentioned plasma medical sources only present relevant sources for this thesis, it does not present an extensive literature review. It is important to note that

numerous additional designs are currently under development for various clinical applications. Here are a few honourable mentions [26]:

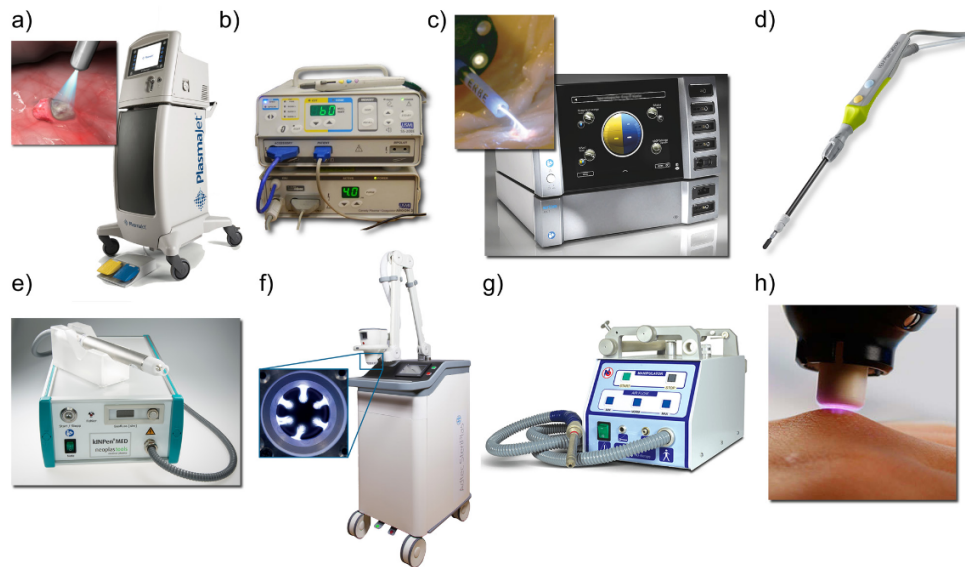


Figure 2.11 Medical plasma sources. a) PlasmaJet, b) Canady Vieira hybrid plasma scalpel, c) the argon plasma coagulator VIO 3/APC 3, d) PlasmaBlade, e) kINPen MED, f) SteriPlas, g) Plason, h) PlasmaDerm. Reproduced from [26]

CHAPTER 3 A MEDICAL-GRADE PLASMA SOURCE DESIGN

This chapter will present the main steps into designing, building, problem-solving, optimizing and assessing the safety made of my own medical plasma jet. The output of the following chapter resulted in the installation of a plasma jet in a biosafety cabinet at the Montreal General Hospital for *in vitro* treatment of cancer cells.

3.1 Plasma Source Design

The plasma source designed and implemented in the Plasma Physics and Spectroscopy Lab (Polytechnique Montréal) and implemented at the General Hospital of Montréal (McGill University Health Center) is a cold atmospheric pressure plasma jet inspired by a coaxial DBD type of plasma jets (see Figure 2.11a). It features a stainless-steel cylinder (OD = 0.078", ID = 0.063", McMaster-Carr) acting as a high voltage electrode inserted inside a quartz tube (OD = 4 mm, ID = 2 mm, Thomas Scientific). The distance between the high voltage electrode and the jet nozzle is 10 cm. The ground electrode is made from a copper coil (gauge 24) wrapped around the dielectric tube for a distance of 5 mm between each loop.

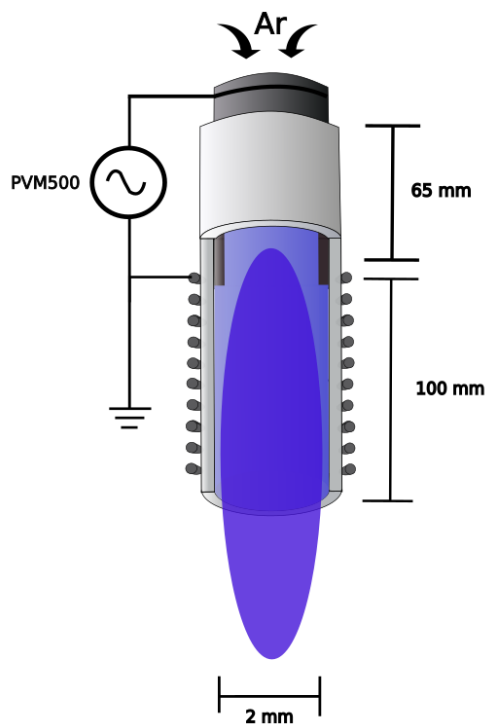


Figure 3.1 CAP-Jet reactor design representation

The power supply used for all the experiments shown in this thesis is a PVM500-1000L (Information Unlimited, US). This device produces a voltage of 3 kV_{pp} at a resonant frequency of 22 kHz. A high purity argon gas ($>99.998\%$, Linde Canada) is injected into the device at 2.5 standard litres per minute (SLM). Similar to the choice of power supply, the choice of argon is mostly related to its cheapness and accessibility. The whole electrical structure was maintained by a high temperature resistant resin (High Temp, FormLabs) that add also the possibility to include a gas curtain around the plasma effluent. The shell was built using stereolithography 3D printing technique. The housing was split into two sections: a support structure and a cap ensuring the ability to access the internal electrical system if maintenance is needed. In Figure 3.2 is represented a cross-sectional view of the plasma support structure for the electrodes and the gas curtain.

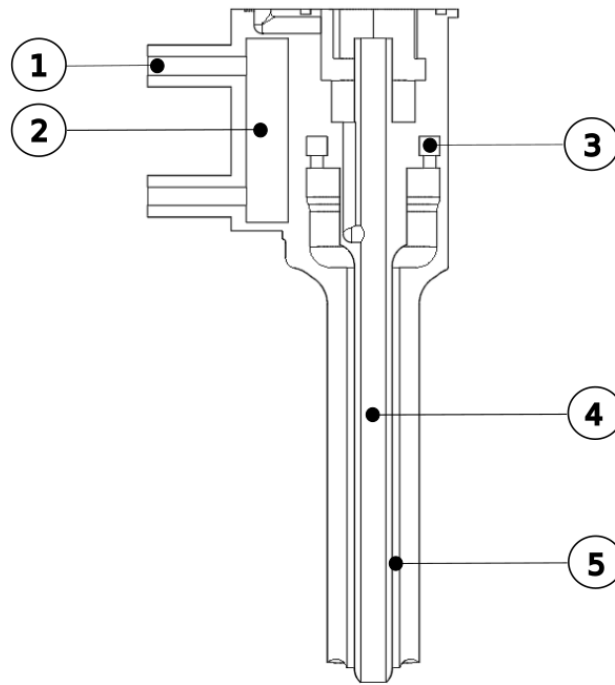


Figure 3.2 Cross-section view of the external jet structure. 1) Gas curtain inlet, 2) Gas curtain mixing chamber, 3) Gas curtain diffuser, 4) Plasma electrode mounting structure, 5) Outlet channels of gas curtain

The plasma jet's overall shape and length were adjusted for direct integration with a computer numerical control (CNC) machine (Genmitsu 3018-PROVer, SainSmart, US), creating a fully automated system both for positioning and ignition: the CNC milling system was modified in order to redirect the TTL signal to the power supply, acting as an on/off signal for the plasma ignition. The overall system was encoded in a GCODE file type and automatized through a home-made GUI python script. The current setup used for biological tests can be

seen in Figure 3.3.

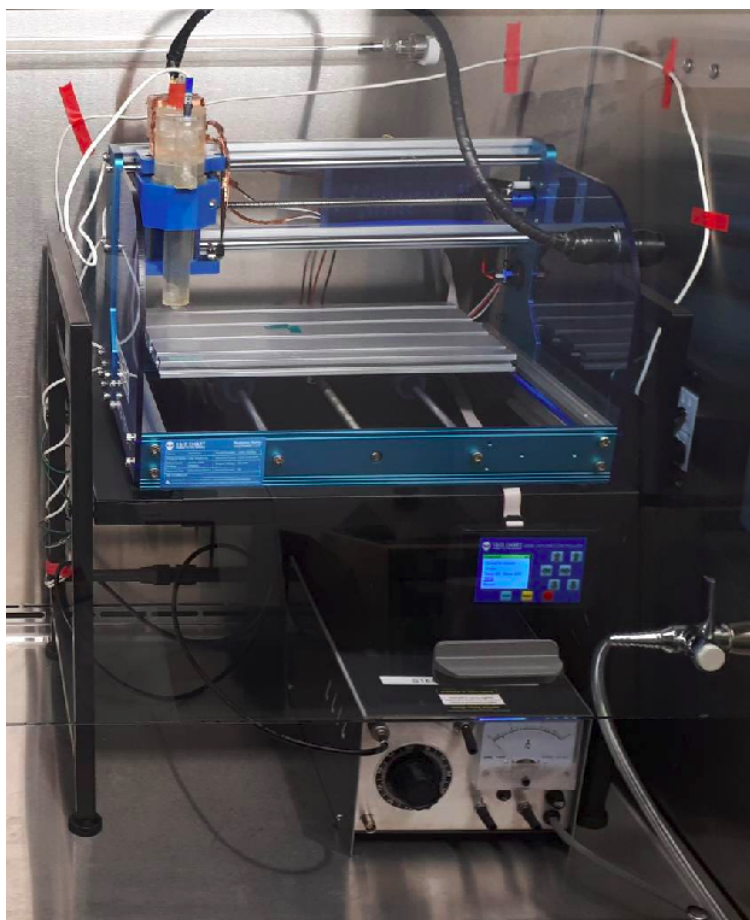


Figure 3.3 Complete plasma treatment setup available at Montreal General Hospital

The primary goal in designing the device was to make it accessible to individuals with less expertise in physics and a stronger background in biology, allowing them to utilize the source without requiring an extensive understanding of plasma science or plasma source engineering. In the context of *in vitro* treatments, it has also been observed that experiments can extend over multiple hours. Therefore, the need for an automated platform became crucial to ensure the well-being of the experimenters.

3.2 Parameter Optimization

Prior to the development of a stable and biocompatible plasma device, numerous iterations of the system were meticulously designed, tested and crafted (over 100 to be more precise). This section will outline the primary challenges encountered and the solutions chosen to overcome them.

3.2.1 Electrical Arcing

One of the most challenging aspects of creating an audiofrequency argon plasma jet is the apparition of arcs when interacting too close or with a conductive surface. As mentioned in Chapter 2.1.1 and 2.2.1, arcing can be a sign of high current and high plasma temperature. On the biological level, the effects of arcing on direct treatment usually result in damaging the surface treated.

One of the initial strategies for minimizing the risk of arcing involves decreasing the overall power input, achieved by lowering the voltage required to induce breakdown in the medium. Since the electric field intensity depend on the distance at which the voltage is applied:

$$E = \frac{V}{d} \quad (3.1)$$

where the electric field E is directly proportional to the applied voltage V divided by the electrode distance d . A straightforward approach to power reduction while preserving the same electric field strength is to minimize the electrode separation distance. This reasoning is reflected by the choice of **employing tubing with a small diameter**.

The second method employed to mitigate arcing risk involves the deliberate introduction of a significant gap between the high voltage electrode and the exit nozzle. Adhering to the principles of the Paschen curve, **an augmented electrode-to-tissue distance** diminishes the likelihood of direct electrical breakdown between these two components. Therefore, in my design, the high voltage electrode is positioned at a distance of 10 cm from the exit nozzle.

Nonetheless, the incorporation of additional distance introduces another significant challenge in plasma behaviour. With a longer distance from the high voltage electrode to the nozzle, conventional grounded electrode configurations often struggle to convey sufficient energy through the dielectric to create a plume or will necessitate more energy, thus creating arcs again. One way proposed to circumvent this problem was to **reshape the grounded electrode to a helical shape similar to a coil**. Experimentally, this distribution has shown great potential in helping propagation of plasma than just a single grounded ring. To the best of my knowledge, no research has been done with the exact same configuration even if helix grounded electrodes have been previously used [92–95]. It has been showcased that the coiled ground was able to create similar plume shapes while significantly lowering the temperature and preventing arcing due to the ability to produce plasma in low current conditions.

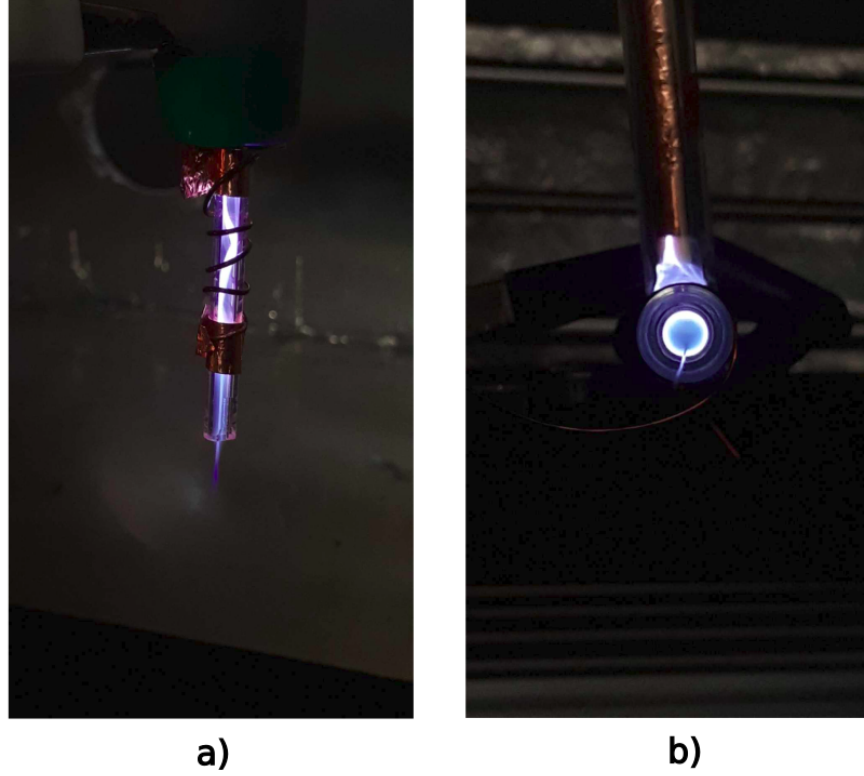


Figure 3.4 Plasma discharge induced by the cascade coiled ground

As we can see in Figure 3.4, the propagation of the discharge inside the tube is not linear and adopt a more helical shape that follows the inner wall of the dielectric tube. The ability of the ground coiled cascade electrode distribution to prevent arcing can be firstly understood through a simple simulation of an electrostatic field with the same geometry as proposed earlier. The COMSOL simulation creates a stationary finite element study pre-breakdown electric field solving the following set of equations:

$$\nabla \cdot \mathbf{D} = \rho_v \quad (3.2)$$

$$\mathbf{E} = -\nabla V \quad (3.3)$$

where equation 3.2 represent Gauss's law in the Maxwell's equation with $\mathbf{D}$ as the electric field density and ρ_v the space charge density. Equation 3.3 express the spatial relation between the electric field $\mathbf{E}$ and the applied voltage V . With an applied voltage of 3 kV at the inner tube and the helical coil grounded, the simulation of the electric field presented in Figure 3.5 shows first a high electric field near the electrode, which could be able to guide small

discharges near the coil themselves. However, the further the distance from the high-voltage electrode is, the lower and more evenly spread out the electric field becomes. It can be thus hypothesized that the plasma start to diffuse more along the tube, which promotes more interaction between the particles and reduces the arcing possibility while still keeping a minimum electric field to propel the plasma further down the tube.

