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**Etude d'une décharge hors équilibre à pression
atmosphérique pour des applications biomédicales.**

Physique de la décharge, cinétique de la production des
espèces réactives lors de l'interaction avec des cellules et des
tissus vivants.

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"It seems impossible until it's done"
Nelson Mandela

"

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Abstract :

Over the past decade, a new type of non-equilibrium discharge at atmospheric pressure has attracted growing interest, given the ability to produce a plasma extending in ambient air close to room temperatures. These plasma jets, often based on a dielectric barrier discharge type of reactor are interesting due to their physicochemical properties. In addition, these cold plasma jets have the advantage of allowing applications to heat sensitive materials, creating the new field of Plasma Medicine research. At GREMI the Plasma Gun has been developed for both the study of the physics of plasma jets and for biomedical applications particularly in the field of cancerology. In a first step, *in vitro* and *in vivo* were performed within a rodent model of pancreatic carcinoma. The anti-tumor action of the plasma has been demonstrated as well as its benefic combination with a chemotherapeutic treatment. Based on these encouraging biomedical results, the main focus of this study is to report on the drastic influence of the application target on the plasma properties (propagation and production of reactive species) and on the strong coupling between gas jet and plasma discharge. Diagnostics such as fast, wavelength-filtered and Schlieren imaging, optical emission spectroscopy as well as Fourier transform infrared spectroscopy were used to characterize the plasma. A quantitative study on spatial and temporal distribution of hydroxyl radicals (OH density ranging between 5.10^{11} and $1.10^{14} \text{ cm}^{-3}$) was performed by laser-induced fluorescence. The study of the OH in combination with a numerical model allowed a better understanding of the moist air penetration into the gas jet and the interaction with wet surfaces. This PhD work enlightened the complex interaction between the gas flow, the plasma and the nature of the target which has to be taken into account for further optimization of biomedical applications.

Keywords : Non-equilibrium plasma, Atmospheric pressure, biomedical application, reactive species

Résumé :

Durant la dernière décennie, un nouveau type de décharge hors équilibre thermodynamique à pression atmosphérique a suscité un engouement croissant compte tenu de sa capacité de produire un plasma s'étendant dans l'air ambiant à une température proche de l'ambiante. Ces jets de plasma, souvent basés sur un réacteur de type décharge à barrière diélectrique, sont intéressants du point de vue de leurs propriétés physico-chimiques. De plus, ces jets de plasmas ont l'avantage de permettre des applications des matériaux thermosensibles, ouvrant ainsi un nouveau domaine de recherche, Plasma Médecine. Au GREMI le Plasma Gun, a été développé tant pour l'étude de la physique des jets de plasma que pour les applications biomédicales notamment dans le domaine de la cancérologie. Dans une première étape, des traitements par Plasma Gun in vitro et in vivo, dans le cadre d'un modèle murin du carcinome pancréatique, ont été effectués. L'action anti-tumorale du plasma a été démontrée ainsi que la combinaison bénéfique avec un traitement chimiothérapique. Fondée sur ces résultats encourageants, l'objectif principal de cette étude porte sur l'influence drastique de la cible de l'application sur les propriétés du plasma (propagation et production des espèces réactives) ainsi que l'interaction du gaz et du plasma. Des diagnostics tels que l'imagerie rapide et filtrée en longueur d'onde, la spectroscopie d'émission optique, l'imagerie Schlieren ainsi que la spectroscopie infrarouge à transformée de Fourier ont été utilisés pour caractériser le jet de plasma. Une étude quantitative de la distribution spatiale et temporelle du radical hydroxyle (densité comprise entre 5.10^{11} et $1.10^{14} \text{ cm}^{-3}$) a été réalisée par fluorescence induite par laser. L'étude de l'OH en combinaison avec un modèle numérique a permis une meilleure compréhension de la pénétration de l'air dans le jet de gaz et de l'interaction avec les surfaces humides. L'interaction complexe entre le comportement du gaz, du plasma et la nature de la cible est mise en avant en vue d'optimiser les applications biomédicales.

Mots clés : Plasma hors équilibre, pression atmosphérique, application biomédicale, espèces réactives

Chapter 1

Introduction

1. Context

The use of plasma in the biomedical field was few years ago, only isolated cases of plasma applications which turned into an independent multi-disciplinary field called Plasma-Medicine. Over the last decade, the growing interest in plasma-medicine is measurable by the number of events linked to this field such as the development of specialized biennial International conference on Plasma Medicine (ICPM), of sessions and workshops in the most important international plasma physics conference devoted to plasma-medicine. Moreover, new laboratories and institutes bringing together plasma scientists with biologists and medical doctors have been created. The number of participants in the different conferences as well as of the scientific publications dedicated to the plasma-medicine field is exponentially increasing.

This PhD work has been performed in the frame of the emerging Plasma-Medicine field. This study is an example of non-thermal plasma jets characteristics investigation toward the optimization of biomedical applications. In this chapter information about non-thermal plasma at atmospheric pressure is presented with non-exhaustive description of the historical development of plasma jets and their biomedical applications potential. Moreover some of the main parameters that modify the plasma properties and consequently influence the plasma treatment efficiency are emphasized. The approach followed in this work will be enlightened, taken as a starting point the work already published.

2. Atmospheric-Pressure Cold Plasmas

A plasma is a ionized gas which in the case of cold plasma has usually low ionization degree. The plasma state on itself is not stable, and energy has to be fed to the plasma in order to sustain it. If the energy is not delivered to the system anymore, the ions and electrons will recombine and the plasma relaxes. One way to produce plasma in laboratory is to provide the energy by inducing a difference of potential between electrodes. The produced electric field accelerates the seeds electrons naturally present in the gas due to cosmic rays and/or from the presence of surfaces [1-2]. Through collisions with the neutral species of the gas,

charges particles (ions and electrons) form new charged particles. The released secondary electrons will in turn ionize more gas particles. Through this principle of electronic avalanches, the gas breakdown can happen which then leads to the formation of a plasma.

There are different types of gas discharges. F. Paschen [3] enlightened that voltage needed for electrical breakdown depend on the gas nature and the product pd , p being the pressure and d the distance between the electrodes. The Paschen curves exhibit a minimum showing that at low pd the voltage needed to sustain the plasma is high due to the low probability of electronic collisions while at high pd the probability of electronic collisions is high and a greater energy is needed for the plasma to become self-sustaining. This allowed defining three types of discharges, Pseudo spark type for low pd , Townsend type around the minimum of the Paschen curve and Streamer type for high pd .

Within the framework of a biomedical application with therapeutic aim, the plasmas at atmospheric pressure are privileged. On one hand, atmospheric-pressure plasmas present the advantage to be easily transportable, handy, and are much less expensive than the said low pressure plasmas. On the other hand, living cells, tissues and organisms requires atmospheric-pressure.

After the primary electron avalanche, the plasma contains electrons, ions and neutrals. If the ionization rate is rather small, the density of neutrals will be significantly higher than the density of electrons and ions. Due to the difference of mass ratio between electrons and heavy particles, energy transfers are more efficient from ions than from electrons, to neutrals. Thus the temperature of the electrons is higher than the temperature of the heavy particles ($T_e \gg T_g$). This type of plasma is not in thermodynamic equilibrium and this is why it is often called non-thermal plasma (NTP) or "cold plasma".

The difference of mobility between theses electrons and ions creates an inverse electric field from space charge which becomes dominant in regards to the applied electric field. The inverse electric field at the head of the avalanche will promote the development of the avalanche. Two types of streamer can be generated, the positive and the negative one whereas the inverse electric field become dominant close to the anode or the cathode, respectively. When the streamer has undergone the propagation to the opposite electrode, a conductive channel is produced. The current can rapidly increase in the conductive channel that forms the plasma it-self between the two electrodes; collisions and consequently the gas temperature increase. The streamer-to-arc transition happens when the current increases [4]. At atmospheric pressure, this high current often induces the transition to thermal plasma. To achieve low temperature plasma at atmospheric pressure, thus to inhibit the streamer-to-arc transition, several approaches can be used [5-6].

The following description focuses on the streamer type of discharge, because it is the one which is mostly involved in plasma jets for biomedical applications.

a. Many sources

One way to maintain the gas temperature low is to modify the reactor configuration such as in Dielectric Barrier Discharges (DBDs). In DBDs, a dielectric material covers one or both of the electrodes to self-limit the increase of current and prevent the transition to thermal plasma. The plasma produced by a DBD is mostly non-uniform and produces random appearing filaments.

Another way to prevent arcing at atmospheric pressure, without dielectric barrier, is to deliver energy in nanosecond and microsecond time scale [4, 7]. Short repetitively pulsed discharges allow keeping the discharge current low by keeping the voltage applied across the electrode for a short duration. By using a shorter pulse duration than the time needed for arc to occur, gas heating is also limited.

In practice, the different ways to maintain the discharge non-thermal are non exclusive and often combined [8].

b. Plasma Jets

A plasma jet in thermal non-equilibrium is usually a "cold" plasma, close to room temperature, and stable at atmospheric pressure. It is characterized by its ability to produce an *in-air* expanding plasma, over usually few cm long, so called "plasma plume". Thus they allow producing a wide range of reactive species outside of the reactor zone. Prior to the development of cold plasma jet technology, cold atmospheric-pressure plasmas were limited in the confined space between the electrodes. Consequently the spectrum of applications was limited.

The first reported plasma jet date of early 90's with the study of Koinuma *et al.* [9]. Their reactor was a DBD equipped with a quartz capillary, flushed with Helium and powered by a RF generator, used to etch of Si. Schütze *et al.* published in 1998 on the development of another plasma jet [10-12] also for etching and depositing silicon.

In 2002, Stoffels *et al.* [13] introduced a new type of plasma reactor, the *plasma needle*, producing a few mm long plasma plume at the tip of the powered electrode. They reported that such plasma could eventually be applied on biomaterials due to its low gas temperature. Although this plasma reactor cannot be classified as a plasma jet, this result starts to gain the interest of the community.

In 2005, four teams, Teschke *et al* [14], Lu and Laroussi [15], Kędzierski *et al.* [16] and Förster *et al.* [17], published on plasma jets characterization by fast imaging and optical emission spectroscopy. Since that, many different reactor geometries were developed. Although most of the jets are based on a DBD reactor with a tubular dielectric barrier, other configurations

were developed such as Single Electrode jet, Dielectric-Free Electrode jet, DBD-like plasma jet. A review [18] on the different configurations developed worldwide was published by Lu *et al.* in 2012.

The plasma jet developed at GREMI called the *Plasma Gun* uses a DBD discharge based reactor which is equipped with long dielectric capillary and flushed with continuous and relatively low Neon or Helium flow rate. The main difference with the previous cited ones is the potentiality of the plasma to propagate through a long capillary of tens of cm to meters in length.

3. Research potentialities: Plasma Medicine

The main advantage of the plasmas created in thermal non-equilibrium is the rich chemistry. They provide an energy efficient way of producing the required reactive species while keeping the gas temperature low, often close to room temperature. Thus treatment of heat sensitive materials such as polymer, paper as well as living cells and tissues is possible. Moreover plasma jets allow transporting the reactive species onto the target to be treated and are adaptable to diverse shapes and sizes of targets.

A wide spectrum of research fields is concerned by the development of plasma jets, such as microelectronics [19], surface functionalization [20] and water treatment [21-23].

The interest for non-thermal atmospheric pressure plasmas for biomedical applications is recently growing [24-27]. At early stage of the use of plasma in biomedical research, the field of sterilization [27-29] since the 70s and modification of surface with biocompatibility properties [30-31] were studied. Since the last decade, other fields are under interest mainly due to the development of the plasma jets. Among them we can cite application fields such as wound healing [32], odontology [28], antitumor action [33-34] as well as more recently, disinfection [35-38], coagulation [39-40], and even cellular proliferation (tissues and bones) [41-42].

At GREMI, applications of non-thermal plasmas focused mainly on the antitumor potentialities of non-thermal plasmas. Traditional cancer therapy techniques are mainly surgery and the use of Radiotherapy and Chemotherapy. Although latest techniques have proved some efficiency, there are still a lot to improve to decrease the mortality and side effects linked to those techniques. Moreover some cancerous cell lines appear to be resistant to such treatments. Another therapeutic approach, which is clinically approved, is the Photodynamic Therapy (PDT) [43]. PDT is based on the use of photo-sensible molecules which can be triggered by luminous excitation at specific wavelength to become active. Excitation of the molecules induces a production of reactive oxygen species (ROS) by implicating the oxygen from the tissue environment. The advantage of this technique is the localized action of the excitation however the type of administration is still systemic as in the chemotherapy.

The plasma treatment is among other reasons, under interest due to its ability to generate a rather important amount of ROS in an energy efficient way, in the vicinity of the target

without prior systemic administration of any compound thus minimizing its invasiveness. To this extend, the plasma treatment is a promising new therapeutic agent.

The first pioneer result attesting for *in vitro* antitumor potentiality of non-thermal plasma on living mammalian cells was published in 2004 by Stoffels *et al.* [44]. Few years later Fridman *et al.* 2007 [45] reported also *in vitro* antitumor potentiality together with a decrease in the pH of the cell medium containing the melanoma cells. Since that, plenty of different cell lines have been treated by non-thermal plasmas, DBDs and plasma jets [45-48] and even combined with nanoparticles [49-51]. The antitumor mechanisms are in most cases found to be linked to cell cycle arrest. The plasma is inducing apoptosis, the process of programmed cell death. Necrosis can be induced if the treatment time or the energy delivered to the system is too important.

4. Toward optimization of plasma jets for biomedical applications

When starting this PhD study, the team at GREMI was enrolled in a regional research project (APR) "PLASMED" dedicated to the evaluation of the potentialities of non-thermal plasmas at atmospheric pressure in oncology. This APR was following a translational research procedure, meaning that laboratory experiments were performed in a back-and-forth procedure between the laboratory and the medical doctors' considerations. The GREMI team is also part of a national research project (ANR) Blanc "PAMPA" since 2010. This ANR project is dedicated to the study of ignition and propagation of plasma together with the produced reactive species.

To performed research as efficiently as possible in the plasma medicine field, the study can be divided in three parts as followed:

1. The study of the physics behind the ignition and propagation of the plasma jet.
2. The study of the production of reactive species when plasma interacts with the biological targets.
3. The study of the biological processes induced by the plasma in the given application field.

In this frame, two parallel PhD thesis, Marc Vandamme (2009-2012) [52] and Vanessa Sarron (2009-2013) [53] allow covering simultaneously the diverse parts and optimizing the treatments. Marc Vandamme and Vanessa Sarron have made major contributions in the understanding of the biological processes *in vitro* and *in vivo* induced by plasma treatment and in the understanding of the physical mechanisms of the plasma produced by the Plasma Gun.

The first aim of this thesis is the characterization of the production of reactive species when interacting with bio-mimicking targets.

The elements described below have been taken as starting points for research in this PhD work. Many results were published in parallel to our own work and will be evaluated and compared in the following chapters.

a. Propagation mechanisms

The plasma jet plume seems, by naked eyes, to be a continuous phenomenon. However first studies performed in 2005 [14-16] using fast imaging, reported the observation of a homogeneous spherical plasma propagation at velocity above km.s^{-1} which seems disconnected for the power source. Teschke *et al.* [14] called this plasma volume "plasma bullet".

Observation of a similar propagation, with related velocity measurements, inside long capillaries was made at GREMI as early as 1991. However the CNRS at this time did not follow this discovery to apply for a patent, which results in the production of a sealed letter "solo" in 1991. Later in 2007, a patent [54] attests of the observation made with the Plasma Gun.

Through the observations of those pioneer groups, it appears that the propagation of the "plasma bullet" is an electrically-driven phenomenon instead of a gas flow one. The first hypothesis to explain such propagation was formulated by Lu and Laroussi in 2006 [55]. They proposed that the mechanisms behind the "plasma bullet" can be associated with a positive streamer model developed by Dawson and Winn [56]. In this model the electric field is considered to be rather small and the photo-ionization plays a major action in the processes of propagation. Although, one of the significant differences between a positive streamer and the "plasma bullet" is the absence of branching.

Since then, many groups focused on the behavior of the plasma plume through parametric studies. The studies [57-62] dedicated to the characterization of the plasma plume enlighten the role of the voltage waveform, its amplitude, time duration and pulse repetition rate, in the length of the plasma plume. It was shown that increasing the voltage amplitude and/or voltage pulse duration time results in an increase of the plasma plume length. Méricam-Bourdet *et al.* [57] reported however a maximal plume length that could be reached by modifying only the voltage waveform. This upper limit was induced, according to them, by interactions between plasma and the ambient air. Other results can be found in literature [58-59, 61-64] on the increase of the length of propagation with increasing gas flow rate.

These results first indicate the potential role of hydrodynamics of the gas jet in the propagation of plasma plume in the ambient air.

b. Hydrodynamics

The role of the gas jet hydrodynamics in the plasma plume behavior is particularly important. As plasma jets are made, in most cases, in a continuous rare gas flow, the fluid dynamics has to be taken into account in the characterization of plasma behavior.

The first results in literature related to this aspect have been proposed experimentally by Zhu *et al.* [66] in 2009. They showed that for same reactor configuration and input parameters, a Helium plasma jet expending in a cell full with Helium behaves differently as the one expending in the ambient air. As matter of fact, the plasma in the Helium cell appeared to diffuse isotropically. They proposed that the plasma jet is propagating only in the noble gas which results in a conical emission shape when expending in the ambient air. Later this behavior has been proposed through numerical models by Breden *et al.* [67] and Boeuf *et al.* [68], where the denomination "guided streamer" was enounced.

Also in 2009, Méricam-Bourdet *et al.* [58] showed, in addition to the increased length of the plasma plume with gas flow rate that when exceeding a certain gas flow rate the plume emission pattern begin to present some fluctuations. This upper limit of the gas flow rate depends on the gas nature and the geometry of the system. According to them [58, 69] this switch to fluctuations in the plasma jet would be linked to the transition to turbulent regime in the gas flow. Recent studies suggest that plasma propagation extends in the gas jet, only in the laminar region where sufficient noble gas/air ratio is present. These observations will be further developed in this manuscript.

To characterize plasma jets, the first requirement is that the diagnostics should be non-intrusive. As described above, the mechanism of propagation of the "plasma bullet" is mainly electrically driven while the gas flow pattern is producing a favorable zone of extension. Characterization of the plasma plume can only be achieved by the use of non-intrusive diagnostics. Using probes, such as Langmuir probes to measure electron densities, thermocouples to measure gas temperature, etc, introduce a surface inside the plasma which alters the electric field and gas flow pattern. For this reason, probes commonly used in low pressure plasmas, cannot be used for plasma jets characterization.

c. Production of reactive species

The plasma as an ionized medium containing excited species, emits light. One way to study spatially and temporally the plasma in a non-intrusive way is to measure its emission by Optical Emission Spectroscopy (OES). Other spectroscopic techniques such as Absorption Spectroscopy and Laser Induced Fluorescence allow for quantification of the species but require an external source of light.

The chemical processes occurring in the NTP are resulting from the delivered energy to the buffer gas under the influence of the induced electric field. The elementary processes can be roughly divided into primary processes and secondary processes. Figure 1.1 summarizes the typical timescale of the elementary processes in NTP.

The primary process includes ionization, excitation, dissociation, light emission, and charge transfer. The efficiency of the primary process is highly dependent on the energy delivery methods and their parameters, such as voltage waveform, voltage rise-time, frequency, geometry of the reactor, etc. Note that in the time scale of the primary processes is the streamer propagation.

The electrons are accelerated by the plasma electric field. Collisions are happening through elastic collisions which result in unchanged internal or potential energy of molecules or through inelastic collisions when the electron energy is sufficiently high, transferring the electron energy to the neutrals. In the case of Helium plasma, the minimum energy to produce inelastic collisions is ~ 19 eV. Then the first excited states can be produced. During the primary process, excitation transfers are happening from excited and metastable states induced by electron collisions. It covers the ionizing transfers and non-ionizing ones through binary and three-body collisions.

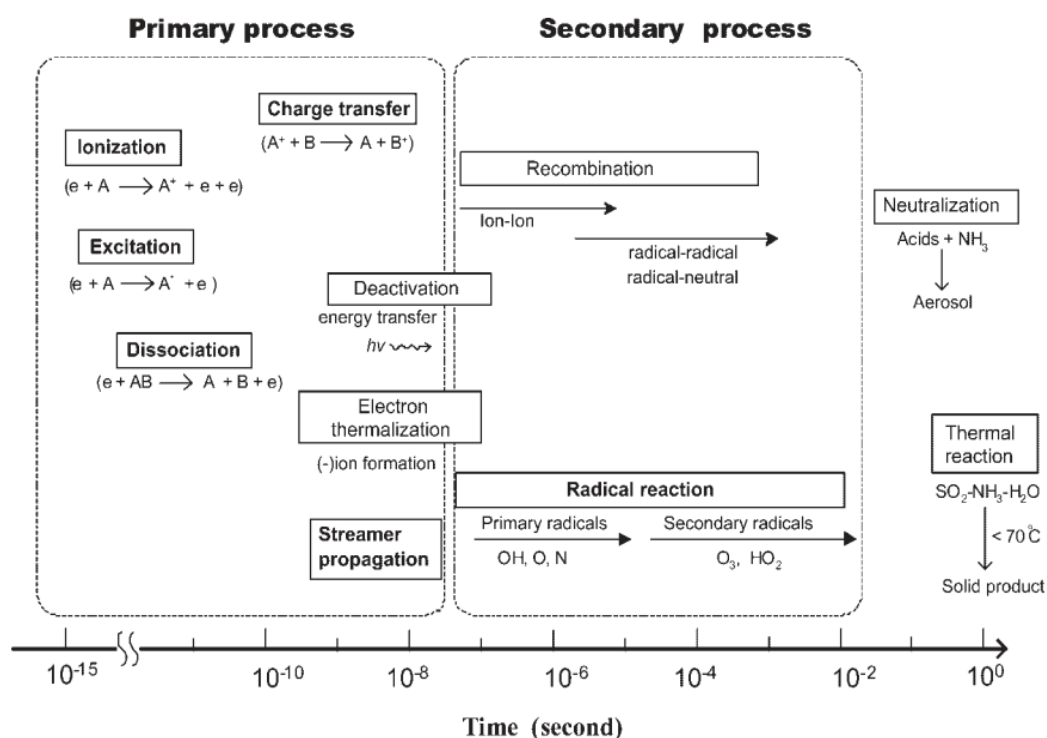


Figure 1.1: Timescale events of elementary processes in non-thermal plasma [From 68]

The secondary process represents the subsequent chemical reactions involving the products of primary processes (electrons, radicals, ions, and excited molecules). Some additional radical species and reactive molecules are also formed by radical-neutral recombination in

second process. Since the typical timescale of the secondary processes is rather fast as compared to the gas residence time, gas flow behavior in the NTP reactor usually has little or no influence on the production rate.

A brief and non-exhaustive description of the main excitation/de-excitation mechanisms responsible for the species emission present in Helium plasma jets are mentioned below. Complementary information can be found in [71-74].

In Figure 1.2, are presented the main energy levels of the excited species together with Helium states. Ne* and Ar* metastable states have been added for comparison. Main Helium lines observable thanks to OES are resulting from transition from n=3 states (3D, 3P and 3S, singlet and triplet) (~ 23 eV) to the n=2 states (2P and metastable states 2S, singlet and triplet). As the energy of those metastable states is around 20 eV, they are highly efficient in transferring their energy on nearly all molecules and especially on N₂⁺ ions.

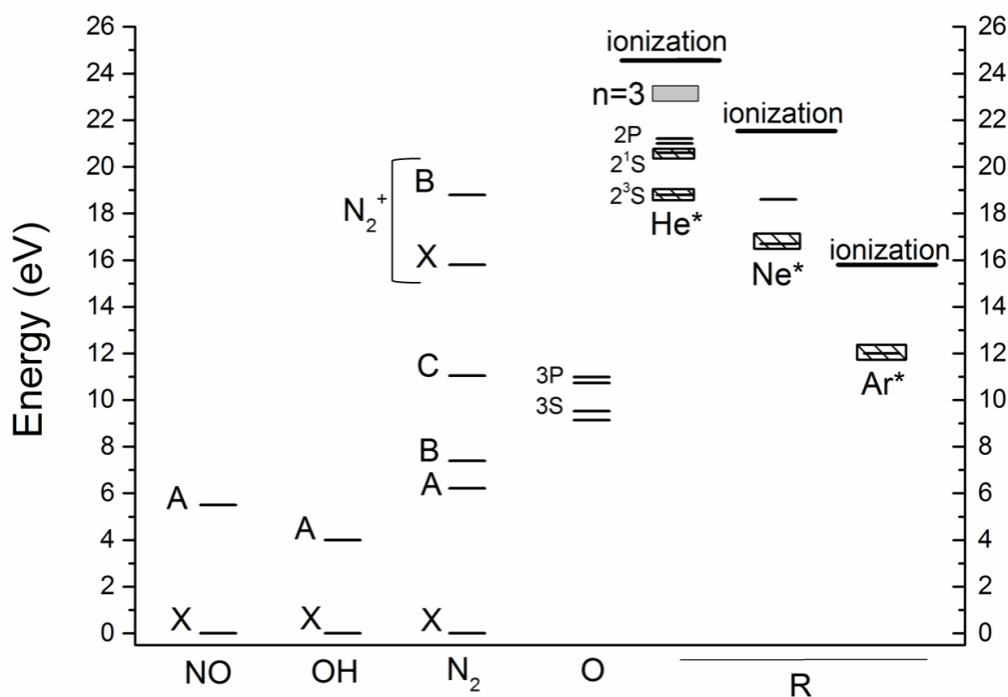
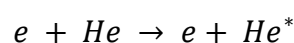


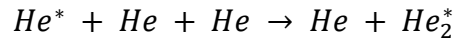
Figure 1.2: Energy levels of the main excited species present in NTP with corresponding ground state. The noble gases are noted R, Ne and Ar metastable states indicated for comparison.

For the plasma jets evolving in a rare gas flow, the main excited and ionized species will come from the neutral gas atoms and in our case from Helium.

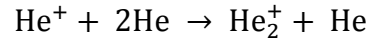
First to report reaction is the inelastic collision of energetic electrons ($e > 19$ eV) with Helium atoms:



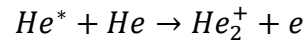
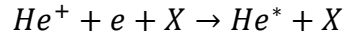
The He^* excited state will quickly react to produce He_2^* by three-body collisions with He atoms:



Such as He_2^* , He_2^+ is produced through three-body collision:

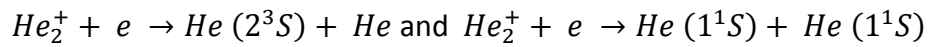


although some recombination plus associative-ionization are also contributing as follows:



At atmospheric pressure and ambient air temperature, the Helium ions clusters He_n^+ are easily and quickly formed. As those clusters are highly reactive, their disappearances are rather fast and play a major role in the plasma chemical processes [75], especially concerning the nitrogen species, which will be developed below. In the specific case of the streamer, the three-body collision reactions mentioned above are mainly happening in the head of the streamer.

A main part of He_2^+ is going to de-excite through recombination processes [76]:



The de-excitation of He_2^+ together with direct electron impact are producing the He metastable states which play a key role in sustaining the ionization in the plasma plume due to the long life time.

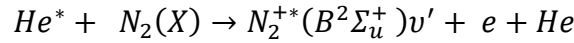
Table 1.1: Life time and excitation energy of He metastable states [77-79].

Helium state	Radiative life time (s)	Excitation energy (eV)
He (2^1S_0)	2.10^{-2}	20.6
He (2^3S_1)	6.10^5	18.8

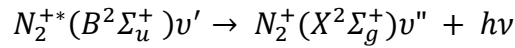
The radiative life time shown in Table 1.1 supposes that Helium is pure. In our condition of experiments where the Helium used is at 99.999% pure, the life time of those metastable states are lower. When characterizing the plasma plume, their life time is even smaller due to the diffusion of ambient air inside the Helium jet. This will be developed later on chapter 4 and 5.

The atomic and molecular Helium excited and metastable states are transferring their energy on the molecules present as impurities in the buffer gas and later in the ambient air. Consequently in the plasma plume will be observed a chemistry dominated by nitrogen, oxygen and water vapor.

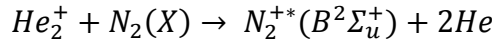
The radiative states of N_2 emit spectral bands of which the most intense are the first negative system of the N_2^+ ion ($B^2\Sigma_u^+ \rightarrow X^2\Sigma_g^+$) (FNS) and the second and first positive systems of N_2 ($C^3\Pi_u \rightarrow B^3\Pi_g$) and ($B^3\Pi_g \rightarrow A^3\Sigma_u^+$) (SPS and FPS). Concerning the N_2^+ FNS, this transition is mainly coming from the fast resonant Penning ionization by Helium metastable states following the transitions scheme bellow:



which is going to de-excite via the emission of the FNS:

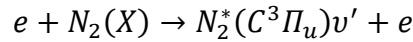


Another way to populate $N_2^{+*}(B)$ is by charge transfer from He_2^+ through:

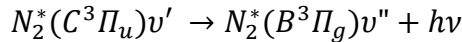


It is worth mentioning that in other commonly used buffer gases for plasma jets, charge transfer on $N_2(X)$ can occur in Neon but not in Argon as their metastable states are at 17 and 11 eV, respectively (Figure 1.2).

The N_2 second positive system (SPS) results mainly from inelastic electron collisions with nitrogen in the ground state, forming $N_2(C^3\Pi_u)v'$:



followed by the de-excitation of $N_2(C^3\Pi_u)v'$ into the $N_2(B^3\Pi_g)v''$ state:



In the specific case of plasma jets, the SPS is mainly linked to the energy transfer from the "plasma bullet" head to the surrounding air. Inelastic electronic collisions are not the only way, to produce $N_2(C)$ state. It is worth noting that pooling reaction can produce $N_2(C)$ by collision between two $N_2(A)$ metastable states and that small cascade contribution from the $E^3\Sigma_g^+$ state (~ 13 eV) is also possible. However those mechanisms are considered in our case negligible.

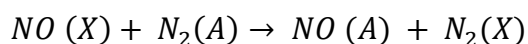
Nitric oxide (NO) is a chemically active species, which plays a well known vital role in many physiological processes inside living organisms [80-81]. Thus, the generation of NO in atmospheric pressure plasmas is an important topic in biomedical plasma applications.

The main relevant mechanisms of production and loss are resumed in Table 1.2 as was described by Dorai and Kushner [82].

Table 1.2: Main reaction of production and disappearance of NO radicals, inspired from [82].

1	$O + N_2 \rightarrow NO + N$
2	$N + O_2 \rightarrow NO + O$
3	$N + OH \rightarrow NO + H$
4	$NO + N \rightarrow O + N_2$
5	$NO + O \rightarrow NO_2$
6	$2NO + O_2 \rightarrow 2NO_2$
7	$NO + OH + M \rightarrow HNO_2 + M$
8	$NO + HO_2 \rightarrow NO_2 + OH$

The production of NO (reactions 1 to 3) requires the presence of atomic O or N which are produced in the active region of the plasma. The reaction 1 and 2 are the so called Zel'dovich reactions known to be an efficient mechanism to produce NO. However the reaction 1 can be neglected at $T_g < 1400$ K [83] consequently NO is formed in areas containing N and/or OH, which suggest the mandatory presence of some humid air or admixture in the buffer gas as well as relatively low energetic electrons. However as shown by reaction 4 and 7, N and OH are also a precursor of NO loss, as well as O (reaction 5). Reactions 6 to 8 are highly influent in the gas effluent when only long living species are present. Further subsequent reactions to produced N_2O_5 , NO_3 and HNO_3 are also happening [82]. Another subsequent production mechanism highly probable in our conditions, involves the N_2 metastable state $N_2(A)$ by excitation transfer as follow:

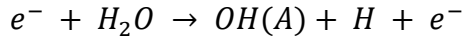


In this work, we mainly observed through OES measurements the $NO\gamma^*$ system which correspond to the (A-X) transition from 200 to 300 nm, although $NO(X)$ would be also mentioned in results concerning FTIR measurements.

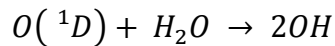
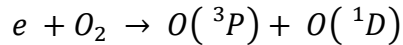
Another important reactive species in terms of biomedical activity is the hydroxyl radical (OH). OH radicals belong to the most strongly oxidative species produced by plasmas when water vapor is present. In plasma jets, the plasma expands in the air where water vapor is naturally present. Moreover when dealing with biomedical applications, the targets are often humid or liquid. As OH radical comes from reaction with water vapor, it is thus

abundant in such biological systems. OH is also one of the building blocks of H₂O₂, which is considered as an important agent in the chemical reactivity of plasmas in contact with liquids [21].

There are many reaction pathways according to the literature [82-84] to produce OH radicals in non-thermal plasmas, which mainly include the electron dissociation of H₂O:

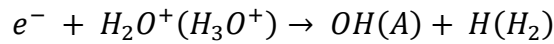


But also dissociation by radicals and metastables,



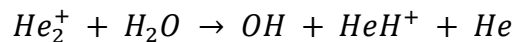
Other metastables such as N₂(A), N(²D) and N(²P) can also dissociate H₂O.

When the electron energy is low, e.g. <2 eV, the dissociative recombination of H₂O⁺ is also an important pathway for OH formation [82]:



It has been suggested by Bruggeman *et al.* [85] that dissociative electron-ion recombination and for electronegative plasmas also positive-negative ion recombination can have a major contribution to the OH production.

Another way to produce OH through charge transfer from He₂⁺, should not be forgotten:



Some of the reactions, listed in this section, produce transitions visible in the UV-visible range which are observable by means of OES and imaging systems. In order to sum up Table 1.3 shows the main transitions in the Helium plasma plume which are going to be discussed in this manuscript. The respective excitation energy of their upper level is also given.

The "plasma bullet" propagation induces fast temporal and spatial evolutions resulting in gradients of the reactive species densities. The complex chemistry in non-thermal plasmas is clearly dependant of the excitation delivery, the density ratio between excited species, neutrals and electrons and the working conditions.

Table 1.3: Transitions and excitation energies of observable spectral system.

Wavelength region	Spectral system	Transition	Excitation energy of the upper level
200 ... 300 nm	NO γ -System	$NO(A^2\Sigma^+)v' \rightarrow NO(X^2\Pi r)v'' + h\nu$	5.5 eV
280 ... 500 nm	2nd positive system of N ₂ (SPS)	$N_2(C^3\Pi u)v' \rightarrow N_2(B^3\Pi g)v'' + h\nu$	11.0 eV
280 ... 310 nm	OH-Radical	$OH(A^2\Sigma^+)v' \rightarrow OH(X^2\Pi)v'' + h\nu$	4.0 eV
325... 585 nm	1st negative system of N ₂ (FNS)	$N_2^+(B^2\Sigma_u^+)v' \rightarrow N_2^+(X^2\Sigma_g^+)v'' + h\nu$	18.8 eV
777...940 nm	O I atomic line	$O(3p) \rightarrow O(3s) + h\nu$	10.7 eV
500... 900 nm	1st positive system of N ₂ (FPS)	$N_2(B^3\Pi g)v' \rightarrow N_2(A^3\Sigma_u^+)v'' + h\nu$	6 eV

d. Influence of the target on plasma properties

As most plasma jets are dedicated to applications, it is important to keep in mind that the target subjected to plasma can have an influence on plasma behavior.

First study to report on the plasma propagation onto a surface was dealing with dielectric material [14]. Teschke *et al.* described that the "plasma bullet" spread onto the surface in an annular distribution before vanishing. They presumed that the plasma emission was following the gas flow spread.

Cao *et al.* [60, 87-88] in 2009-2010 presented jet arrays on treatment conditions, top down and with surfaces in front of the jets. They showed that the plasma plume is well adapting to different angles and distances between capillary outlet and surface, especially in the case of medical forceps. They reported strong plasma-surface interaction when using a glass covered with grounded ITO however no clear results, temporal evolution neither spatial distribution of the reactive species through the gap, were presented on this topic. Nwankire *et al.* [89] in 2010 reported on the influence of the gap distance, between plasma jet outlet and a polymer sheet positioned on a grounded metal plate, such as a shorter gap induced a higher discharge current and power due to enhanced ground electric return.

Despite those few studies, there is a lack of scientific research on this specific interaction. This can be understood by the complexity of the plasma jets experiments dealing with what has been mentioned above, the time and spatial scale of the phenomena undergoing as well as influences of the hydrodynamics of the gas. Although from biomedical treatment point of

view, the nature and shape of the target is of prime importance. As Plasma Gun was directly design toward biomedical applications, in the frame of the translational research mentioned above, the experiments performed in this PhD work mainly focus on the influence of the presence of the target in front of the plasma plume.

5. Aim and outline of the thesis

As mentioned above, the study of the plasma jet physics started only few years. When this work begun, the behavior of the so called "plasma bullet" and the induced production of reactive species were still far from being understood. Moreover no experiments were reported on *in vivo* **plasma jet** antitumor activity and estimation of plasma jets antitumor potential needed to be investigated.

The main aim of this work was to estimate qualitatively the influence of input plasma and external parameters on the production of reactive species in order to better promote plasma antitumor actions. For this purpose, antitumor efficiency of the Plasma Gun was under investigation in a back and forth procedure between plasma characterization and biomedical experiment. The idea behind this multi-disciplinary work was the evaluation of the external parameters, linked to the particular experimental conditions, which could be influent in the use of a plasma jet for biomedical treatments. This evaluation of the external condition influences on the plasma behavior expect to give material for further control of the production of reactive species and biological induced effects of plasma treatment.

The studies presented in this PhD thesis were carried out in collaborations with many French and foreign research labs. Main part of the experiments was performed in the frame of one APR and one ANR projects:

The APR PLASMED (2008 - 2011) was dedicated to the evaluation of the antitumor potentialities as a new treatment strategy in oncology. The APR PLASMED brings together two laboratories of biology, the CIPA¹ and CBM² (Orleans), the GREMI (Orleans) and the Hospital Regional Center (CHR) of Tours.

The ANR Blanc "PAMPA" (2010 - 2014) was dedicated to the study of physical mechanism behind the ignition, propagation and topology of the plasma produced by plasma jets, from experimental and numerical point of views. Five French laboratories are associated to this project: LPGP³ (Orsay), LIPhy⁴ (Grenoble), LAPLACE⁵ (Toulouse), ISAE⁶ (Toulouse) and GREMI⁷ (Orleans).

¹ CIPA: Centre d'Imagerie du Petit Animal, UPS CNRS, Orléans

² CBM: Centre de Biophysique Moléculaire, UPR CNRS, Orléans

³ LPGP: Laboratoire de Physique des Gaz et des Plasmas, UMR CNRS, Orsay

⁴ LIPhy: Laboratoire Interdisciplinaire de Physique, UMR CNRS, Grenoble

Moreover some experiments were performed abroad in the frame of two Short-Term Scientific Missions (STSMs) thanks to the European COST action MP1101 "Biomedical Applications of Atmospheric Pressure Plasma Technology". During one month, experiments were carried on at Loughborough University (Loughborough, England) in collaboration with Prof. Michael Kong and Dr. Hai-Lan Chen with the assistance of Dr. Gilbert Shama. These experiments were focusing on developing a procedure for E. Coli sterilization and pH modification of aqueous solutions. Considering the preliminary stage of the results, they will not be reported in this manuscript. Finally the results related to OH distribution by Laser Induced Fluorescence were carried out during two months in collaboration with Dr. Giorgio Dilecce and his team, Dr. Paolo Francesco Ambrico and Prof. Santolo De Benedictis, at the CNR-IMIP (Bari, Italy).

This manuscript is constituted of 6 chapters and a conclusion with perspectives. In addition to this introduction, the following 5 chapters are organized as follows:

Chapter 2 is describing the experimental setup and techniques used during this work.

Chapter 3 focus on the Plasma Gun biomedical applications. Some Plasma Gun antitumor activities *in vitro* and *in vivo* are presented and discussed.

Chapter 4 and 5 treat about characterization of the Plasma Gun in two specific conditions, without (Chapter 4) and with (Chapter 5) the presence of a target in front of the plasma plume. They follow the same structure using Optical Emission Spectroscopy and imaging techniques to characterize the plasma in the two conditions.

Chapter 6 is dedicated at the qualitative and quantitative study of the hydroxyl radical ground state by Laser induced Fluorescence. Spatial distribution patterns of OH ground state are discussed in regards of the results presented in Chapter 4 and 5.

⁵ LAPLACE: Laboratoire PLASMA et Conservation d'Energie, UMR CNRS, Toulouse

⁶ ISAE: Institut Supérieur de l'Aéronautique et de l'Espace, EPSCP – GE, Toulouse

⁷ GREMI: Groupe de Recherches sur l'Énergétique des Milieux Ionisés, UMR CNRS, Orléans

French summary of Chapter 1

L'utilisation des plasmas dans le domaine biomédical, était il y a encore quelques années un cas isolé d'application plasma qui maintenant est devenu un domaine indépendant, appelé Plasma-Médecine. Dans la dernière décennie, la création d'une conférence internationale biannuelle ainsi que des sessions dans les plus importantes conférences internationale sur la physique des plasmas, dédié au domaine Plasma médecine avec un nombre croissant de participants, montrent l'intérêt grandissant pour ce domaine. De plus, des nouveaux laboratoires et instituts regroupant des experts du plasma avec des biologistes et des médecins, se crée et les publications scientifiques dédiées au domaine ne cessent de croître.

C'est dans l'émergence de ce nouveau domaine de recherche que mon travail de thèse s'est effectué. Cette étude est un exemple d'investigation des caractéristiques d'un jet de plasma non-thermique à la pression atmosphérique dirigé vers l'optimisation des applications biomédicales. Dans ce chapitre des informations caractéristiques des plasmas à la pression atmosphérique sont résumées. Puis brièvement l'historique du développement des jets de plasma et de leurs potentialités en termes d'applications biomédicales sont mentionnés. De plus, quelques paramètres pouvant modifier les propriétés du plasma et en conséquence les effets biologiques induits sont mis en avant. Pour finir, l'approche suivie pour ce travail de thèse et l'organisation du manuscrit sont décrits.

Le plasma est un gaz partiellement ionisé, contenant des électrons libres, des ions, des neutres, radicaux et radiation UV. Le plasma est un état nécessitant une source d'énergie extérieure pour être maintenu, faute de quoi les ions et les électrons recombinent rapidement. Dans le cas des plasmas de laboratoire, l'énergie est apportée en général en créant une différence de potentiel entre deux électrodes, qui par la suite accélère les électrons "germes" naturellement présent dans le gaz. Cette accélération conduit à des collisions avec les particules neutres. La différence de mobilité entre les électrons et les ions génère un champ électrique induit. S'en suit une cascade de collisions et de transfert d'énergie pouvant conduire au claquage, formant ainsi le plasma.

Lorsque le taux d'ionisation est faible, les transferts d'énergie entre les particules lourdes ne sont pas très efficaces ce qui engendre une différence de température entre les électrons et les atomes et molécules ($T_e \gg T_g$). Dans ce cas le plasma n'est pas à l'équilibre thermodynamique et est souvent appelé plasma non-thermique ou "froid". A haute pression le plasma a tendance à devenir instable et filamentaire. Ceci est lié au faible parcours moyen des électrons et à la formation de streamers entre les électrodes. La transition "streamer" à "l'arc électrique" se produit quand le courant devient trop important. Pour éviter cette transition et conserver un plasma à température basse, plusieurs techniques sont utilisées.

Une de ces techniques consiste à concevoir une géométrie de réacteur limitant les risques de transition, tel que les décharges à barrière diélectrique (DBD). Une autre consiste à délivrer des impulsions de tensions d'une durée de l'ordre de la nanoseconde ou quelques centaines de nanoseconde. En pratique ces différentes méthodes sont souvent combinées.

Les jets de plasma froid ont permis l'extension des applications grâce à la possibilité de produire un plasma froid s'étendant dans l'air sur des distances de quelques centimètres, appelé "plume plasma". Le premier jet de plasma a été signalé au début des années 90 par Koinuma *et al.* suivi à la fin des années 90 par l'équipe de Schütze au même moment où le Plasma Gun fut développé au GREMI. En 2002, Stoffels *et al.* introduise un nouveau réacteur plasma et signale que celui-ci, grâce à sa température proche de l'ambiante, peut être utilisé pour des applications biomédicales. A partir de 2005, plusieurs études paraissent sur la caractérisation des jets de plasma froids. Les études de Teschke *et al.*, Lu and Laroussi, Kędzierski *et al.* et Förster *et al.* sont le tournant de l'intérêt de la communauté plasma pour ce type de jet et depuis de multiples configurations ont été développées.

Le jet de plasma développé au GREMI, s'appelant le Plasma Gun, est un jet basé sur un réacteur DBD équipé d'un long capillaire pouvant aller jusqu'au mètre, ce qui fait sa principale différence avec les autres jets développés dans le monde.

L'intérêt principal des jets de plasma froids est la chimie produite par ceux-ci. Ils permettent de produire des espèces réactives d'une manière efficace avec relativement peu d'énergie tout en conservant une température proche de l'ambiante. Des matériaux sensibles à la température tels que les polymères, le papier ou les cellules et tissus vivants peuvent ainsi être traité par plasma. De plus, la plume plasma s'adapte au traitement de matériaux de diverses morphologies et tailles. Outre les matériaux sensibles à la température, des applications dans les domaines de la microélectronique, la fonctionnalisation de surface, les traitements des gaz d'échappement et de l'eau sont fortement développés.

Récemment l'intérêt pour les applications biomédicales a explosé. Premièrement ce sont les domaines de la stérilisation et de la modification de surface biocompatible qui ont été développés. Puis, plus récemment vinrent d'autres domaines tels que l'odontologie, le traitement des plaies chroniques, le traitement des cancers ainsi que la désinfection, la coagulation et l'aide à la prolifération cellulaire, des tissus mais aussi des os.

Au GREMI, l'application du Plasma Gun est particulièrement focalisée sur les traitements anticancéreux. En effet, malgré les agents thérapeutiques communément utilisés telles que la radio et la chimio thérapie, le plasma offre la possibilité de traiter les tumeurs de manière localisé. De plus il génère des espèces réactives d'oxygènes et d'azotes bien connues des biologistes pour leurs effets au sein des tissus.

Les premiers résultats attestant de l'effet anti-tumoral des plasmas froids, par DBD, a été publié en 2004 par Stoffels *et al.* suivi par Fridman *et al.* en 2007, sur des cellules de

mélanome *in vitro*. Depuis d'autres études ont été effectuées montrant un mécanisme d'action lié à l'arrêt du cycle cellulaire, produit par une modification des protéines régulant ce cycle. Toutes les études s'accordent sur l'induction de l'apoptose, la mort programmée de la cellule et la possibilité de nécrose.

Au GREMI, une recherche dite translationnelle a été mise en œuvre dans le cadre d'un projet APR, l'APR "PlasMed". Elle a permis la première démonstration *in vivo* par DBD en 2009. Le but d'une telle méthode est de lier la recherche fondamentale et l'application thérapeutique clinique pour une plus rapide mise au point d'agent thérapeutique. Un tel va-et-vient nécessite la présence d'acteurs tels que des physiciens, chimistes, biologistes et des médecins, permettant une réelle prise en compte des applications. C'est dans ce cadre que ma thèse a débutée et tout particulièrement dans le but d'estimer la production des espèces réactives suivant les paramètres appliqués au plasma lors de l'interaction avec des cibles vivantes, telles que des cellules et des tissus.

Les notions énoncées ci-dessous, relatant les propriétés des jets de plasma, ont servi de matériel de départ à l'étude présenté dans ce manuscrit.

La plume plasma semble à première vue être un phénomène continu. Or les premières études effectuées en 2005 utilisant l'imagerie rapide rapportent l'observation d'un plasma de forme sphérique homogène se propageant à une vitesse supérieure au km.s^{-1} qui semblait alors déconnecté de la source de tension. Ce volume de plasma fut appelé "balles de plasma" (en anglais "plasma bullet"). Ces premières expériences de caractérisation montrèrent que la propagation de ce plasma est gouverné par des phénomènes électrique plus que par le flux de gaz.

Une observation similaire, morphologique et de mesure de la vitesse de propagation, au sein de long capillaire fut dès le début des années 90 faite au GREMI. Malheureusement le CNRS à cette époque n'a pas suivi pour déposer un brevet ce qui amena à la création d'une lettre "solo" en 1991. Un brevet fut déposé en 2007, attestant des observations faites avec le Plasma Gun.

Depuis lors de nombreuses équipes se sont penchées sur l'étude de la plume plasma et de ces dites "balles de plasma". Il apparaît notamment que la forme ainsi que l'amplitude de tension ainsi que le débit de gaz jouent un rôle dans la longueur de la plume plasma. Il apparaît donc que malgré que le phénomène soit majoritairement de nature électrique le flux de gaz porteur doit être considéré dans l'étude de la plume plasma. D'après la littérature, le jet de gaz porteur permettrait de produire une zone favorable pour l'expansion du jet de plasma, d'où la dénomination de "streamer guidé".

Pour ne pas perturber le jet de plasma et de gaz, la caractérisation de la plume plasma doit s'effectuer par des diagnostics non-intrusifs, tels que la spectroscopie et l'imagerie. Dans ce chapitre une description non-exhaustive des mécanismes de production et de disparition

des espèces réactives produites par les jets de plasma dans l'Hélium sont énoncées et discutées (se reporter à la version en anglais). La cinétique réactionnelle liée aux "balles de plasma" induit de rapides gradients de densité, temporellement et spatialement. Il est important de rappeler que cette chimie est dépendante du type d'excitation, de la géométrie du réacteur, du rapport de densité entre les espèces excitées, les neutres et les électrons ainsi que des conditions de l'extérieur, comme la présence d'une cible en face de la plume plasma.

Comme la plus part des jets de plasma sont dédiés à des applications mettant en œuvre une cible, l'influence que celle-ci peut avoir sur les propriétés du plasma ne doit pas être négligée. Il apparaît dans la littérature relativement peu d'étude spécifique à cet aspect. Toutefois, une première distinction émerge quant à la nature de la cible et plus particulièrement sa conductivité électrique. Dans le cadre des applications biomédicales, la nature et la forme de la cible est de prime importance. Pour cette raison ce travail a été dirigé en vue d'estimer l'influence de celle-ci sur les propriétés de la plume plasma et sa production d'espèces réactives.

L'étude des jets de plasma est, comme mentionné plus haut, récent. Quand ce travail de thèse débuta, le comportement de la dite "balle de plasma" et la production d'espèces réactives induites était encore mal connus. De plus, aucune étude anti-tumorale *in vivo* n'avait été effectuée avec un jet de plasma.

Le but principal de ce travail de thèse était d'estimer de manière qualitative l'influence des paramètres appliqués au plasma et des paramètres extérieurs, sur la production d'espèces réactives pour ainsi tenter d'optimiser l'action anti-tumorale du plasma. Dans ce but, la potentialité anti-tumorale du Plasma Gun a été à l'étude dans un protocole de va-et-vient entre la caractérisation du plasma et les expériences biologiques. L'idée derrière ce travail multidisciplinaire était l'évaluation des paramètres extérieurs, liés aux conditions expérimentales des essais biologiques, qui pouvaient être influents dans l'utilisation du jet de plasma. Les conclusions tirées de cette évaluation se veulent permettre un meilleur contrôle de la production des espèces réactives et des effets biologiques induits par le traitement par jet de plasma.

Le travail présenté dans ce manuscrit a été effectué en collaborations avec de nombreux laboratoires, en France et à l'étranger. Une grande partie des expériences ont été exécutées dans le cadre de deux projets, un APR et un ANR:

L'APR PLASMED (2008-2011) était voué à l'évaluation des potentialités antitumorale du plasma comme nouvelle stratégie thérapeutique en cancérologie. Cet APR regroupait deux laboratoires de biologie, le CIPA et le CBM (Orléans), le GREMI (Orléans) et le CHR de Tours.

L'ANR Blanc PAMPA (2010-2014) était voué à l'étude de la physique du jet de plasma, de l'amorçage, de la propagation et de la topologie du plasma, par une comparaison entre expériences et modèle numérique. Cinq laboratoires Français étaient regroupés dans ce projet: le LPGP (Orsay), le LIPhy (Grenoble), le LAPLACE (Toulouse), l'ISAE (Toulouse) et le GREMI (Orleans).

De plus des expériences ont été effectuées à l'étranger dans le cadre de deux missions scientifiques de court-terme (STSM) grâce à l'action du COST Européen MP1101 "Biomedical Applications of Atmospheric Pressure Plasma Technology". Pendant un mois, des expériences ont été effectuées à l'Université de Loughborough (Loughborough, Angleterre) en collaboration avec le Prof. Michael Kong, le Dr. Hai-Lan Chen avec l'assistance du Dr. Gilbert Shama. Ces expériences ciblaient à la mise en place d'une procédure de stérilisation des E. Coli et la modification du pH de solutions aqueuses induites par deux types de réacteur plasma. Considérant le caractère préliminaire de cette étude, les résultats ne seront pas rapportés dans ce manuscrit. Pour finir, les résultats relatifs à la distribution de la densité du radical hydroxyle par fluorescence induite par laser ont été effectués pendant un STSM de deux mois en collaboration avec Dr. Giorgio Dilecce et son équipe, Dr. Paolo Francesco Ambrico et Prof. Santolo De Benedictis, au CNR-IMIP à Bari (Italy).

Ce manuscrit est composé de 6 chapitres et d'une conclusion où des perspectives à cette étude seront données. En plus de cette introduction, les 5 chapitres suivants sont organisés comme suit:

Le **chapitre 2** décrit les conditions expérimentales et les techniques utilisées pendant ce travail de thèse.

Le **chapitre 3** focalise sur les applications biomédicales du Plasma Gun. Des études *in vitro* et *in vivo* sont présentées et discutées.

Le **chapitre 4 et 5** se concentrent sur la caractérisation du Plasma Gun dans deux conditions distinctes, sans (chapitre 4) et avec (chapitre 5) la présence d'un cible en face de la plume plasma. Ces chapitres sont organisés de manières similaires, utilisant plusieurs diagnostics de spectroscopie et d'imagerie pour caractériser le plasma dans ces deux conditions.

Le **chapitre 6** est dédié à l'étude qualitative et quantitative du radical hydroxyle dans son état fondamental par fluorescence induite par laser. Les distributions spatiales de OH ainsi que celles de l'air et de l'eau sont discutées comparativement aux résultats présentés dans les chapitres 4 et 5.

References

- [1] Loeb, L.B. Basic processes of gaseous electronics. University of California Press, Berkeley CA, 2nd ed., revised edition, 1960.
- [2] Meek, J. M. and Craggs, J. D. Electrical breakdown of gases. Wiley, New York, 1978.
- [3] Paschen, F., 1889. Wiedemann Annales de physique 37, 69-96.
- [4] Raizer, Y.P., 1991 Gas Discharge Physics, Springer. Berlin
- [5] Fridman, A., Chirokov, A., Gutsol, A., 2005. Non-thermal atmospheric pressure discharges. J. Phys. D: Appl. Phys. 38, R1. doi:10.1088/0022-3727/38/2/R01
- [6] Tendero, C., Tixier, C., Tristant, P., Desmaison, J., Leprince, P., 2006. Atmospheric pressure plasmas: A review. Spectrochimica Acta Part B: Atomic Spectroscopy 61, 2–30. doi:10.1016/j.sab.2005.10.003
- [7] Pai, D.Z., Lacoste, D.A., Laux, C.O., 2010. Transitions between corona, glow, and spark regimes of nanosecond repetitively pulsed discharges in air at atmospheric pressure. Journal of Applied Physics 107, 093303. doi:10.1063/1.3309758
- [8] Laroussi, M., Akan, T., 2007. Arc-Free Atmospheric Pressure Cold Plasma Jets: A Review. Plasma Processes Polym. 4, 777–788. doi:10.1002/ppap.200700066
- [9] Koinuma, H., Ohkubo, H., Hashimoto, T., Inomata, K., Shiraishi, T., Miyanaga, A., Hayashi, S., 1992. Development and application of a microbeam plasma generator. Applied Physics Letters 60, 816–817. doi:10.1063/1.106527
- [10] Schutze, A., Jeong, J.Y., Babayan, S.E., Park, J., Selwyn, G.S., Hicks, R.F., 1998. The atmospheric-pressure plasma jet: a review and comparison to other plasma sources. IEEE Transactions on Plasma Science 26, 1685–1694. doi:10.1109/27.747887
- [11] Jeong, J.Y., Babayan, S.E., Tu, V.J., Park, J., Henins, I., Hicks, R.F., Selwyn, G.S., 1998. Etching materials with an atmospheric-pressure plasma jet. Plasma Sources Sci. Technol. 7, 282. doi:10.1088/0963-0252/7/3/005
- [12] Babayan, S.E., Jeong, J.Y., Tu, V.J., Park, J., Selwyn, G.S., Hicks, R.F., 1998. Deposition of silicon dioxide films with an atmospheric-pressure plasma jet. Plasma Sources Sci. Technol. 7, 286. doi:10.1088/0963-0252/7/3/006
- [13] Stoffels, E., Flikweert, A.J., Stoffels, W.W., Kroesen, G.M.W., 2002. Plasma needle: a non-destructive atmospheric plasma source for fine surface treatment of (bio)materials. Plasma Sources Sci. Technol. 11, 383. doi:10.1088/0963-0252/11/4/304

- [14] Teschke, M., Kedzierski, J., Finantu-Dinu, E.G., Korzec, D., Engemann, J., 2005. High-speed photographs of a dielectric barrier atmospheric pressure plasma jet. *IEEE Transactions on Plasma Science* 33, 310–311. doi:10.1109/TPS.2005.845377
- [15] Laroussi, M., Lu, X., 2005. Room-temperature atmospheric pressure plasma plume for biomedical applications. *Applied Physics Letters* 87, 113902. doi:10.1063/1.2045549
- [16] Kędzierski, J., Engemann, J., Teschke, M., Korzec, D., 2005. Atmospheric Pressure Plasma Jets for 2D and 3D Materials Processing. *Solid State Phenomena* 107, 119–124. doi:10.4028/www.scientific.net/SSP.107.119
- [17] Förster, S., Mohr, C., Viöl, W., 2005. Investigations of an atmospheric pressure plasma jet by optical emission spectroscopy, *Surf. Coatings Technol.*, 200, 827.
- [18] Lu, X., Laroussi, M., Puech, V., 2012. On atmospheric-pressure non-equilibrium plasma jets and plasma bullets. *Plasma Sources Sci. Technol.* 21, 034005. doi:10.1088/0963-0252/21/3/034005
- [19] Jeong, J.Y., Babayan, S.E., Schutze, A., Tu, V.J., Park, J., Henins, I., Selwyn, G.S., Hicks, R.F., 1999. Etching polyimide with a nonequilibrium atmospheric-pressure plasma jet. *Journal of Vacuum Science Technology A: Vacuum, Surfaces, and Films* 17, 2581–2585. doi:10.1116/1.581999
- [20] Schröder, K., Meyer-Plath, A., Keller, D., Besch, W., Babucke, G., Ohl, A., 2001. Plasma-Induced Surface Functionalization of Polymeric Biomaterials in Ammonia Plasma. *Contrib. Plasma Phys.* 41, 562–572. doi:10.1002/1521-3986(200111)41:6<562::AID-CTPP562>3.0.CO;2-Y
- [21] Sato, M., Ohgiyama, T., Clements, J.S., 1996. Formation of chemical species and their effects on microorganisms using a pulsed high-voltage discharge in water. *IEEE Transactions on Industry Applications* 32, 106–112. doi:10.1109/28.485820
- [22] Foster, J.E., Weatherford, B., Gillman, E., Yee, B., 2010. Underwater operation of a DBD plasma jet. *Plasma Sources Sci. Technol.* 19, 025001. doi:10.1088/0963-0252/19/2/025001
- [23] Lukes, P., Brisset, J-L., Locke, B.R., 2012. Plasma Chemistry and Catalysis in Gases and Liquids. Parvulescu, V.I., Magureanu, M., Lukes, P., chapter 8, John Wiley & Sons.
- [24] Fridman, G., Friedman, G., Gutsol, A., Shekhter, A.B., Vasilets, V.N., Fridman, A., 2008. Applied Plasma Medicine. *Plasma Processes Polym.* 5, 503–533. doi:10.1002/ppap.200700154
- [25] Kong, M.G., Kroesen, G., Morfill, G., Nosenko, T., Shimizu, T., Dijk, J. van, Zimmermann, J.L., 2009. Plasma medicine: an introductory review. *New J. Phys.* 11, 115012. doi:10.1088/1367-2630/11/11/115012

- [26] Morfill, G.E., Kong, M.G., Zimmermann, J.L., 2009. Focus on Plasma Medicine. *New J. Phys.* 11, 115011. doi:10.1088/1367-2630/11/11/115011
- [27] Laroussi, M., 2005. Low Temperature Plasma-Based Sterilization: Overview and State-of-the-Art. *Plasma Processes Polym.* 2, 391–400. doi:10.1002/ppap.200400078
- [28] Lee, H.W., Nam, S.H., Mohamed, A.-A.H., Kim, G.C., Lee, J.K., 2010. Atmospheric Pressure Plasma Jet Composed of Three Electrodes: Application to Tooth Bleaching. *Plasma Processes Polym.* 7, 274–280. doi:10.1002/ppap.200900083
- [29] Pointu, A.-M., Ricard, A., Dodet, B., Odic, E., Larbre, J., Ganciu, M., 2005. Production of active species in N₂–O₂ flowing post-discharges at atmospheric pressure for sterilization. *J. Phys. D: Appl. Phys.* 38, 1905. doi:10.1088/0022-3727/38/12/009
- [30] Sarra-Bournet, C., Turgeon, S., Mantovani, D., Laroche, G., 2006. A study of atmospheric pressure plasma discharges for surface functionalization of PTFE used in biomedical applications. *J. Phys. D: Appl. Phys.* 39, 3461. doi:10.1088/0022-3727/39/16/S03
- [31] Brétagnot, F., Valsesia, A., Ceccone, G., Colpo, P., Gilliland, D., Ceriotti, L., Hasiwa, M., Rossi, F., 2006. Surface Functionalization and Patterning Techniques to Design Interfaces for Biomedical and Biosensor Applications. *Plasma Processes Polym.* 3, 443–455. doi:10.1002/ppap.200600015
- [32] Isbary, G., Morfill, G., Schmidt, H. u., Georgi, M., Ramrath, K., Heinlin, J., Karrer, S., Landthaler, M., Shimizu, T., Steffes, B., Bunk, W., Monetti, R., Zimmermann, J. I., Pompl, R., Stolz, W., 2010. A first prospective randomized controlled trial to decrease bacterial load using cold atmospheric argon plasma on chronic wounds in patients. *British Journal of Dermatology* 163, 78–82. doi:10.1111/j.1365-2133.2010.09744
- [33] Vandamme, M., Robert, E., Pesnel, S., Barbosa, E., Dozias, S., Sobilo, J., Lerondel, S., Le Pape, A., Pouvesle, J.-M., 2010. Antitumor Effect of Plasma Treatment on U87 Glioma Xenografts: Preliminary Results. *Plasma Processes Polym.* 7, 264–273. doi:10.1002/ppap.200900080
- [34] Fridman, G., Shereshevsky, A., Jost, M.M., Brooks, A.D., Fridman, A., Gutsol, A., Vasilets, V., Friedman, G., 2007. Floating Electrode Dielectric Barrier Discharge Plasma in Air Promoting Apoptotic Behavior in Melanoma Skin Cancer Cell Lines. *Plasma Chem Plasma Process* 27, 163–176. doi:10.1007/s11090-007-9048-4
- [35] Heinlin, J., Isbary, G., Stolz, W., Morfill, G., Landthaler, M., Shimizu, T., Steffes, B., Nosenko, T., Zimmermann, J., Karrer, S., 2011. Plasma applications in medicine with a special focus on dermatology. *Journal of the European Academy of Dermatology and Venereology* 25, 1–11. doi:10.1111/j.1468-3083.2010.03702.x
- [36] Li, Y.-F., Shimizu, T., Zimmermann, J.L., Morfill, G.E., 2012. Cold Atmospheric Plasma for Surface Disinfection. *Plasma Processes Polym.* 9, 585–589. doi:10.1002/ppap.201100090

- [37] Koban, I., Matthes, R., Hübner, N.-O., Welk, A., Meisel, P., Holtfreter, B., Sietmann, R., Kindel, E., Weltmann, K.-D., Kramer, A., Kocher, T., 2010. Treatment of *Candida albicans* biofilms with low-temperature plasma induced by dielectric barrier discharge and atmospheric pressure plasma jet. *New J. Phys.* 12, 073039. doi:10.1088/1367-2630/12/7/073039
- [38] Alkawareek, M.Y., Algwari, Q.T., Laverty, G., Gorman, S.P., Graham, W.G., O'Connell, D., Gilmore, B.F., 2012. Eradication of *Pseudomonas aeruginosa* Biofilms by Atmospheric Pressure Non-Thermal Plasma. *PLoS One* 7. doi:10.1371/journal.pone.0044289
- [39] Priya Arjunan, K., Morss Clyne, A., 2011. Hydroxyl Radical and Hydrogen Peroxide are Primarily Responsible for Dielectric Barrier Discharge Plasma-Induced Angiogenesis. *Plasma Processes Polym.* 8, 1154–1164. doi:10.1002/ppap.201100078
- [40] Arjunan, K.P., Friedman, G., Fridman, A., Clyne, A.M., 2011. Non-thermal dielectric barrier discharge plasma induces angiogenesis through reactive oxygen species. *J. R. Soc. Interface* rsif20110220. doi:10.1098/rsif.2011.0220
- [41] Miletić, M., Mojsilović, S., Đorđević, I.O., Maletić, D., Puač, N., Lazović, S., Malović, G., Milenković, P., Petrović, Z.L., Bugarski, D., 2013. Effects of non-thermal atmospheric plasma on human periodontal ligament mesenchymal stem cells. *J. Phys. D: Appl. Phys.* 46, 345401. doi:10.1088/0022-3727/46/34/345401
- [42] Freeman, T., Steinbeck, M., Fridman, G., Zhang, N., Shainsky, J., and Friedman, A., Fridman, G., Nonthermal dielectric barrier discharge plasma enhances skeletal cell differentiation and autopod development. In 4th International Conference on Plasma Medicine (ICPM4), Orléans, France, June 17–21 2012.
- [43] Huang, Z., 2005. A Review of Progress in Clinical Photodynamic Therapy. *Technol Cancer Res Treat* 4, 283–293.
- [44] Stoffels, E., Sladek, R.E.J., Kieft, I.E., 2004. Gas Plasma Effects on Living Cells. *Phys. Scr.* 2004, 79. doi:10.1238/Physica.Topical.107a00079
- [45] Lee, H.J., Shon, C.H., Kim, Y.S., Kim, S., Kim, G.C., Kong, M.G., 2009. Degradation of adhesion molecules of G361 melanoma cells by a non-thermal atmospheric pressure microplasma. *New J. Phys.* 11, 115026. doi:10.1088/1367-2630/11/11/115026
- [46] Yan, X., Zou, F., Zhao, S., Lu, X., He, G., Xiong, Z., Xiong, Q., Zhao, Q., Deng, P., Huang, J., Yang, G., 2010. On the Mechanism of Plasma Inducing Cell Apoptosis. *IEEE Transactions on Plasma Science* 38, 2451–2457. doi:10.1109/TPS.2010.2056393
- [47] Kim, C.-H., Kwon, S., Bahn, J.H., Lee, K., Jun, S.I., Rack, P.D., Baek, S.J., 2010. Effects of atmospheric nonthermal plasma on invasion of colorectal cancer cells. *Applied Physics Letters* 96, 243701. doi:10.1063/1.3449575