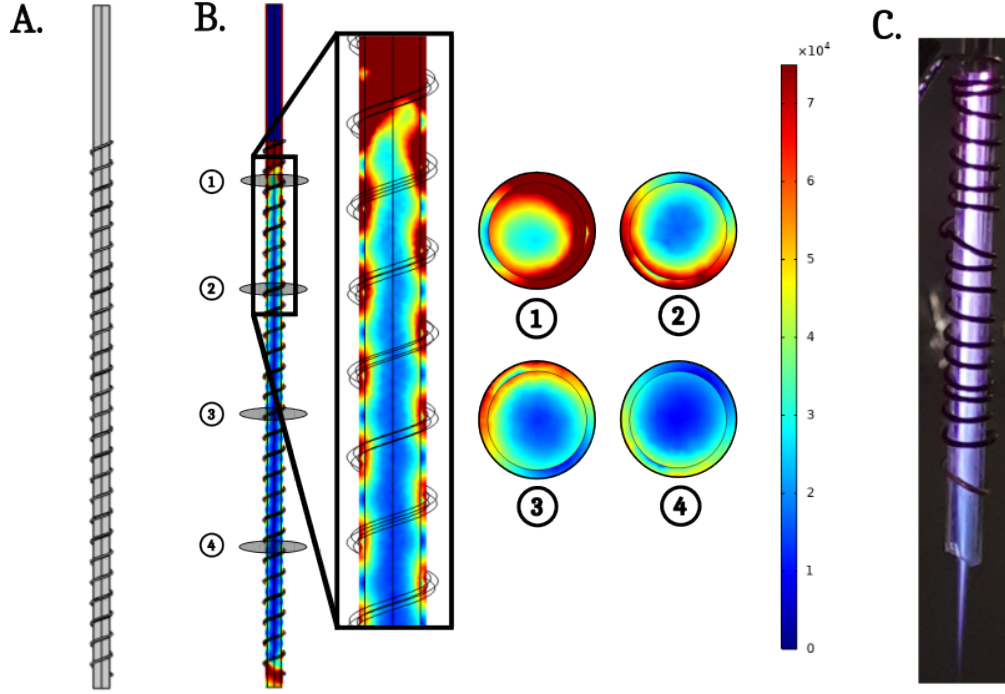


Figure 3.5 Simulation and comparison of electric field (V/m) inside the CAP-Jet. A) 3D model of the dielectric and electrodes, B) Sectional cut of the electric field value and C) Upgraded prototype version of the CAP-Jet with more loops and longer grounded electrode

In comparison to the previous model developed at the PPSL (see Figure 3.6 B) and inspired by [22,96,97], the interaction of the helical ground on a grounded metallic surface is able to propagate the electric field on a longer distance without creating high electrical field gradient on the surface. As expressed by the Figure 3.6, the effect of a close high voltage electrode to a grounded surface, even with a grounded ring, enhance the electric field which can propagate the plasma more easily and create an arc. In contrast, a far high voltage electrode with a grounded ring will not be able to propagate the plasma far enough to interact with a surface.

The Figure 3.6 not only express the improvement on electric field containment with the design of the grounded electrode, but also confirm our requirement for increasing the electrode-to-tissue distance. It is able to prevent formation of high electric field on the treated surface while keeping a minimum requirement of the electric field to propagate the plasma further

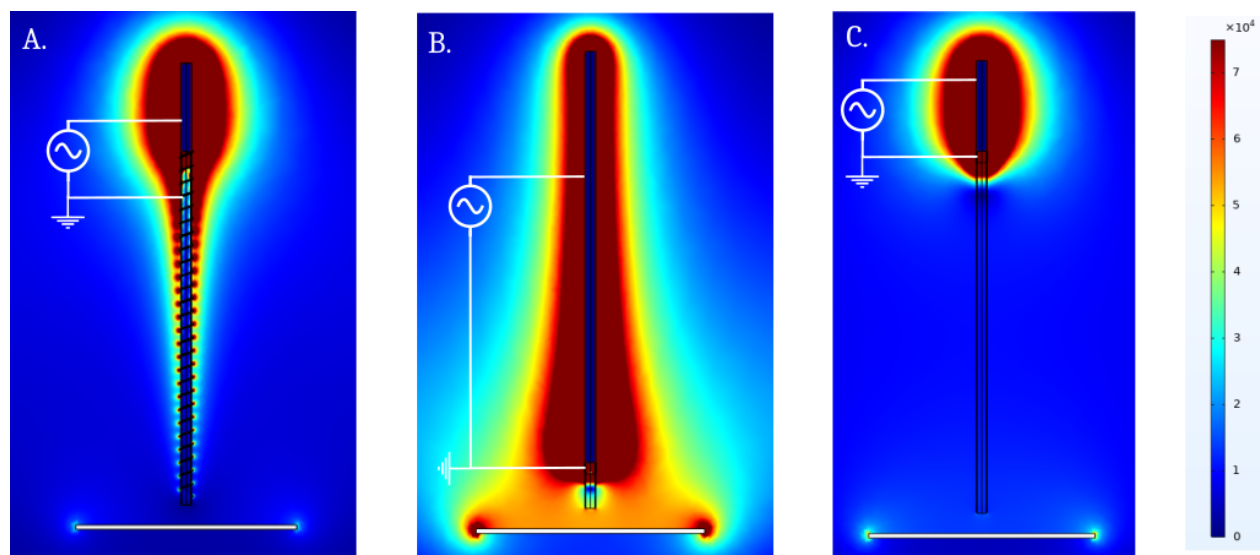


Figure 3.6 Simulation of the plasma jet electric field (V/m) for different configurations when close to a grounded metallic surface. A) Helical ground with a distant high voltage electrode, B) Close high voltage electrode to the surface treated with a grounded ring and C) Far high voltage electrode with a grounded ring

into the dielectric tube.

3.2.2 Optimal Flow

In order to maximize the direct interaction between plasma active zone and tissue, the flow must be thoroughly adjusted and the temperature associated with it must be kept under tolerable conditions for biological interaction.

As Figure 3.7 can show, the modification of flow affects multiple parameters in a nonlinear fashion. The temperature of the plasma was measured with an insulated thermocouple and the plume length were estimated with a ruler. Especially for the plume length, a low flow rate may not provide sufficient thrust to expel the plasma from the dielectric tube, while an excessively high flow rate is more likely to extinguish the plasma rather than helping its propagation. Experimentally, the optimal flow is around 2.5 SLM and respect our criteria for having a temperature lower than 43°C . It is important to note that biological tests conducted on 2D liquid cell culture plates exposed to the plasma jet revealed flow-related issues such as overflowing, rapid evaporation and cell dispersion. Consequently, the flow rate was adjusted to 2 SLM.

To gain better control over plasma properties, the implementation of a gas curtain becomes essential. The flow was characterized using a Schlieren Imaging technique, which is further

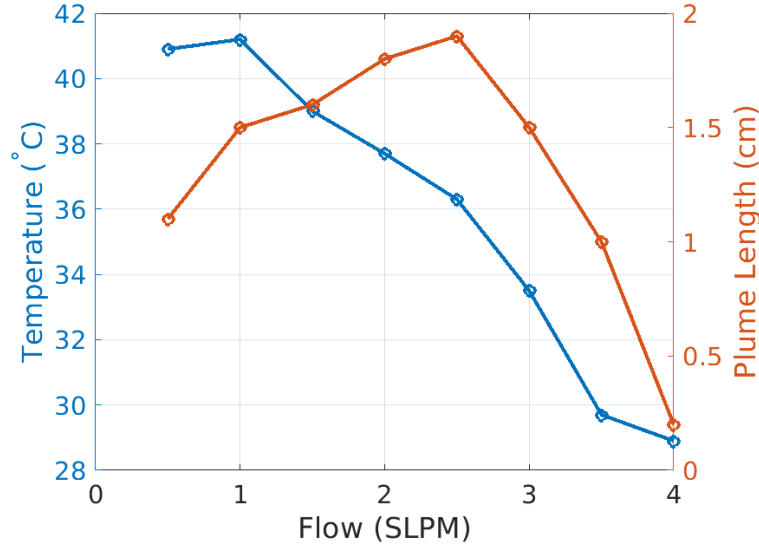


Figure 3.7 Parametric study of the flow effect on temperature and plume length

described in Chapter 4. As we can observe in Figure 3.8, a strong laminar flow is only observed for a flow of 5 SLM due to high contrast and overall laminar behaviour. This result can also be compared when using an inner gas of argon of 2 SLM, where the flow of the curtain remained unaffected by the inner gas source.

For future experiments on the gas curtain, it will thus be preferable to choose at least a value of 5 SLM in order to prevent any mixing with external perturbation such as air currents. Higher values of gas curtain flow could also be chosen, but it can lead to the apparition of a turbulent regime and less available operational time of the plasma source.

3.3 Safety Assessment

3.3.1 Electrical Safety

To certify devices using electrical-type of therapy for medical use, many can base their safety standard on the International Electrotechnical Commission, also called IEC section 60601-1, in their document called "Medical electrical equipment – Part 1: General requirements for basic safety and essential performance" [98]. This document outlines many basic safety protocols to get completely certified. One of the first tests to do in order to ensure the safety of a person exposed to a medical electrical-based source is to measure the patient leakage current by the method following Figure 3.9.

The principle of this experiment is to recreate the circuit equivalent of a human body through electrical components. For devices working at frequencies higher than 1 kHz, the maximum

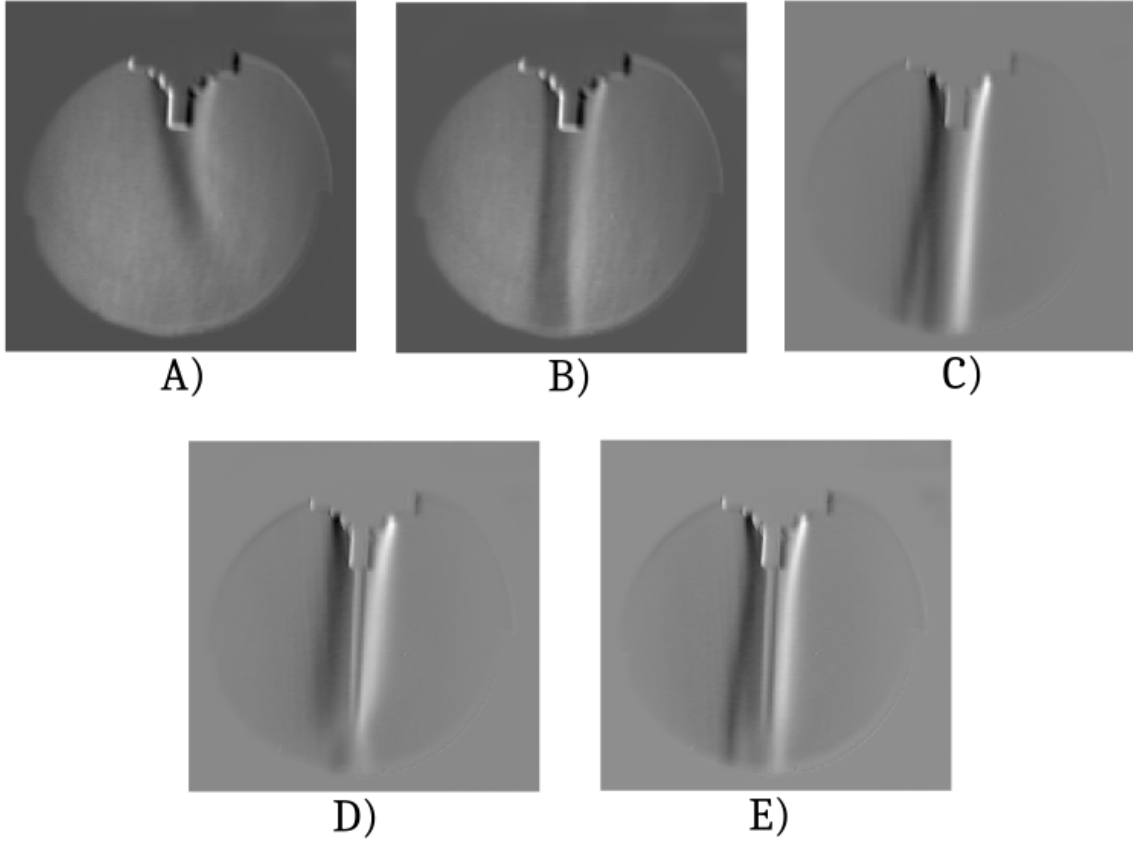


Figure 3.8 Schlieren imaging of gas curtain shape for different flow. A) Oxygen curtain flow of 1 SLM, B) Oxygen curtain flow of 3 SLM, C) Oxygen curtain flow of 5 SLM, D) Oxygen curtain at 3 SLM and argon inner flow at 2 SLM and E) Oxygen curtain at 5 SLM and argon inner flow at 2 SLM

current calculated to have low probability of ventricular fibrillation is $150 \mu\text{A}$ in normal condition. To calculate the current from our voltage measurement of the capacitor, it is possible to use the following expression [99]:

$$I = \left[\sum_{t=0}^{t_{int}} \frac{|V(t)|}{R_2} \Delta t \right] f \quad (3.4)$$

where I is the patient leakage current, R_2 is the value of the resistor equal to $1 \text{ k}\Omega$, $V(t)$ is the voltage measured on the capacitor, t_{int} is the integration time of a single pulse, Δt is the inverse count rate and f is the pulse repetition rate. From this formula, it has been estimated that the patient leakage current is $136 \mu\text{A}$, thus respecting the criteria emitted by the IEC.

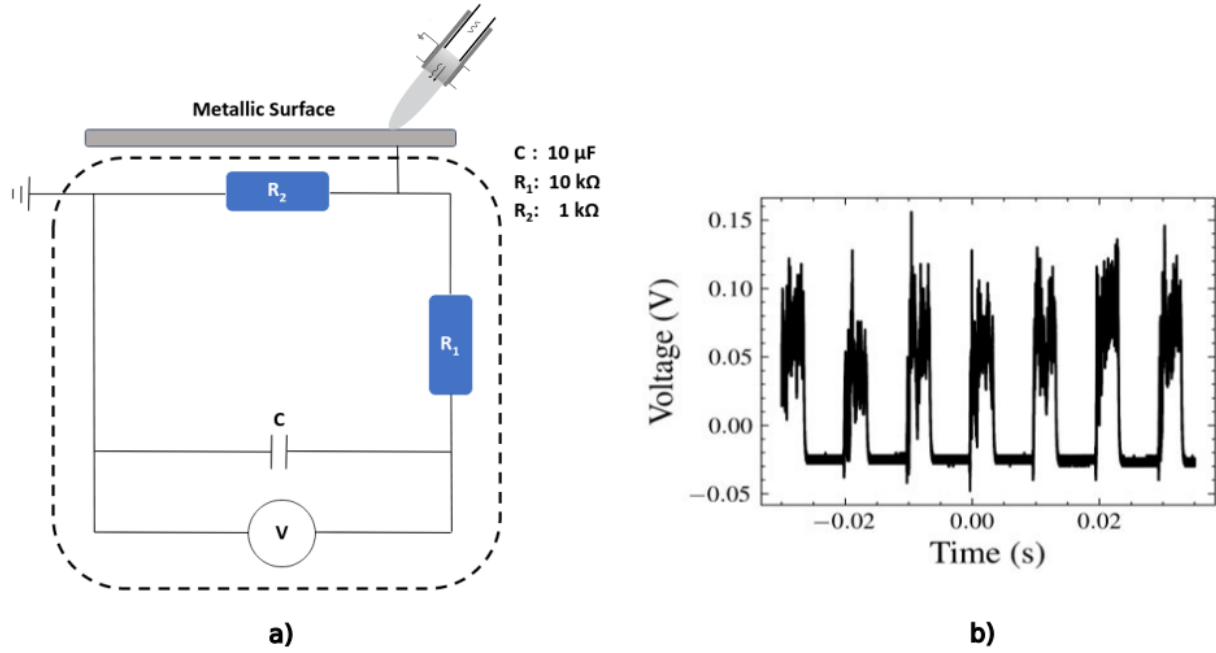


Figure 3.9 Sketch and results of patient leakage measurement for the CAP-Jet. A) Sketch of the equivalent circuit of a human body resistance and B) Measured voltage signal at the capacitor

It is also important to note that the test was made on a highly conductive surface, which represents the most extreme case of interaction with the plasma source.

Similarly, another crucial criterion outlined by the IEC for electrical safety is linked to the earth leakage current. In standard operating conditions, this current should remain below 5 mA. Detailed current measurement procedures are elaborated in Chapter 4, which enables us to confirm our compliance with this criterion, as evidenced by the measured value of 2.58 mA_{RMS} .

3.3.2 Thermal Safety

As stated in the literature [100], the temperature where thermal damage is observed start at around 43 °C. In order to prevent these types of damage, the overall gas temperature of the plasma must be characterized. While it is thoroughly explored in Chapter 4, one parametrization study was able to clearly showed which parameters can be used for temperature control. As seen in Figure 3.10, the variation of the duty cycle linearly affects the gas temperature of the plasma.

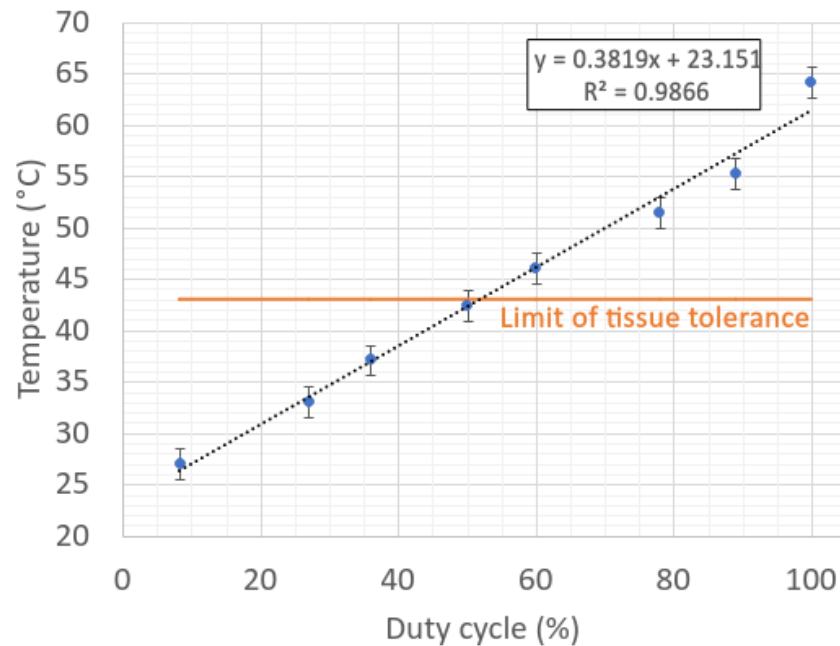


Figure 3.10 Effect of duty cycle variation on temperature. The flow of argon here is set at 2 SLM

By utilizing an insulated thermocouple, it becomes possible to estimate a reliable threshold for a safe duty cycle in biological treatments. To establish a secure margin between the tissue damage threshold, a duty cycle of 40% has been selected.

In summary, this chapter highlights the design steps and meticulous characterization that led to the implementation of a safe, efficient and biocompatible medical device. The Figure 3.11 exemplifies the success of the process.



Figure 3.11 Interaction between a finger and the CAP-Jet source

CHAPTER 4 ARTICLE 1: A COMPARATIVE STUDY OF NON-INVASIVE FIBRE BRAGG GRATING FOR SPATIALLY AND TEMPORALLY RESOLVED GAS TEMPERATURE MEASUREMENTS IN COLD ATMOSPHERIC PRESSURE PLASMA JETS

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4.1 Preamble

Combined with the aforementioned section on temperature requirements for plasma-tissue interaction safety, this article provides a detailed study on multiple techniques available for measuring plasma gas temperature. It provides thorough details on the experimental parameters and results of the temperature safety assessment, thus completing the global picture of the biosafety requirement of the plasma source. The article has been submitted in the peer-reviewed Plasma Source Science and Technology journal [101]. The analysis, the majority of the writing, editing and an important part of data acquisition and experimental manipulations were made by myself.

4.2 Abstract

Cold atmospheric pressure plasma jets (CAP-Jet) are successfully used in medical therapy for healing of chronic wounds. Their biological effects are widely researched in inactivation of pathogens and in assisting in cancer therapy. A fundamental parameter for these plasma applications are that CAP-Jets operate at temperatures that are tolerable for biological tis-

sues. Therefore, a first step to render plasmas capable of treating biological organisms is to guarantee limited heat dissipation and efficient power distribution in the plasma to generate the relevant reactive species. While tools characterizing the plasma’s gas temperature are well developed, there are only a few methods that work with an agreeable limit of uncertainty, complexity and non-perturbative properties to accurately determine that the studied plasma jet operates at tissue tolerable temperatures at all times. In the current work, time resolved measurements of the gas temperature in the effluent of a CAP-Jet are performed using the innovative technique of a Fibre Bragg Grating (FBG). The temperature dynamics is measured by a shift of the resonant wavelength of the FBG through its thermo-optic coefficient. Based on previous works using the technique of FBG on different types of plasmas and other temporal and spatial diagnostic tools such as thermocouple measurement and Schlieren imaging, we showcase reliable calorimetric measurements at different plasma conditions, and we precisely characterize the heat response during plasma ignition and extinction. The plasma source maintains tissue tolerable temperatures inside the plasma active zone with values below 35°C at 1 cm distance from the jet nozzle. The calorimetric measurements have revealed that the heat power dissipation in comparison to electric energy of our plasma source is at least 50 %. Our study advances the methodology of reliable tools of gas temperature and calorimetric characterization for biocompatible plasma sources.

4.3 Introduction

Over the past two decades, plasma medicine has emerged as a exciting novel therapy for wounds healing, and as promising tool for infection treatment, as well as for cancer and immunotherapy [31, 32, 102–107]. Cold atmospheric pressure plasma jets (CAP-Jets) have gained much attention for their application potential in medicine, primarily through delivering of an oxy-nitroso stress burst [55]. The complex interaction process of plasma and biological systems generates reactive species of oxygen and nitrogen (RONS), triggering cell death mechanisms and modifying cell signalling pathways [108]. By controlling parameters like electric fields, gas composition and energy input, various biological responses can be induced. Particularly for thermally sensitive biological tissues, an exact knowledge of the plasma’s gas temperature becomes paramount for controlling RONS production while mitigating any adverse thermal effects [109].

Conventional gas temperature measurements typically are performed with electrical thermocouples [110, 111], by emission and absorption spectroscopy [112–114], by laser-induced fluorescence [115–117], with fluoroptic probing [117, 118], and with Schlieren imaging [119, 120]. However, these techniques often introduce substantial uncertainties to the overall tempera-

ture evaluation or necessitate complex manipulations and analysis. The present work proposes for an electrically non-invasive type of temperature measurement a more approachable measurement of the plasma's gas temperature made for the first time in CAP-Jet with a Fibre Bragg Grating (FBG). The inherent dielectric property of the FBG renders it resilient to potential interferences from high voltage, high frequency and gas composition variations, thereby enhancing measurement accuracy. As an optical method, FBG distinguish itself from other fibre-based method to its ability to integrate multiple sensors in a single fibre, thus increasing its sensitivity through wavelength-division multiplexing [121]. In order to validate the FBG temperature measurements, we compared the measurements to those with an electrically insulated thermocouple as well as a sensitive Schlieren imaging system.

4.4 Methodology

4.4.1 Cold Atmospheric Pressure Plasma Jet

The plasma jet used in this experiment is a self-designed coaxial dielectric barrier plasma jet inspired by [122, 123]. This CAP-Jet is operated with dry argon as feed gas, operated with a high voltage at a frequency of 22 kHz. It is powered by a flyback power supply (PVM500-1000L, Information Unlimited, US) at up to 3 kVpp operating voltage. Its driven electrode consists of a stainless-steel tube ($\varnothing$ 2 mm) snugly mounted inside a borosilicate dielectric tube ($\varnothing$ 4 mm). The grounded electrode consists of a wire which is helically wrapped around the dielectric tube as seen in Figure 4.2. The plasma jet can produce a plume length of up to 2 cm for an argon flow of 2.5 SLM. This agrees well with the findings for non-turbulent plasma flow at higher frequencies of Darny et al. [124]. The current and voltage were monitored with a set of voltage probe with a resistor of 2 Ω and a CT4028 voltage probe (Cal Test Electronics, US) respectively.

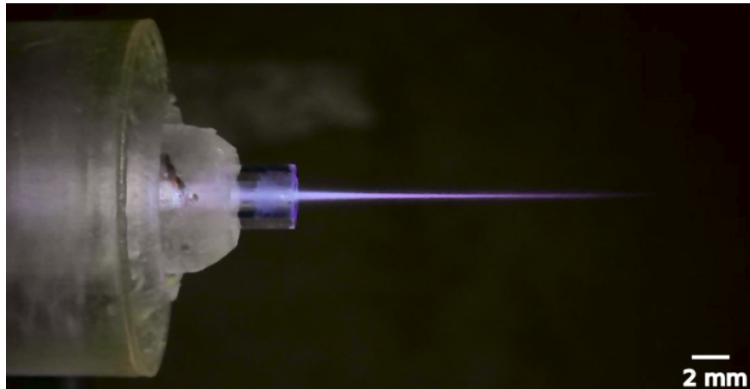


Figure 4.1 Image of a CAP-Jet plume

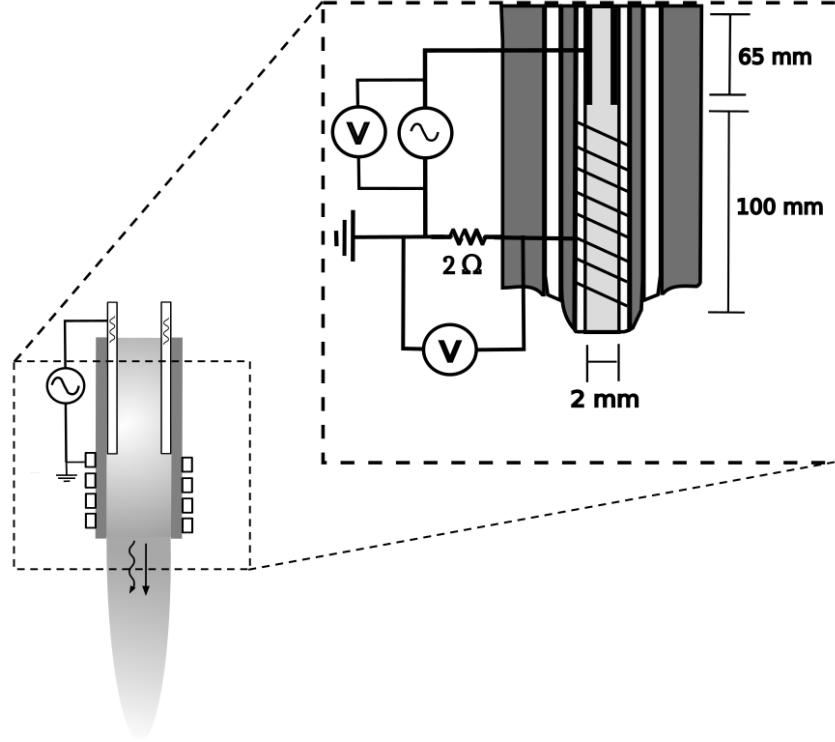


Figure 4.2 Sketch of a CAP-Jet. The design is based on a coaxial dielectric barrier discharge using a helicoidal grounded electrode

4.4.2 Fibre Bragg Grating

The fibre Bragg grating (FBG) is home-made by periodically structuring an optical fibre using a phase-mask technique as described in detail in [125].

The FBG temperature sensor technique is based on the phenomenon of Bragg wavelength shift, which occurs in response to temperature changes. This wavelength shift is a result of a shift of the thermo-optic coefficient of the optical fibre [126]. To actively monitor temperature changes, we integrated a C-band Broadband Source (BBS, JDS Uniphase, US) into the FBG setup via a fibre-optic circulator. The reflected signal was directed to an ANDO Optical Spectrum Analyzer (OSA), enabling real time monitoring of Bragg reflection wavelength variations.

The FBG sensor's response to temperature, characterized by a shift of 11 pm/°C, is measured. To ensure stable and unperturbed plasma operation, we performed optical emission spectroscopy. The comparison of the emission spectra of the plasma with and without the FBG has shown no significant difference and we can conclude that the FBG does not perturb the plasma.