- [48] Kim, S.J., Chung, T.H., Bae, S.H., Leem, S.H., 2010. Induction of apoptosis in human breast cancer cells by a pulsed atmospheric pressure plasma jet. *Applied Physics Letters* 97, 023702. doi:10.1063/1.3462293
- [49] Cheng, X., Murphy, W., Recek, N., Yan, D., Cvelbar, U., Vesel, A., Mozetič, M., Canady, J., Keidar, M., Sherman, J.H., 2014. Synergistic effect of gold nanoparticles and cold plasma on glioblastoma cancer therapy. *Journal of Physics D: Applied Physics* 47, 335402. doi:10.1088/0022-3727/47/33/335402
- [50] Kim, G.C., Kim, G.J., Park, S.R., Jeon, S.M., Seo, H.J., Iza, F., Lee, J.K., 2009. Air plasma coupled with antibody-conjugated nanoparticles: a new weapon against cancer. *Journal of Physics D: Applied Physics* 42, 032005. doi:10.1088/0022-3727/42/3/032005
- [51] Kong, M.G., Keidar, M., Ostrikov, K., 2011. Plasmas meet nanoparticles—where synergies can advance the frontier of medicine. *Journal of Physics D: Applied Physics* 44, 174018. doi:10.1088/0022-3727/44/17/174018
- [52] Marc Vandamme. "Les plasmas froids, nouvelle stratégie thérapeutique en cancérologie." PhD thesis, Université d'Orléans, Orléans, 2012.
- [53] Vanessa Sarron " Étude et optimisation d'une décharge « Plasma Gun » à pression atmosphérique, pour des applications biomédicales" PhD thesis, Université d'Orléans, 2013.
- [54] Pouvesle, J.M., Cachoncinlle, C., Khacef, A., Viladrosa, R., Robert, E., Dozias, S., Patent : Transient plasma ball generation system at long distance in INPI, CNRS, Editor 2007: FR.
- [55] Lu, X., Laroussi, M., 2006. Dynamics of an atmospheric pressure plasma plume generated by submicrosecond voltage pulses. *Journal of Applied Physics* 100, 063302. doi:10.1063/1.2349475
- [56] Dawson, G. A. and Winn, W. P. A model for streamer propagation. *Zeitschrift für Physik A Hadrons and Nuclei*, 183(2) :159–171, 1965.
- [57] Jarrige, J., Laroussi, M., Karakas, E., 2010. Formation and dynamics of plasma bullets in a non-thermal plasma jet: influence of the high-voltage parameters on the plume characteristics. *Plasma Sources Sci. Technol.* 19, 065005. doi:10.1088/0963-0252/19/6/065005
- [58] Mericam-Bourdet, N., Laroussi, M., Begum, A., Karakas, E., 2009. Experimental investigations of plasma bullets. *J. Phys. D: Appl. Phys.* 42, 055207. doi:10.1088/0022-3727/42/5/055207
- [59] Chen, L., Zhao, P., Shu, X., Shen, J., Meng, Y., 2010. On the mechanism of atmospheric pressure plasma plume. *Physics of Plasmas* (1994-present) 17, 083502. doi:10.1063/1.3465665

- [60] Cao, Z., Nie, Q., Bayliss, D.L., Walsh, J.L., Ren, C.S., Wang, D.Z., Kong, M.G., 2010. Spatially extended atmospheric plasma arrays. *Plasma Sources Sci. Technol.* 19, 025003. doi:10.1088/0963-0252/19/2/025003
- [61] Zaplotnik, R., Kregar, Z., Bišćan, M., Vesel, A., Cvelbar, U., Mozetič, M., Milošević, S., 2014. Multiple vs. single harmonics AC-driven atmospheric plasma jet. *EPL* 106, 25001. doi:10.1209/0295-5075/106/25001
- [62] Jiang, N., Ji, A., Cao, Z., 2009. Atmospheric pressure plasma jet: Effect of electrode configuration, discharge behavior, and its formation mechanism. *Journal of Applied Physics* 106, 013308. doi:10.1063/1.3159884
- [63] Li, Q., Li, J.-T., Zhu, W.-C., Zhu, X.-M., Pu, Y.-K., 2009. Effects of gas flow rate on the length of atmospheric pressure non-equilibrium plasma jets. *Applied Physics Letters* 95, 141502. doi:10.1063/1.3243460
- [64] Karakas, E., Koklu, M., Laroussi, M., 2010. Correlation between helium mole fraction and plasma bullet propagation in low temperature plasma jets. *J. Phys. D: Appl. Phys.* 43, 155202. doi:10.1088/0022-3727/43/15/155202
- [65] Chen, Q., Zhang, Y., Han, E., Ge, Y., 2005. Atmospheric pressure DBD gun and its application in ink printability. *Plasma Sources Sci. Technol.* 14, 670. doi:10.1088/0963-0252/14/4/005
- [66] Zhu, W.-C., Li, Q., Zhu, X.-M., Pu, Y.-K., 2009. Characteristics of atmospheric pressure plasma jets emerging into ambient air and helium. *J. Phys. D: Appl. Phys.* 42, 202002. doi:10.1088/0022-3727/42/20/202002
- [67] Breden, D., Miki, K., Raja, L.L., 2012. Self-consistent two-dimensional modeling of cold atmospheric-pressure plasma jets/bullets. *Plasma Sources Sci. Technol.* 21, 034011. doi:10.1088/0963-0252/21/3/034011
- [68] Boeuf, J.-P., Yang, L.L., Pitchford, L.C., 2013. Dynamics of a guided streamer ("plasma bullet") in a helium jet in air at atmospheric pressure. *J. Phys. D: Appl. Phys.* 46, 015201. doi:10.1088/0022-3727/46/1/015201
- [69] Karakas, E., Koklu, M., Laroussi, M., 2010. Correlation between helium mole fraction and plasma bullet propagation in low temperature plasma jets. *J. Phys. D: Appl. Phys.* 43, 155202. doi:10.1088/0022-3727/43/15/155202
- [70] Kim, H.-H., 2004. Nonthermal Plasma Processing for Air-Pollution Control: A Historical Review, Current Issues, and Future Prospects. *Plasma Processes Polym.* 1, 91–110. doi:10.1002/ppap.200400028
- [71] Fridman, A., 2008. *Plasma Chemistry*. Cambridge University Press.

- [72] Ricard, A., 1996. Reactive Plasmas. Société Française du Vide.
- [73] Liu, D.-X., Rong, M.-Z., Wang, X.-H., Iza, F., Kong, M.G., Bruggeman, P., 2010. Main Species and Physicochemical Processes in Cold Atmospheric-pressure He + O₂ Plasmas. *Plasma Processes Polym.* 7, 846–865. doi:10.1002/ppap.201000049
- [74] Murakami, T., Niemi, K., Gans, T., O’Connell, D., Graham, W.G., 2013. Interacting kinetics of neutral and ionic species in an atmospheric-pressure helium–oxygen plasma with humid air impurities. *Plasma Sources Science and Technology* 22, 045010. doi:10.1088/0963-0252/22/4/045010
- [75] Lee, F.W., Collins, C.B., Waller, R.A., 1976. Measurement of the rate coefficients for the bimolecular and termolecular charge transfer reactions of He²⁺ with Ne, Ar, N₂, CO, CO₂, and CH₄. *The Journal of Chemical Physics* 65, 1605–1615.
- [76] Pouvesle, J.M., Bouchoule, A., Stevefelt, J., 1982. Modeling of the charge transfer afterglow excited by intense electrical discharges in high pressure helium nitrogen mixtures. *The Journal of Chemical Physics* 77, 817–825.
- [77] Van Dyck, R.S., Johnson, C.E., Shugart, H.A., 1971. Radiative Lifetime of the 2 S₀₁ Metastable State of Helium. *Phys. Rev. A* 4, 1327–1336. doi:10.1103/PhysRevA.4.1327
- [78] Drake, G.W.F., 1971. Theory of Relativistic Magnetic Dipole Transitions: Lifetime of the Metastable 2S₃ State of the Heliumlike Ions. *Phys. Rev. A* 3, 908–915. doi:10.1103/PhysRevA.3.908
- [79] Lin, C.D., Johnson, W.R., Dalgarno, A., 1977. Radiative decays of the n=2 states of He-like ions. *Phys. Rev. A* 15, 154–161. doi:10.1103/PhysRevA.15.154
- [80] Weller, R., 1997. Nitric oxide — a newly discovered chemical transmitter in human skin. *British Journal of Dermatology* 137, 665–672. doi:10.1046/j.1365-2133.1997.19332063.
- [81] Knowles, R.G., Moncada, S., 1994. Nitric oxide synthases in mammals. *Biochem J* 298, 249–258.
- [82] Dorai, R., Kushner, M.J., 2003. A model for plasma modification of polypropylene using atmospheric pressure discharges. *J. Phys. D: Appl. Phys.* 36, 666. doi:10.1088/0022-3727/36/6/309
- [83] Baulch, D.L., Cobos, C.J., Cox, R.A., Frank, P., Hayman, G., Just, T., Kerr, J.A., Murrells, T., Pilling, M.J., Troe, J., Walker, R.W., Warnatz, J., 1994. Evaluated Kinetic Data for Combustion Modeling. Supplement I. *Journal of Physical and Chemical Reference Data* 23, 847–848. doi:10.1063/1.555953

- [84] Tiny Verreycken "Spectroscopic investigation of OH dynamics in transient atmospheric pressure plasmas", PhD Thesis, Technische Universiteit Eindhoven, 2013
- [85] Bruggeman, P., Schram, D.C., 2010. On OH production in water containing atmospheric pressure plasmas. *Plasma Sources Sci. Technol* 19, 045025.
- [86] Liu, D.X., Bruggeman, P., Iza, F., Rong, M.Z., Kong, M.G., 2010. Global model of low-temperature atmospheric-pressure He + H₂O plasmas. *Plasma Sources Sci. Technol.* 19, 025018. doi:10.1088/0963-0252/19/2/025018
- [87] Cao, Z., Walsh, J.L., Kong, M.G., 2009. Atmospheric plasma jet array in parallel electric and gas flow fields for three-dimensional surface treatment. *Applied Physics Letters* 94, 021501. doi:10.1063/1.3069276
- [88] Nie, Q.Y., Cao, Z., Ren, C.S., Wang, D.Z., Kong, M.G., 2009. A two-dimensional cold atmospheric plasma jet array for uniform treatment of large-area surfaces for plasma medicine. *New J. Phys.* 11, 115015. doi:10.1088/1367-2630/11/11/115015
- [89] Nwankire, C.E., Law, V.J., Nindrayog, A., Twomey, B., Niemi, K., Milosavljević, V., Graham, W.G., Dowling, D.P., 2010. Electrical, Thermal and Optical Diagnostics of an Atmospheric Plasma Jet System. *Plasma Chemistry and Plasma Processing* 30, 537–552. doi:10.1007/s11090-010-9236-5

Chapter 2

Plasma Gun and experimental techniques

1 Plasma Gun

In this work different power supplies have been used to ignite plasma in the Plasma Gun reactor. The power supplies are mainly differentiated by the rising time of the voltage pulse, the voltage pulse polarity and the pulse repetition rate range. Each of them is described in this section.

1.1 Reactor design

The Plasma Gun [1-2] is a DBD-based reactor flushed by a continuous rare gas flow. Our reactor is constituted by an inner electrode, a dielectric capillary and an outer cylindrical electrode as shown in Figure 2.1.

The inner electrode is a cylinder with a hole of 1 mm through which rare gas is flushed. It is made of brass ended by a tungsten piece to avoid any, even small, possibility of corrosion and ablation at the plasma ignition zone. The inner electrode is connected to the High Voltage (H.V.). It is inserted into the dielectric capillary and airtightness is made thanks to o-seal rings. The dielectric capillary is of borosilicate with inner diameter of 4 mm and outer diameter of 6 mm. Its length can vary from cm to tens of cm, however in this work the length of the capillary has been fixed to 10 cm from the inner electrode tip to the capillary outlet. The outer electrode is made of concentric cylinders of different nature, a cylindrical brass piece (5 mm thickness) surrounded by 2 cylindrical dielectric pieces, Delrin® (4 mm thickness), to avoid corona discharge, and maintained on the borosilicate capillary by o-seal rings.

The Plasma Gun is flushed with Neon, Argon or Helium at moderate gas flow rate (from few tens of Standard Cubic Centimeter per Minute (sccm) to 2 000 sccm). In this work most of the experiments were carried on with Helium AlphaGaz1 (AirLiquide, France) at either 500 or 1000 sccm.

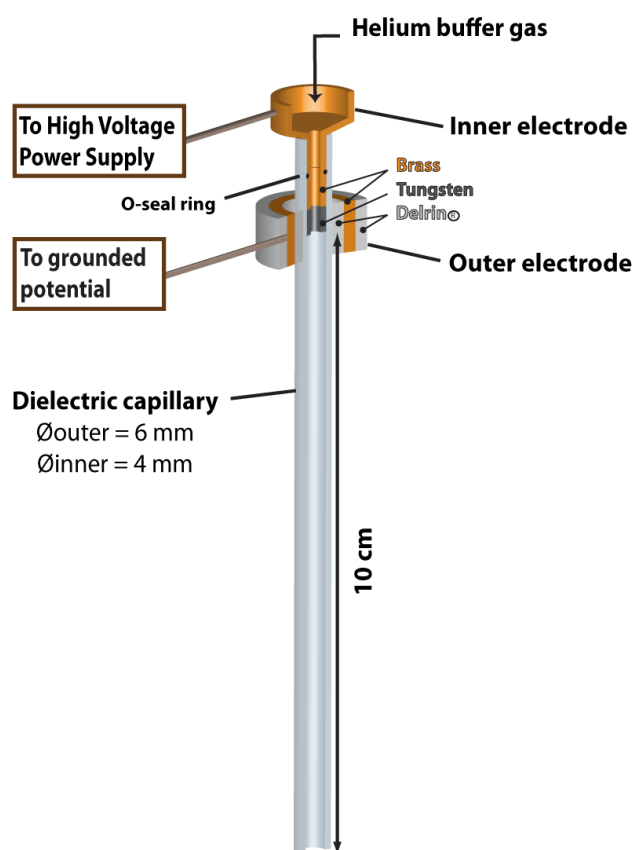


Figure 2.1: Scheme of the Plasma Gun

In the aim of endoscopic plasma treatments, the borosilicate capillary can be equipped with a flexible and thin plastic capillary of various diameters. For external treatments as well, the flexible capillary helps reaching the tumor when hard to reach. *In vivo* experiments described in Chapter 3 are in this configuration (see Figure 2.2).

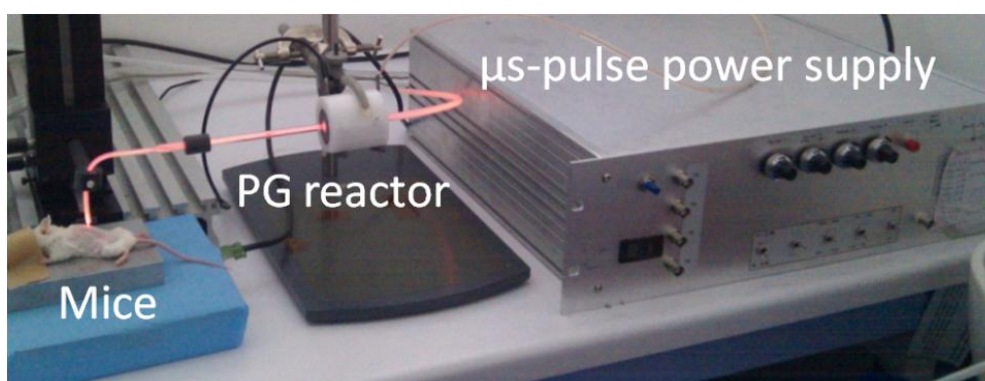


Figure 2.2: *In vivo* setup with Neon fed Plasma Gun.

1.2 Power supply

Three different high voltage power supplies have been used during this study. The main difference between them is the rising time and the polarity of the voltage waveform which is expected to modify the production of reactive species and the reduced electric field.

ns power supplies: thyatron and spark gap

Prior studies needed the use of a voltage pulse characterized by nanosecond rising time and high voltage amplitude delivery. This power supply was mainly used during the parallel PhD thesis of Vanessa Sarron [3]. The electrical circuit can be found in Figure 2.3.

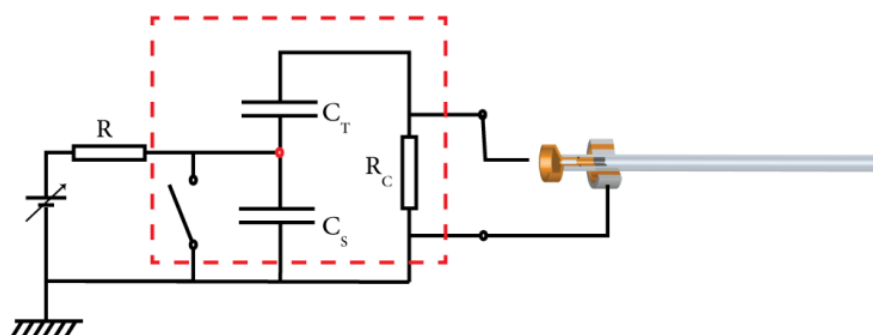


Figure 2.3: Scheme of the ns power supply.

With $C_s = C_t = 400$ pF and $R_c = 10$ k Ω , the red square defining the zone of the Blumlein system.

A.D. Blumlein developed a system based on two coupled transmission lines positioned at the exit of a fast commutating switch such as a Thyatron or a spark gap. The system at GREMI makes use of two capacitors (C_s and C_t) charged in parallel which are connected to the fast commutator (Thyatron HY 3202 MFR 25506). The capacitor C_s as storage capacitor, allows the ignition of the plasma inside the Thyatron while the C_t capacitor as transfer capacitor, allows the storage of the energy which will be delivered to the Plasma Gun. At Thyatron commutation time, an voltage inversion happens at the C_s edges which induces a difference of potential across the electrodes of the Plasma Gun.

This power supply is characterized by a pulse repetition rate from single shot to 500 Hz with voltage amplitude going from -5 to -50 kV. The Blumlein-line pulse forming network equipped with the Thyatron is expensive and voluminous. In the frame of biomedical applications, we tend to make the system more compact. Thus the Thyatron in the Blumlein line has been replaced for some experiments, such as FTIR presented in Chapter 4, by a spark gap. With use of the spark gap, this power supply generated voltage pulse with fast rising time (~ 20 ns) in positive or negative polarity. In both cases, the voltage is applied to

the inner electrode and the outer electrode is maintained at the grounded potential. This power supply allowed pulse repetition rate from 10 Hz to 500 Hz and was much more compact but still expensive with a limited pulse repetition rate. However it was used until the work presented in [4], determining the relatively low influence of the rising front duration, except for the possibility of overvoltage, as compared to the total duration of the voltage pulse, for long distance plasma propagation. As a matter of fact, to ensure plasma propagation a μs duration pulse is mandatory. This phenomenon will also be described briefly in Chapter 4.

μs power supply

The μs duration pulse device developed at GREMI is called "Abiopulse". A scheme of the electric circuit can be seen in Figure 2.4. It has been designed following a model of on/off use for biologists which gives a convenient interface for biomedical applications.

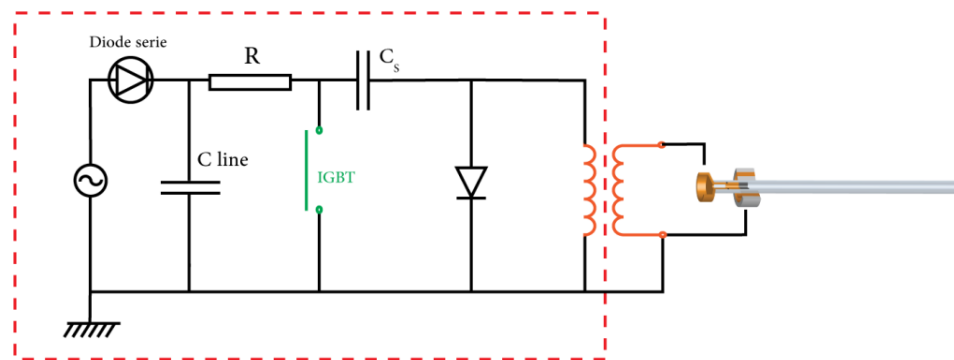


Figure 2.4: Scheme of the Abiopulse μs power supply.

Abiopulse is constituted by the following components (Figure 2.4):

- a voltage source,
- a capacitor C_s (470 nF),
- a bench of capacitors C ,
- a Switch (Transistor IGBT),
- and a step-up transformer.

When the switch is open, the capacitor C_s and the bench of capacitors are accumulating the energy and the current moves in the conventional direction. When the switch is closed an inversion of the voltage and current is undergoing, which leads to the transmission of the voltage into the transformer. This transformer multiplies the primary voltage and transfers it into the Plasma Gun where the discharge is ignited.

It is characterized by a pulse repetition rate from single shot to 8 kHz and voltage amplitude from 1 to 25 kV in negative or positive polarities. The rising front is of 2.3 μs for a FWHM of 4 μs . The voltage waveform keeps the same form, including the FWHM, whatever the voltage

amplitude. Together with the compactness of this power supply, it enables an increase of the pulse repetition rate. Moreover this power supply is reliable and stable during hours.

The polarity of the voltage pulse is influencing the plasma properties such as to cite only two of them its velocity [4-8] together with the mode of propagation and transfer [9]. More detailed analysis about polarity influence on plasma characteristics can be found in [3]. Further experiments are carried on by T. Darny (PhD thesis 2012-2015) to complete the analysis of the polarity influence on other plasma features. In this work, most of the presented experiments have been performed in positive polarity.

During the ignition, two plasmas are produced [3, 10], upstream from the reactor zone through the gas pipes and downstream from the reactor zone to the end of the capillary. In this work only the downstream plasma is studied.

2 Physical and Chemical analysis

Different diagnostics have been used during this work. In this sub-chapter, the techniques and corresponding setups used during this work are described and organized as follow:

1. Electrical characterization;
2. Imaging characterization including fast-imaging in the ns time-scale, wavelength-filtered imaging and Schlieren imaging;
3. Optical Emission Spectroscopy (OES);
4. Tunable Diode Absorption Spectroscopy (TDAS);
5. Fourier Transform InfraRed spectroscopy (FTIR).

Some experiments such as absorption spectroscopy and the high-resolution OES were performed in the frame of the ANR Blanc PAMPA in collaboration with Nader Sadeghi at LIPhy, Grenoble, France; the Schlieren imaging performed at ISAE Toulouse, France by E.Robert, V.Sarron and T. Darny in collaboration with L. Joly and J. Fontane.

2.1 Electrical characterization

The voltage waveform delivered to the Plasma Gun is measured by a high voltage probe from Tektronix (P6015A 75MHz) connected to an oscilloscope (Tektronix TDS 3054, 500MHz, 5GS/s). Voltage waveforms obtained with the Abiopulse and ns power supply can be found in Figure 2.5.

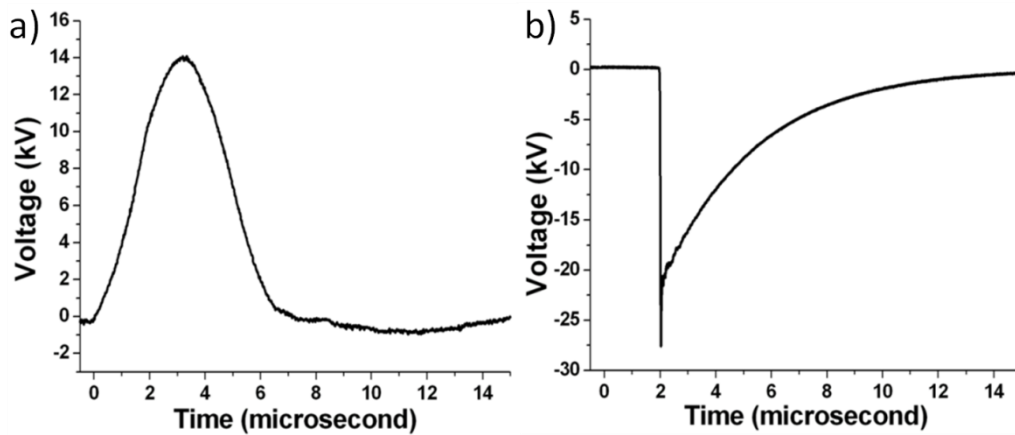


Figure 2.5: Voltage waveform of a) the Abiopulse μ s driver and b) the ns power supply.

Current measurements were performed with help of two Pearson probes Model 4100, one around the grounded wire to control the influence of the second which is placed around PG dielectric capillary (Figure 2.6).

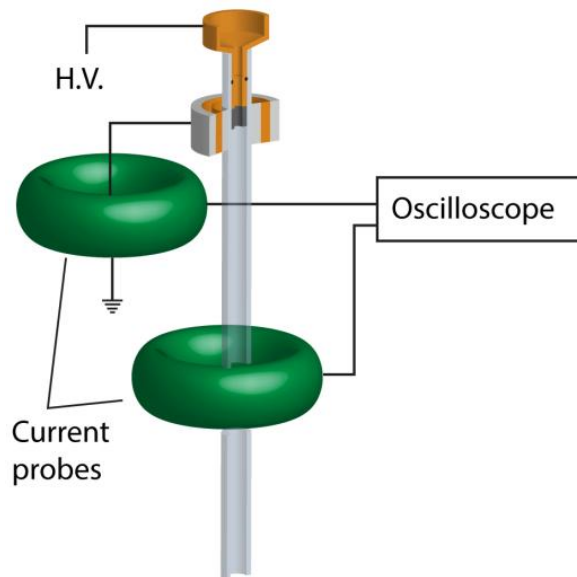


Figure 2.6: Experimental setup for current measurements.

It is important to notice that no influence from the current probe has been observed on the control current probe monitoring the current delivered by the pulse power driver. However the current probes induces a localized perturbation of the plasma [3, 11] by acting for example on the propagation velocity due to a modification of the electric field. In practice, discharge current is inferred from by subtracting the capacitive current measured when no plasma is generated, without buffer gas (plasma off), from the current signal (plasma on) (Figure 2.7). An estimation of the electron density (n_e) in the plasma has been conducted through the electric current measurements. n_e calculation from current pulse integration over time requires the assumption of a homogeneous plasma volume.

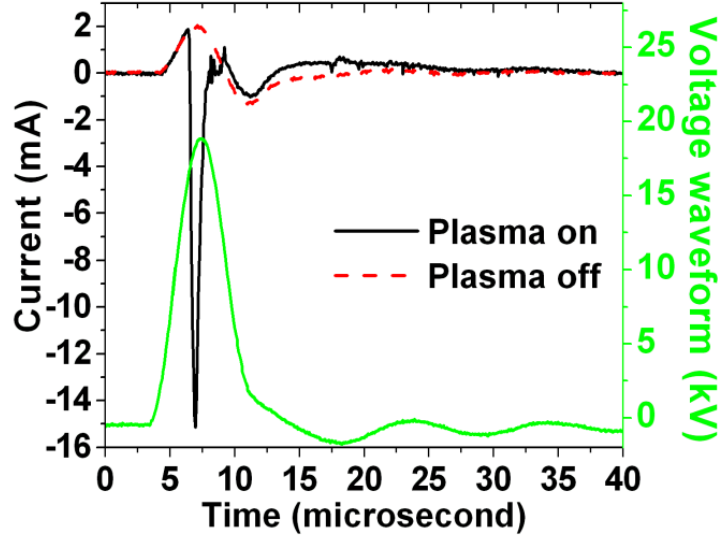


Figure 2.7: Current signals and voltage waveform, for He fed PG at 1 kHz, 1 l.min⁻¹ and 18 kV. Measurement performed at 7 cm from the inner electrode tip inside the capillary.

The volume considered is the intense emission of the plasma inside the capillary and has been estimated from fast-imaging experiment. With the PIMax3 ICCD camera, the smallest volume was observed with a 1 ns time-exposure image. The volume is approximated to 50 mm³. The electron density can be defined as the integral of dissipated current over a time period divided by the sampled volume such as:

$$n_e = \frac{\int i dt}{V \cdot e^-}$$

Where n_e is the electron density, $\int i dt$ the charge, V the volume scanned and e^- the elementary charge.

The calculated electron density is with this method of the order of few 10^{10} cm^{-3} with rather large error due to the experimental procedure. Uncertainties are linked to the estimation of the plasma volume plus the influence of the probes on the plasma.

Due to the difficulties in reducing the uncertainties, no other current measurements were performed during this work. Efforts were made on more convincing techniques of characterization.

2.2 Imaging system

Two ICCD cameras had been used at GREMI for the imaging and OES measurements. Both are from Princeton Instruments and are characterized by a resolution of 512 x 512 and 1024 x 1024 pixels and a minimal time exposure of 2 ns and 310 ps, respectively for the PIMax 2 and PIMax 3.

Those cameras were equipped with different lenses from Nikon, depending on the experiment, with characteristics described below:

- AF micro Nikkor, 60mm, f/2.8 D;
- AF micro Nikkor, 105mm, f/2.8 G; which zooms allowing observation of details in the plasma plume region;
- UV-NIKKOR, 105mm, f/4.5 (Nikon); which allows transmitting the UV range of plasma emission.

a) Fast imaging system

The ICCD camera was used to follow the fast propagation of the plasma within the capillary (Chapter 4) and when expanding in the air (Chapter 4 and 5).

The ICCD snapshot was synchronized and delayed with regards to the commutation of the power supply or to the light emitted by the plasma within the capillary when looking at the *in-air* expansion. As the detector is synchronized with the plasma, for one voltage pulse a unique snapshot is performed. Variation of the delay allows following the propagation of the plasma. Thus the study of the plasma propagation is a combination of multiple events. To ensure a good correlation between the different measurements, two optical fibers connected to a PMT were placed along the PG capillary to measure the plasma velocity between two points and respective intensity ratio. Small variations in the plasma propagation could be controlled and the snapshots presented in Chapter 4 and 5 are dealing with plasma events of same velocity and emission intensity.

b) Wavelength filtered imaging system

The Pimax2 equipped with the UV-Visible lens allows to capture all emissions from UV to near infrared. Filters have been positioned in front of the lens to select specific excited species emissions.

Different types of filters have been used

- Bandpass filters from LOT, also called interferential filters, which transmit light of a specific wavelength range, characterized by its centered wavelength and its bandwidth;
- High-pass filters from Schott which transmit light above a certain wavelength value, note as $\lambda > x$ nm, x being the wavelength where 10% of the light is transmitted.

The high-pass filters allow selecting part of the visible light emitted by the plasma. As will be shown in Chapter 4, in some experimental conditions, the light captured can be approximated as mainly revealing He* lines due to the low contribution of other components. However this approximation is no longer valid in the case developed in Chapter 5, when a conductive target is placed in front of the plasma plume. In this case N₂* (B-A) emits intensively in the same wavelength range as He* peaks.

Table 2.4 presents the list of the different filters used with their characteristics and the selected excited species.

Table 2.4: List of the filters with their characteristics

Filters type	Wavelength (nm)	Bandwidth (FWHM)	Excited species
Bandpass	239	25 nm	NO*
Bandpass	341	10 nm	N ₂ * (C)
High-pass	$\lambda > 515$	/	Mainly He*, O*, N ₂ * (B) if applicable
High-pass	$\lambda > 715$	/	Mainly O*, N ₂ * (B) if applicable

c) Schlieren imaging

As the plasma jet is evolving in a continuous rare gas flow, it is of great interest to evaluate its hydrodynamic behavior. The use of Schlieren imaging allows visualization of the gas flow. It is a qualitative diagnostics where the black and white zones observed in the gas flow correspond to variations of refractive index gradient due to density gradients between air rich and helium rich regions. Some measurements performed at ISAE Toulouse in the frame of the ANR Blanc "PAMPA", are presented in Chapter 4 and 5.

The difference of refractive index is obtained by the shine of a collimated light source onto the target object (Figure 2.8). The object distorts the light due to variation of density and a mirror reflects the distorted light onto a knife edge positioned at the focal point of the mirror. The knife edge cut off part of the signal before reaching the camera and light which has been distorted appears as darker or as lighter than the undistorted light.

In fluid dynamics, two regimes can be enlightened, laminar and turbulent, which have characteristic behaviors. The two zones are easily differentiable due to the weak gradients of density of the laminar zone and the contrasting strong and random gradients of the turbulent zone. As already mentioned in Chapter 1, the plasma is considered to extend only in the laminar regime of the gas flow. In this regime, the ambient air diffuses gradually in the rare gas flow, such as the excited nitrogen species at the mixing interface gives the conical shape of the plasma jet plume emission. Thus at the nozzle outlet, the rare gas flow is "supposed" to be 100 % pure.

In this work, the Plasma Gun is positioned vertically with reactor zone on top and tube outlet at the bottom. Reasons of this configuration are explained later in this Chapter. In this condition, some other phenomena have to be considered. Due to the fact that Helium is lighter than air, the gas jet is subjected to buoyancy forces.

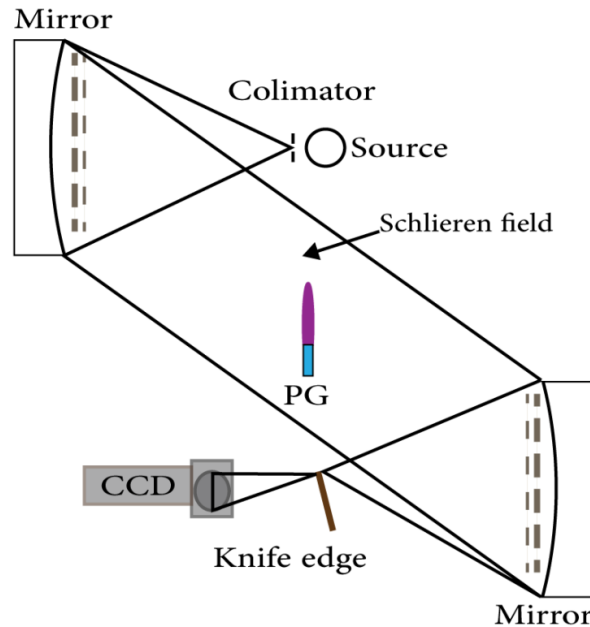


Figure 2.8: Schlieren imaging scheme.

The "normal" behavior of such top-down laminar gas jet exiting a nozzle can be described as follows. First the gas exiting the capillary will extend downward, in the same direction that inside the capillary. In this laminar channel, the gas is unchanged as compared to the one inside the capillary. At the border of the channel however changes of index can be observed representing the gradual diffusion of ambient air inside the channel. Gradually the buoyancy forces become stronger than the force produced by the gas flow rate. Thus depending on the gas flow rate, as well as the density of the gas and the diameter of the nozzle, the zone where the gas jet expansion will be stopped can vary. A bending of the jet can be observed in this zone and finally the gas flows upward.

2.3 Spectroscopic diagnostics

a) Optical Emission Spectroscopy (OES)

The optical emission spectroscopy defines the technique allowing for measurement of dispersed light emission. The instrumentation is constituted of a monochromator and a

detector. The resolution depends on the monochromator used (focal distance and grating), of the wavelength range (determined by the Blaze angle of the grating) and the order of dispersion in which the emission is detected. After the dispersion of the light, a detector is mandatory to observe the dispersed emission.

Two types of detector have been used, the first one is a photomultiplier tube (PMT) which convert the received photons into electrons and multiplies them. The PMT used in this work is a PMT R955 from Hamamatsu which has a high temporal resolution (5 ns). The produced current is observable thanks to an oscilloscope (Tektronix TDS 3054, 500 MHz, 5GS/s). The second one is an intensified charge-couple device (ICCD) camera (PIMax 2) which is connected to a computer.

Each measurement presented in Chapter 4 and 5 has been performed at least 3 times and have shown a good reproducibility. Uncertainties on the measurements increase with the decrease of the signal-to-noise ratio and are estimated to be 15 % of the measured signal. Error bars are not presented for readability purposes.

To resolve molecular bands and observe their rotational structures, a mid to high resolution is needed. However in a qualitative procedure the intensity of the molecular systems can be followed by the emission of the most intense transition. In this case, a low resolution can be used which usually allows for observation of a wider wavelength range.

In this work, six spectrometers were used to match the experiment requirements.

For high-resolution OES experiments performed at LIPhy spectra were acquired using a high resolution 2 m focal length monochromator (Sopra SA), equipped with a 1200 grooves/mm grating working in the third diffraction order. A 1600x400 pixel, 16 μm pitch charge coupled device CCD camera (Andor Technology, Newton EMCCD) used as detector.

The monochromators, VM505, VM504 (Acton Research) as well as the Jobin-Yvon, have been used at GREMI for low to mid resolution OES depending on the grating used. Those monochromators need to be linked to a detection system such as a photomultiplier tube or a (I)CCD camera. Those monochromators allow spectra acquisition together with time-resolved emission analysis.

Table 2: Characteristic of the 4 monochromators.

Spectrometers	Focal length	Grating	Spectral range	Laboratory
Jobin Yvon	10 cm	1200 gr/mm	200-800 nm	GREMI
VM504	30 cm	300 gr/mm	200-900 nm	GREMI
VM505	50 cm	3600gr/mm	200-400 nm	GREMI
Sopra	200 cm	1200gr/mm	200-1100 nm	LIPhy

The Maya and HR2000 Ocean Optics spectrometers allowed acquisition of spectra with relatively low resolution. However those spectrometers are convenient due to their ability to measure the whole UV-visible range from 200 to 1100 nm without scanning.

Two different types of measurements will be presented in the next chapters, the spatially-integrated and spatially resolved measurements. Integration of the signal over the whole plasma plume has been captured by positioning the monochromator directly in front of the plasma gun plume. In the case of spatially resolved measurements, an UV optical fiber has been used. The optical fiber was moving on the PG axis at 2 mm from the capillary (Figure 2.9). The position 1 is an example of optical fiber positioning for measurement of plasma emission inside the capillary and position 2 one for the detection of plasma emission at the target surface.

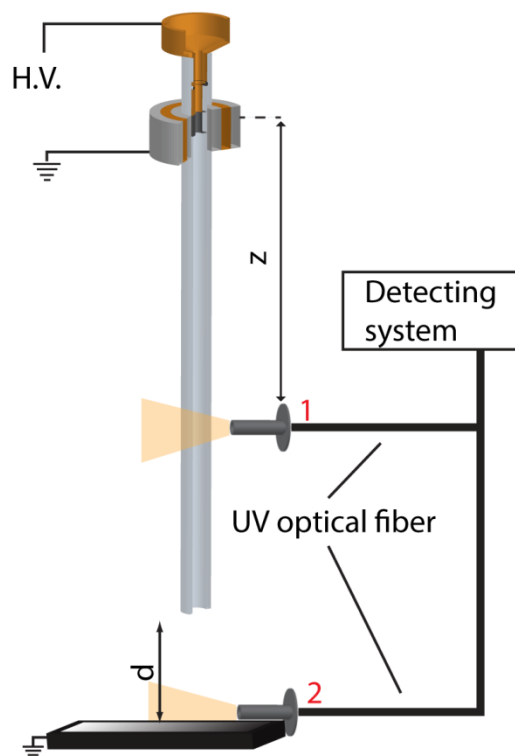


Figure 2.9: Scheme of the spatially resolved OES measurements.

At the surface of the metal plate, some reflections may happen which modify slightly the plasma emission intensity in this zone. To prevent such reflections, some mat black paint has been deposited on the plate. However no differences could be observed in the plasma emission pattern and intensity measurements either with the ICCD or with the PMT. As the chemical reactions between the paint and the plasma are unknown, we decided to use the uncovered metal plate.

Three examples of OES measurements are presented as preliminary experiments for plasma characterization or biomedical applications.

At low –resolution OES, the emission of OH*(A-X) has been followed versus time. This preliminary experiment focus on influence of the presence of impurity in our system.

At mid-resolution OES, rotational resolution, the gas temperature has been estimated by fitting the N₂* (SPS) band head with synthetic spectra.

At high- resolution OES, in the pm range, the broadening of the H_β line was studied to estimate the electron density present in the plasma.

Water vapor contain

To estimate the stability of the system in regards of excited species emissions, optical emission spectroscopy has been conducted directly with the first gas flush of the day of Helium at 500 sccm and simultaneous plasma ignition. Signal was retrieved thanks to monochromator VM505 (Princeton instrument) equipped with a PMT R955 (Hamamatsu) selecting the OH* (A-X) band emission at 306 nm. OH* emission has been chosen as an indicator of water impurities contained in the gas pipes.

Figure 2.10 shows the evolution of OH* emission intensity over a period of one hour.

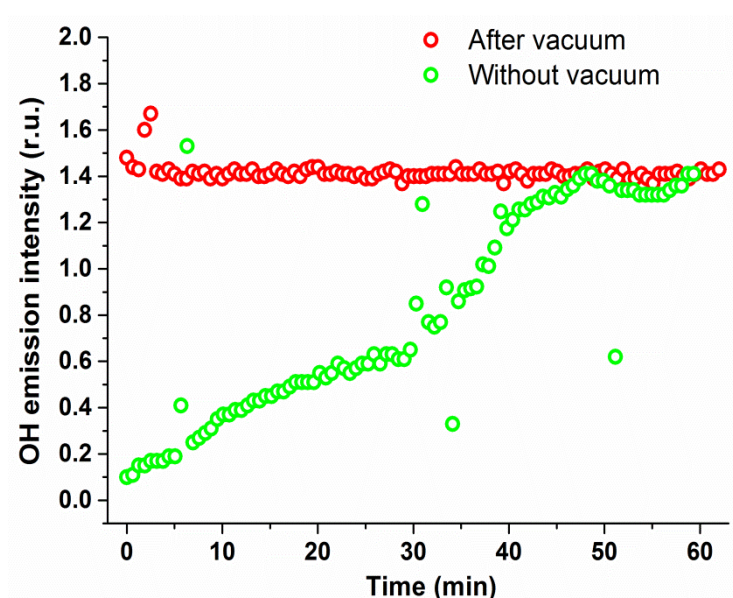


Figure 2.10 : Comparison of OH emission intensity versus time. After Vacuum (red empty dots) and without vacuum (green empty dots). In both case, plasma was On during the whole acquisition, 2 kHz, 14 kV, 500 sccm.

OH* emission was followed when no vacuum was preliminary performed (empty green dots). In this condition, an increase of OH* emission is observed during the first 45 min after

ignition and gas flow. After 45 min, the emission appears to be stable. To discriminate between possible plasma and gas flow effects, OH* emission was followed another day by setting the whole system under vacuum (from the buffer gas bottle to the Plasma Gun tube outlet). To achieve vacuum in the system, a seal with two o-seal rings is fixed at the tube outlet, and pumps are turned on. After reaching a 5.10^{-4} mbar, pumps are turned off and gas flow pushed in. The seal is ejected when the pressure becomes too high and plasma is ignited. The observed OH* emission intensity (red empty dots of Figure 2.0), in this case, is stable over the whole period. This experiments shows that the ignition of discharges in the kHz range is not responsible for the emission increase, rejecting the role of temperature in this process and consequently pointing out the effect of the gas flow. As a matter of fact, the gas flow acts as a progressive cleaner of the system and water impurities are decreased in the pipes which induces lower quenching of OH*.

The vacuum system is constituted of the two pumps plus pipes and controller. Together with the power supply, the vacuum system makes difficult to transport the whole system. Thus to avoid the use of the vacuum system, another set of experiments (data not shown) have been performed flushing different gas flow rates into the system showing a quicker stabilization of the signal with increasing gas flow. Those experiments lead us to clean of the system by flushing a 5 l.min^{-1} gas flow during 10 min before each operation of the Plasma Gun.

Temperature estimation

One plasma parameter essential to be considered for bio-medical application of plasma is the gas temperature. As a matter of fact, the aim of treatments is to induce selective response of cancer cells or tumors compared to healthy cells and tissues and not thermal effects which would be undesirable.

In non-equilibrium atmospheric plasmas, gas temperature is associated to rotational temperature of molecular excited species due to fast rotational energy transfers. Then gas temperature can be estimated by comparing rotational structures of molecular excited species with synthetic spectra. Rotational structures of N_2^* ($\text{C}^3\Pi_u - \text{B}^3\Pi_g$, $v'=0 \rightarrow v''=0$) second positive system (SPS) at 337.13 nm, measured by use of the VM505 monochromator has been fitted by synthetic spectra as shown in the Figure 2.31. The fit is performed with a home-made software developed by Nader Sadeghi and Esmaeil Eslami [12-13].

At 2 kHz and 14 kV input voltage, temperature has been estimated to be $320 \pm 10 \text{ K}$ [14]. This estimation will be confirmed by another technique in Chapter 6 by fitting of Laser induced fluorescence measurements on OH ground state with synthetic spectra. This temperature measurement is giving an upper limit of the real gas temperature due to extremely fast rotational energy transfers happening in non-equilibrium discharges at atmospheric pressure. As a matter of fact, this procedure assumes that the system is at

equilibrium between the different rotational levels of N_2 which may not be the case in such rapidly transient plasmas.

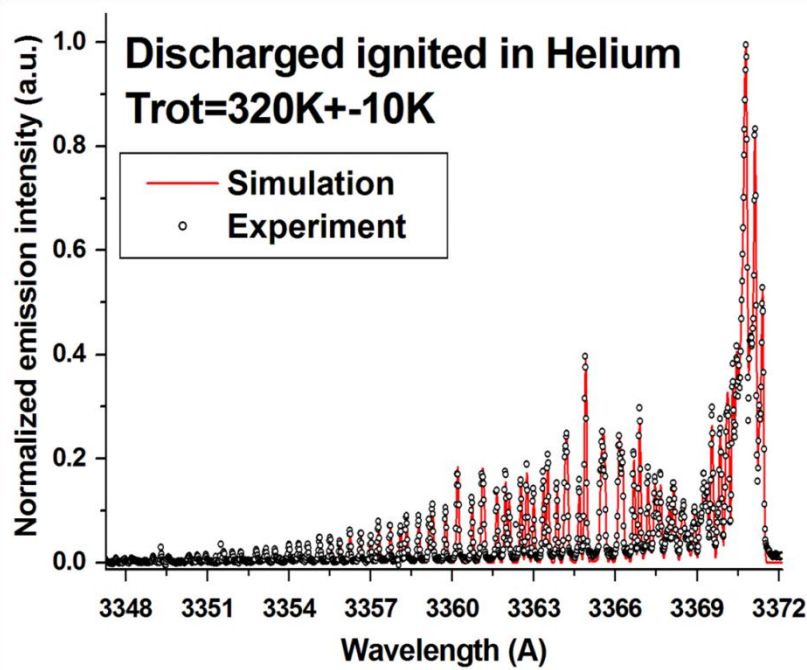


Figure 2.31 : Estimation of rotational temperature by comparison of synthetic spectrum (red line) and experiment (black empty dots) of N_2 SPS band head. Plasma parameters: power supply Abiopulse, 2 kHz, 14 kV, 500 sccm of Helium. Measurement performed at 2 cm from inner electrode tip inside the capillary.

Figure 2.42 shows synthetic spectra at different rotational temperatures from 320 to 380 K compared to experimental spectrum. Two wavelength ranges are presented, in Figure 2.4 a) rotational structure of N_2^* SPS at high rotational numbers (J) and in Figure 2.4 b) the band head corresponding to the degenerated triplet $J = 0$.

Figure 2.4 shows that spectra fitting are highly dependent on rotational temperature at high J while no difference between synthetic spectra could be observed for the SPS band head. From 340 K the synthetic spectrum is no longer fitting the experimental measurement confirming the low uncertainty (± 10 K) linked to this method. It is worth noting that experimental spectra are time and space integrated. Thus the estimated temperature is averaging the possible temperature gradients in time and in space, which could be present in the plasma plume. Those gradients are thought to mainly result from the plasma propagation and the gradual mixing of the buffer gas with the ambient air. Regarding of the biomedical applications, the temperature variations might be too fast and too small to interfere with cell and tissue response [15-16].

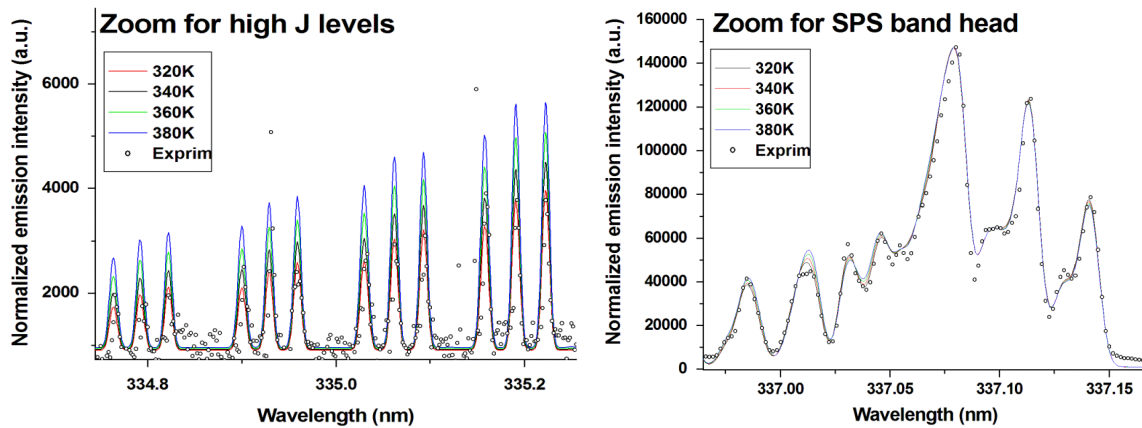


Figure 2.4 : Comparison of synthetic spectra at various rotational temperatures. Left: for high rotational levels Right: for the band head.

Another remark is that even if this value is close to room temperature, average temperature of 47° C is a high temperature for biological samples which are very sensitive to averaged temperature changes. By placing ones hand below the Plasma Gun plume, it suggests that the average temperature is under 47° C. Moreover, living tissues can temporally adapt to temperature gradients, such as localized high temperature of short duration. Temperature determined by synthetic spectrum model giving upper temperature together with the considerations listed above, suggest that heating may be a negligible parameter in antitumor Plasma Gun efficiency.

Estimation of electron density by H_{β} Stark broadening

The study to estimate the electron density has been performed in collaboration with Nader Sadeghi at LIPhy, Grenoble, in the frame of the ANR Blanc PAMPA. The setup used is the same as developed in [12].

The hydrogen Balmer-beta line at 486.1 nm has been selected to estimate the electron density through the stark broadening effect. The Stark broadening effect is a collisional broadening mechanism which affects spectral lines. In plasmas, this mechanism is due to the influence of the electric field of charged particles in movement on the emitting atom which splits its sub-atomic levels creating a broadening of the observed line. There exist other type of line broadening which need to be taken into account to evaluate the electron density from Stark broadening, such as the instrumental, the Doppler and the pressure broadenings, resonant and Van Der Waals ones. The Stark broadening effect conserves statistical information about the electric field and the relative charged particles distribution interfering with the emitting atom, allowing for the estimation of the electron density. Moreover for the H_{β} line, as well as for all other hydrogen line, the Stark broadening is linear with little interaction of the electron temperature [17].

At atmospheric pressure, the pressure broadening is relatively high and the Stark broadening becomes low in regards of the other broadening effects. The lower limit of detection of the H_β line is $5 \times 10^{13} \text{ cm}^{-3}$ which can be calculated. In our experiments, the Stark broadening of H_β line was not detected which informs that the electron density is under the detection limit.

To conclude, two different estimation of the electron density in the plasma produced by the Plasma Gun has been undertaken. The experimental determination of this quantity is in our conditions, not trivial. The two techniques performed have intrinsic limits and are not relevant in our case, for Stark broadening due to the high pressure broadening and for the current measurements due to the electrical influence of the measurement it-self. Due to these difficulties, another technique available at LiPHY could be used to estimate the electron density by following the He (2^3S) metastable state, as described below.

b) Tunable Diode Laser Absorption Spectroscopy (TDLAS)

Tunable Diode Laser Absorption Spectroscopy (TDLAS) experiments were performed at LiPHY, Grenoble in collaboration with Nader Sadeghi in the frame of the ANR Blanc "PAMPA".

A metastable state is an excited atom which has no allowed dipolar radiative transition [18], its ionization cross section are rather high [19-21]. The metastable state (2^3S) (He^m) can be used to determine the order of magnitude of the electron density considering that its main excitation process is by electron impact [19, 22]. However it is worth noting that this technique is highly sensitive to impurities which modify the kinetics of the system and interfere with the production and relaxation mechanisms of the He^m . In this experiment, the Helium gas was 99.99 % pure.

The setup used is the same as developped in [23] (Figure 2.5). The use of a tunable-wavelength laser diode allow scanning of the two components of the He ($2^3S_1 \rightarrow 2^3P_J$) transition for $J = 1$ and 2 separated by 2.3 GHz [24-25]. The laser diode is fixed at the maximum of absorption $\lambda = 1082.91 \text{ nm}$, and the average absorption of the He ($2^3S_1 \rightarrow 2^3P_J$) transition for $J = 1$ and 2 is measured.

In the Figure 2.64, He^m is followed versus time and plotted as a function of the ratio of the signal intensity received by the diodes with and without absorption. This parameter is linked to the He^m density by an exponential as shown in the formula below:

$$\langle N_i \rangle = \frac{\Delta \nu_L}{l} 1.24 \times 10^{15} \times \ln \frac{I_0}{I}$$

With $\langle N_i \rangle$ the He^m average density in m^{-3} , $\Delta \nu_L$ the FWHM of the lorentzian profile of the peak in GHz , l the length of absorption, I_0 the signal without absorption and I the signal with

absorption. The coefficient $1.24 \cdot 10^{15}$ is calculated from the oscillator forces of $J = 1$ and 2 ($f_{11} = 0.1797$ et $f_{12} = 0.2994$ [24]).

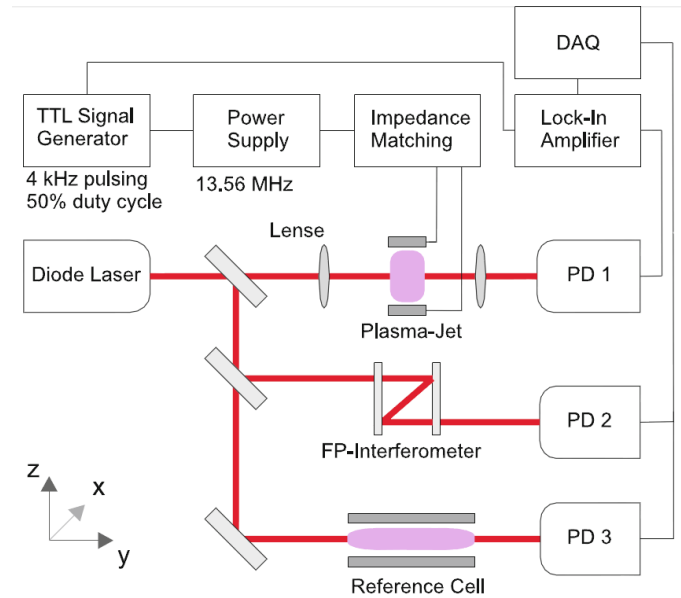


Figure 2.5: Experimental Setup for TDLAS measurements. PD1 to PD3 are Photodiodes to measure the transmitted beam intensity. From [23].

Figure 2.64 shows that He^m absorption signal, linked to the density by an exponential, is increasing with gas flow rate. Inside the tube, at 12 mm from the outlet, the increase of the gas flow rate is linked to a reduction of the air penetration inside the Helium flow in the plume and a relative decrease of impurities in the capillary. Thus at higher gas flow rate, the collision of He^m with gas impurities is lowered which results in a higher density. In Figure 2.6, we can also observed a second production of He^m , $4.5 \mu\text{s}$ after the first peak which is linked to the plasma produced during the second front of the pulse voltage [4] and will be discussed in chapter 4.

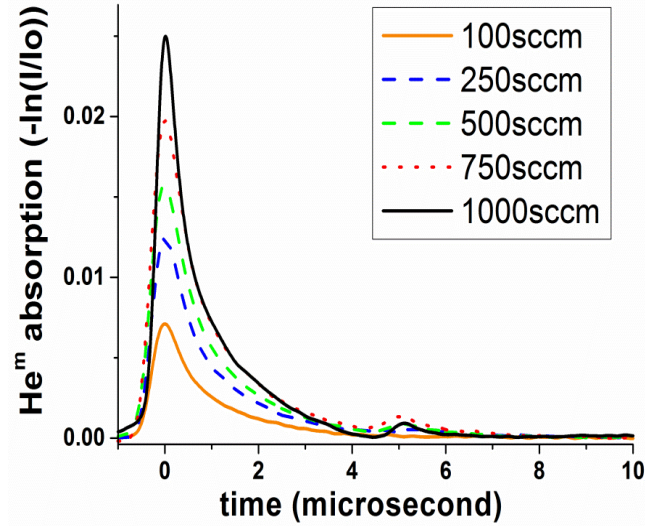


Figure 2.64 : Temporal evolution of He (2^3S) absorption for various gas flow rates. Measurement inside the capillary at 88 mm from inner electrode tip, at 2 kHz and 18 kV.

He^m density is scanned spatially along the PG capillary and in the *in-air* expending plume (Figure 2.7). The spatial resolution is obtained by the use of a diaphragm of 4x4 mm² positionned on the beam optical path and uncertainties on measurements have been estimated to be 20 %.

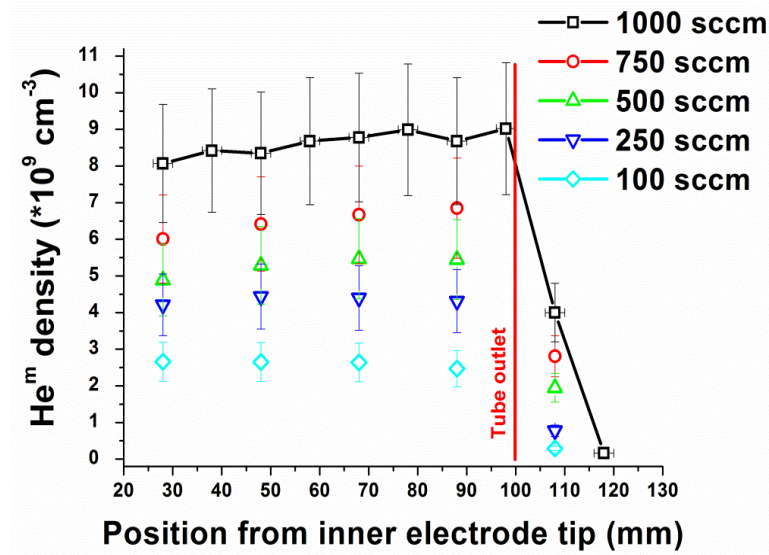


Figure 2.7 : Spatial evolution of He (2^3S) density within and outside the capillary for different gas flow rates. 2 kHz, 18 kV.

Figure 2.7 shows that He^m density is stable along the propagation in the capillary and decrease drastically when expending in the air. It tends to indicate that He^m are quenched by air components more than by collisions with Helium atoms or derivatives (ions and molecular states) which is coherent with literature [22]. Moreover Douat *et al.* observed a difference of one order of magnitude in the He^m density while using different purities of Helium gas,

namely He Standard (99.99%) and He alphagaz1 (99.999%) (Air Liquide). This observation supports the importance to consider the role of air impurities for quantification of produced plasma species. The density of $\sim 8.10^9 \text{ cm}^{-3}$, found with the 1 l.min^{-1} He Standard flow at 2 kHz and 18 kV, is thus coherent (one order of magnitude difference) with the estimation of the electron density by current measurements performed with the He alphagaz1.

To perform a full study concerning the He metastable states in the plasma jet, it would be interesting to improve the spatial resolution in the plasma plume along the axis and in radial direction. It would be then possible to discriminate between the roles of impurities present in the gas and the ambient air diffusion inside the jet. Development of numerical models taking into account the hydrodynamics of the jet would be of great help in determining Helium metastable distribution and quantification as well as the quantification of electron density in non-thermal plasma jets.

c) Fourier Transform Infrared spectroscopy (FTIR)

A molecule can be excited to a higher vibrational state by absorbing IR radiation. The probability of a particular IR frequency being absorbed depends on the actual interaction between this frequency and the molecule. In general, a frequency will be strongly absorbed if its photon energy coincides with the vibrational energy levels of the molecule. IR spectroscopy is therefore a very powerful technique which provides fingerprint information on the chemical composition of the sample. This requires that the radiation source covers a broad spectral range and the individual frequencies of the radiation are analyzed. FTIR spectrometer is constituted of a Michelson interferometer leading to obtain an interferogram which will be mathematically modified into a spectrum via Fourier transform.

The results presented in this work come from two different FTIR setups.

In Chapter 4, the results are obtained at GREMI with the FTIR spectrometer Magna-IR 550 series II (Nicolet) from Olivier Aubry group, working in the diagnostic range 500 to 4000 cm^{-1} with an internal resolution of 1 cm^{-1} . The measurement cell is of 1 L with optical path length of 10 m. In this setup the whole system is under atmospheric pressure. The PG capillary is linked to a gas pipe of 1 m long allowing the gas effluent to reach the measurement cell.

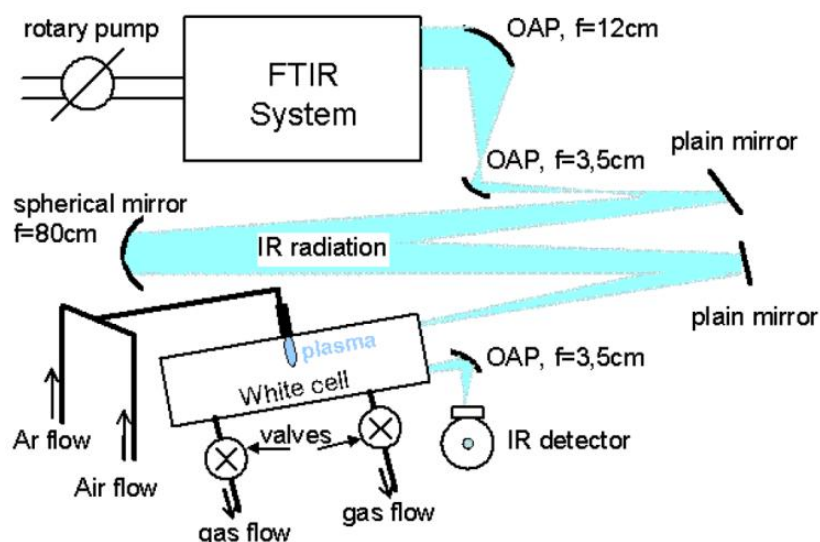


Figure 2.8: Experimental FTIR setup of the INP Greiswalt instrument from [29]

In Chapter 5, the results are obtained at INP Greiswald with the FTIR (Bruker IFS 66v/s) from Stephan Reuter group, working in the diagnostic range $700\text{ cm}^{-1} - 4000\text{ cm}^{-1}$ with an internal resolution of 0.5 cm^{-1} [29]. In this setup, a special chamber (the white cell) has been built to connect the Plasma Gun working zone at atmospheric pressure to the measurement cell at 100 mbar as shown in Figure 2.8. The measurement cell is of 4.5 L with optical path length of 30 m. The whole system, except the white cell, is placed into a container which is purged with nitrogen. The main advantage to decrease the pressure in the measurement cell is to freeze the chemical reactions before measurements.

A known problem in FTIR analysis is linked to the high absorbance of CO_2 and H_2O [21, 26-28]. As the quantity of those species often changes between the background acquisition and the measurement it-self, the background substraction process induces artefacts such as negative absorbance. Another problem linked to the presence of those species is the induced lost of information. Due to the extended absorbance wavenumber range of those molecules, the weaker signals in these ranges may be overlapped and difficult to identify. In our experiments, especially H_2O bands overlaps with molecules of interest, such as NO , NO_2 , HNO_2 and HNO_3 . Thus the results presented in this work are of qualitative nature.

3 Plasma Gun configuration for biomedical applications

As described in the last section of Chapter 1, the plasma medicine field is in need of *in situ* plasma jet characterization for optimization of biomedical applications. While in literature, studies can be found on one side about plasma jet physical and chemical properties and on the other side on biological effect after plasma treatment, characterization considering the whole system, jet plus treated target are pretty rare.