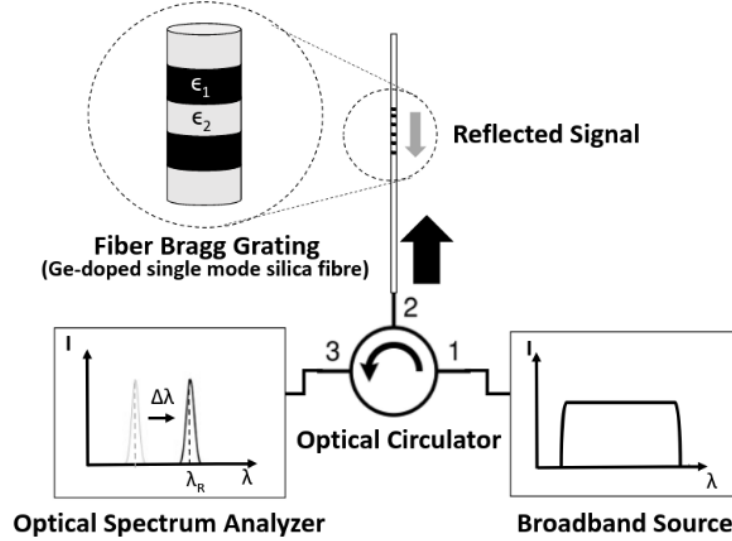


Figure 4.3 Diagram of FBG Temperature Measurement Setup. A broadband source is sent into a FBG with the use of an optical circulator. When the light reaches the FBG, a sharp, thermally-sensitive signal is reflected and subsequently measured by an Optical Spectrum Analyzer

4.4.3 Schlieren Imaging System

The second method we used to measure the temperature is based on Schlieren imaging. The imaging system is set up in the so-called z-shape configuration (see Figure 4.4) [127]. As point light source, a collimated Laser-Diode-Pumped DPSS Laser Module (532 nm, 0.9 mW) is used. In the z-shape setup, the light is collimated from the point source with an aspherical parabolic mirror. A second aspherical parabolic mirror focuses the light onto a razor blade, which acts as a spatial filter in the Fourier plane. The plasma source is installed in the collimated section of the light beam. The system images any variation in the index of refraction. The light is collected by a CMOS camera (Allied Vision Alvium 1800 U-240m, Edmund Optics, US).

The temperature is derived from an analysis of the relationship between the derivative of the refractive index (n) and the density of the gas, employing both the Clausius-Mossotti equation and the Gladstone-Dale relation. This analysis is performed through the Abel inversion of our signal $\hat{c}$ and can be described by the following expression [119]:

$$\hat{c}(r) = S(n(r) - n_0) = \int_r^\infty \frac{c(y)dy}{\pi\sqrt{y^2 - r^2}} \quad (4.1)$$

Here, S represents the contrast signal within our image. Utilizing this expression, we can

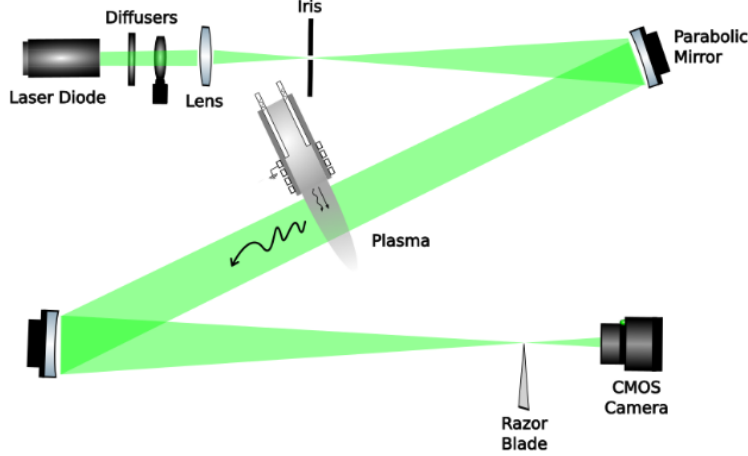


Figure 4.4 Schlieren Measurement System. A point source light is shining into a spherical mirror where it is collimated on a second spherical mirror. At the focal point of the second mirror, a razor blade is used to filter spatially the signal before being detected by the camera.

subsequently derive the value of our effective relative refractive index as follows:

$$n_0 + \frac{\hat{c}}{S} - 1 = \frac{N}{N_r} \sum_i \epsilon_{r,i} \chi_i \quad (4.2)$$

Finally, by assuming a linear correlation between temperature and relative refractive index, we utilize the following expression to determine the gas temperature based on Schlieren measurements.

$$T = T_0 \frac{n_0 + \frac{\hat{c}_{fl.}}{S} - 1}{n_0 + \frac{\hat{c}_{pl.}}{S} - 1} F \quad (4.3)$$

where F , the factor for change in air mole fractions, is assumed to be nearly equal to 1. Numerically, the Abel inversion was computed using a discretization technique, and each figure was averaged over 200 images.

4.4.4 Thermocouple

The thermocouple measurements were chosen to compare the temperature measurement of the FBG with standard measurement techniques. To avoid major interference between the instrument and the plasma source, the thermocouple was insulated with a dielectric; a heat shrink tube (polyolefin) was wrapped around the probing part of the thermocouple (Type K thermometer, EXTECH Instrument, US). The temperature was recorded every 15 seconds by

hand and its position was fixed and controlled via a XYZ positioning system (CNC Genmitsu 3018-PROVer, SainSmart, US) within the accuracy of $100\text{ }\mu\text{m}$.

4.4.5 COMSOL Simulation

The thermal energy dissipation presented in Figure 4.5 was simulated using COMSOL Multiphysics. The simulation was generated using the non-isothermal physics module (k- ϵ turbulent flow and heat transfer in fluids). Leveraging the inherent inner rotational symmetry of our plasma source, we configured the model to operate in a two-dimensional space. In this setup, we maintained a steady flow rate of argon at 2.5 SLM and established a constant plasma output temperature of $38\text{ }^{\circ}\text{C}$. This meticulously controlled simulation was executed to mimic the operation of the plasma source over the duration of 3 minutes. This extended runtime allowed us to reach a steady-state condition, where the system's parameters and behaviour stabilize, providing a comprehensive understanding of the plasma source's thermal performance over an extended period of time.

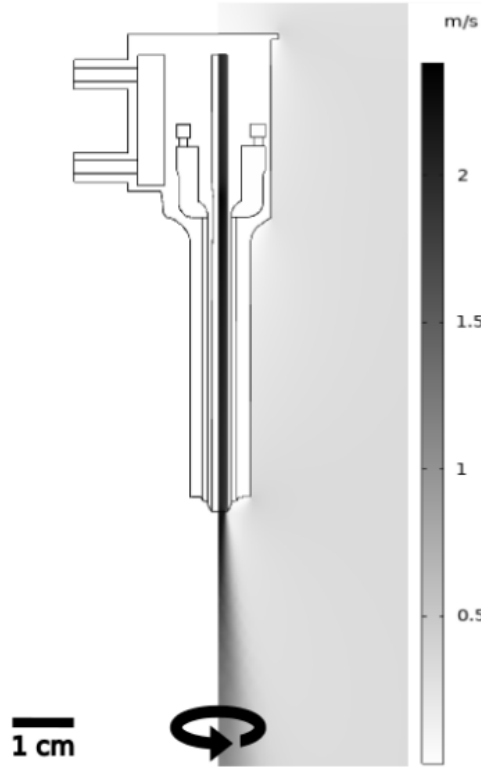


Figure 4.5 COMSOL simulation of thermal energy dissipation through convection and turbulent diffusion. The velocity of the feed gas is plotted on the right side.

4.5 Results and Discussion

4.5.1 Temporal Resolution

In Figure 4.6, we present a comparative analysis of the probe's responses to the temporal evolution of the gas temperature using both the FBG technique and the thermocouple. At a distance of 1 cm from the nozzle of the CAP-Jet, the overall temperature of the CAP-Jet saturates near 34 °C. Notably, when the plasma is initially ignited at time $t = 0$ s, two distinct heating phenomena become apparent. The first arises from the plasma's self-heating, leading to a rapid and discernible increase in the recorded temperature. The second phenomenon, associated with the probe's own heat inertia, contributes to the signal in an exponential fashion. The latter effect can be aptly described by Newton's Law of Cooling:

$$T(t) = T_f - (T_f - T_0) \exp(-t/\tau) \quad (4.4)$$

where T_f is the terminal temperature, T_0 the initial temperature and τ the response time of the probe. Similarly, once the plasma ceases at $t = 800$ s, the cooling process exhibits a comparable effect. This observation indicates that both temperature probes experience thermal inertia. Although both techniques exhibit a commendable degree of correlation, the thermocouple introduces significantly more uncertainties (± 0.75 K) compared to the FBG (± 0.1 K). It is important to note that this uncertainty primarily stems from the quality of the instrument employed.

Characteristic Time and Effective Heat Capacity

A fitting procedure on the data shown in Figure 4.6 offers us the means to determine the characteristic time constants inherent to the measurement probes. Remarkably, this procedure unveils a characteristic time of 70.7 seconds for the Fibre Bragg Grating (FBG) and 87.1 seconds for the thermocouple. These time constants encapsulate the instruments' temporal response to transient temperature variations, reflecting their thermal inertia which can lead us to a calorimetric analysis [128]:

$$\tau = R_e C_e \quad (4.5)$$

where R_e is the thermal resistance and C_e is the thermal inertia, also expressed by:

$$C_e = m_e c_p \quad (4.6)$$

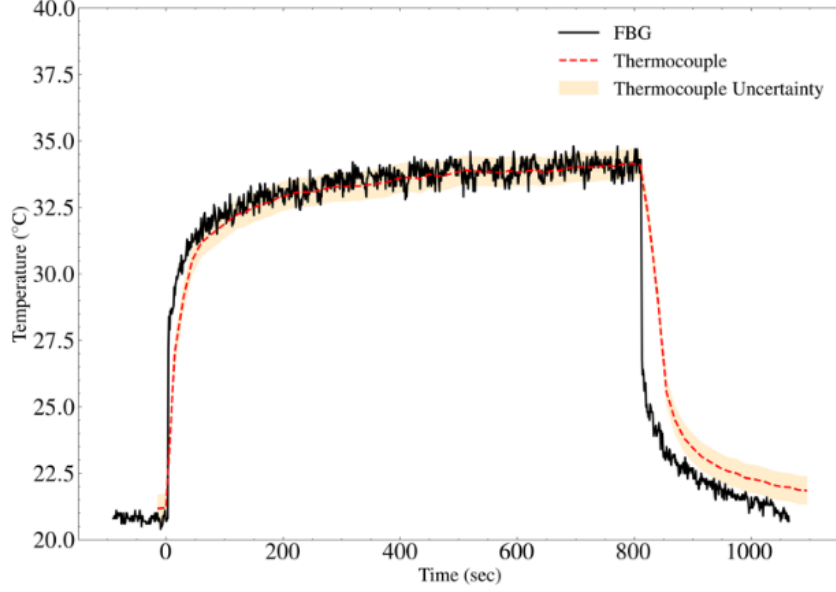


Figure 4.6 Comparative analysis of the temporal evolution of the gas temperature measured with FBG and a thermocouple.

where m_e is the mass of the probe and c_p its heat capacity. Similarly, the heat resistance can be obtained through the following equation:

$$R_e = \frac{L}{ka} \quad (4.7)$$

where L is the length perpendicular to the heat flow, k the heat conductivity and a the cross-section perpendicular to the heat flow. By combining both equation 4.6 and 4.7 into equation 4.5, we can obtain the following expression for the effective heat capacity:

$$c_p = \tau \frac{ka}{Lm_e} \quad (4.8)$$

For our K-type thermocouple, the thermal resistance can be approximated as a series of interconnected thermal resistors comprised of chromel, alumel, and insulator materials. Leveraging values from [129], we estimate an effective heat capacity of 3.812 J/K.

With these important parameters, we can undertake a succinct yet comprehensive calorimetric analysis. This methodology, inspired by the pioneering work of [130], enables us to assess the dissipation of heat power within the system. This critical calculation is formulated as:

$$P_{in} = c_p \left(\frac{dT_h}{dt} - \frac{dT_c}{dt} \right) \quad (4.9)$$

where P_{in} denotes the input heat power, c_p signifies the effective heat capacity, and T_h and T_c represent the temperature signals during both the heating and cooling phases of the thermocouple. Through meticulous analysis and a linear fit applied to the thermocouple's heating and cooling phases, we attain a heat power input value of 1.3 W (± 0.5 W). Similarly, with a specific heat capacity for the Fibre Bragg Grating rated at 680 J Kg⁻¹K⁻¹, we can estimate the value of 1.2 W (± 0.6 W), providing a similar value to our power input. The measurement uncertainty yields the error term associated with the covariance of the linear fit. The difference between both values can be explained by the heating of the insulator of the thermocouple that modifies its heat capacity during the measurement.

From these values, insights into the efficiency of the plasma source can be used to adapt treatments for better biological response while reducing the heat dissipation.

To assess the significance of our data, the overall power of our source needs to be quantified. By measuring both current and voltage as seen in Figure 4.7, we can get a rough estimate of the power input in the plasma source.

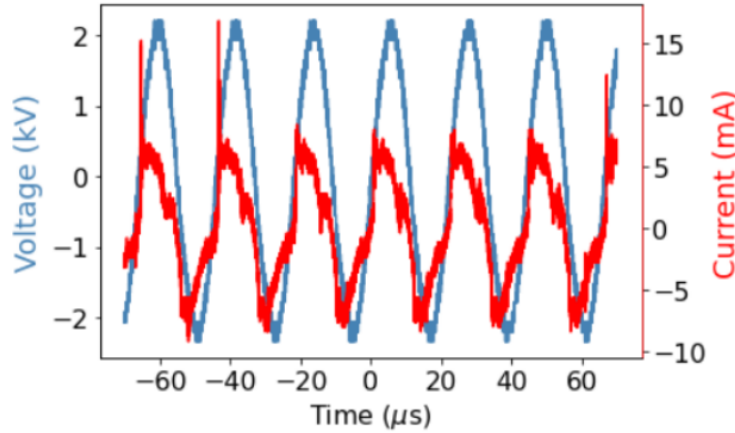


Figure 4.7 Current and voltage measurements of plasma source

By using the following relation, we can estimate the power input of our source.

$$P_{average} = V_{RMS} I_{RMS} e^{i\theta} \quad (4.10)$$

where the root mean square (RMS) of both the voltage V and the current I are multiplied together via a scalar product at an angle θ . For an angle estimated at 3 degrees through

the analysis of the time step maximizing correlation between both signals and a duty cycle of 40 %, we can estimate our total power to be roughly 2.61 W. This brief power analysis reveals that at least about 50 % (both for FBG and thermocouple measurements) of the total power input is dissipated into heat. Due to the approximated nature of the total power measurement, the determined percentage of the power dissipated into heat can only be taken as a minimum estimation.