It is worth noting that most studies are performed with the jet positioned horizontally during plasma jet *in-air* expansion. As we will see in this work, many parameters such as, to cite only two of them, the gas flow hydrodynamics and the electromagnetic environment, are significantly influencing the plasma properties. To estimate the antitumor effect of the plasma, some *in vitro* and *in vivo* studies are carried on. *In vitro* studies are dealing with liquid cell media placed in well and *in vivo* experiments with tumors growing on diverse organs in mice. Both experiments need to bring the plasma on a surface which, in our experiments, is lying on a conductive grounded metal plate. Especially in the case of the liquid cell media, plasma should arrive from top. Thus we first defined the conditions mandatory for biological trials and kept them for the plasma characterization. In practice the Plasma Gun has been positioned vertically with the reactor zone on top and the tube outlet at bottom.

During *in vivo* studies, the distance between the inner electrode tip and the capillary outlet was set at 20 cm (10 cm borosilicate plus 10 cm adapter and flexible capillary) while for *in vitro* experiments and plasma characterization, the borosilicate capillary was used alone and measured 10 cm length.



Figure 2.9: Setup of the PG with the target. d been the variable distance between the capillary outlet and the target.

During *in vitro* and *in vivo* experiments, a grounded metallic plate was placed under the target, culture wells or mice (Figure 2.9). In order to keep maximum parameters constant, the same metallic plate was also used for spectroscopic and imaging experiments. To vary the humidity contains and shape of the target; some experiments make use of a potato, potentially mimicking the mice. The gap between the tube outlet and the target has been varied depending on the experiment.

4 Biological techniques and methods

During the first year of my PhD thesis, I was involved in the biomedical application side of the APR project "Plasmed". This study was performed in collaboration with Laura Brullé (PhD thesis 2009-2012) [30] from CIPA, Orléans, France. The main target of those experiments was the pancreatic carcimona.

Pancreatic tumors are the gastrointestinal cancer with the worst prognostic in humans and with a survival rate of 5%, 5 years after the first diagnosis. Nowadays, no chemotherapy has demonstrated efficacy in terms of survival for this cancer. The reference chemotherapy is Gemcitabine and experiments comparing its anti-tumor activity to plasma treatment are presented in Chapter 3.

These experiments are following the work of Marc Vandamme (2009-2012) [10], although he mainly used the Floating-Electrode DBD (FE-DBD) and focused on other tumor cell lines such as colorectal, lung and brain tumors.

In this section the methods and techniques used for the evaluation of the antitumor efficiency of the Plasma Gun over the pancreatic carcimona are given. First the *in vitro* and *in vivo* experimental details will be described then the technique of detection used, namely the bioluminescence, will be resumed.

4.1 *In vitro*

MiaPaCa2 pancreatic cancer cell line (American Type Culture Collection) was used for *in vitro* and *in vivo* studies.

For *in vitro* assays, cells (5×10^4) were seeded in 24 well culture plates in cell culture medium (VWR International). The Dulbecco Modified Eagle medium is supplemented with various nutriments. Cells are proliferating in a humid incubator at 37 °C overnight before treatment. Culture plates were placed on a grounded metallic plate and a gap of 2 mm was kept constant between the end of the dielectric capillary and the top of the cell medium (500 μ l).

Half maximal inhibitory concentration (IC_{50}) is a quantitative measure of the effectiveness of a treatment. In this work the IC_{50} has been determined by *in vitro* assays using Plasma Gun device to compare treatment parameters such as rising front duration and repetition rate

This is worth mentioning that the choice of the culture medium used for incubation should be consider for treatment efficiency. As described in [10], the different additive contained in the medium can specifically or un-specifically interfere with plasma produced reactive oxygen and nitrogen species. A recent study confirmed this observation on multi-cellular tumor spheroids [31]. Another remark is that those additives can modify the conductivity of the solution which induces a modification of the coupling between plasma and target as will be described in Chapter 5 and 6.

4.2 *In vivo*

In vivo experiments were performed at CIPA and surgery was undertaken by L. Brullé. All experiments were performed according to the national animal care guidelines (EC directive 86/609/CEE, French decree no 87-848) and mice were under general anesthesia administered by gaseous anesthesia, 2.5% isoflurane in air (Aerrane).

Prior to antitumor evaluation experiments, some tolerance studies are needed. Tolerance studies are performed on 2 groups of 4 mice each, first on ears and then directly on healthy pancreas. Parameters such as voltage amplitude and repetition rate are varied to determine best conditions with maximum "dose-rate" in regards of mice tolerance. Visual determination of inflammation was followed directly after the 10 min of NTP treatment and after 48 hours.

Tumor induction is performed on Female Swiss nude mice injected by a needle contenting $2 \cdot 10^6$ MIA PaCa2 luc cells in 50 μ l of PBS 1X. Orthotopic implantation was achieved surgically directly into the pancreas.

Orthotopic implantation has the advantage to show a response of cancerous cells in the organ where it is supposed to grow. However for human cancerous cells, such as the MIA PaCa2 cells, to be tolerated and grow inside the mice organs, the mice should be immunodeficient. This immunodeficiency can impact the response of the organism to treatment.

To treat the pancreas, a surgery procedure is needed. The pancreas is externalized directly after the anesthesia and a 10 min plasma treatment is performed. After treatment, the pancreas is replaced inside the mouse and a three point suture is done. The surgery implies a stress in the mouse which can impact on the cells behavior. To take this phenomenon into account, the control group also suffers surgery although no plasma treatment is undertaken.

Caliper measurement is the reference procedure to measure tumor volume (V in mm^3) which is calculated from the formula of an ellipsoid. When dealing with tumors positioned sub-cutaneously, the caliper measurements can be performed directly from outside the mice. Indeed in the case of tumors positioned on deep organs, such as pancreas, caliper measurement needs an externalization of the tumor which is often performed after mice sacrifice. In our protocol, caliper measurements have been performed at each externalization of the pancreas.

4.3 Bioluminescence detection system

To control tumor activity *in vivo* and evaluate antitumor potentialities of plasma treatment, a non-invasive technique is used in addition to caliper measurement. Bioluminescence imaging (BLI) is an imaging technique based on photon releasing chemical reactions catalyzed by luciferase. BLI enables real time and non invasive imaging of tumor evolution and cell growth. One major advantage of BLI is to give insights of the metabolic activity of the tumor and cells. As a matter of fact, the volume of a tumor may contain some necrotic or hypoxic areas which are not, or less, actives and would in both cases, not exhibit BLI signal. Thus BLI allows differentiating between the tumor activity and the tumor volume. This technique is also used *in vitro* to access cell survival rates.

To perform BLI, cells were transfected with firefly luciferase gene. L. Brullé reported [30] that this transfection did not alter the cell proliferation rate and response to conventional chemotherapy treatment.

This enzymatic reaction needs Adenosine-5'-triphosphate (ATP) and O_2 to form the oxidized complex which release photons. Unlike for *in vitro* growth, *in vivo* the vascular system surrounding the tumor tries to adapt to its increasing needs by developing vessels. Nevertheless, the cancerous cells proliferate faster than the adaptation of the vascular system, meaning that not enough ATP and O_2 can reach the entire tumor volume. This phenomenon called hypoxia impacts on the bioluminescence visualization for large volume tumors. In the case of hypoxic tumors, the BLI will not reflect the tumor metabolism anymore due to the lack of O_2 in the cells environment. The pancreatic model developed by L. Brullé *et al.* [30, 32] shows a decrease in BLI intensity from day 42 after induction due to hypoxia. For this reason and to increase the pertinence of such diagnostic, our experiments were limited to 36 days.

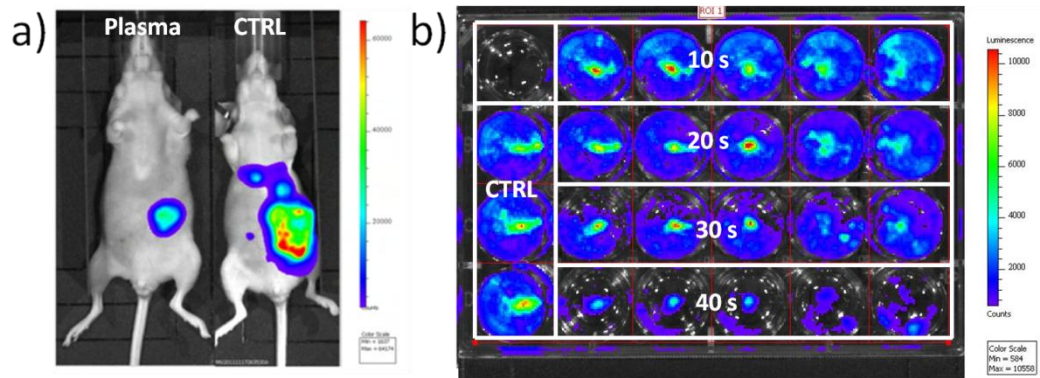


Figure 2.18: Bioluminescence imaging of a) *in vivo* experiments showing mice with pancreatic tumors, from [10] and b) *in vitro* experiments showing a response to different PG treatment duration, from [30].

Bioluminescence imaging allows also mice randomization prior to plasma treatment. The mice randomization was carried out 4 days after cancerous cells inoculation to constitute groups of equivalent average bioluminescence intensity.

Figure 2.18 shows examples of raw BLI *in vivo* and *in vitro* measurements. For *in vitro* experiments, bioluminescence was measured after a 24 h and 48h incubation period and an injection of 300 $\mu\text{g/ml}$ luciferin potassium salt per well. For *in vivo* experiments, bioluminescence was followed weekly. For *in vitro* assays, results are plotted as percentage of viable cells from untreated cells while *in vivo*, results are presented as percentage of increase of bioluminescence intensities in photons/s from untreated target.

French summary of chapitre 2

Dans ce chapitre, la configuration du réacteur plasma utilisé durant ce travail, appelé le Plasma Gun, ainsi que les générateurs de tension utilisés sont décrits. De plus les diagnostics de caractérisation du plasma sont détaillés. Cette partie comprend:

1. Les mesures électriques, courant et tension;
2. L'imagerie incluant l'imagerie rapide, l'imagerie filtrée en longueur d'onde et l'imagerie Schlieren;
3. La spectroscopie d'émission optique (OES)
4. La spectroscopie d'absorption laser (TDLAS)
5. L'infrarouge à transformée de Fourier (FTIR)

Les mesures ont été majoritairement effectuées au GREMI mais certaines ont été rendues possibles grâce à des collaborations dans le cadre de l'ANR Blanc PAMPA. L'imagerie Schlieren a été effectuée à l'ISAE, Toulouse par E. Robert, V. Sarron et T. Darny en collaboration avec L. Joly et J. Fantane; la TDLAS ainsi que des mesures OES à haute résolution ont été réalisées pour leur part en collaboration avec N. Sadeghi au LIPhy, Grenoble.

Certaines mesures telles que la stabilité de l'émission du radical hydroxyle au cours du temps, l'estimation de la température gaz et de la densité d'Hélium métastable (2^3S), sont détaillées et discutées dans ce chapitre en tant qu'études préliminaires à la caractérisation du plasma.

Ensuite une description des contraintes imposées à l'étude du plasma par les applications biomédicales, nous amène à définir le positionnement du Plasma Gun lors de la caractérisation ainsi que l'utilisation d'une cible imitant les cibles biologiques.

Dans une dernière partie, les méthodes liées à l'étude biologique, *in vitro* et *in vivo*, de l'effet du plasma sur les cellules et tissus cancéreux ainsi que le système de détection de leur activité, l'imagerie de Bioluminescence, sont détaillées.

References

- [1] E. Robert, E. Barbosa, S. Dozias, M. Vandamme, C. Cachoncinlle, R. Viladrosa, and J-M. Pouvesle. "Experimental study of a compact nanosecond plasma gun." *Plasma Processes and Polymers*, 6(12) :795–802, 2009.
- [2] Pouvesle, J.M., Cachoncinlle, C., Khacef, A., Viladrosa, R., Robert, E., Dozias, S., Patent : Transient plasma ball generation system at long distance in INPI, CNRS, Editor 2007: FR.
- [3] Vanessa Sarron. " Étude et optimisation d’une décharge « Plasma Gun » à pression atmosphérique, pour des applications biomédicales" PhD thesis, Université d'Orléans, Decembre 2013
- [4] Robert E, Sarron V, Riès D, Dozias S, Vandamme M and Pouvesle J M, “Characterization of pulsed atmospheric-pressure plasma streams (PAPS) generated by a plasma gun” *Plasma Sources Sci. Technol.***21** (2012) 034017.
- [5] Jiang C, Chen MT and Gundersen MA 2009 Polarity-induced asymmetric effects of nanosecond pulsed plasma jets. *J. Phys. D: Appl. Phys.* **42** 232002.
- [6] Urabe, K., Morita, T., Tachibana, K., Ganguly, B.N., 2010. Investigation of discharge mechanisms in helium plasma jet at atmospheric pressure by laser spectroscopic measurements. *J. Phys. D: Appl. Phys.* **43**, 095201. doi:10.1088/0022-3727/43/9/095201
- [7] Walsh, J.L., Iza, F., Janson, N.B., Law, V.J., Kong, M.G., 2010. Three distinct modes in a cold atmospheric pressure plasma jet. *J. Phys. D: Appl. Phys.* **43**, 075201. doi:10.1088/0022-3727/43/7/075201
- [8] Xiong, Z., Lu, X., Xian, Y., Jiang, Z., Pan, Y., 2010. On the velocity variation in atmospheric pressure plasma plumes driven by positive and negative pulses. *Journal of Applied Physics* **108**, 103303. doi:10.1063/1.3511448
- [9] Xiong Z, Robert E, Sarron V, Pouvesle J-M and Kushner M J 2013 “Atmospheric-pressure plasma transfer across dielectric channels and tubes” *J. Phys. D: Appl. Phys.***46** 15520
- [10] Marc Vandamme. "Les plasmas froids, nouvelle stratégie thérapeutique en cancérologie." PhD thesis, Université d'Orléans, Orléans, 2012.
- [11] G V Naidis and J L Walsh "The effects of an external electric field on the dynamics of cold plasma jets—experimental and computational studies" *J. Phys. D: Appl. Phys.* **46** (2013) 095203

- [12] S.G. Belostotskiy, T. Ouk, V.M. Donnelly, D.J. Economou, D. J. and N. Sadeghi, "Gas temperature and electron density profiles in an argon dc microdischarge measured by optical emission spectroscopy." J. Appl. Phys. 107 053305 (2010).
- [13] E. Eslami, "Etude des mécanismes de formation de la zone d'ionisation secondaire en post-décharge d'un plasma micro-onde d'azote," PhD thesis, Grenoble I, (2005).
- [14] D Riès, E Robert, S Dozias, M Vandamme, V Sarron, JM Pouvesle, "Characterization of plasma sources for biomedical applications" 20th International Symposium on Plasma Sciences, Philadelphie (USA), 24–29 Juillet 2011 (POSTER)
- [15] Hildebrandt, B., et al., The cellular and molecular basis of hyperthermia. Critical Reviews in Oncology Hematology, 2002. 43(1): p. 33-56.
- [16] Song, C.W., et al., Improvement of tumor oxygenation status by mild temperature hyperthermia alone or in combination with carbogen. Semin Oncol, 1997. 24(6): p. 626-32.
- [17] H.R. Griem "Spectral line broadening by plasma", Academic Press, New-York & London, 1974
- [18] E. Biémont, Spectroscopie Atomique, De boeck. (2006).
- [19] Biondi, M.A., 1952. Diffusion, De-Excitation, and Ionization Cross Sections for Metastable Atoms. I. Phys. Rev. 88, 660–665. doi:10.1103/PhysRev.88.660
- [20] L. L. Alves, K. Bartschat, S. F. Biagi, M. C. Bordage, L. C. Pitchford, C. M. Ferreira, G. J. M. Hagelaar, W. L. Morgan, S. Pancheshnyi, A. V Phelps, V. Puech, and O. Zatsarinny, "Comparisons of sets of electron–neutral scattering cross sections and swarm parameters in noble gases: II. Helium and neon," J. Phys. D: Appl. Phys., 46 (33), 334002, (2013).
- [21] Y. Ralchenko, R. K. Janev, T. Kato, D. V. Fursa, I. Bray, and F. J. de Heer, "Electron-impact excitation and ionization cross sections for ground state and excited helium atoms," At. Data Nucl. Data Tables, 94 (4), 603, (2008).
- [22] Claire Douat " Étude d'un micro-jet de plasma à pression atmosphérique" PhD thesis, Université Paris-Sud, February 2014
- [23] B. Niermann, M. Böke, N. Sadeghi, and J. Winter, "Space resolved density measurements of argon and helium metastable atoms in radio-frequency generated He-Ar micro-plasmas," Eur. Phys. J. D, 60 (3), 489, (2010).
- [24] B. M. G. W.L. Wiesen, M.W Smith, "Atomic Transition Probabilites." U.S. Government Printing Office, (1962).
- [25] "NIST Atomic Spectra Database Lines Form." [Online]. Disponible :http://physics.nist.gov/PhysRefData/ASD/lines_form.html.

- [26] PerkinElmer, CMSResources, " Atmospheric Vapor Compensation on Spectrum Two and Frontier FT-IR Spectrometers" Technical manual
- [27] Materson Tri.Gas, FTIR-PG19 Purge Gas Generator, Technical manual
- [28] R Daly, D Connaughton, Parker Hannifin Corporation, "Effect of Purging a Sealed and Desiccated FTIR Spectrometer Sample Compartment", Technical study.
- [29] Pipa, A., Ropcke, J., 2009. Analysis of the Mid-Infrared Spectrum of the Exhaust Gas From an Atmospheric Pressure Plasma Jet (APPJ) Working With an Argon-Air Mixture. IEEE Transactions on Plasma Science 37, 1000–1003. doi:10.1109/TPS.2009.2013865
- [30] Laura Brullé. "Développement de stratégies d'imagerie multimodalités pour la pharmacologie des agents anticancéreux." PhD thesis, Université d'Orléans, Orléans, 2012.
- [31] Plewa, J.-M., Yousfi, M., Frongia, C., Eichwald, O., Ducommun, B., Merbahi, N., Lobjois, V., 2014. Low-temperature plasma-induced antiproliferative effects on multi-cellular tumor spheroids. New J. Phys. 16, 043027. doi:10.1088/1367-2630/16/4/043027
- [32] Laura Brullé, Marc Vandamme, Delphine Riès, Eric Martel, Eric Robert, Stéphanie Lerondel, Valérie Trichet, Serge Richard, Jean-Michel Pouvesle, and Alain Le Pape. "Effects of a non thermal plasma treatment alone or in combination with gemcitabine in a MIA PaCa2-luc orthotopic pancreatic carcinoma model." PLoS ONE, 7(12) :e52653, 2012.

Chapter 3

Antitumor activity of the Plasma Gun

1. Introduction

This chapter is concerned with the application of cold atmospheric pressure plasma jet to estimate its antitumor efficiency. This study to be described here grew out of work on biological assessments performed by M. Vandamme with an FE-DBD [1-4] where *in vitro* and *in vivo*, on sub-cutaneously and heterotopically grafted tumors in a murin model, were involved. Some biological processes induced by plasma treatment such as cell cycle arrest [1, 4-6], induction of apoptosis [1, 4, 7-13] and migration velocity reduction [1, 14-15] have been identified at GREMI and by other teams worldwide. Those promising application abilities gave first insights of cold atmospheric pressure plasma antitumor actions.

As mentioned in Chapter 1 and 2, the biomedical experiments presented here are performed at the beginning of this work (2010-2011). These experiments have been performed in the frame of APR "PLASMED" in collaboration with CIPA, Orléans and especially with Laura Brullé (PhD Thesis 2012). The parallel development of non-thermal plasma jets in regards to biomedical applications, led us to perform, to best of our knowledge, first *in vivo* study of plasma jet antitumor effect on orthotopic tumor [16-17]. The pancreatic carcinoma has been chosen for its low survival prognostic and the low efficiency of classical chemo- and radio-therapies.

As in any medical procedure, it is essential to first determine the tolerance to the treatment of the chosen target. Then the effect of Plasma Gun and of those chosen parameters on cultured cells *in vitro* will be presented followed by three *in vivo* studies. After the observation of *in vivo* antitumor effects of the PG working in Neon (ns driver at 200 Hz) and Helium (μ s driver at 2000 Hz), a study has been devoted to the comparison of the PG efficiency with that of gemcitabine, the conventional chemotherapy, and to the combination of those two.

The results which are presented along with the multiple applications of the developed non-thermal plasma jets worldwide [18-19] give rise to the concern of "dose" definition. This

chapter will end with a discussion summarizing observations linked to the interaction between the plasma and the treated target nature.

2. Tolerance studies

To estimate the tolerance of the pancreatic tissue, the plasma plume produced by the Plasma Gun was directed onto the surface of FVB mice healthy pancreas (FVB = Friend leukemia Virus B, is a type of mice strain). Parameters under study are mainly voltage amplitude and pulse repetition rate as these two quantities are obviously modifying the energy delivered to the targeted tissue. Duration of exposure was fixed to 10 min as the upper time limit of anesthetic medication administration (isofluorane).

Helium gas flow rate has been fixed at 500 sccm. In the case of the ns driver, voltage amplitude is -35 kV and repetition rates going from 50 Hz to 200 Hz by 50 Hz steps. For the μ s driver, voltage amplitudes are set at 13 and 16 kV while repetition rates are 500 Hz, 2 kHz, 3 kHz and 6 kHz. Each mouse has received a unique treatment with a define set of parameters.

Visual determination of inflammation is followed directly after plasma treatment and 48 hours later. A second pancreas externalization has been performed for observation.

In the case of the ns driver, no damages are observed for the tested conditions. Visual observations following μ s driver treatment has revealed no damages for the tested voltage amplitudes up to 2 kHz repetition rate while above this limit black dots with white halo were produced. For 3 kHz these traces induced no visual and texture modifications in the pancreatic tissue 48 hours after plasma exposure on the contrary 6 kHz plasma induced a white and solid volume where plasma impinged. This volume was assimilated to a chemical burn inducing necrosis of tissues. To avoid possible necrosis, plasma generation parameters were settled to 14 kV, 2 kHz and -35 kV, 200 Hz for the μ s and ns drivers respectively in the following *in vivo* studies.

The range of parameters defined by the tolerance studies defines, to some extent, the parametric plasma characterization experiments described in this manuscript.

3. *In vitro* antitumor estimation

In this section *in vitro* experiments on Mia PaCa 50 cell line are described. The aim of this study was on one hand, to determine if the plasma produced by the Plasma Gun induces

antitumor activity and on the other hand, to identify the influence of the parameters in the previously determined tolerance range.

In vitro experiments were conducted as described in chapter 2. Cell response to plasma is dependent on various parameters from the production of the plasma to the cell environment such as:

- pulse repetition rate;
- voltage waveform, its amplitude and pulse rising time;
- feeding gas flow rate and its nature;
- capillary geometry, length and diameter;
- nature of cell medium;
- geometry of the culture well.

It has been previously reported [1] that ROS scavengers present in some cell media are lowering the effect of plasma on cells and recently confirmed on multi-cellular tumor spheroids [20]. Culture well geometry, surface and depth of medium, is also influencing the concentration of species which are in the surrounding of the cells by dilution processes. Moreover, modification of the coupling between plasma and the grounded target can influence the production of reactive species as will be discussed in Chapter 5 and 6.

The nature of the feeding gas flushed in the plasma jets is modifying the nature of the reactive species produced. Moreover the gas flow rate influences the hydrodynamics of the jet and its behavior on the target, phenomena which will be developed in the following chapters.

Studying the cell response to all the parameters listed above was not under the scope of this work and will not be developed further. Some studies can be found in literature on *in vitro* antitumor activities of non-thermal plasma treatment with DBDs [10], needles jets [21] and plasma jets [22-23].

From the prior studies performed at GREMI, the pulse repetition rate appeared to be highly influent in the antitumor efficiency of plasma produced by the PG. Moreover historical expertise of our team in power supplies producing voltage pulses of ns rising time leads us to perform *in vitro* assays with both drivers (ns and μ s) allowing for observation of voltage waveform influence. Figure 3.10 shows three conditions of Helium flushed PG with repetition rates set as 200 Hz for the ns driver and 200 Hz and 2 kHz for the μ s driver, as determined by tolerance studies.

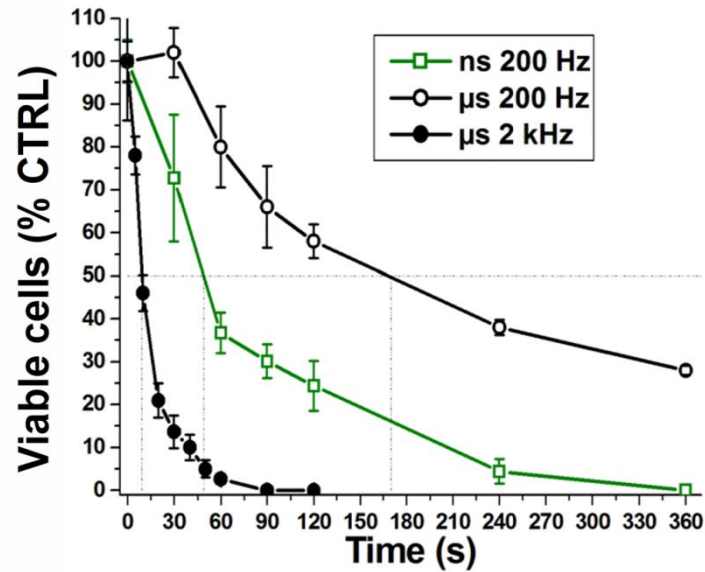


Figure 3.10 Inhibitory effect of plasma treatment on cell proliferation *in vitro* for different repetition rates and drivers versus time of treatment. Cell viability was determined 24 h after treatment by BLI assays and normalized to untreated cells. Each point represents the mean ($\pm$ SEM), $n=5$ for each group.

As shown in the Figure 3.10, significant cell growth inhibition is observed in the three NTP tested conditions.

The half maximal inhibitory concentrations (IC_{50}) determined using the Hill slope method, are 48 s for NTP ns driver at 200 Hz, 167 s and 12 s for NTP μ s driver at 200 Hz and 2 kHz respectively. Literature reports plasma jets *in vitro* assessments such as those performed by Barekzi *et al.* (2012) [24] on leukemia cells showing IC_{50} s around 3 minutes for jet sustained in the kilohertz range while cells treated with plasma needles and torch-like jets [21, 25] induce antitumor activities in few seconds probably due to a temperature increase in treated media. However the sensibility of the cells to plasma treatment depends either on the type of plasma used as well as the type of the cells it-self [1, 26].

Two main items can be described by observing Figure 3.10, on one hand the plasma produced by the ns driver is more efficient than the μ s driver from the same repetition rate, at 200 Hz. This higher antitumor efficiency is suspected to be due to the strong electric field produced by the ns rising-time pulse.

On the other hand, the cell viability is clearly reduced by the treatment at 2 kHz compared to the 200 Hz μ s driver plasma. Increasing the repetition rate ten times induces a decrease of the IC_{50} more than this factor (14 times lower) which could suggest a repetition rate dependence of the cell response.

The μ s driver due to its ability to work at higher repetition rate, together with its compactness, seems more likely to be used for biological experiments. Then in the following part of this *in vitro* chapter only experiments performed with the μ s driver will be presented.

To estimate if the repetition rate increase allows for a higher antitumor efficiency, BLI is followed for two sets of *in vitro* assays. One set is performed by fixing the exposure time to 30 s and varying the repetition rate while in the other the repetition rate is 2 kHz with varying time of exposure (Figure 3.2). In both conditions the PG is flushed with Helium 500 sccm.

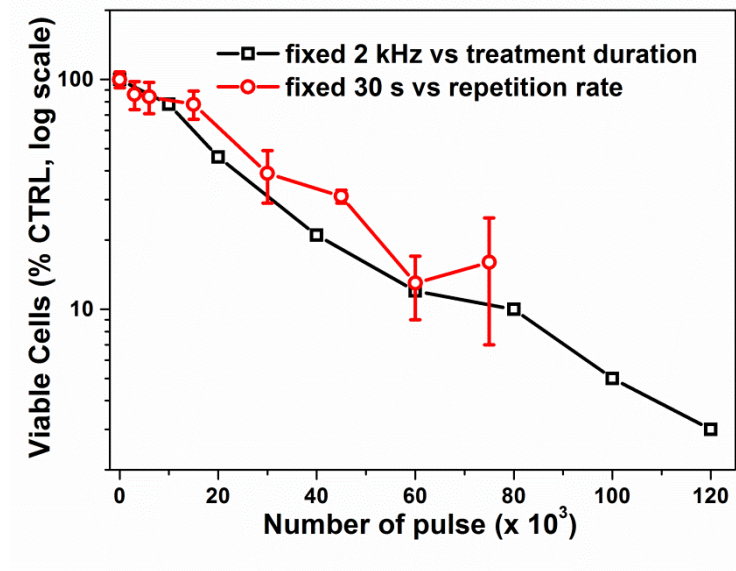


Figure 3.2 : Inhibitory effect of plasma treatment on cell proliferation *in vitro* versus number of pulses. Cells were treated at fixed time of treatment or at fixed repetition rate. Cell viability was determined 24 h after treatment by BLI assays and normalized to untreated cells. Each points represents the mean ($\pm$ SEM), $n=5$ for each group.

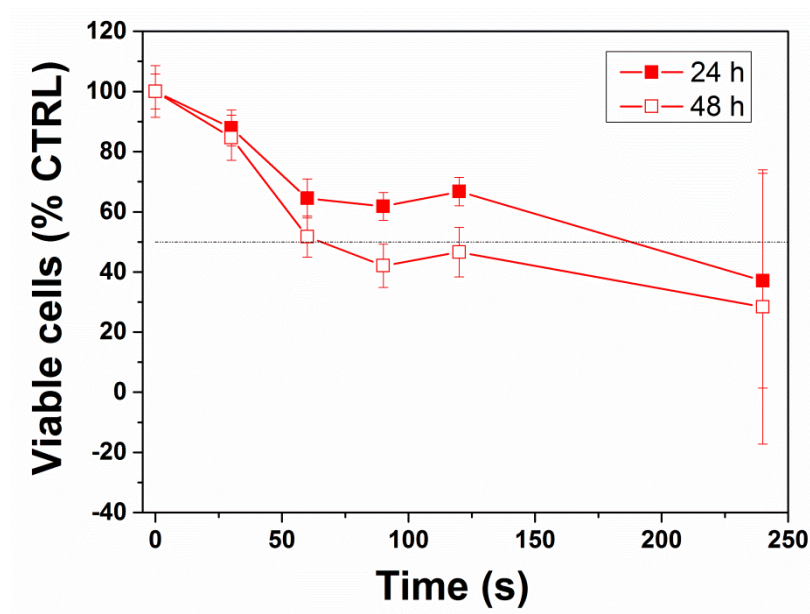


Figure 3.3: Inhibitory effect of Neon plasma treatment 24h and 48h after treatment. Cell viability was determined 24 h after treatment by BLI assays and normalized to untreated cells. Each points represents the mean ($\pm$ SEM), $n=5$ for each group.

This additional experiment (Figure 3.2) shows that antitumor activity *in vitro* is dependant of the number of pulse delivered to the cells and not repetition rate-dependant, as the two cases exhibit similar tendencies.

Figure 3.3 shows cell viability for different treatment time of μ s driver plasma fed with Neon 200 sccm at 200 Hz. It appears that cell response is higher 48 h after treatment compare to 24 h which is typical for apoptosis as described elsewhere [1, 4, 7-10]. This figure gives us the opportunity to illustrate that Neon feed PG induces antitumor activities as previously shown in Helium. The apoptotic behavior has been also observed in Helium at 200 Hz and 2 kHz however it is less pronounced.

Figure 3.4 shows a comparison between the plasma treatment with the two buffer gases, Neon and Helium for same operating conditions.

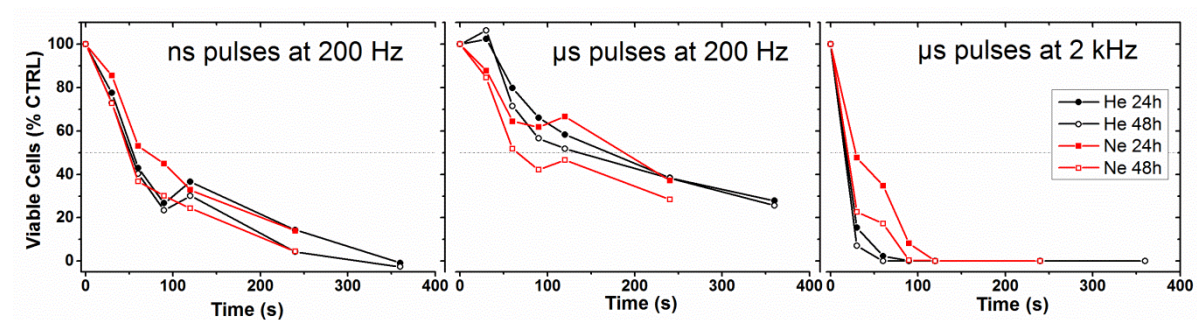


Figure 3.4: Comparison of inhibitory effects between Helium and Neon flushed PG treatments. Three conditions: the ns driver at 200 Hz and the μ s driver at 200 and 2000 Hz. Each points represents the mean ($\pm$ SEM), n=5 for each group.

In the three cases, either by using the ns driver at a pulses repetition rate of 200 Hz or by using the μ s one with pulse repetition rates 200 Hz and 2 kHz, we observe similar tendencies in the survival curves *in vitro*. Except for the μ s driver at 2 kHz, even the IC₅₀ are comparable when using Neon or Helium flushed PG. These experiments did not allow determining whether the buffer gas used to transport the plasma has or not an importance in the antitumor plasma efficiency.

We can conclude that for *in vitro* experiments, plasma produced by the Plasma Gun induces antitumor activity with the two drivers, ns and μ s, as well as with two feeding gas, Neon and Helium. Moreover the number of pulses appears to be the main parameter acting on cell viability.

4. *In vivo* antitumor estimation

Simultaneously to the exploration of antitumor efficiency of the Plasma Gun *in vitro*, we performed *in vivo* experiments on a murin model with female Swiss nude mice.

4.1 Antitumor efficiency of plasma gun

To begin with *in vivo* experiments, the historical expertise of GREMI dealing with ns rising front pulses enabled us to use in a first step the ns driver. The plasma was generated at maximum pulse repetition rate of 200 Hz, determined by the tolerance study as suitable. Also for historical reason Neon was used as the plasma carrier gas with a gas flow rate of 200 sccm.

As described in Chapter 2, plasma treatments were performed with three consecutive plasma deliveries of 10 min on externalized pancreas with 10 days interval. Bioluminescence intensities were monitored weekly to determine the tumor activity in each group. Two randomized groups of 8 mice according to their bioluminescence, were studied. Figure 3.5 shows the evolution of the bioluminescence variation until day 36 with reference intensity taken 4 days post-induction.

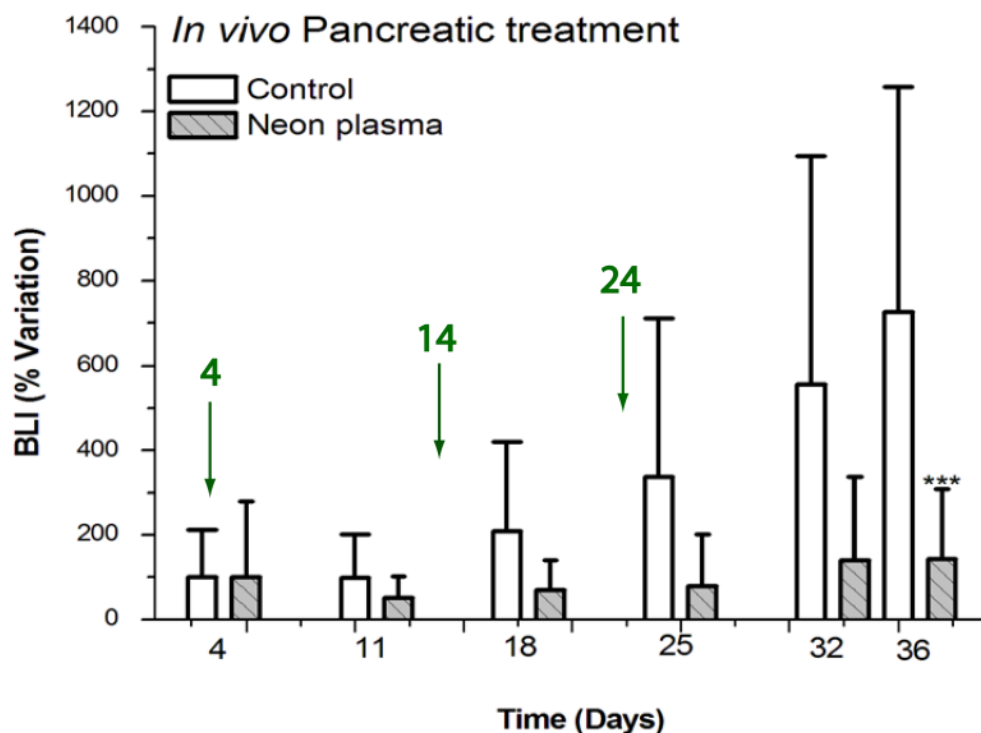


Figure 3.5: Neon plasma treatment effects on tumor growth in an orthotopic pancreatic model. Tumor growth followed by bioluminescence. Each point represents the mean ($\pm$ SEM). Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The BLI of the control group increased continuously during the whole study which is in good agreement with tumor volume increase assessed by caliper measurements. This observation indicates that BLI is relevant to follow tumor growth in this study. Plasma treatment of pancreatic carcinoma allows for a significant tumor activity decrease. After the second plasma delivery the inhibition tendency can already be observed while the difference between the treated group and the control one is highly significant at D36.

Development of the μ s power supply with its ability to generate plasma at higher repetition rate enables to optimize plasma treatment of pancreatic tumors. In this second study, the plasma generated with the μ s driver at 2 kHz and 14 kV flushed with Helium gas at 400 sccm was used with the same biological protocol. The BLI measurements are similar to the one which will be presented in Figure 3.6a) for the plasma treated group, thus the data not presented.

From this other study same conclusions can be drawn, highly significant inhibition of tumor activity and tumor volume reduction is induced by plasma delivery. Comparison between those studies does not allowed for clear conclusion about the best efficiency of the voltage pulse, repetition rate and carrier gas. As a matter of fact Neon plasma at 200 Hz and Helium plasma at 2 kHz induce tumor activity inhibitions which are not significantly different between them.

Moreover, these studies show that *in vivo* tumors do not respond the same way to plasma treatment than *in vitro* cancer cells, as it does not generate same difference in antitumor activity as previously described thanks to *in vitro* experiments (figure 3.1). This observation is consistent with literature [27-28].

This difference can be explained by the wide range of biological mechanisms in action *in vivo* which are induced by the plasma induced stresses such as endoplasmic reticulum (ER) stress and consequent organism responses. All these mechanisms are not fully understood nowadays however role of intracellular ROS production, hypoxia, angiogenesis, voltage dependant Calcium canals and protein modification are enlightened to be inseparable from tumor response [1, 6, 17, 29-31]. From these observations and theoretical considerations, the plasma treatment could be thought as a stimulus inducing biological processes inside the living organism.

4.2 Combination of Chemotherapy and Plasma Gun

In order to compare the efficiency of plasma treatment to the reference chemotherapy, a study involving one control group, one group of mice treated with the reference chemotherapy namely gemcitabine and one with plasma treatment was performed. Moreover another mice group has received a combination of the two treatments, gemcitabine and plasma. The gemcitabine is administered 24h before plasma treatment.

Figure 3.6 shows the evolution of bioluminescence variation of the 4 mice groups versus time (Figure 3.6.a)) and the tumor volume at day of necrosis normalized to control group (Figure 3.6.b)).

The tumor activity of the control group increases continuously with time while in the group receiving the gemcitabine, the bioluminescence stabilizes from day 17. In the group treated by the Plasma Gun, the bioluminescence stabilization occurs from day 10 and presents a significant reduction compare to the control group at the end of the study at day 36. The group treated by the combination of gemcitabine with plasma follows the same behavior than the plasma treated group although more efficiently as a highly significant inhibition of tumor activity at D36 is expressed.

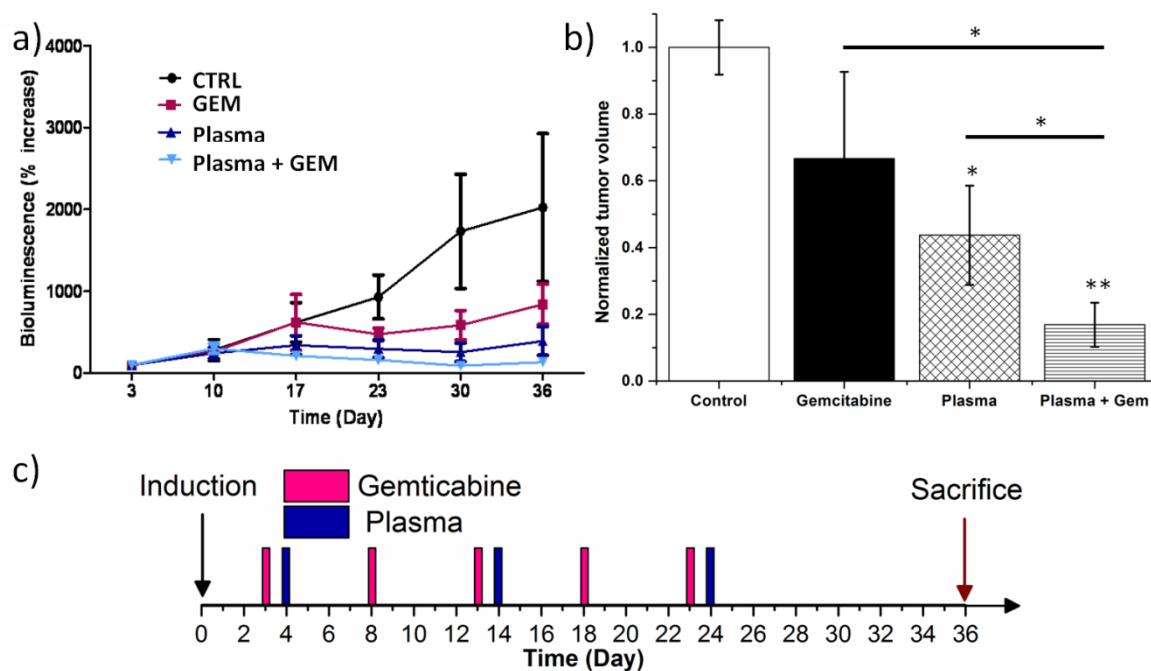


Figure 3.6: Plasma Gun and gemcitabine treatments effects on tumor growth in an orthotopic pancreatic model. Four groups: control, gemcitabine (GEM), Plasma Gun (plasma), and bi-therapy (Plasma + GEM), eight mice per group. a) Tumor activity followed by BLI. b) Tumor volume was determined using caliper on the day of euthanasia. c) Therapeutic scheme of bi-therapy treatment. Each star represents the mean ($\pm$ SEM).

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The tumor volume (Figure 3.6.b)) and tumor weight (data not shown) measurements performed at euthanasia day confirm the bioluminescence evolution with a significant reduction of the volume for the plasma and plasma-gemcitabine groups compared to the control group. The gemcitabine group do not induced significantly reduction tumor volume. These results showed the first insights of combination treatment efficiency and to the best of our knowledge the first treatment with a plasma jet on orthotopic tumors.

For this experiment, a choice in the protocol of the two therapeutic agents' delivery has been made. In our protocol, the protocol has been settled in such a way that plasma treatments happened one day after Gemcitabine administration (Figure 3.6c)). Other

protocols are possible. It is worth noting that it could have significant influence on the anti-tumor activity as one of the therapeutic agents may better enhance the potentiality of the other.

The results of this study led us to propose that plasma treatment was enhanced by gemcitabine activity due to its radiosensitization properties [32]. Further experiments thus should be conducted with other protocols, in order to estimate a possible synergic effect.

5. Toward standardization

Despite the growing interest toward the wide range of cold plasma at atmospheric pressure potentials [18-19] difficulties are encountered to define plasma treatments protocols.

At a first glance, plasma treatments were quantified in function of the energy delivered per unit area in J/cm^2 . Historically this notion of “dose” grew out from radiotherapy where the Gray ($1 \text{ Gy} = 1 \text{ J/Kg}$) is commonly accepted. Later the photo therapy and finally the photodynamic therapy quantified the treatment in J/cm^2 . However the energy deposited onto the treated target must not be assimilated to the one delivered to the plasma reactor as each reactor geometry and voltage pulse waveform enable a different transfer of energy to the plasma. For example, treatments performed with a DBD and a plasma jet at similar input energy lead to different deposition onto the target. Moreover, the treated surface alone cannot reflect the treated target such as for *in vitro* studies, depth of the culture medium plays a role in the concentration of reactive species [10, 33], or for *in vivo* studies, it has been shown that while treating the tumor surface, apoptotic behavior is induced in the whole tumor volume [1, 4].

Due to the fact that plasma is a combination of reactive oxygen and nitrogen species, UV (A-B-C) radiations, electric field, temperature, charged particles and excited species, experiments were performed to try to isolate component or group of component [4, 29, 34] in the aim of identifying mechanisms induced by each of those. However mechanisms relative to reactive oxygen and nitrogen species produced by the plasma cannot be discriminated from those induced by culture media and/or intracellular ROS and RNS. Glancing at our experiments *in vivo*, the induced antitumor activity obtained thanks to the ns and μs drivers is a good example of such problem with a quasi-equivalent inhibition of tumor activity for different plasma parameters (in our case dealing especially with generated electric field). Thus synergetic effect between plasma components has to be considered. It is worth noting that all developed plasma sources produce a mix between all these components in different ratios inducing a real difficulty in comparison of used sources and results obtained worldwide. From these non-exhaustive reasons, it appears that trying to define the delivered plasma quantity is currently not possible and each biological treatment

has to refer to the “target exposure time” **in function of each plasma source in a set of fixed parameters.**

6. Discussion

In this Chapter, it is shown that the significant difference in antitumor activity *in vitro*, induced mainly by changing the number of delivered pulses, cannot be directly compare to effects *in vivo*. *In vivo* studies enlightened that whatever the gas nature, the voltage rising time and the pulse repetition rate used, the tumor activity decrease is significant compared to the control group. As a matter of fact, cell response to plasma treatment is different while being *in vitro* or *in vivo* partially due to regulation factors present in the vascularized organism *in vivo*.

Not only cellular response differs for *in vitro* to *in vivo* but also plasma plume color and shape with some unexpected behaviors. Thus it is expected that plasma it-self is influenced by the type of treated target. These visual observations of plasma modifications are the starting point of considerations focusing on interactions between plasma and nature of the treated target as will be discussed further in this manuscript.

French summary of Chapter 3

Ce chapitre traite des potentialités anti-tumorales du Plasma Gun. Ces expériences font suite au travail de doctorat de M. Vandamme effectué avec une FE-DBD [1-4]. Dans le cadre de L'ANR "PlasMed", et en collaboration avec les laboratoires du CIPA et du CBM, un développement en parallèle du Plasma Gun et des expériences biomédicales nous ont conduits à effectuer ces expériences alors que relativement peu d'informations sur les jets de plasma étaient disponibles. À notre connaissance les expériences présentées dans ce chapitre sont les premières sur tumeur orthotopique *in vivo* [16-17]. Le carcinome pancréatique a été choisi pour cible en raison du développement simultané d'un modèle animal au CIPA. Cette cible est particulièrement intéressante en lien avec son faible pronostic de survie et de l'efficacité relativement limitée des traitements classiques.

Dans une première partie, une étude de tolérance sur tissus sains est présentée, suivie d'une étude paramétrique *in vitro* focalisant sur la forme de l'impulsion de tension et la fréquence de répétition de l'impulsion. Les études *in vitro* ont montré que le nombre d'impulsion reçues par les cellules cancéreuses jouait un rôle fondamental.

Trois études *in vivo* sont décrites. Tout d'abord, une comparaison de deux traitements, avec le PG ns en Néon à 200 Hz puis le PG μ s en Hélium à 2000 Hz, montrant que quelque soit le gaz, la nature de l'impulsion de tension et la fréquence de répétition de celle-ci, une diminution de l'activité tumorale est observée. Finalement une étude comparative entre le PG μ s en Hélium et la chimiothérapie de référence, la gemcitabine, ainsi qu'une combinaison des deux traitements montre un effet prometteur du plasma seul comparé à la chimiothérapie. De plus la combinaison des deux traitements augmente l'efficacité du traitement anti-tumoral.

Ce chapitre se termine avec une discussion sur la notion de dose et de standardisation des traitements dans la communauté plasma médecine pour un passage plus propice à l'utilisation des jets de plasma dans le domaine médical. De plus des observations concernant le comportement du plasma lors des traitements *in vitro* et *in vivo*, suivant la nature de la cible, nous amène à définir les directions de recherche développées dans ce manuscrit. Il apparaît en vue de ces expériences que l'interaction plasma-cible modifie les propriétés du plasma ainsi que les effets biologiques induits.

References

- [1] Vandamme Marc. "Les plasmas froids, nouvelle stratégie thérapeutique en cancérologie." PhD thesis, Université d'Orléans, Orléans, 2012.
- [2] Vandamme, M., Robert, E., Dozias, S., Sobilo, J., Lerondel, S., Le Pape, A., Pouvesle, J.-M., 2011. Response of Human Glioma U87 Xenografted on Mice to Non Thermal Plasma Treatment. *Plasma Medicine* 1, 27–43. doi:10.1615/PlasmaMed.v1.i1.30
- [3] Vandamme, M., Robert, E., Pesnel, S., Barbosa, E., Dozias, S., Sobilo, J., Lerondel, S., Le Pape, A., Pouvesle, J.-M., 2010. Antitumor Effect of Plasma Treatment on U87 Glioma Xenografts: Preliminary Results. *Plasma Processes Polym.* 7, 264–273. doi:10.1002/ppap.200900080
- [4] Vandamme, M., Robert, E., Lerondel, S., Sarron, V., Ries, D., Dozias, S., Sobilo, J., Gosset, D., Kieda, C., Legrain, B., Pouvesle, J.-M., Pape, A.L., 2012. ROS implication in a new antitumor strategy based on non-thermal plasma. *Int. J. Cancer* 130, 2185–2194. doi:10.1002/ijc.26252
- [5] Kim, C.-H., Bahn, J.H., Lee, S.-H., Kim, G.-Y., Jun, S.-I., Lee, K., Baek, S.J., 2010a. Induction of cell growth arrest by atmospheric non-thermal plasma in colorectal cancer cells. *Journal of Biotechnology* 150, 530–538. doi:10.1016/j.jbiotec.2010.10.003
- [6] Landsberg, K., Scharf, C., Darm, K., Wende, K., Daeschlein, G., Kindel, E., Weltmann, K.-D., von Woedtke, T., 2011. Use of Proteomics to Investigate Plasma-Cell Interactions. *Plasma Medicine* 1, 55–63. doi:10.1615/PlasmaMed.v1.i1.50
- [7] Kim, S.J., Chung, T.H., Bae, S.H., Leem, S.H., 2010. Induction of apoptosis in human breast cancer cells by a pulsed atmospheric pressure plasma jet. *Applied Physics Letters* 97, 023702. doi:10.1063/1.3462293
- [8] Yan, X., Zou, F., Zhao, S., Lu, X., He, G., Xiong, Z., Xiong, Q., Zhao, Q., Deng, P., Huang, J., Yang, G., 2010. On the Mechanism of Plasma Inducing Cell Apoptosis. *IEEE Transactions on Plasma Science* 38, 2451–2457. doi:10.1109/TPS.2010.2056393
- [9] Ahn, H.J., Kim, K.I., Kim, G., Moon, E., Yang, S.S., Lee, J.-S., 2011. Atmospheric-Pressure Plasma Jet Induces Apoptosis Involving Mitochondria via Generation of Free Radicals. *PLoS ONE* 6, e28154. doi:10.1371/journal.pone.0028154
- [10] Fridman, G., Shereshevsky, A., Jost, M.M., Brooks, A.D., Fridman, A., Gutsol, A., Vasilets, V., Friedman, G., 2007. Floating Electrode Dielectric Barrier Discharge Plasma in Air Promoting Apoptotic Behavior in Melanoma Skin Cancer Cell Lines. *Plasma Chem Plasma Process* 27, 163–176. doi:10.1007/s11090-007-9048-4

- [11] Fridman G, Shereshevsky A, Jost MM, Brooks AD, Fridman A et al. (2007) Floating electrode dielectric barrier discharge plasma in air promoting apoptotic behavior in melanoma skin cancer cell lines. *Plasma Chem Plasma Process* 27: 163-176. doi:10.1007/s11090-007-9048-4.
- [12] Zhang XH, Li MJ, Zhou RL, Feng KC, Yang SZ (2008) Ablation of liver cancer cells in vitro by a plasma needle. *Appl Phys Lett* 93: 021502. doi:10.1063/1.2959735.
- [13] Kim CH, Bahn JH, Lee SH, Kim GY, Jun SI et al. (2010) Induction of cell growth arrest by atmospheric non-thermal plasma in colorectal cancer cells. *J Biotechnol* 150: 530-538. doi:10.1016/j.jbiotec.2010.10.003. PubMed: 20959125.
- [14] Kim, C.-H., Kwon, S., Bahn, J.H., Lee, K., Jun, S.I., Rack, P.D., Baek, S.J., 2010. Effects of atmospheric nonthermal plasma on invasion of colorectal cancer cells. *Appl Phys Lett* 96. doi:10.1063/1.3449575
- [15] Shashurin, A., Keidar, M., Bronnikov, S., Jurjus, R.A., Stepp, M.A., 2008. Living tissue under treatment of cold plasma atmospheric jet. *Applied Physics Letters* 93, 181501. doi:10.1063/1.3020223
- [16] Laura Brullé, Marc Vandamme, Delphine Riès, Eric Martel, Eric Robert, Stéphanie Lerondel, Valérie Trichet, Serge Richard, Jean-Michel Pouvesle, and Alain Le Pape. Effects of a non thermal plasma treatment alone or in combination with gemcitabine in a MIA PaCa2-luc Orthotopic pancreatic carcinoma model. *PLoS ONE*, 7(12) :e52653, 2012.
- [17] Laura Brullé, "Développement de stratégies d'imagerie multimodalités pour la pharmacologie des agents anticancéreux." PhD thesis, Université d'Orléans, Orléans, 2012.
- [18] Laroussi, M., Kong, M.G., Morfill, G., Stolz, W., 2012. *Plasma Medicine: Applications of Low-Temperature Gas Plasmas in Medicine and Biology*. Cambridge University Press.
- [19] Isbary, G., Zimmermann, J.L., Shimizu, T., Li, Y.-F., Morfill, G.E., Thomas, H.M., Steffes, B., Heinlin, J., Karrer, S., Stolz, W., 2013. Non-thermal plasma—More than five years of clinical experience. *Clinical Plasma Medicine* 1, 19–23. doi:10.1016/j.cpme.2012.11.001
- [20] Plewa, J.-M., Yousfi, M., Frongia, C., Eichwald, O., Ducommun, B., Merbahi, N., Lobjois, V., 2014. Low-temperature plasma-induced antiproliferative effects on multi-cellular tumor spheroids. *New J. Phys.* 16, 043027. doi:10.1088/1367-2630/16/4/043027
- [21] Lee, H.J., Shon, C.H., Kim, Y.S., Kim, S., Kim, G.C., Kong, M.G., 2009. Degradation of adhesion molecules of G361 melanoma cells by a non-thermal atmospheric pressure microplasma. *New J. Phys.* 11, 115026. doi:10.1088/1367-2630/11/11/115026

- [22] Kim, S.J., Chung, T.H., Bae, S.H., Leem, S.H., 2010. Induction of apoptosis in human breast cancer cells by a pulsed atmospheric pressure plasma jet. *Applied Physics Letters* 97, 023702. doi:10.1063/1.3462293
- [23] Kim, C.-H., Kwon, S., Bahn, J.H., Lee, K., Jun, S.I., Rack, P.D., Baek, S.J., 2010. Effects of atmospheric nonthermal plasma on invasion of colorectal cancer cells. *Applied Physics Letters* 96, 243701. doi:10.1063/1.3449575
- [24] Barekzi, N., Laroussi, M., 2012. Dose-dependent killing of leukemia cells by low-temperature plasma. *J. Phys. D: Appl. Phys.* 45, 422002. doi:10.1088/0022-3727/45/42/422002
- [25] Kieft, I., Darios, D., Roks, A.M., Stoffels, E., 2005. Plasma treatment of mammalian vascular cells: a quantitative description. *IEEE Transactions on Plasma Science* 33, 771–775. doi:10.1109/TPS.2005.844528
- [26] Haertel, B., Volkmann, F., von Woedtke, T., Lindequist, U., 2012. Differential sensitivity of lymphocyte subpopulations to non-thermal atmospheric-pressure plasma. *Immunobiology* 217, 628–633. doi:10.1016/j.imbio.2011.10.017
- [27] Partecke, L.I., Evert, K., Haugk, J., Doering, F., Normann, L., Diedrich, S., Weiss, F.-U., Evert, M., Huebner, N.O., Guenther, C., Heidecke, C.D., Kramer, A., Bussiahn, R., Weltmann, K.-D., Pati, O., Bender, C., Bernstorff, W. von, 2012. Tissue Tolerable Plasma (TTP) induces apoptosis in pancreatic cancer cells in vitro and in vivo. *BMC Cancer* 12, 473. doi:10.1186/1471-2407-12-473
- [28] Keidar, M., Walk, R., Shashurin, A., Srinivasan, P., Sandler, A., Dasgupta, S., Ravi, R., Guerrero-Preston, R., Trink, B., 2011. Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy. *Br J Cancer* 105, 1295–1301. doi:10.1038/bjc.2011.386
- [29] Zhao, S., Xiong, Z., Mao, X., Meng, D., Lei, Q., Li, Y., Deng, P., Chen, M., Tu, M., Lu, X., Yang, G., He, G., 2013. Atmospheric Pressure Room Temperature Plasma Jets Facilitate Oxidative and Nitrative Stress and Lead to Endoplasmic Reticulum Stress Dependent Apoptosis in HepG2 Cells. *PLoS ONE* 8, e73665. doi:10.1371/journal.pone.0073665
- [30] Collet, G., Robert, E., Lenoir, A., Vandamme, M., Darny, T., Dozias, S., Kieda, C., Pouvesle, J.M., 2014. Plasma jet-induced tissue oxygenation: potentialities for new therapeutic strategies. *Plasma Sources Sci. Technol.* 23, 012005. doi:10.1088/0963-0252/23/1/012005
- [31] Ninomiya, K., Ishijima, T., Imamura, M., Yamahara, T., Enomoto, H., Takahashi, K., Tanaka, Y., Uesugi, Y., Shimizu, N., 2013. Evaluation of extra- and intracellular OH radical generation, cancer cell injury, and apoptosis induced by a non-thermal

- atmospheric-pressure plasma jet. *Journal of Physics D: Applied Physics* 46, 425401. doi:10.1088/0022-3727/46/42/425401
- [32] Azria, D., Jacot, W., Prost, P., Culine, S., Ychou, M., Lemanski, C., Dubois, J.-B., 2002. Gemcitabine and ionizing radiations: radiosensitization or radio-chemotherapy combination. *Bulletin du Cancer* 89, 369–79.
- [33] Kalghatgi, S., Kelly, C.M., Cerchar, E., Torabi, B., Alekseev, O., Fridman, A., Friedman, G., Azizkhan-Clifford, J., 2011. Effects of Non-Thermal Plasma on Mammalian Cells. *PLoS ONE* 6, e16270. doi:10.1371/journal.pone.0016270
- [34] Fridman, G., Brooks, A.D., Balasubramanian, M., Fridman, A., Gutsol, A., Vasilets, V.N., Ayan, H., Friedman, G., 2007. Comparison of Direct and Indirect Effects of Non-Thermal Atmospheric-Pressure Plasma on Bacteria. *Plasma Processes Polym.* 4, 370–375. doi:10.1002/ppap.200600217

Chapter 4

Characterization of the Plasma Gun in the free-jet mode

1. Introduction:

This chapter is dedicated to the study of Plasma Gun in terms of plasma propagation and production of reactive species when operating in the “free-jet mode” condition. The “free-jet mode” is here defined as the case where plasma plume is expanding in open-air region at floating potential.

To obtain the Plasma Gun plume, plasma first requires to be ignited at the PG reactor and to propagate inside a long capillary from cm to hundred of cm in length. In this section, the plasma properties inside the capillary will be studied via a brief comparison of experiments and model. On one hand, plasma propagation description inside the capillary will summarize some results obtained during the parallel PhD thesis of Vanessa Sarron [1]. On the other hand, the model was developed by Zhongmin Xiong and Mark Kushner [2]. The comparison between those experiments and the model was the starting point of a collaboration with the University of Michigan [1, 3-4]. The propagation will be also described in the *in-air* expanding jet, the so-called plasma plume.

As the plasma jet is developed in a buffer gas, some details of hydrodynamics of gas jet will be given. Schlieren photography will show an interplay between gas and plasma.

Plasma produced by the PG gives rise to diverse excited species emission during its propagation. Plasma emission is first observed inside the capillary. Inside the capillary, the emission corresponds to He* and excited states of the impurities present in the buffer gas and the gas pipes. Then plasma emits transitions from different excited species when propagating in the ambient air region. The following section will focus on the identification of those species, their spatial distribution and temporal evolution using optical emission spectroscopy (OES) and wavelength-filtered imaging. Influence of parameters such as gas flow rate and pulse repetition rate will be reported.

Among the various components of plasma jet that interact with the cells and living tissues, species such as radicals and long living molecules are not observable by OES. Some long-living molecules have been measured by means of Fourier Transform Infra-Red Spectroscopy.

2. Plasma propagation

2.1. Inside the capillary

As described in Chapter 1, plasma jets are discontinuous phenomena, made of plasma volumes propagating at high velocity ($\sim 10^6 - 10^8 \text{ cm.s}^{-1}$) which seem to evolve freely after ignition. This hypothesis lead the pioneer team of Teschke *et al.* to define this plasma volume as “plasma bullets” (see Chapter 1) [5].

Plasma propagation inside the capillary is shown in Figure 4.1 composed of five ICCD 10 ns snapshots corresponding to different delays with respect to the voltage onset. In this case the plasma gun was powered with the ns driver at voltage amplitude equal to 22 kV in negative polarity. It was fed with Neon, at 200 sccm, due to its intense emission in the visible range although same phenomena are observable with Helium Fed PG.

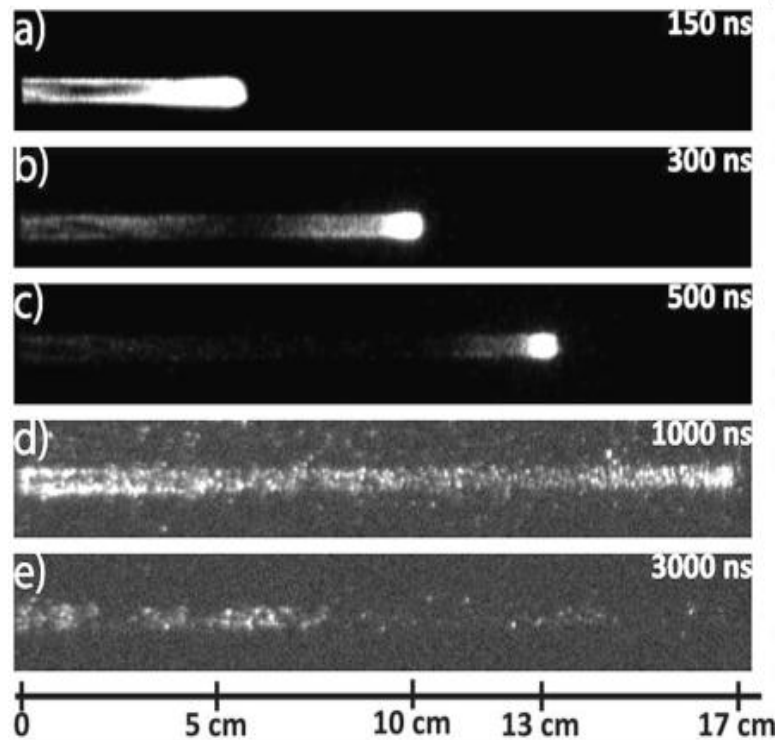


Figure 4.1: a), b), c): 10 ns snapshots of plasma propagation in the borosilicate capillary in horizontal configuration with labeled delays with respect to the voltage onset. d) and e) are 1 μ s snapshots for the indicated delays with respect to the voltage onset. [6]

Plasma is ignited between the inner electrode tip and the outer electrode and it propagates over the first cm in an inhomogeneous, filamentary mode as shown in the 150 ns delay snapshot by the bright and dark zone reflecting the difference of emission intensity signal of excited Neon atoms. From 300 ns corresponding in this case to a 10 cm propagation length the so called “plasma bullet” can be observed, showing an intense emission zone followed

by a lower signal connecting this ionization front to the reactor zone which had been called the "plasma tail". The homogeneous plasma propagates until vanishing in this case at 17 cm, leaving only the plasma tail emission (delay 1000 ns). At longer time, the emission of the plasma tail is decreasing backward to the reactor zone until vanishing completely. The presence of a tail following an ionization front propagation had been suggested earlier without clear demonstration of the connection to the reactor zone [7]. In our study, the ionization front is clearly shown to be never disconnected from the primary power source whatever the voltage amplitude and waveform, the pulse repetition and gas flow rates, length of propagation as well as for any delay of observation.

Plasma velocity (Figure 4.2) has been followed by use of a photomultiplier tube and a bench of UV optical fibers allowing its determination along full length of propagation on a single event. In the figure below, results are corresponding to the PG powered with the ns driver at voltage amplitude equal to 22 kV in negative polarity, 200 Hz pulse repetition rate and with Neon at 200 sccm.