4.5.2 Control Over the Temperature

To control the temperature, the energy input is modified by duty cycle control. As shown in Figure 4.8, it becomes considerably easier to monitor the temporal variations in three distinct energy input percentages applied to the plasma jet device. This set of measurements demonstrate the capability to effectively distinguishing between distinct thermal plasma conditions. In comparison to higher energetic conditions, e.g. 50 % of duty cycle, the temperature is drastically reduced at a substantially lower energy input scenarios, such as 10 % of the total power. Furthermore, our analysis reveals a fairly constant response time of the probe for the different conditions, with an uncertainty of around 4 seconds. This results express consistency of our probe over multiple conditions. These findings are in line with Newton's law of cooling as the characteristic time is independent of the thermal conditions.

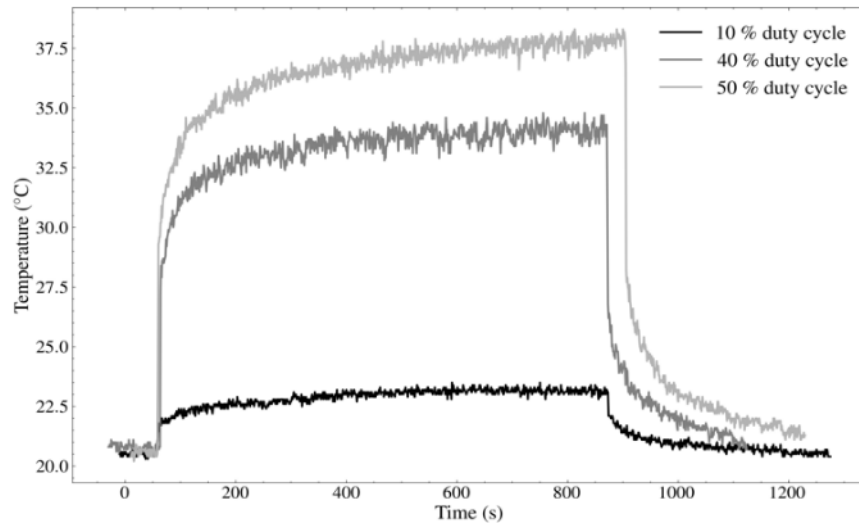


Figure 4.8 Temporal measurement of plasma jet gas temperature for different energy inputs using the FBG probe. The electric signal input is compared to duty cycle at 10 %, 40 % and 50 %.

4.5.3 Spatial Distribution and Temperature Measurement Correlation to FBG and Simulation

In Figure 4.9, a spatial distribution analysis of the plasma gas temperature is presented. This measurement provides essential insight into the temperature evolution and how it interacts with its environment. On the right side of the figure, a Schlieren imaging measurement provides 2D information on the gas temperature of the plasma jet, mirroring the results obtained via FBG and thermocouple measurements. As expected, the temperature obtained at 1 cm of distance consistently measures 35 ± 5 °C, reinforcing the reliability and repeatability of our temperature measurements. On the left side of the figure, a COMSOL simulation of a non-isothermal flow has been created as a tool to compare the Schlieren measurements to a simpler case of a heated gas flow for a fixed temperature. A strong correlation between the two sets of results is evident, extending at least 15 mm downstream the nozzle. Beyond this region, a noticeable divergence in the plasma plume's shape can be observed. Coincidentally, these regions of divergence correspond with the transition zones between laminar flow and turbulent regime, indicating the influence of flow dynamics on temperature distribution. These findings underscore the importance of considering flow characteristics when employing Schlieren imaging for temperature measurements, particularly in scenarios where the transitions from laminar to turbulent flow occurs, which introduces substantial deviations in the recorded data.

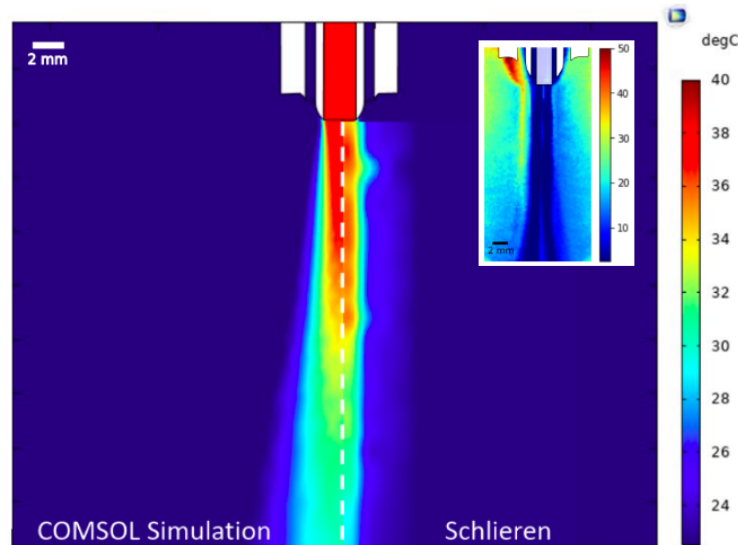


Figure 4.9 Spatial distribution of a CAP-Jet source with a simulation comparison. A study of the uncertainty is also presented on the top right of the image.

4.6 Conclusion

In this work, we used the thermo-optic properties of a fibre Bragg grating as a tool for measuring the gas temperature of a cold plasma jet through direct effluent interaction. We compared the FBG temperature measurements with thermocouple measurements (temporal) and Schlieren Imaging measurements (spatial). For a distance of 1 cm, set as a reference for biological treatments, the temperature measurement techniques show strong correlation at a value of 35 °C. This result confirms the tissue tolerable operating regime of our plasma jet. From the temporal resolution, a simple calorimetric analysis technique was used to provide information on the heat efficiency of the source. Overall, the ability to distinguish and manipulate these thermal trends allow for customization of plasma-based therapy, ultimately expanding the potential application of the technology.

CHAPTER 5 ARTICLE 2: COHERENT HOMODYNE DETECTION FOR AMPLIFIED CROSSED-BEAM ELECTRIC-FIELD INDUCED SECOND HARMONIC

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5.1 Preamble

This article has been submitted for publication in the peer-reviewed Optics Letters journal [131]. It is furthermore published in Optica Open [132]. The analysis, the majority of the writing, editing and an important part of data acquisition and experimental manipulations were made by myself. It acts as a thorough investigation of the electric-field nature of the plasma and present an important improvement on the directionality and spatial resolution of the well-known E-FISH technique. This article shifts our attention toward fundamental electric processes happening in the plasma that could impact strongly the chemical output of plasma sources.

5.2 Abstract

The Electric Field Induced Second Harmonic (E-FISH) signal sensing is commonly used as non-linear optical technique to probe electric fields inside a plasma region. Cross-beam E-

FISH is being investigated to improve spatial resolution by defining the interaction volume via a controlled geometry of two overlapping non-collinear optical beams. This drastic reduction in interaction length of electric field and laser region results in a significant signal reduction. To overcome this signal reduction, we introduce coherent amplification of cross-beam E-FISH signal by mixing the low E-FISH signal with a phase-locked bright local oscillator. We demonstrate enhancement of the signal. By introducing a local oscillator we can now derive the polarity of the measured electric field through the phase of the homodyne signal. To illustrate the technique, we, for the first time, measure magnitude and the direction of electric field in a cold atmospheric pressure plasma jet, which dynamically follows the profile of the applied bias current.

5.3 Introduction, fundamentals, and challenges

Electric field detection has long been source of important information in plasma science. From microfabrication [133, 134] to agriculture [135, 136] and medicine [137], the ability of cold plasma technology to generate different excitation processes by control over the reduced electric field (E/N), i.e. the electric field normalized to the number density of neutral species, has become highly relevant. With a deep understanding of the plasma kinetics involving fast ionization waves, tailoring of plasma reactivity could answer various needs in the domain [138].

The investigation of electric field dynamics in plasma requires non-perturbative and non-resonant techniques in order to get highly reliable information without modifying the excitational processes. By using the property of electric fields to induce asymmetries in the polarizability of otherwise centrosymmetric media, second harmonic light generated from the interaction of laser light with the non-linear optical medium, so-called Electric Field Induced Second Harmonics (E-FISH), can provide electric field information in any type of gas mixture [139]. The technique can thus be adapted as a tool for electric-field diagnostic in plasma [140, 141]. This third order nonlinear optic process exploits hyperpolarizability properties of a medium to create a quadratic dependency of the intensity of the generated second harmonic signal on the applied electric field amplitude, thus enabling high-resolution probing over a large range of types of plasma. The underlying process can be described by [139, 142]:

$$P_i^{2\omega} = \frac{3}{2} N \chi_{i,j,k,l}^{(3)}(-2\omega, 0, \omega, \omega) E_j^{(F)} E_k^{(\omega)} E_l^{(\omega)} \quad (5.1)$$

where $P_i^{2\omega}$ is the induced polarization at frequency 2ω , N is the number density of the gas, $\chi_{i,j,k,l}^{(3)}(-2\omega, 0, \omega, \omega)$ is the nonlinear susceptibility, $E_j^{(F)}$ and $E_{k,l}^{(\omega)}$ are amplitudes of the applied

electric field and the electric field of the laser beam(s) at frequency ω , respectively.

While E-FISH provides important information on the electrical state, the technique still faces major challenges: the quadratic dependency on the electrical field limits both sensing small values of electric fields and probing into low spatial dimensions. The electric field signal assumed to originate from the Rayleigh range is highly dependent on the global distribution of the electric field along the laser beam path [139,143]. An estimation of the Rayleigh length is given by:

$$z_R = \frac{\pi w_0^2}{\lambda}, \quad (5.2)$$

where z_R is limited by the wavelength λ of the beam and the squared radius of the minimum beam waist w_0 . For common reported parameters of E-FISH diagnostics, involving a beam waist of 65 microns at a wavelength of 800 nm, the Rayleigh range is approximately 1.65 cm. Approaches to increase the spatial resolution of E-FISH by generating second harmonic light from two crossed laser beams come at a steep cost of dramatically reduced signal intensity [144, 145]. Typical beam crossing angles of 0.5° largely precludes the increase in spatial resolution [146]. Motivated by these challenges, our solution is to amplify the second harmonic signal from the cross-beam E-FISH with a local oscillator. In our previous work using single beam configuration, we demonstrated homodyne amplification method to give linear, instead of quadratic, sensitivity to the field, resulting in an improved signal-to-noise ratio (SNR) by a factor of 7 [147]. In this work we use two advantages of local oscillator E-FISH: 1) signal amplification provides access to a smaller interaction length and hence better spatial resolution and 2) the linear dependency of the amplified second harmonic light enables measuring the directionality of the electric field. The amplification of the signal compensates for the reduced efficiency when increasing the angle of the fundamental beams, allowing us to obtain an estimated factor of 5 improvement in comparison to other experiments. The signal measured with our technique of amplified cross-beam E-FISH (ACE-FISH) can be described mathematically by:

$$S_{ACE-FISH} \propto E_{LO}^2 + E_{E-FISH}^2 + 2E_{LO}E_{E-FISH} \cos(\phi), \quad (5.3)$$

where the signal S is a result of interference of the electric field E_{LO} of the local oscillator and E-FISH field, and ϕ is the angle between their polarizations. From Eq. 5.3, two important observations can be made. Firstly, the overall signal is directly impacted by the sign of the applied electric field, where E-field polarized parallel/antiparallel to E_{LO} results in maximal positive/negative signal, while orthogonal case of $\phi = 90$ yields no amplification.

Secondly, low amplitude of E_{E-FISH} is amplified by a large amplitude of E_{LO} , which can be experimentally increased up to the point of detector saturation. In the context of small applied electric fields, linear scaling of ACE-FISH improves sensitivity in comparison to a vanishing sensitivity of the quadratic scaling in a conventional E-FISH [147]. By adjusting $E_{LO} \gg E_{E-FISH}$ and subtracting the constant offset E_{LO}^2 from S in Eq. 5.3, we therefore isolate our signal to be proportional only to the amplified E_{E-FISH} and the angle ϕ . From this reasoning, the variation of the polarity of the signal can be directly translated to a directionality of the applied electric field. Starting with this approach, we address in this Letter, the problem of lack of electric field polarization sensitivity in cross-beam E-FISH technique through the ACE-FISH method.

5.4 Setup

The configuration for ACE-FISH setup is presented in Figure 5.1.

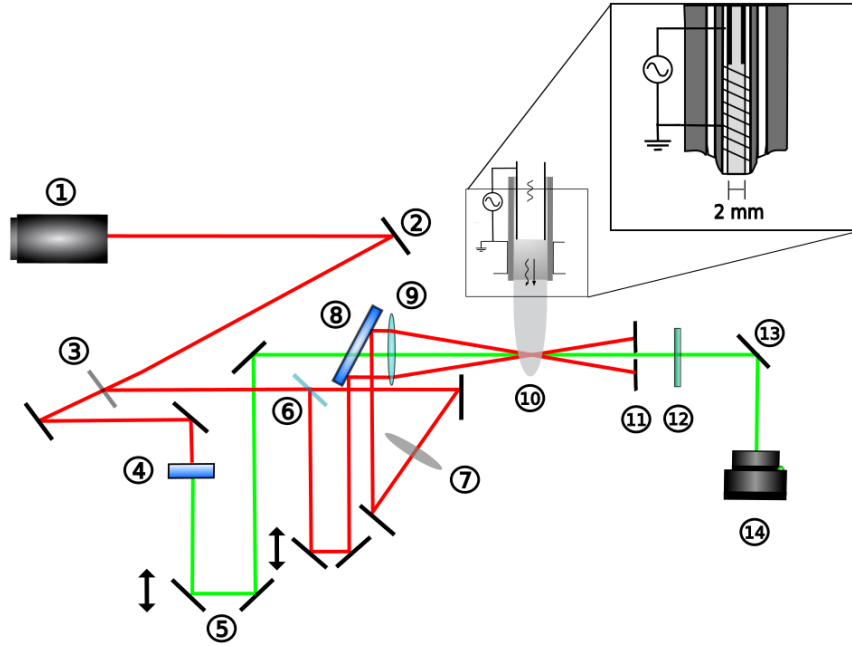


Figure 5.1 **Amplified Cross-Beam E-FISH Setup:** 1. Ti:Sapphire-based femtosecond optical amplifier (Solstice Ace), 2. Mirror, 3. 97.5 % Reflective Dielectric Mirror, 4. BBO crystal, thickness $200 \mu\text{m}$, Type-I phase-matching for local oscillator signal generation, 5. Translation stage, 6. 50/50 Beam splitter, 7. Variable ND filter, 8. Dielectric mirror at 800 nm , 9. Lens ($f = 50 \text{ cm}$), 10. Cold atmospheric pressure plasma jet, 11. Iris, 12. 405 nm filter (10 nm of bandwidth) and 13. 400 nm HR mirror, 14. Hamamatsu CCD camera with intensifier/Scientific CMOS camera

As a light source, we used the Solstice Ace femtosecond amplified Ti:Sapphire laser system at

a repetition rate of 333 Hz, wavelength of 800 nm, with a pulse width of 50 fs and pulse energy of up to 6 mJ. The local oscillator was generated from a small fraction of the fundamental beam which was sampled and directed into a BBO crystal to provide seed beam at the same frequency as the cross-beam E-FISH signal. To create both branches of the fundamental cross-beam geometry, a 50/50 beam splitter was used and a variable ND filter wheel was chosen on the higher-powered branch to perfectly match the power output of the other twin branch. This process is crucial as it maximizes the second harmonic generation along the bisector between the cross-beams. The beams were crossed at an angle of 1.7° via either a spherical convex lens with a focal length of 50 cm during the characterization phase of the polarization and later changed to a cylindrical convex lens of 40 cm of focal length during plasma experiments. The temporal overlap of all three branches was insured by delay stages inserted in two of the beam lines. The second harmonic generated by each of pump beams was eliminated by using an iris, allowing only the E-FISH signal produced by both beams to propagate towards the acquisition part of the optical system. In order to keep only the second harmonic signal into the optical system, the residual fundamental frequency was filtered by a bandpass filter with a bandwidth of 10 nm centered around 405 nm. To acquire the images, an ICCD (Figure 5.2 and Figure 5.4) and a sCMOS (Figure 5.3) camera were used depending on the intensity of our local oscillator.

For the reference electric field, we used a high-voltage DC power supply (ranging from 0 to 3 kV) to create an electric field between two rounded metal plates of 2 and 3 inches respectively separated by about 5 mm. For the calibration curve, the voltage was monitored by a CT4028 voltage probe (Cal Test Electronics, US). The plasma source used in this experiment is a homemade coaxial dielectric barrier discharge plasma jet with a cylindrical inner electrode and a dielectric tubing similar to the one shown in [101, 148, 149]. In our setup, a set of stainless-steel tube connected to a high voltage source and a grounded copper coil were separated by a borosilicate glass tube acting as a dielectric barrier. The cold atmospheric pressure plasma jet (CAP-Jet) uses an argon gas fed into the inner electrode tube at 2 standard litres per minute. The plasma operates at a voltage of up to 3 kV_{p-p} . The plasma jet's operating frequency is 22 kHz. The electrical field measurements were made at a distance of 5 mm from the exit nozzle of the jet. To monitor the current, the voltage drop over a 2Ω resistor between the anode and the ground was measured. The value of the resistor is assumed to be small enough not to create perturbations into the plasma. The timing adjustment between laser and the camera gate was made by a pulse/delay generator (DG535, Stanford Research System) synchronized with the laser system.

5.5 Results

To assess the performance of the ACE-FISH technique in comparison to the cross-beam E-FISH method, we measured a standard calibration curve (depicted in Figure 5.2) at known electric field values.

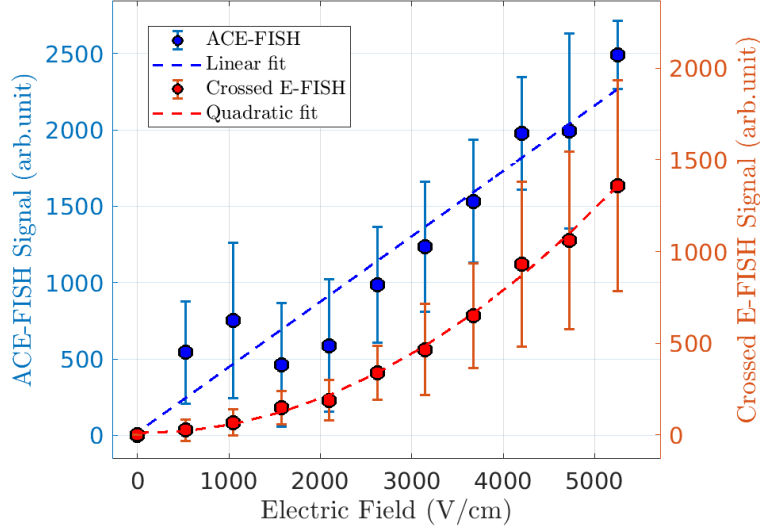


Figure 5.2 Comparison of the signals between ACE-FISH and cross-beam E-FISH as a function of the applied electric field. Linear behaviour from the ACE-FISH signal and quadratic trend from the E-FISH signal are observed.

We collected each data point from the sum of each pixel position from pixels with a signal greater than a specific threshold for the highest electric field value. This approach allowed us to monitor the amplified pixels consistently throughout the variation in electric field amplitude. Each image was acquired in triplicates with the same optical setup, using an on-chip average over 256 images and an exposure time of 50 μ s. The standard deviation is represented by the error bars. To establish our baseline, we corrected both signals by subtracting their respective signals obtained at zero-field conditions. The instabilities of the linear trend in the ACE-FISH signal are thought to be affected by a $\chi^{(2)}$ type of parasitic effect due to the high photoionization yield present at the focal point.

To prove the sensitivity of our system to the direction of the electric field, an interferometric pattern (as seen in Figure 5.3) was characterized by two opposing electric field directions under a DC applied voltage of 2000 V (equivalent to 4200 V/cm).

The acquired signal was averaged over 256 pictures for three different sets at 20 μ s exposition time. To obtain the raw images (cropped to the region of interest) of the interference pattern of our beam, the signal of the local oscillator was subtracted from the ACE-FISH signal. The

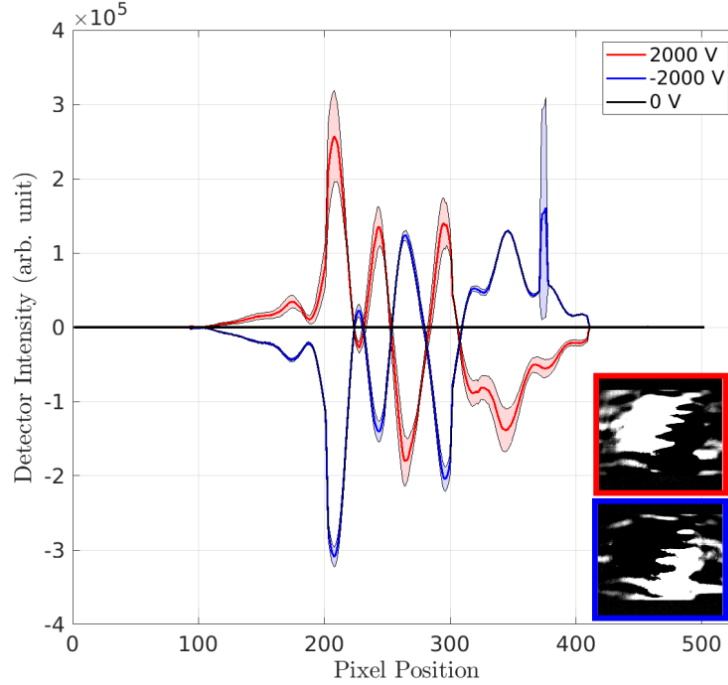


Figure 5.3 Interferometric pattern created by the ACE-FISH method for positive and negative polarization between electrodes. The raw images of the ACE-FISH signal beam are shown in inset (bottom right).

curves represent a vertical sum of the intensity of all pixels, and the uncertainty depicted as a pale area around the curves represents the standard deviation across the three sets of summed data. The direction of the electric field was set to be parallel with the polarization of both the fundamental beam and the local oscillator.

Finally, we demonstrate our ability to measure the time resolved electric field of a cold plasma jet. For this, we performed time resolved single shot ACE-FISH measurements in the effluent of the plasma jet (see Figure 5.4). Single shot measurements were achieved through a random sampling triggered by the laser pulse. By systematically monitoring both the current and the electric field of the plasma source at the same time, it is possible in post-processing to rearrange the data in a time sequence as shown in Figure 5.4.

The data was divided into 18 distinct bins that cover the entire period of the electrical signal. The bins were selected to be equidistant in time, yielding a bin-duration of $1.7 \mu\text{s}$. The signals in each bin have all been averaged to attain the final signal. The uncertainty corresponds to the standard deviation of the images contained in each bin. The plasma signal was converted to an electric field value using a calibration determined in the same way as the one presented previously in Figure 5.2. In order to retrieve a similar zero-field value

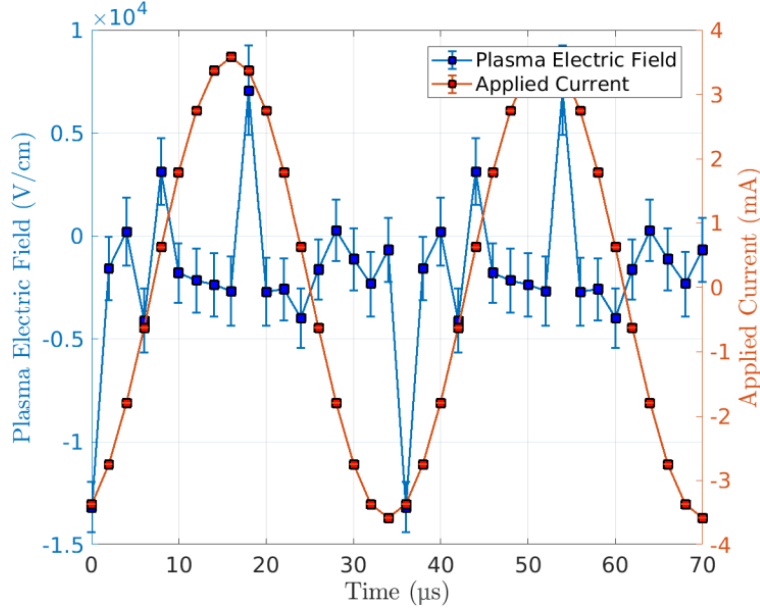


Figure 5.4 Monitoring of a cold atmospheric pressure plasma jet electric field dynamic and its applied current. The signal was treated using a binning technique with bins of $1.7 \mu\text{s}$ long. A equivalent calibration curve was made to convert intensity signals into electric field values.

to the other experiments, the data was subtracted by the means of each signal intersecting at a current equal to zero. While cylindrical focusing was used instead if spherical for this experiment, the weak nature of our signal forced us to consider the entire acquired signal instead of using it as a tool for 1D spatial resolving.

5.6 Discussion

The main result of this series of experiments is our ability to efficiently sense the polarization of small electric field in a cross-beam type laser setup. This optical amplification process is very important since, as stated in [147], it enables the usage of less powerful and more affordable laser, allowing for high repetition rate measurements, but also enabling us to measure at reduced interaction length between laser and electric field in the cross-beam configuration to enhance the spatial resolution of the region of interest.

Considering that in our demonstration experiment, our current angle between the two fundamental beam is only 1.7° , an amplification of the data means that we could be able to investigate the cross-beam technique into larger angles, thus increasing our spatial resolution. From the Rayleigh length of the initial laser focus of 1.65 cm, and an interaction length of 3 mm in the cross-beam configuration, we have improved the spatial resolution by a factor of

5. While still limited in terms of noise due to photoionization at the focal point as seen in Figure 5.2, the Figure 5.3 demonstrates a highly promising pathway in terms of amplification and electric field directionality sensitivity. By measuring the signal-to-noise ratio for both signals, we are able to estimate a maximum value of around 220 of SNR. Considering the approximation that the minimum signal-to-noise ratio detectable is at a value of 1, one could potentially measure time- and polarity-resolved electric field values as low as 19 V/cm. The amplification by the local oscillator can also be translated into an increase in the accessibility of the cross-beam E-FISH system. Due to the bright LO beam in the ACE-FISH technique, affordable acquisition systems such as CMOS cameras can be sensitive enough to detect the E-FISH signal. In terms of electric field directionality sensitivity, the interferometric pattern clearly shows a mirroring of both curves which can be directly linked to a phase change during the variation of the electric field directionality. In our case, we are able to identify a strong negative electric field (-13.1 kV/cm) and a weaker positive signal (7.2 kV/cm).

While the ACE-FISH technique shows highly promising results, this technique has some drawbacks that still need to be taken into account. As previously stated, we could potentially improve the E-FISH signal by indefinitely increasing the local oscillator intensity. However, to make sure that we are under interference conditions, it should not saturate the E-FISH signal. Furthermore, the presence of laser-induced ionization and plasma filament formation influences the measurement system overall which would make use of MHz laser a preferable choice for future experiments, since they have low laser pulse energy and high statistics through high repetition rate. Overall, the ability of ACE-FISH to offer superior and more sensitive data opens up new exciting possibilities that have remained largely uncharted.

CHAPTER 6 CHEMISTRY OF PLASMA-HYDROGEL INTERACTIONS

In this chapter, I will cover analysis of relevant long-lived chemical species present in plasma-treated hydrogels. Typically, *in vitro* treatment represents the first step toward clinical applications as it is an accessible way to gather important data at the cellular level. In comparison to *in vivo* or *in ovo* treatment, 2D *in vitro* treatments relies on a simple model to isolate cellular mechanisms. With the use of 3D *in vitro* modelling enabled by bioprinting technology, it is possible to consistently reproduce more complex interactions such as presence of oxygen, nutrient gradients, cell-to-cell interactions and drug penetration [150], thus reducing the gap between *in vitro* and *in vivo* studies [151,152]. For plasma-hydrogel interactions, the intricate mechanism of reactive species production pathways can be studied to understand precise biological response. The first step toward chemical analysis usually aims to measure long-lived species as their measurement technique are more accessible than their short-lived counterparts. However, the measurement of long-lived species can still provide some insights into the internal reaction mechanism happening inside the 3D bioprinted model.

6.1 Measurement of pH

In general, pH decrease in bodily fluid, or acidosis, can affect epigenetics of tumours and RNA processing, which ultimately helps it escape immune defence systems and promote tumour proliferation [153, 154]. For RONS production, a decrease in pH enhance the quantity of protons (H^+) in a material, which enhance the overall quantity of RONS. The analysis of pH in hydrogels is thus helpful in characterizing the effect at the cellular level, but also provide crucial information on the conditions in which the reactive species are produced.

For pH measurements, the methodology was based on the work of Busco *et al.* [155]. By linking the degradation of fluorescein to pH decrease, it is possible to create a calibration curve correlating fluorescence intensity to its pH. Given the pH sensitivity of fluorescein and its established usage in angiography [156], it has proven to be a reliable choice for tissue-related applications. To image fluorescence (as seen at Figure 6.1), a 25mW, 405nm Violet Alignment Laser was used as an excitation source. The resulting emitted fluorescence was acquired via a set of a CMOS camera (Allied Vision Alvium 1800 U-240m, Edmund Optics, USA) and a 475 nm longpass optical filter (Edmund Optics, USA).