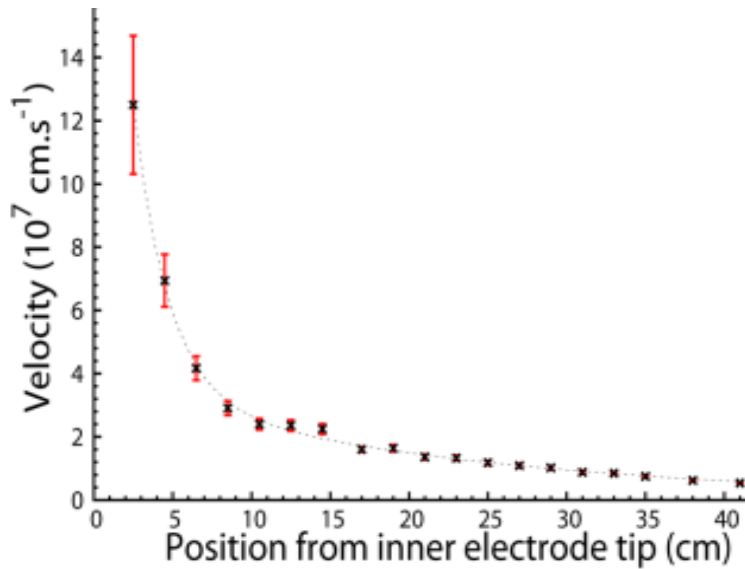


Figure 4.2 Evolution of the PAPS velocity versus the position from the inner electrode tip, for the same experimental conditions as in Figure 4.1. Dotted trace is a fit of experimental data, which only clarify the analysis proposed in the text. [6]

The curve is fitted by a combination of two exponential curves which correspond to different plasma modes. The transition between the two modes is progressive over the length of propagation. In the first 5 cm the first plasma velocity behavior is mainly following the first exponential law characterized by a high velocity amplitude and decay. From 5 to 15 cm the two modes are contributing while after 15 cm the plasma is following mainly the second exponential law which results in a lower amplitude and decay of the plasma velocity. Those distances depend on the input parameters such as voltage front rising time and amplitude. The plasma velocity behavior follows the emission distribution seen in the Figure 4.1, namely

a first high speed inhomogeneous and intense plasma called the *Wall-Hugging* mode followed by a shift to a homogeneous plasma volume at lower velocities called the *Homogeneous* mode. More details can be found in [1]. Together with the fact that the ionization front is shown to be always connected to the power source through the tail, these two modes of propagation led E. Robert *et al.* in 2012 to the denomination of the observed plasma as Pulsed Atmospheric-pressure Plasma Stream (PAPS) [1,6] instead of the historical term "plasma bullet".

It has been shown in [6] that the PAPS propagation, velocity and propagation length inside the capillary, is directly influenced by the voltage pulse amplitude and duration. Figure 4.3 shows plasma propagation inside the capillary of Neon fed Plasma Gun for three different voltage pulse durations with identical peak voltage amplitude. Parameters used were similar as for Figure 4.1 and Figure 4.2.

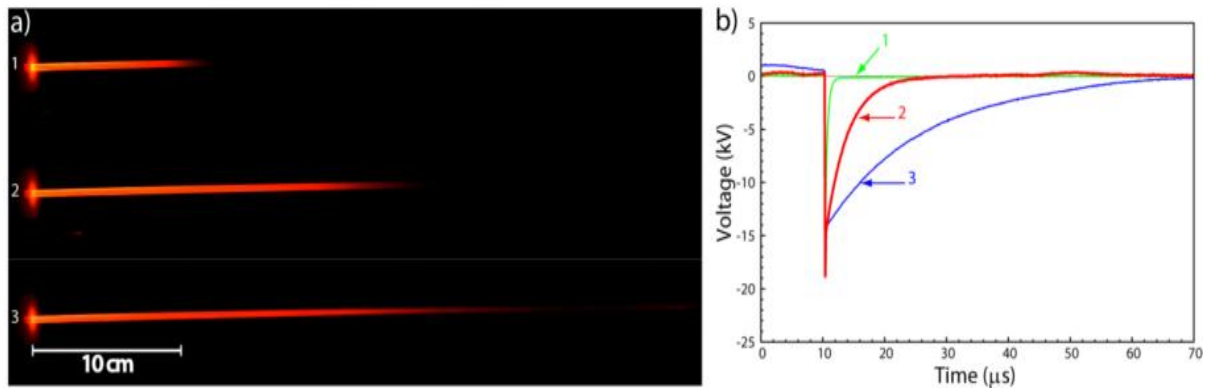


Figure 4.3 a) Digital camera pictures of neon PAPS propagation in 45 cm long borosilicate capillary for the three voltage waveforms plotted in b). The 1, 2, and 3 labels connect the neon PAPS pictures with the corresponding voltage waveform applied to the DBD reactor. Extracted from [6].

By varying the voltage pulse duration, the PAPS propagation length is controlled as the longer propagation path in a) is obtained for corresponding longer pulse duration in b). PAPS propagation stops whatever the dumping of the voltage pulse when voltage amplitude reaches around -4 kV. The plasma front propagation carries on as long as the plasma tail, connecting the plasma stream front with the powered electrode, is sufficiently ionized to ensure ionization wave propagation. The transfer of voltage to the ionization front is reduced by the progressive increase of the tail impedance with the PAPS propagation. These experiments clarified the control of the PAPS propagation through the crucial role of the plasma tail.

Another particular consequence of the voltage waveform is linked to its rising and falling fronts. It has been previously demonstrated that for each rising-falling front of ns and μ s duration time [6] as well as for oscillating voltage waveform [8] a PAPS is generated. The literature mentioned above makes reference to observation of the second PAPS in Neon fed PG. Moreover this feature is observed with Helium buffer gas and as can be found in

experiments using different Helium fed plasma jets for a sinusoidal voltage [9] and pulsed voltage [10]. Figure 4.4 shows emission captured at the electrode tip for PG powered by the μ s driver at +12 kV and pulse repetition rate of 200 Hz for a gas flow rate of 200 sccm of Neon.

Plasma emission presents an intense peak for the first rising front of the voltage waveform while a second weaker emission can be observed for the falling front. The two contributions are narrow and last for around 200 ns linked to the ionization front propagation in front of the optical fiber. It is worth nothing that in Helium, the second PAPS emission is almost not observable.

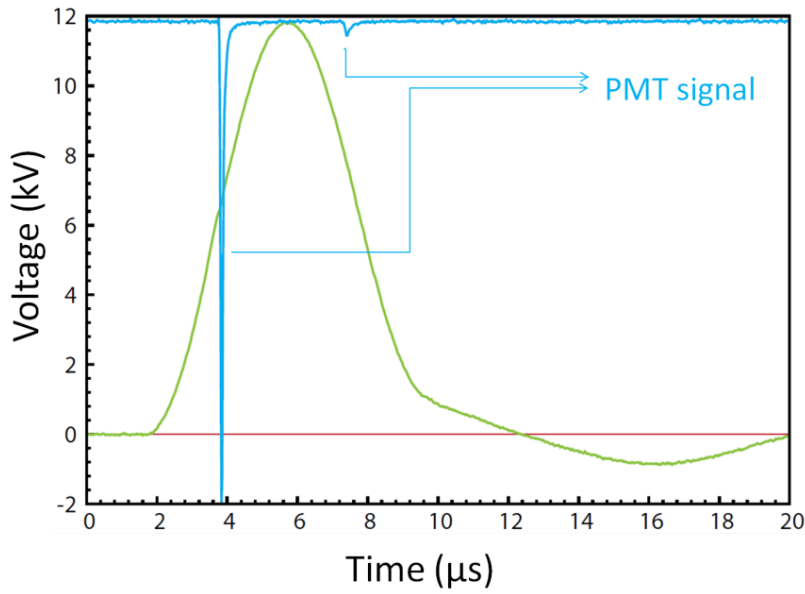


Figure 4.4: Voltage waveform delivered by the μ s driver, and PMT signal captured by a UV optical fiber positioned at the electrode tip, adapted from [1].

Figure 4.5 is extracted from [2] and shows the results of the model developed by Xiong *et al* in the case of plasma propagating in a small diameter capillary with high aspect ratio. In this model the voltage pulse used to ignite the plasma is similar to the one produced with the ns pulse power supply (± 50 kV voltage amplitude and a 20 ns rising time). The buffer gas is a mix of Ne/Xe, with Xe standing for impurities present in the gas. The modelling platform used is *nonPDPSIM*, a 2D plasma hydrodynamics model with radiation-photon transport, based on solving continuity equations for charged and neutral species, and Poisson's equation [11].

This model reveals that the ionization front development inside capillaries leads to a two stages behavior as shown in the right part of the figure. This behavior is clearly identified by the electron density n_e (Figure 4.5 b)) and ionization source function S_e (Figure 4.5 e)) modulations along the propagation. From point A, corresponding to the electrode section, to D, around 5 cm away; the n_e and S_e decrease rapidly and appear to be strongly influenced by

the voltage source. From D to H, the plasma characteristics are reaching a quasi-steady state. This result can be linked to the change of PAPS mode during the propagation, *Wall-hugging* and *Homogeneous*, as well as to the experimentally obtained velocity shown in Figure 4.2.

The general trend of the other plasma characteristic such as electron temperature T_e and reduced electric field E/N , shows a conservation of the plasma source function along the length of propagation which is in good agreement with experimental observations. Those characteristics oscillate around a mean value with small variations due to the relatively high curvatures chosen in the model, which results in additional plasma-surface interactions.

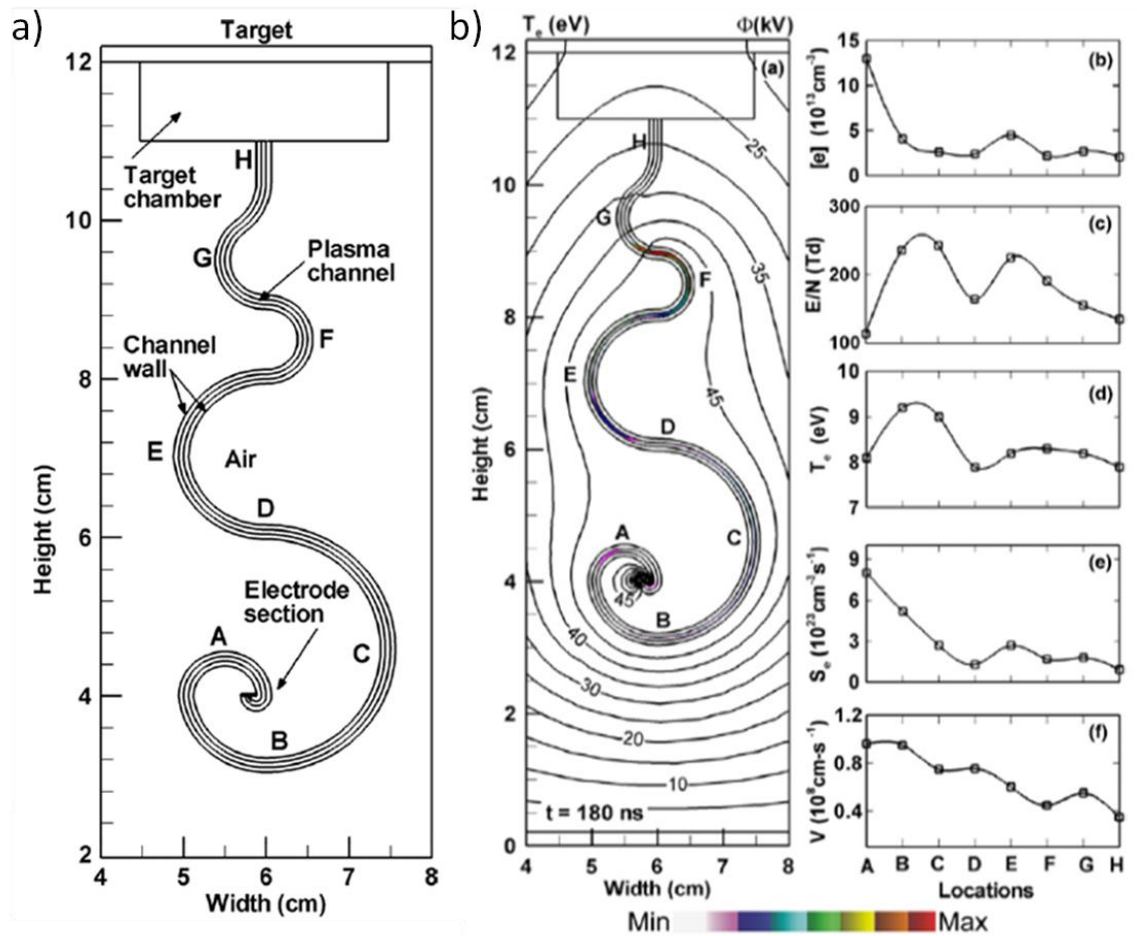


Figure 4.5: a) Discharge configuration of ionization waves propagating through high ratio flexible dielectric capillary channel. b) Plasma characteristics of an ionization wave produced by a + 50 kV voltage pulse. Extracted from [2]

The model has proved to support, and also predict, experimentally observed features when dealing with splitting and mixing of PAPS as well as transfers through metallic section and dielectric barriers [1, 3-4]. Full description of the model and similarities with experiments

performed with the PG are out of the scope of this thesis. However it allowed clearer understanding of the physics behind the PAPS propagation inside the Plasma Gun capillary.

Xiong and Kushner enounced an increase of the impedance of the plasma with propagation and hence an increase of the electric field behind the ionization front due to space charge produced by the ionization front it-self. Although the plasma is conductive and allowed transmitting the potential along the path of the plasma, there exist a voltage drop in the plasma channel. Thus the smooth decrease of n_e , S_e , T_e and E/N from point D is largely a result of the increasing voltage drop across the plasma along the length of the tube as the plasma column extends inside the capillary.

More details about the PAPS propagation properties inside the capillary as well as the comparison of experimental results to Xiong *et al.* model can be found in V. Sarron's thesis [1] and papers [2-4].

2.2. In the plasma plume

The plasma propagation in the plasma plume is shown in Figure 4. composed of five ICCD 5 ns snapshots corresponding to different delays, with time origin set when PAPS reaches the capillary outlet. In this case, the plasma gun was powered with the μ s driver at voltage amplitude equal to +14 kV and flushed with 1000 sccm Helium flow rate.

The observed propagation in the air reaches, in this condition, 2 cm (Figure 4.). To visualize the plasma dynamics, 20 ns time exposure snapshots are used. This is induced by the rather low plasma emission intensity, especially when PG is flushed with Helium and allows minimizing the contribution of the inherent jitter of the discharge.

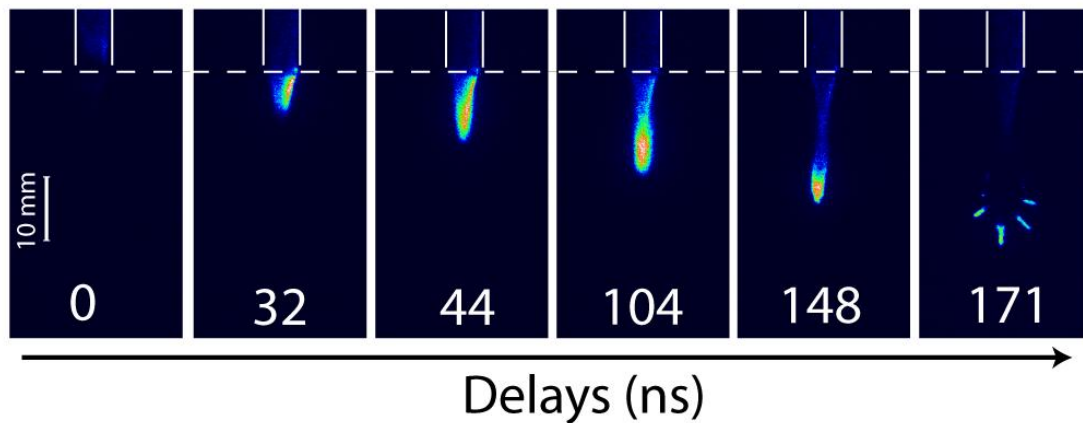


Figure 4.6: 20 ns snapshots of plasma propagation in the plasma plume in vertical configuration with labeled delays with respect to the PAPS reaching the tube outlet.

When exiting the capillary, the PAPS continues its propagation in the helium channel developed in the ambient air similarly as along the capillary. This shows that there is a conservation of the mode of propagation; a *Wall-hugging* PAPS will exit the capillary following its propagation in *Wall-hugging* mode while an *Homogeneous* PAPS will stay *Homogeneous* in the plume. In Figure 4., the PAPS is homogeneous after the 10 cm long propagation inside the capillary. It propagates downstream until reaching a maximum propagation length observable for the 148 ns delay snapshot. Until 104 ns delay snapshot, the ionization front conserves its shape while the PAPS tail gets thinner at ~ 5 mm from the tube outlet resulting in a droplet-like shape. At 148 ns delay, the ionization front emission stops and plasma spreads on the border of the maximum propagation position to finally split into multiples small plasma volumes going in random direction before vanishing (171 ns delay). It is worth noting that the observed sub-PAPSs created are always connected to the voltage source by low intensity tails similarly as the PAPS propagating inside the capillary, as already described in section 2.1.

The propagation of the PAPS produced during the falling front of the voltage pulse could not be followed by fast imaging due to its weak emission when studying the Helium fed PG expansion in the plume region.

Velocities of the in-air expending PAPS have been evaluated by ICCD imaging as the diagnostic consisting of the PMT with the series of optical fibers is not applicable in this situation because plasma and gas flow is modified by their presence. As a matter of fact, plasma plume tends to bend when objects are close to it. This feature will be developed further on in next paragraphs. Our measurement performed thanks to fast imaging tends to support observations made in the literature [10, 12-15]. Plasma velocity is first slightly increasing at capillary outlet over the first mm and then decrease until plasma vanishes whatever the voltage amplitude, pulse repetition rate and gas flow rate. The *in-air* expansion is characterized by a drop of velocity of about one order of magnitude compared to the propagation within the capillary, from 15 to 5.10^6 cm.s^{-1} which is in good agreement with velocities reported elsewhere [10, 16-20]. This result confirms that plasma is propagating at much higher velocity than the gas in the confined environment of the capillary as well as in the *in-air* expending jet. We can remind that for a gas flow rate of 1 l.min^{-1} , the gas exiting a 4 mm inner diameter capillary has a mean velocity of $1.33.10^2 \text{ cm.s}^{-1}$.

2.3. Plasma-gas flow interplay

The plasma jet exiting the capillary is generated thanks to a buffer gas jet thus the fluid dynamics should be considered. As described in Chapter 2, the gas flow can have different flow modes, namely laminar, transitional and turbulent. The gas zones can be observed by the use of Schlieren photography, which captures gradient of refractive indices between the

flushed gas and the ambient air (see Chapter 2). It is worth noting that this observation is possible in Helium due to the difference of density between the latest and the air.

It has been shown in the pioneer work by Jiang *et al.* [21] in 2011, that the Helium plasma jet is modifying the gas jet behavior. As a matter of fact by following the gas flow behavior of the horizontally positioned nozzle and superimposed the plasma jet emission, they described a tendency of the plasma to modify the curvature of the gas flow. The natural upward flow is, in some conditions of voltage amplitude and gas flow rate, shifted toward the axis of the nozzle.

In Figure 4.7, 2 Schlieren images show the PG fed with Helium 1 l.min^{-1} comparing the behavior of the gas flow alone (Figure 4.7a)) and when plasma is ignited (Figure 4.7b)). PG is here driven by the μs power supply at 14 kV in positive polarity, 2 kHz pulse repetition rate and a gas flow of 1 l.min^{-1} . As in previously shown experiments the PG is positioned top-down.

When PG is not powered, the gas jet behaves as expected. The normal behavior of such top-down gas jet has already been described in Chapter 2. To resume briefly the helium flow expands downwards over around 7 mm, in our conditions, before bending and diluting in the upper direction due to the buoyancy forces (see chapter 2).

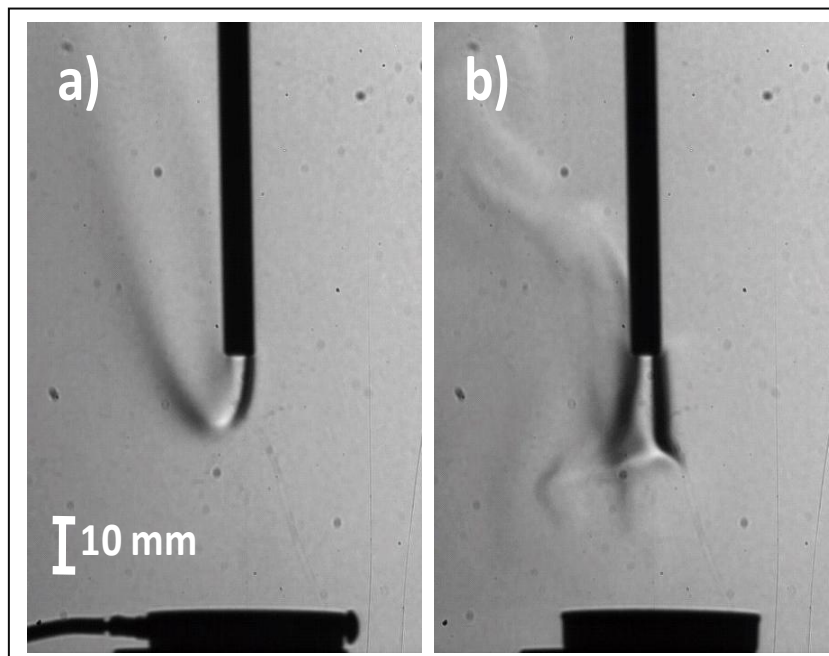


Figure 4.7: Schlieren images when helium is flushed at 1 l/min through a 15 cm long vertical capillary. The dark disk at the bottom of the images is the 40 mm in diameter metallic target. a) The PG is not powered while in b) the PG is powered at 2 kHz, 14 kV with floating potential target. Reported from [21]

When PG discharges are ignited, the flow is channeled by the plasma and the effect of buoyancy forces is significantly shifted downward for few more mm. When using a floating

potential target, the helium flows on a longer distance downstream, up to the middle of the gap between the outlet and the target, at 22 mm from tube outlet. In the zone where the gas channeling is stopped, some smaller gas channels are observable on radial directions or downward. Helium flow is then flowing upward and diffusing with ambient air. The upward flow seems slightly perturbed, as shown by the gradients increased as compared to Figure 4.7a). This upward behavior is probably resulting from the spreading in multiple small gas channels. It is worth noting that recent Schlieren imaging, performed by T. Darny, E. Robert, L. Joly and J. Fontane, shows that this behavior is specific to the PG operating in positive polarity.

This experiment shows that in addition to the previously described gas guiding effect on the plasma (see Chapter 1), the plasma is acting as a guide for the gas jet. In other words, there are two simultaneous effects, guiding and guided phenomena of the plasma on the gas flow [1, 21-22]. Parametric studies that will be discussed furthermore in the forthcoming chapters, showed that this channeling of the gas flow is influenced by multiple parameters such as voltage amplitude, pulse repetition and gas flow rates as well as a presence, nature and distance of a target in front of the PG.

By going deeper into the analysis of the Figure 4. thanks to the Schlieren imaging, the end of the *in-air* propagation suggests that PAPS spreads apart in the bending zone following the multiple Helium channels created by gas guided effect of the PAPS.

3. Production of reactive species

3.1. Identification of excited species

In the frame of biomedical applications, all components of plasma produced by plasma jets could be biologically active (see Chapter 1). Among these components, reduced electric field, ions, UV radiations; the reactive species produced, reactive oxygen and nitrogen species, are extensively studied by biologists and are under special interest for the plasma medicine applications. The nitric oxide NO and the hydroxyl radical OH especially are thought to play a major role in biological processes [23-24]. In this study we use UV-visible optical emission spectroscopy to identify the excited species produced by the Plasma Gun.

The spectrum shown in Figure 4.8 is performed at 6 mm from the tube outlet when the μ s driven PG is working at 2 kHz, 14 kV and 1 l.min⁻¹ He flow rate and is representative of emission of plasma jets working with Helium feeding gas [25-27]. In the Figure 4.8 the main emission contribution is coming from the second positive system of N₂^{*} (SPS) from 290 to 500 nm. Relatively intense emission is also observed for N₂⁺⁺ (FNS) from 390 to 480 nm, He^{*} lines at 388, 501, 587, 667, 706, 728 and 1083 nm and O^{*} lines at 777, 844 and 926 nm. One

can note the presence of the NO^* and OH^* systems from 200 to 300 nm and from 280 to 315 nm, respectively. Although these systems are observed their intensities are rather small.

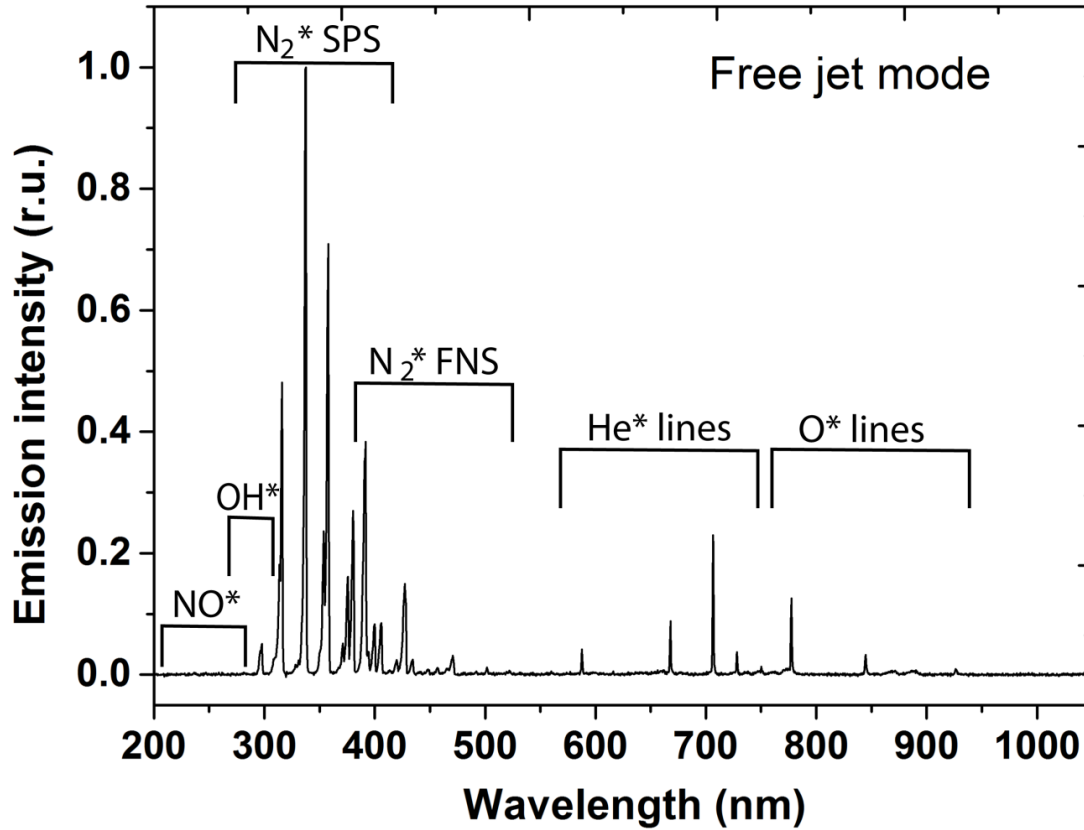


Figure 4.8: UV-visible spectra in the PG plume at 6 mm from the tube outlet. Flushed with Helium 1 l.min^{-1} , 14 kV, 2 kHz and PG vertical configuration.

Population of excited species depends on the energy delivery (see Chapter 1) which is linked to the ionization source function (S_e). The model from Xiong *et al.* (Figure 4.55) describes the huge decrease of S_e in the first cm of propagation inside a capillary, when comparing its value at the electrode zone (point A) to the mid-capillary distance (point D). This aspect plus the energy loss happening when PAPS exists the capillary, mainly due to the progressive mixing with ambient air, is inducing a change in the population of the excited species when comparing the plasma ignition zone and the plasma plume emissions.

Figure 4.119 presents spectra recorded at 1 cm from the inner electrode tip and at 2 mm from the capillary outlet. Both spectra are normalized to the emission of the He line at 587 nm because at 2 mm from the capillary outlet in our condition (see Figure 4.7), the gas jet is still laminar so mainly constituted of Helium.

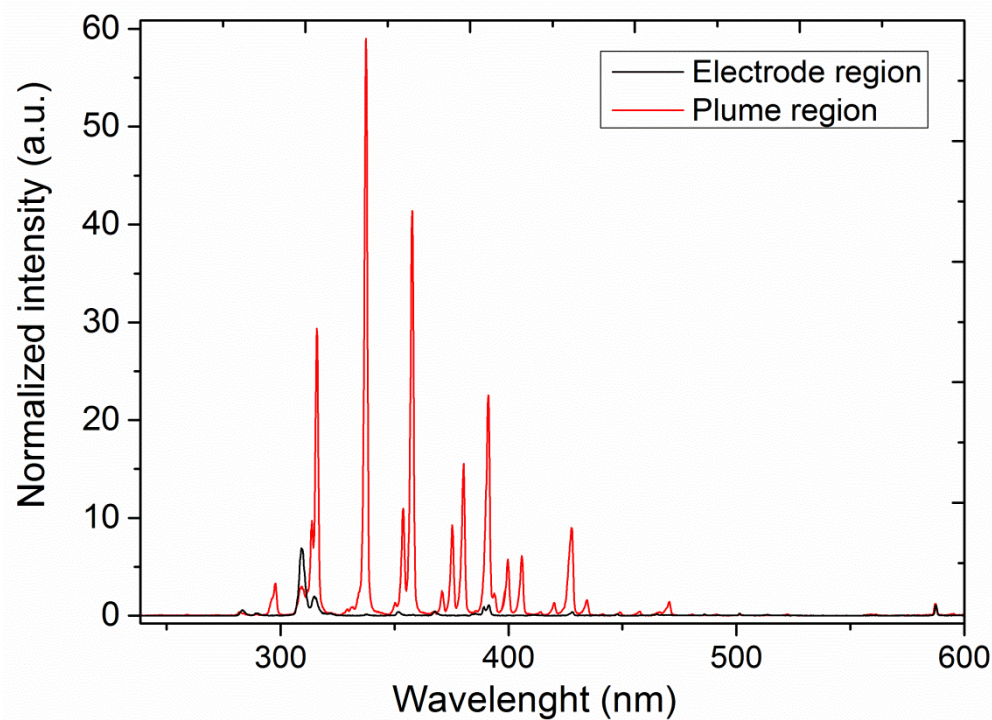


Figure 4.11: UV-visible spectra at two different locations of the PAPS propagation. Flushed with Helium 500 sccm, 14 kV, 2 kHz and PG vertical configuration. Normalized on the He* line at 706.5 nm.

When exiting the tube, transfers are happening between He* and N₂ from ambient air to produce N₂* SPS and N₂⁺* FNS which become the main contributions of the global emission. The strong emission of N₂⁺* in the plasma plume is mainly coming from transfers from He metastable states and charge transfer from He₂⁺, when the ambient air gradually diffuse in the Helium jet. The long life time of the Helium metastable (2³S) (see Chapter 1 table 5.2) decrease drastically when mixing with ambient air, to be of the order of few μs [28-29] in the high He/air ratio region of the plume and even shorter in low He/air ratio regions. It appears that their life time is highly dependent of the ratio of He versus air (see Chapter 2 and [28-29]) suggesting that main de-excitation is the transfers with N₂ which are highly more efficient than recombination by three-body collision with He atom.

In the electrode region, main contribution is coming from He* lines and OH* bands with a minor contribution of N₂⁺*. OH* and N₂⁺* are mainly coming from impurities present in the Helium Alphagaz 1. They are thought to be excited by direct electron impact (see chapter 1) and penning ionization respectively. Due to the fact that OH* emission decreases drastically at 2 mm from the tube outlet as compared to the ignition zone, the direct electron dissociation of H₂O is suspected to be an important mechanism in the plasma ignition zone. Moreover at 2 mm from tube outlet, the diffusion of humid ambient air into the Helium jet is low. This implies that other mechanisms which may happen further downstream such as H₂O dissociation by radicals and metastables as well as dissociative recombination of H₂O⁺, are limited due to the lack of H₂O in this region.