The pH measurements were made with sodium fluorescein (Sigma-Aldrich, USA) and calibrated with a pH probe (LabSen 241-6 Glass-body pH Electrode, Apera) and acquisition

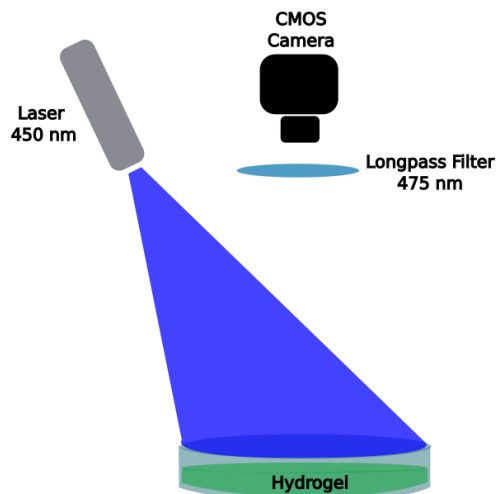


Figure 6.1 Fluorescence imaging system for fluorescein-doped hydrogel

system (Mi 150, Martini Instruments). A solution of hydrogel (1 % alginate, 7 % gelatin) mixed at 6 different concentrations with HCl (Sigma-Aldrich, USA) was used to create the calibration curve as seen at Figure 6.2. The experimental parameters for direct plasma treatment include a 1 cm distance, a gas flow rate of 2 SLM of argon and a 40 % duty cycle.

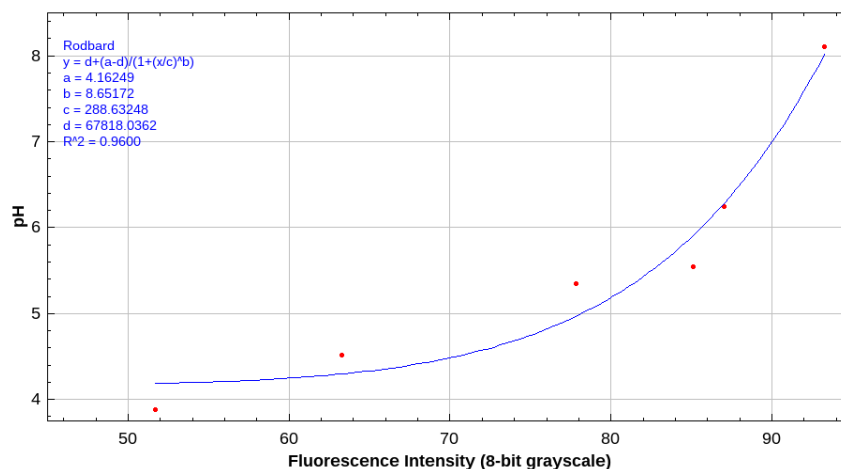


Figure 6.2 Calibration curve of fluorescence decrease of pH-sensitive fluorophore. The fit was made with ImageJ with the Rodbard function.

From the results obtained at Figure 6.3, the degradation of fluorescein resulting from the anticipated pH decrease is clearly observable. Similar to the literature [155], the scale has been modified to reduce the noise produced by the inhomogeneity of the hydrogel surface. For an increase time of plasma treatment, two main observations can be made. Firstly, and

as expected, the longer the time of treatment is, the lower the pH gets. The distance of interaction seems to follow a radial diffusive pattern limited to a diameter of around 9.25 mm. The second observation can be made on the direct interaction zone with the plasma. For extensive time of treatment, it appears that the plasma has a desiccating effect on the hydrogel, causing opacification in the treated zone. This opacification hinders our ability to obtain accurate fluorescent measurements. More tests still need to be done in order to characterize well the effect of prolonged treatment on hydrogel.

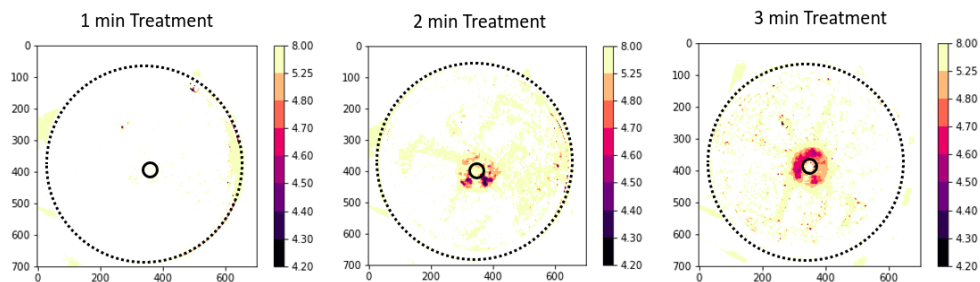


Figure 6.3 Spatial comparison of plasma-induced pH for different plasma treatment time

One way to be more representative of clinical application and to prevent drying of the hydrogel is to add motion to the plasma treatment. With the help of a CNC, it is possible to design a precise pattern of treatment at a fixed velocity.

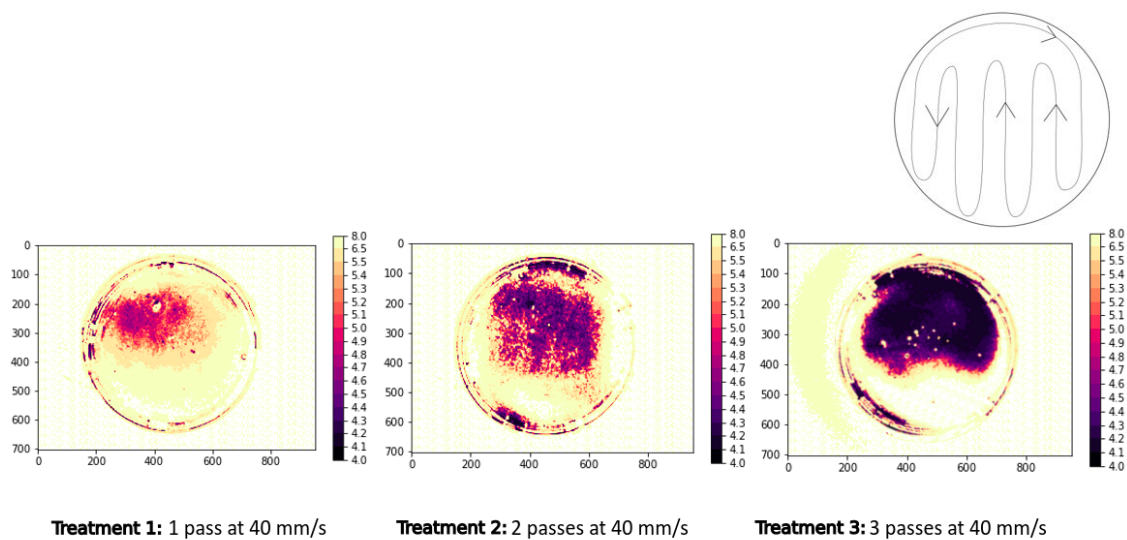


Figure 6.4 Study of the spatial distribution plasma-induced pH for different passes of a pattern at fixed velocity

The results presented in Figure 6.4 can show clearly the effect on the pH decrease for multiple

passes over the same pattern. Similar to Figure 6.3, it is possible to observe a strong decrease in pH value as a function of the amount of time the plasma goes over a region. With a meandering path design, a zone of uniform pH distribution can also be observed, which could be very useful information when using the plasma source for treating a larger surface area.

Another relevant parameter to study for clinical applications is the effect of different interfaces during plasma treatment of tissue models. More precisely, the Figure 6.5 shows the effect of adding a liquid layer of media on top of hydrogels during plasma-induced pH decrease.

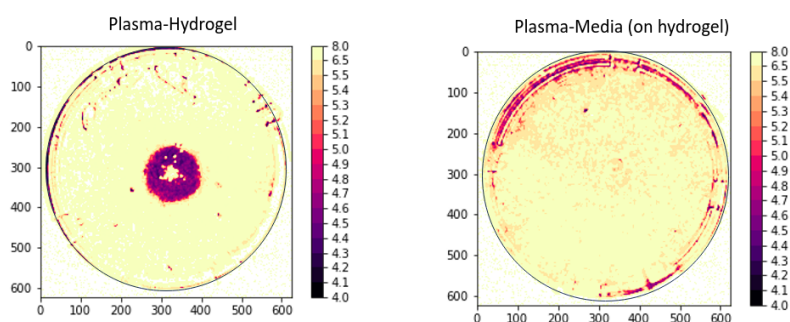


Figure 6.5 Comparison of plasma-induced pH decrease for different interfaces at 5 minutes of treatment

For a layer of only 2 mm in height, a noticeable discrepancy in pH between the two interfaces is evident. The area of low pH seems to have completely vanished, leaving only a small global reduction of the pH. This intriguing observation underscores strongly the effect of liquid layers, such as blood, on the distribution of reactive species. For clinical applications, this result could be an indication that plasma treatment interact highly at the interface and that its penetration capacity is fairly limited.

6.2 Nitrite

Given the abundant presence of nitrogen and oxygen in atmospheric conditions, nitrite (NO_2^-) serves as a crucial and long-lived species produced by plasma, making it essential to characterize. A precise technique for assessing the presence of nitrite in a hydrogel involves subjecting it to chemical scavengers. One highly specific method is the analysis of the hydrogel absorbance when it interacts with a Griess reagent, resulting in the formation of a diazo dye with a strong absorbance at 540 nm.

For the preparation of the nitrite detection reagent (Griess reagent), sulfanilamide (Sigma-Aldrich, USA), N-(1-naphthyl)ethylenediamine dihydrochloride (Sigma-Aldrich, USA) and

phosphoric acid 10 % (Sigma-Aldrich, USA) were used. Calibration curves were made with sodium nitrite solution (NaNO_2 , Sigma-Aldrich, USA). Nitrite measurements were carried out by exposing a solution of Griess reagent onto the surface of hydrogel treated by plasma (factor of 1:2, 200 μL of hydrogel) with an incubation time of 15 minutes. The 96 well-plate was then placed in a spectrophotometer (Infinite® 200 PRO, TECAN) for absorption measurement at 540 nm. The calibration curve is presented in Figure 6.6.

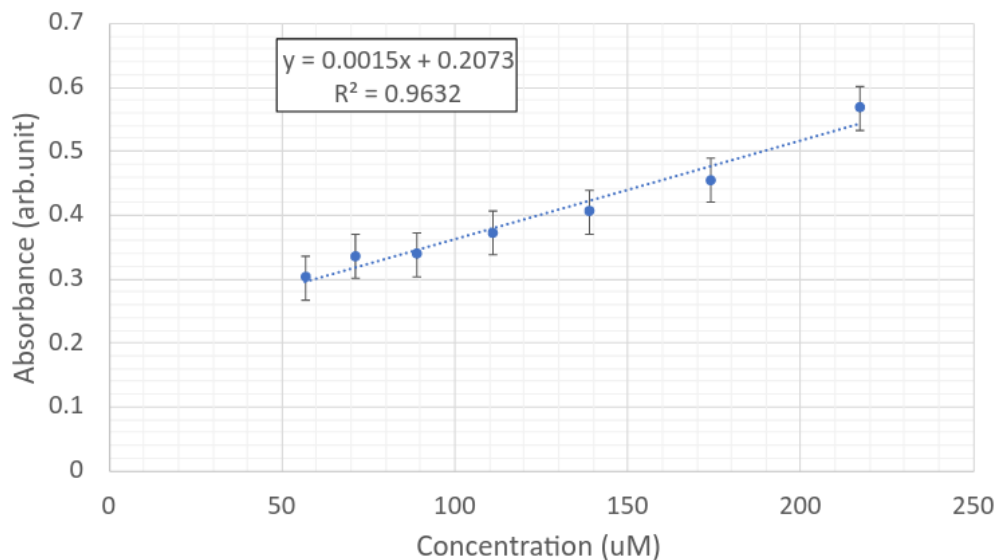


Figure 6.6 Calibration curve of known nitrite concentration as a function of its absorbance

With a high correlation coefficient, it is possible to translate linearly values from absorbance to concentration. This technique enables a parametric study to observe different effects of plasma parameters on its capability to produce nitrite in hydrogels. Figure 6.7 showcase the effect of duty cycle, time and distance of treatment on the production of nitrite. The uncertainty yields the standard deviation of a triplicate experiment.

As we can observe, it becomes apparent that over half of the nitrite production occurs in less than 15 seconds. Additionally, the production curve exhibits a non-linear behaviour, resembling more of an inverse exponential trend as the nitrite concentration tends to saturate rapidly for increased distance or energy input (duty cycle). For reduced distance and increased energy, the overall trend does not appear to be saturating as quickly as the other experimental conditions, which highlights the intricate dynamic behaviour of nitrite production mechanisms.

In concert with the pH measurements, a 2D spatial distribution study of nitrite in hydrogel can also be done. By using a Petri dish in 5 cm of diameter, 4 mL of hydrogel and 3 mL of

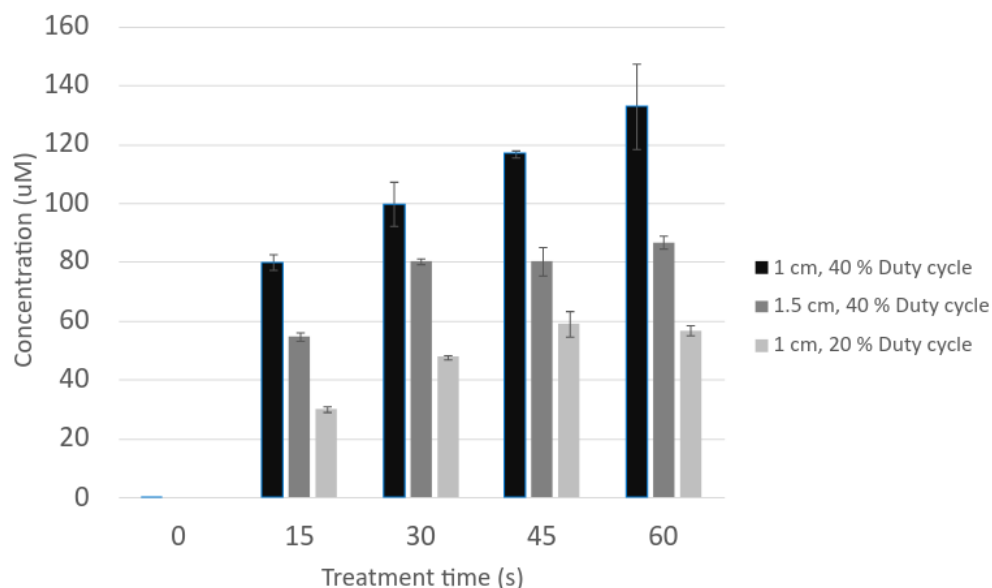


Figure 6.7 Parametric study of nitrite concentration as a function of time of treatment, distance of treatment and duty cycle

Griess reagent, a similar pattern to the pH distribution can be observed at Figure 6.8.

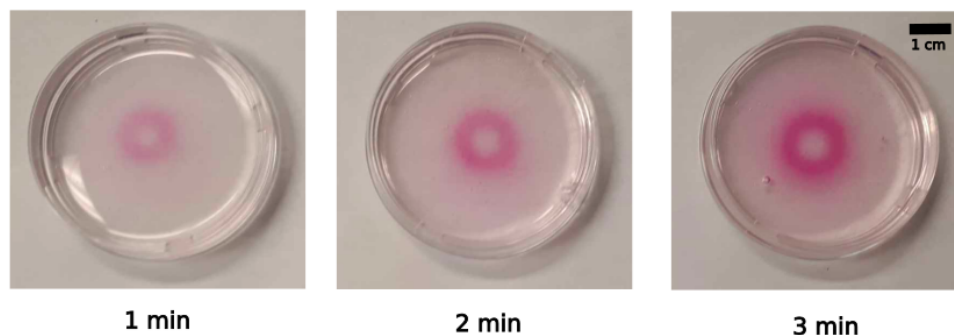


Figure 6.8 Spatial distribution of nitrite in hydrogel

For the spatial distribution, the surface radius of nitrite propagation seems to be consistent as a function of time of treatment. The donut-shaped region where nitrite seems to be produced can be estimated to be ID: 5 mm and OD: 20 mm and the overall shape seem to be consistent to what has already been observed in literature [157]. From a qualitative perspective, the region directly treated with plasma appears not to be generating significant amounts of nitrite. This observation could potentially be attributed to the phenomenon of electrophoresis happening in the gel due to the high electric field of the plasma source [158]. Since nitrite are charged molecules, strong electric field could influence the motion of usual

diffusive processes happening in the gel into a more radial pattern. This idea can also be linked to the asymmetric electric field measurements made in Chapter 5 on the plasma source, which shows that negative electric field are stronger than the positive ones. This results could be correlate to the reason why positive ion species, such as H^+ , have a lower inner radius than the negative ion species, like NO_2^- .

6.3 Nitrate

Similar to nitrite, nitrate (NO_3^-) is an important subproduct of plasma interacting with air. It can also be detected through colorimetric assays using chemical scavengers. In the case of nitrate, specific enzymes like nitrate reductase are employed to convert nitrate into nitrite. This enzymatic conversion facilitates detection using the same technique as outlined in Section 6.2.

For the conversion of nitrate to nitrite, a Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical, US) was employed. The nitrate measurements involved the addition of nitrate reductase enzymes (10 μ L) and nitrate reductase cofactors (10 μ L) to a volume of 200 μ L of crosslinked hydrogel (1% alginate, 7% gelatin), followed by incubation for 1 hour. Subsequently, Griess reagent (100 μ L) was applied and allowed to incubate for 15 minutes before measuring absorbance using a spectrophotometer at 540 nm. The calibration curve presented in Figure 6.9 was made using known concentrations of sodium nitrate ($NaNO_3$) mixed with hydrogel.

From the linear behaviour, it is now possible to estimate the concentration as function of once again the time of treatment, distance of treatment and duty cycle. It is important to note that a subtraction of known nitrite concentration with Griess reagent absorbance measurements at exact same conditions were made. The results can be seen in Figure 6.10.

Despite the high uncertainties in the data (represented by the standard deviation of a triplicate of the experiment) due to being close to the limit of detection, trends can still be discernable. Surprisingly, the preliminary results show an increase in nitrate production for more distant treatment, which might be explained by the longer interaction length between the plasma and its environment. In contrast to the nitrite measurements, it is also possible to observe that lower nitrate concentration seems to be linked to an increase in power or decrease in distance of treatment. An increase of enzyme incubation time could help lead to reduced threshold of detection and overall uncertainties.

In conclusion, the identification of chemical production response to plasma parameters change

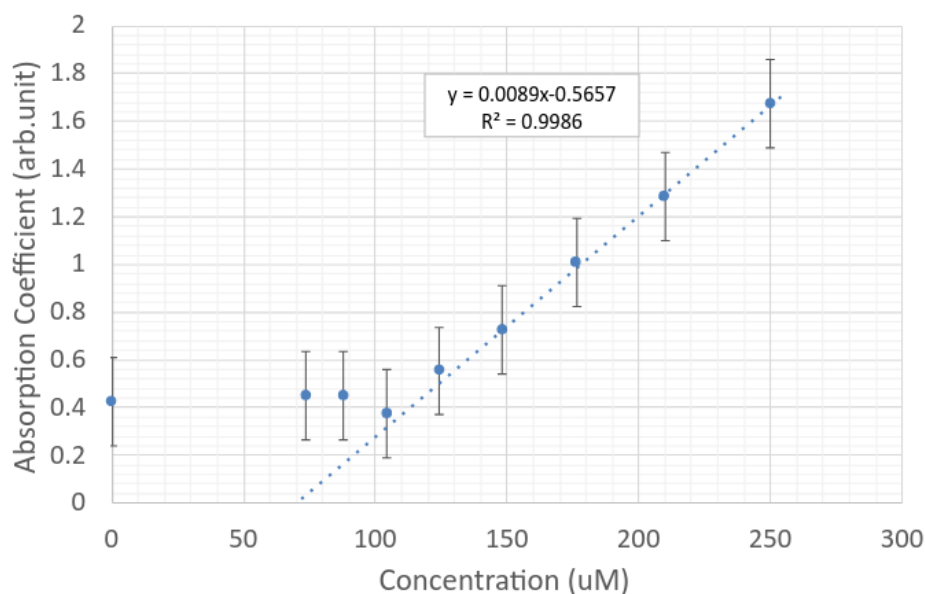


Figure 6.9 Calibration curve of known nitrate concentration as a function of its absorbance. The three first points ignored can be attributable to saturation indicating the threshold of detection.

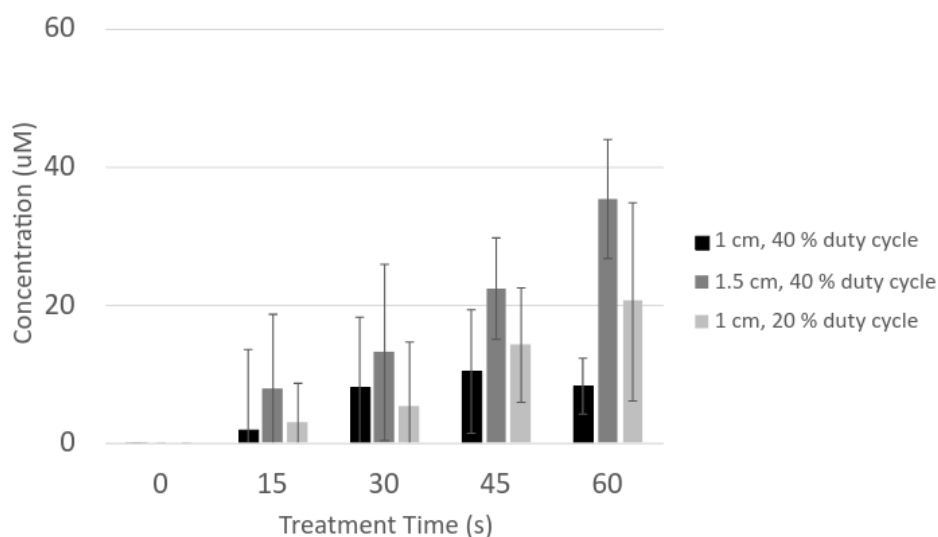


Figure 6.10 Preliminary results of nitrate detection in hydrogel for different conditions

can help us understand more their inherent relation and lead to appropriate dosage. Especially for selective treatment, the type of surface treated and treatment operational mode must be well characterized and tailored to ensure the maximum efficiency.

CHAPTER 7 GENERAL DISCUSSION

Each chapter presented in this thesis has made remarkable improvements toward my research objectives. This chapter will outline all the impacts associated with the objectives and will discuss more in detail their respective contribution.

7.1 Outcome of the Thesis

With the first objective of this thesis to create a biologically safe plasma source, Chapter 3 and 4 were able to show in detail the many steps taken to construct and characterize thermally and electrically the properties of the plasma source. By building a cold plasma jet respecting the electrical safety demands of the International Electrotechnical Commission through a limited patient leakage current of less than $150\ \mu\text{A}$ and safe temperature mode of operation of $35\ ^\circ\text{C}$, the plasma source was proven to efficiently interact with biological material. The success of these objectives not only enabled me to create reliable bio-plasma experiments, but also to set up a robust tool for physicists and biologists to use. Furthermore, with an article comparing a new gas temperature detection method of plasma jets with established methods, I was also able to showcase reliably the safety of the temperature produced by the plasma source. The FBG technique offers another alternative to previous less accurate technique of gas temperature measurement.

Chapter 5 reflect well on the second objective of characterization of electrical amplitude and directionality of the plasma jet as it demonstrates the ability of an improved technique of electric-field measurement to detect accurately polarization changes. The ability to image the electrical dynamic of the plasma in a crossed-beam disposition also greatly enhance the ability to spatially resolve our plasma sources. This significative advancement made with the E-FISH technique enables a whole community of cold plasma scientists to learn more precisely the dynamic behaviour of their plasma source, which would lead to new research in the effect of polarization effect on plasma chemistry and better tailoring possibilities for personalized medicine. While some improvements on the technique still need to be made to perfect it, the proof-of-concept delivered by my co-authors and I reflect a new avenue for profound understanding of plasma behaviour.

For the final objective on the creation of tools enabling probing the chemical nature of plasma-hydrogel interaction, Chapter 6 has shown significative promises in terms of imaging pH and nitrite concentrations as well as characterizing the nitrate production. The ability to

identify trends occurring for different plasma parameters offers a very interesting opportunity to correlate chemical production pathways to biological response. This ability will enable the implementation of better and more appropriate treatment procedures that could respond to different interfaces and cell types, as well as optimizing the overall efficiency of the plasma treatment.

CHAPTER 8 CONCLUSION

8.1 Summary of Works

The work described in this thesis proposes the implementation of a cold atmospheric pressure plasma jet for biological applications to answer the research question: **How can we build a fully tailored plasma source suitable for clinical applications of cancer treatment and ensure its maximum treatment efficiency?** Through the design choices, electrical characterization and temperature diagnostics I was able to showcase how we can adapt a plasma source to interact safely with tissues while still providing sufficient energy to induce precise and reproducible chemical output. With a new gas temperature diagnostic of the plasma, the measurements offer a reliable approach for assessing the thermal safety. In terms of maximum treatment efficiency, this work also presents an improved type of characterization for the electric field, using the ACE-FISH technique, which inherently offers more accurate information spatially and in terms of the directionality of the field. This information can provide better knowledge over the chemical pathways induced during the plasma dynamic behaviour. Finally, this work showcase a few important tools for characterizing long-lived chemical species in hydrogel models, such as pH, nitrite and nitrate. This could help bridge the gap between understanding plasma physical processes and accurate biological response, and therefore enabling the tailoring possibilities for enhance treatment.

8.2 Limitations

While this work proposes many important tools for plasma characterization, some improvements still need to be made. Firstly, in terms of temperature diagnostic, it is important to note that the plasma state is not constant when interacting with a surface since it was experimentally observe to behave differently through the conductivity of the material and the distance of treatment. The temperature diagnostics made in this thesis only presented ways to measure the plasma gas temperature without any surface interactions, thus providing limited knowledge over the temperature evolution during treatment with a surface. A second limitation in this thesis, more closely linked to the electric field diagnostic, is toward the instability of the calibration curve of the ACE-FISH technique due to high photoionization yield. This instability prevents the technique to precisely convert the raw intensity values into electric field values and limit the ability of the local oscillator to showcase amplification of the SNR. Further steps can also be done to improve the single-shot cylindrical focussing as

a way to obtain a 1D spatial distribution of the electric field. This technique could be applied for more thorough studies on the dynamic behaviour of the electric field, after plasma breakdown, completing the electro-static simulation presented in Chapter 3. Finally, for chemical diagnostics, important long-lived species such as hydrogen peroxide have not been able to be characterized due to colorimetric technique having similar absorbance spectrum than the hydrogel used.

8.3 Future Research

This work can be placed in a top-down approach for plasma characterization and tailoring. With this thesis, I was able to provide information on the characterization of heat and some of the radical chemistry happening in the during plasma-hydrogel interaction. To get a complete picture of the whole plasma processes, as represented in Figure 8.1, more advanced tools need to be developed in order to probe faster chemical and electronic processes happening in the gas phase and at the interface of the plasma.

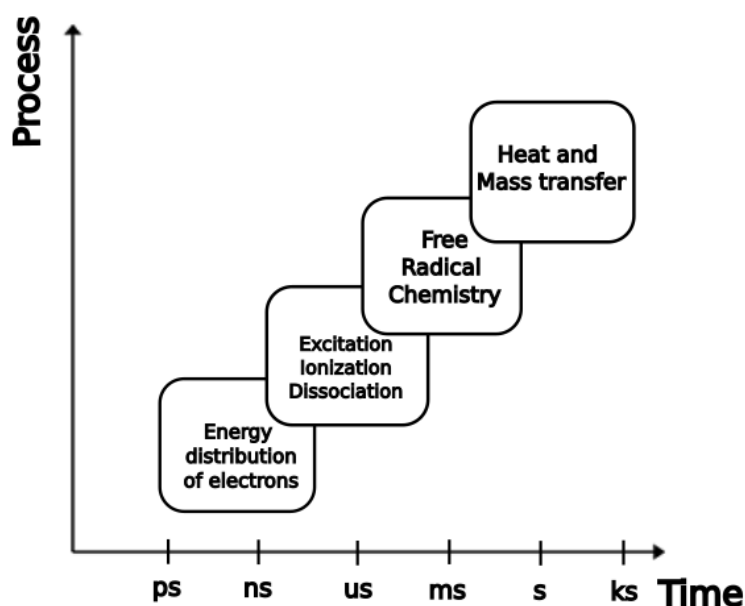


Figure 8.1 Timeline of the dynamic response for different processes inside a plasma source. Reproduced from [27]

Furthermore, once the tools developed for plasma diagnostic, biological research need to be conducted to correlate the cell response to plasma properties. The next steps toward using this technology in clinical environment would be to correlate response of 3D co-cultured model, in order to isolate cellular response and migration of tumour, and start *in vivo* studies

on small animals for studying more complex biological model. In a more global approach, the steps taken to correlate physical and biological results can be then taken as a data training set for artificial intelligence algorithms who could adapt the plasma properties in real time during treatment to maximize the efficiency and selectivity of the treatments. In a clinical situation, one could imagine this technology to be coupled with an optical probing system such as OCT or Raman spectroscopy, to send information on the type of tissue treated and using a feedback loop to tailor the plasma reactivity instantly.

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