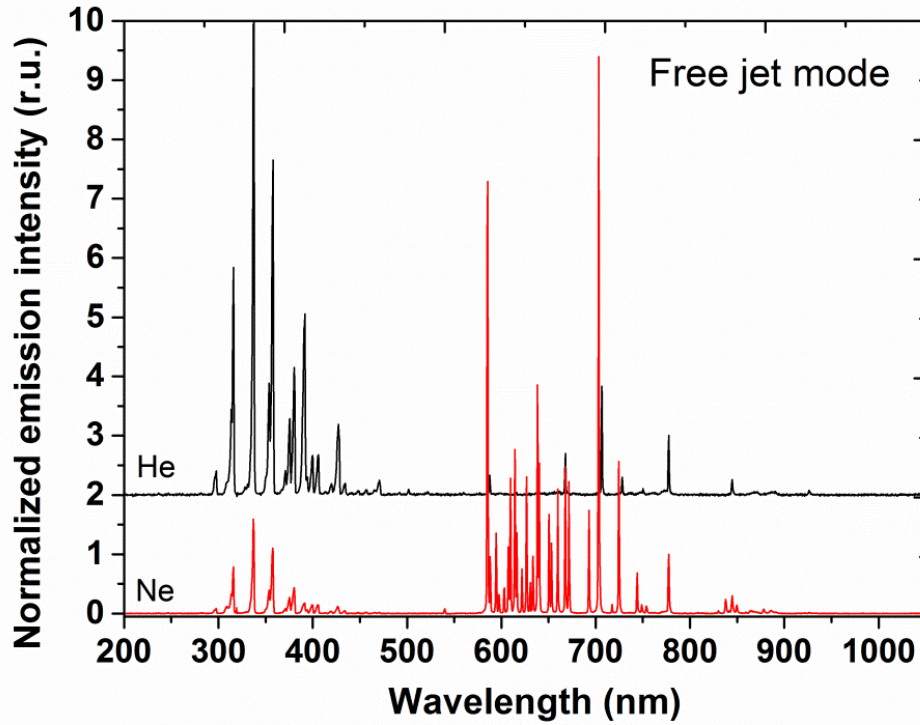


Figure 4.10: UV-visible spectra of the PG plume for two different buffer gas: Neon 250 sccm and Helium 500 sccm. 14 kV, 2 kHz and PG vertical configuration. Normalized on the O*I line at 777 nm.

In the plume region, the reactive oxygen and nitrogen species produced depend on the buffer gas used. Figure 4.10 presents a comparison of the PG with the μ s driver at 2 kHz and 14 kV with Helium (500 sccm) and Neon (250 sccm). These spectra are normalized to the emission of O*I line at 777 nm to estimate the relative emission intensity of UV species and rare gases lines.

Although N_2 SPS and FNS are visible in the two spectra their relative intensity is different, more N_2 FNS is observed in the case of Helium PG than in Neon PG. This is due to the high efficiency of penning ionization in the case of He metastable states which reaction is almost resonant with $N_2^{+*}(B)$. While in the case of Ne PG, the excitation may come from direct excitation of ground states $N_2(X)$ and $N_2^+(X)$ or by transfers from higher excited states of $Ne^{+*}(> 21.5 \text{ eV})$ (Figure 1.2).

In the 580-750 nm range, the rare gases emission lines are visible. Neon lines are more intense than the Helium ones which facilitate the observation of PAPS dynamics when performing fast-imaging. Among other reasons, this is why Neon was used during the experiments presented in 5.2.1.

3.2. Spatial distribution

3.2.1. Optical emission spectroscopy

By measuring spectra along the plasma plume axis with an UV optical fiber, we can plot emission intensities of present excited species normalized to their emissions at the capillary outlet. Indeed emission of excited species does not give information about the concentration of the species in their fundamental state however some mechanisms can be identified from such procedure. Figure 4.11 shows the normalized emission intensities of main excited species along the jet from the capillary outlet to 18 mm in standard condition of the discharge, 2 kHz, 14 kV in positive polarity and 1 l.min⁻¹ Helium gas flow rate.

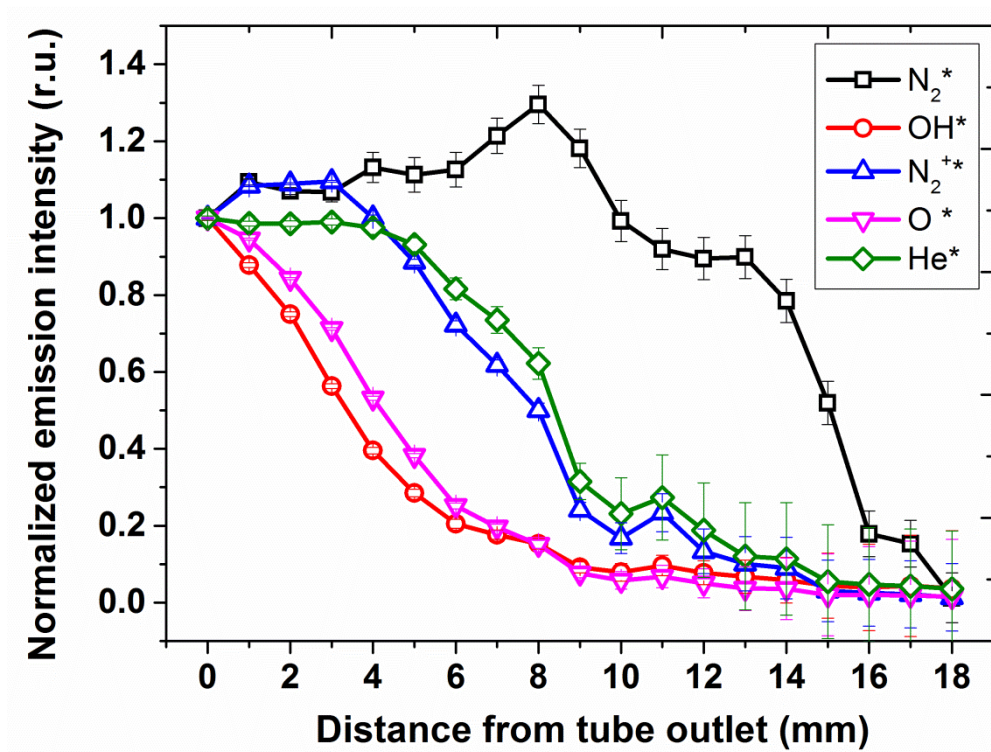


Figure 4.11: Emission intensities of plasma species along its propagation from the tube outlet (set at 0 position). PG operating at 2 kHz and 14 kV in positive polarity with a He gas flow rate of 1 l.min⁻¹.

As can be seen in this figure, the concentration of excited species is evolving spatially during *in-air* propagation. During the expansion in the plasma plume, emissions are displaying the energy transfers to the surrounding air. Energy transfers to N₂ is clearly shown by the increase of N₂^{*} (C) over the distance. O^{*} and OH^{*} emissions which are mainly coming from direct electron impact of impurities as previously described (Figure 4.11), are decreasing gradually. Helium and N₂^{+*} emissions follow a different trend, first being stable over a 4 mm distance before decreasing. Douat *et al.* showed [28] that He metastable states are following the same spatial distribution in the plasma plume as He^{*} and N₂^{+*}. The fact that those

emissions are similar tends to confirm that penning ionization is a favored transition to produce N_2^+ . The visible length of the plasma plume is here around 9 mm while PAPS in fact propagates over a distance of 14 mm as shown by the excitation of N_2^+ . Further away from the tube outlet, energy losses are becoming too high for the propagation of the PAPS and emission decreases drastically before vanishing.

3.2.2. Wavelength filtered imaging

In order to set appropriate distance between tube outlet and target to be treated during biomedical applications, we focused on spatial distribution of three excited species, He^+ as the representative of the observable plasma plume by naked eyes, N_2^+ to approach the Helium plasma/air mixing zone and NO^+ as one of the main biologically active species [23-24]. For this purpose, we used an ICCD equipped with a UV lenses (Nikon) 2 band-pass filters (LOT-Oriel) centered on 239 nm and 341 nm (FWHM 25 and 10 nm, respectively), to track the NO^+ emission at 236 nm and the N_2^+ emission at 337 nm. A filter ($\lambda > 515$ nm) allows detection of mainly He^+ peaks plus some additional, but weak, emission from O^+ (see Figure 4.8). Exposure time is 10 μs and He^+ and N_2^+ images are averaged on 100 pulses and NO^+ on 1000 pulses due to its lower emission intensity.

Influence of the gas flow rate

Figure 4.12 shows wavelength-filtered imaging for the two species N_2^+ and NO^+ plus the emission with $\lambda > 515$ nm filter dominated by He^+ , for gas flow rates from 200 sccm to 1000 sccm for PG working at 2 kHz and +14 kV.

Plasma emission length is growing with increasing gas flow rate for the three species of interest. In the case of He^+ , emission pattern length extends from 6 to 18 mm over this gas flow range while N_2^+ and NO^+ emission patterns are detected from 18 to 21 mm and 18 to 26 mm respectively. Moreover, for each gas flow rate, emission of the species has different distribution. For low gas flow rate, 200 sccm, emission patterns have needle shapes, really narrow pencil mainly visible for N_2^+ and NO^+ while for He^+ it is almost not visible. When increasing the gas flow rate from 400 sccm a conical-emission like is observable for He^+ which become longer and longer up to 1000 sccm. At 300 sccm a transition is observable between the needle shape and the conical-like pattern. He^+ emission in the plasma plume can be separated into two areas of emission intensity, one of high intensity (shown in green) where He/air ratio is high and a diffuse lower intensity emission (shown in blue) downstream and at the border of the high intensity pattern, where He/air is low.

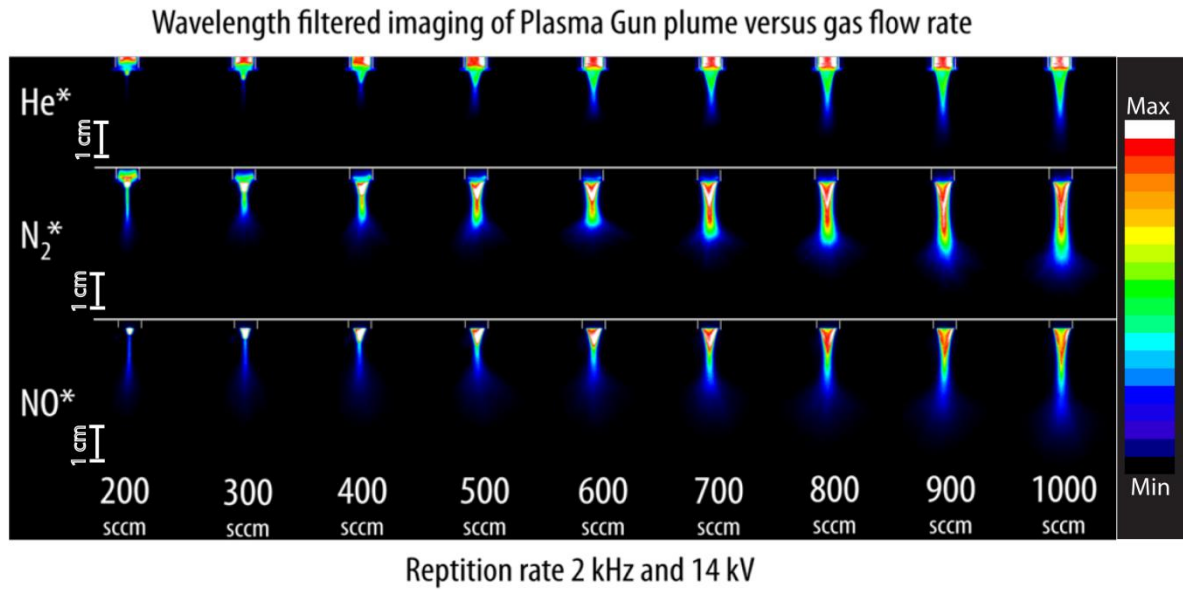


Figure 4.12 : Wavelength filtered imaging versus gas flow rate from 200 sccm to 1000 sccm in false colors. Pulse repetition rate 2 kHz and voltage amplitude 14 kV.

N_2^* and NO^* emissions patterns are a bit more complex and can be separated into two contribution for clarity purpose, the first one linked to the intense He^* emission pattern and the second downstream linked to the weaker He^* emission pattern. By looking at N_2^* spatial distribution, we can see that the first conical-like contribution is larger than the He^* one with maximum intensity at the border of the He^* maximum emission region (shown in white to red). This conical-like emission is surrounded by a middle intensity emission (shown in green). This observation is coherent with literature [16, 28, 30] where N_2^* emission was observed to have an annular shape by using either, head-on imaging, Abel inversion on wavelength-filtered imaging or spatially resolved OES. The second contribution which is observed downstream is characterized by a wide diffusion of mid to low intensities (shown in green to blue) over 1.5 cm. NO^* emission pattern, compared to N_2^* emission one, has narrower first conical-like emission and wider diffusion downstream over 2 cm.

The emission patterns are linked to the gas flow behavior (see Figure 4.7). As a matter of fact the intense plasma emission zone of the N_2^* pattern is linked to laminar Helium flow while the less intense one (the diffusion zone) is linked to the increased admixture of ambient air to the Helium, probably corresponding to the multiple small Helium channels produced by the discharge observed in Figure 4. and Figure 4.7b). Those small channels are here averaged in time. By increasing the gas flow rate, the plume emission length increases following previously reported phenomena. Karakas *et al.* [20] described that the plasma plume length was first increasing with gas flow rate before decreasing when gas flow rate (and associated Reynolds number) reaches a critical value. They suggested that this was the transition from laminar to turbulent regime of the gas flow hydrodynamics. Thus the increasing length behavior of PG plume observed in Figure 4.12 may only be valuable in this range of gas flow

rate, always in laminar flow. While for gas flow rates exceeding 1 l.min^{-1} other behaviors can be expected. From [20] it would be expected that a decrease in the plasma plume length will happen with increasing gas flow rate. However Robert *et al.* has shown [1, 22] that plasma plume behavior cannot be resumed only to fluid dynamics, by presenting experiments with opposite behavior (shift of the transition point from laminar to turbulent for larger distances). The interplay between plasma and gas flow appears to be influenced by various parameters such as the gas flow rate, the capillary length, the presence or not of a grounded conductive target and the pulse repetition rate.

Influence of the pulse repetition rate

At fixed gas flow rate (500 sccm), Figure 4.13 shows the influence of the pulse repetition rate on plasma plume configuration and species distributions. Pulse repetition rate are varying over the range 100 Hz - 3 kHz, at voltage amplitude of +14 kV and a gas flow rate of 500 sccm.

As shown here, for low pulse repetition rate, the emissions can be separated into two contributions, first a downstream expansion with conical shape and second a part directed horizontally (here directed on the right) or even upstream. This bending is happening around 7 mm downstream. In this condition the plasma plume seems to undergo spatial instability around the Plasma gun axis. As seen previously (see Figure 4.7), this second contribution is directly linked to the hydrodynamics of the gas jet. When buoyancy effects are stronger than the guiding effect of the plasma on the gas flow, or in other words when more influent than the plasma channeling, (see Figure 4.7) the helium gas flows upward. As pulse repetition rate increase, emissions are more and more centered on the axis and expend further away from the tube outlet. This observation is linked to an enhanced plasma channeling due to shorter time between pulses. As a matter of fact, the gas flow has shorter time where it can evolve without ionization induced by the plasma. He^* emission reaches around 13 mm while N_2^* and NO^* expend up to 18 and 20 mm respectively at 2 kHz.

Length of emissions for the three species is however stabilizing after 2000 Hz, phenomenon which will need further experiments and is presently studied in the thesis of T. Darny at GREMI [31]. It can be suggested that the plasma length stabilization reaches a maximum which might be linked to the limited guiding effect of the ionic species. Following this hypothesis to enhance channeling of the gas, one can play on other parameters such as voltage amplitude and gas flow rate.

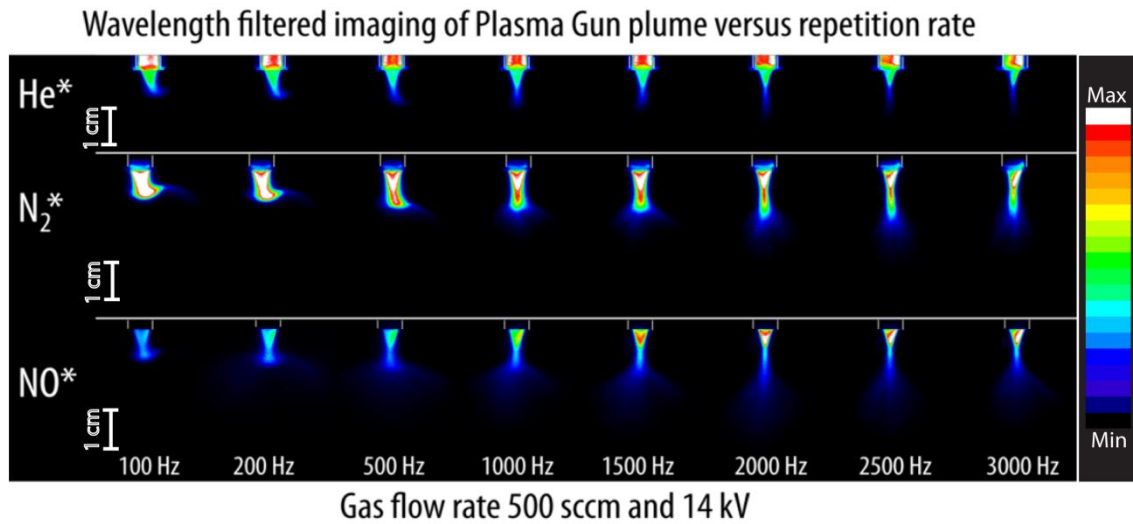


Figure 4.13 : Wavelength-filtered imaging versus pulse repetition rate from 100 Hz to 3000 Hz in false colors. Gas flow rate 500 sccm and voltage amplitude 14 kV.

The behavior of plasma distribution versus pulse repetition rate indicates that gas hydrodynamics is drastically influenced by plasma parameters. The interdependence of plasma and gas or in other words between the guiding effect of the gas and the plasma, had to be considered to characterize RONS production of the plasma jet.

Moreover Figure 4.12 and Figure 4.13 suggest as well that reactive species produced in the plasma jet are not confined in the visible region of the jet (c.f. He^* emission). The observation of the different distributions should be taken into account for the biomedical applications as we can expect treatment to be modulated, i.e. changing the ratio of reactive species deposited on the surface, by changing the gap between the PG outlet and the targeted surface.

3.3. Temporal evolution

In order to gain insight into kinetics undergoing in the plasma plume, temporal evolutions have been captured using a Jobin Yvon spectrometer equipped by a photomultiplier tube (PMT) R955 (Hamamatsu). Figure 4.14 presents temporal evolutions for O^* (777,1 nm), He^* (706.5 nm), N_2^* (C-B) at 337.1 nm, N_2^{+*} (391.4 nm), OH^* (306,8 nm) and NO^* (247,7nm). Signals are spatially integrated over the whole plasma plume in a way that PAPS displacement over this region has to be taken into account. Considering the mean PAPS velocity ($\sim 7.10^6$ cm/s), and length of the plume around 9 mm as seen in Figure 4.7, Figure 4.12 and Figure 4.13, this spatial integration is inducing a maximum of 130 ns broadening which has influence mainly on the observation and interpretation of the first peak.

Figure 4.14 enlightens fast decay for He^* and N_2^* (C-B) with emissions vanishing ~ 400 ns after the excitation while N_2^{+*} and NO^* emissions vanish lately, ~ 1200 ns and 1500 ns respectively. Short N_2^* and He^* emission FWHM are coherent with the ionization front propagation time, taken from fast imaging, in the scanned zone thus indicating that direct electron impact excitation is mainly involved. Population of N_2^{+*} states is mainly attributed to transfer of charges from He_2^+ and transfer of excitation from He metastable states with typical half life of the order of μs in a plasma jet; average value of $4.7 \mu\text{s}$ in [28] and $2 \mu\text{s}$ in [29].

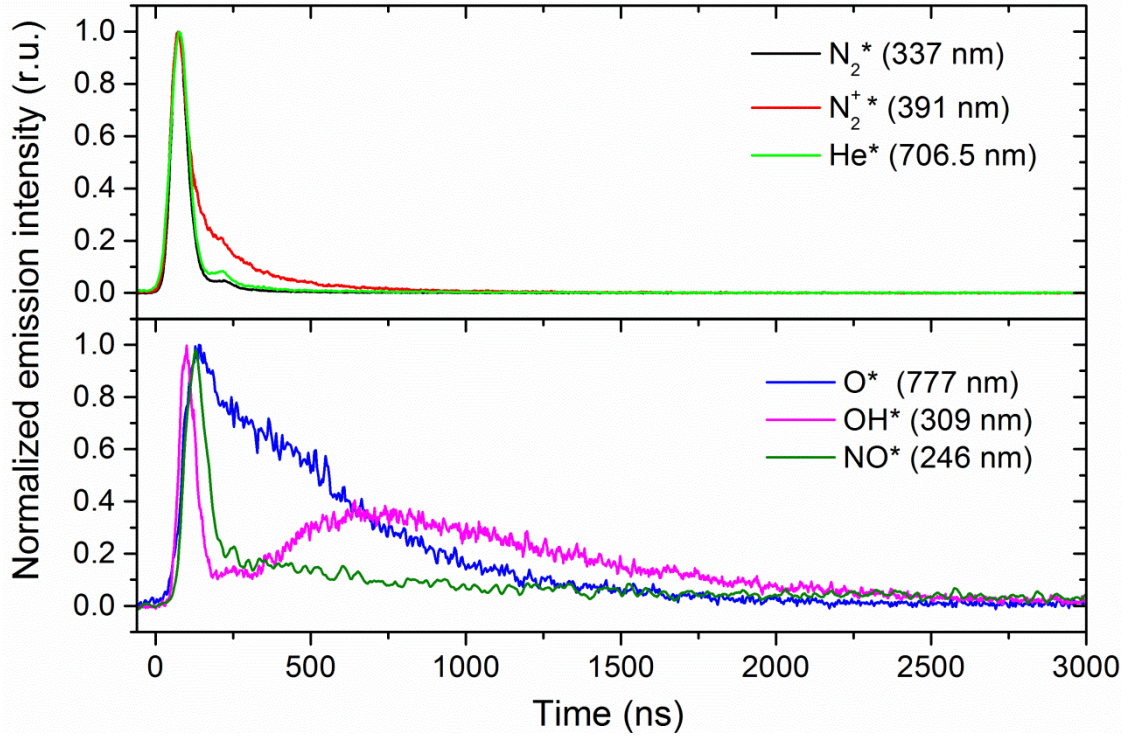


Figure 4.14: Temporal evolution of the normalized intensity of different emissions of ROS and RNS. Top for Nitrogen systems and He^* line and bottom for O^* , OH^* and NO^* . PG parameters: 14 kV, 2 kHz and $1 \text{ l} \cdot \text{min}^{-1}$ Helium gas flow rate.

This is why the first really fast decay of N_2^{+*} is associated to the charge transfer from reactive He_2^+ and the second longer one to the penning ionization from He metastable states. Long lifetime of NO^* emission is also attributed to energy transfer, mainly from N_2 (A) metastable state, which follows the ionization front. Note that a small "bump" can be observed ~ 150 ns after the first peak for all species. This feature would need further experiments to be understood.

To better understand the O^* and OH^* behaviors, their temporal evolutions have been plotted in logarithmic scale in Figure 4.15.

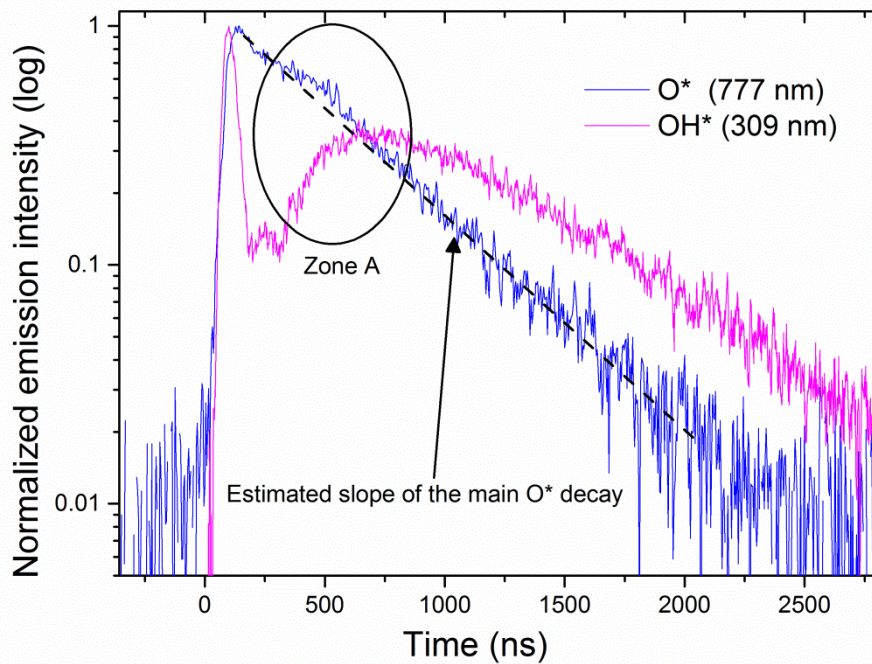


Figure 4.15: Temporal evolution of the normalized intensity of O* and OH* in logarithmic scale. PG parameters: 14 kV, 2 kHz and 1 l.min⁻¹ Helium gas flow rate.

O* emission can be shared into two contributions, a main smooth decay with emission vanishing ~ 2000 ns after the excitation and a second contribution which overlaps the first contribution with an emission peaked around 480-500 ns. The first contribution has been illustrated in Figure 4.15 by a dotted black line while the second contribution is departing from the one-slope tendency (see Zone A of the figure). The difference between the estimated decay of the main O* disappearance mechanism with the experimental temporal evolution of O* emission, informs about the presence of a repopulation of the O* excited state.

OH* emission exhibits as well two contributions however for this excited species the two contributions are separated in early and late emissions. OH* early emission follows He* and N₂* (C) emissions (Figure 4.14) and is proposed to results from direct electron impact due to its high decay rate. Its late emission exhibits a maximum between 550 and 700 ns and vanishes lately around 3000 ns. The synchronicity (see Zone A) between the O* repopulation and OH* late emission suggests that repopulation of OH* (A) excited state and O* are probably originated from the same mechanisms. This suggestion is also supported by similar spatial distribution of these two species along the axis (see Figure 4.11).

4. Production of long living species, FTIR

In this chapter we, for now, focused on description of short time scale plasma propagation and reactive species emissions. However in the frame of biomedical applications, most of

the species mentioned above will have relaxed or recombined before reaching the sample to be treated. Thus Fourier Transform InfraRed Spectroscopy (FTIR) experiments have been carry out to estimate molecular products formed from the reactive oxygen and nitrogen species. As described in Chapter 2, the effluent of the plasma gun plume is confined and directed into the FTIR cell through plastic tubes of 1 meter length approximatly. The whole system is under atmospheric pressure. These experiments were performed during premilinary experiments using the ns Spark driven PG described in Chapter 2.

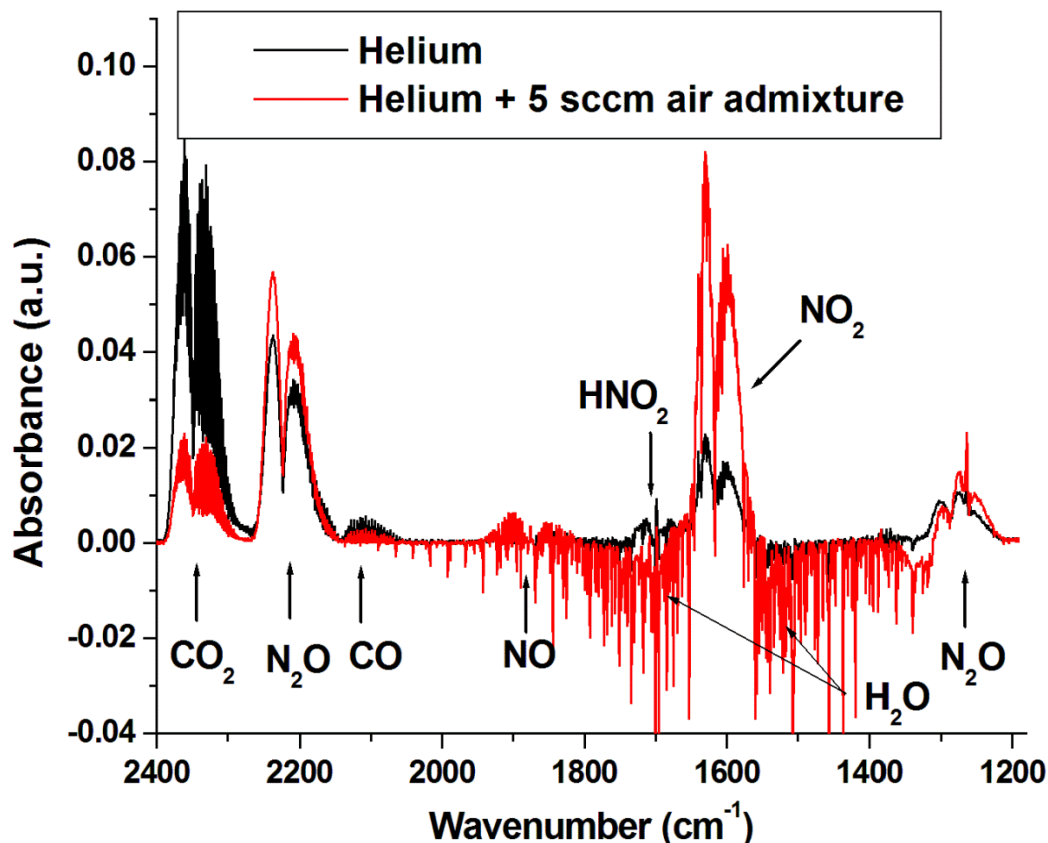


Figure 4.16: Infrared Absorption spectra performed by FTIR of the effluent of PG for Helium and He/air mixture feeding gases. PG parameters 22 kV, 160 Hz.

FTIR measurements allow observing transformed species such as, acids (HNO_2 and HNO_3) as well as NO_2 , N_2O and NO in its ground state and ozone (not shown in Figure 4.). The presence of these molecules in the effluent of the PG plume is coherent with the literature for other plasma jets [32] and for DBD [33] identified by FTIR as well as through numerical model [34]. It is worth emphasizing that even after 1 meter propagation in the gas pipe and at atmospheric pressure; molecules that are rather reactive are detected in the measurement cell.

CO_2 , CO and H_2O species are also observed in the spectra; however their intensity were varying a lot, during background and plasma effluent measurements. This results in an

artifact of negative absorbance for the H_2O bands which has indeed no physical meaning. This feature is a commonly known problem in FTIR measurements. In our case, it can be due to a change in the pressure in the measurement cell between experiments and background as well as an interaction with the pressured air used to clean the mirrors of the spectrometer (see chapter 2). Due to the time scale of this experiment, 2 min per spectrum in our experimental procedure, we have to keep in mind that chemical reactions may modified the concentration of the species present in the effluent. For example, it is expected that an oxidation of NO with mainly O_2 and/or O_3 to form NO_2 and to some extent acids, can influence the observed species in the measurement while in the real biomedical application condition, those species may have already react with the target surface and induce biological reaction cascades.

Figure 4. compares the species produced by the PG when fed with Helium (200 sccm) or a mixture of Helium with 5 sccm of dry air. It can be shown that such small quantity of dry air increases N_2O , NO and NO_2 . However water bands overlap lots of bands of interest (NO, HNO_2 and NO_2) and should be minimized to allow parametric studies. In this FTIR setup, gas mixing is happening in a confined environment and is homogenous when reaching the measurement cell unlike in the real biomedical conditions. Non-homogeneity surely modifies the species production.

As mentioned above, these experiments were devoted to the observation whereas long-living species could be observed after a certain time and distance of propagation in gas pipes. Indeed if we would like to performed experiments in biological conditions of treatment, we would need to find solutions to include ambient air in the process; by for example using an open-path FTIR instrument.

5. Conclusion

In this chapter, the plasma produced by the plasma gun in the free-jet mode was under analysis. Description of the Pulsed Atmospheric-pressure Plasma Stream (PAPS) with its two modes of propagation inside the capillary, namely *Wall-Hugging* mode and *Homogeneous* mode is reminded [1]. The PAPS propagation can be controlled by modifying the plasma ionization, by acting on the voltage waveform and on the pulse repetition rate. Indeed the PAPS will continue to propagate until the voltage amplitude is important enough to ensure a sufficient transfer of energy to the ionization front though the plasma tail.

It has been shown that when PAPS propagated freely in the air, conservation of propagation mode is happening. Energy losses are however increasing, linked to a velocity decrease, and PAPS is spreading apart in multiple sub-PAPS before vanishing which is specific of the positive polarity.

By mean of Schlieren imaging, we observed a channeling of the gas jet by the plasma. The interplay between gas and plasma is also evidenced by wavelength-filtered imaging. Parameters such as gas flow and pulse repetition rates are modifying hydrodynamics of the jet and species distributions, influence that should be taken into account during biomedical applications.

Optical emission spectroscopy gave us information about the reactive species produced at different stage of the propagation. First comparison between the reactor region and the integrated plume zone enlightens the energy transfer between buffer gas excited species (O^* , N_2^{+*} and OH^* coming from impurities) and the surrounding air, producing all nitrogen excited species and nitric oxide. During the biomedical protocol establishment, we have compared emission spectra in the plume for PG fed with Helium or Neon gases. This comparison allowed us to choose the PG with Helium buffer gas as major treatment tool regarding the cost of Helium versus Neon gas as well as its ability to produce relatively high ratio of reactive species especially considering N_2^{+*} and NO^* .

The spatial scan, along the plume, of the excited species enlightened the energy transfer to the surrounding nitrogen component of air and allowed to gain insights of the chemical kinetics between species. Temporal evolution of the excited species confirms that most excitations are from direct electron impact in the vicinity of the ionization front. However emission of some species such as O^* , OH^* and NO^* lasts longer which suggests that transfers are happening after the propagation of the ionization front.

In a first approach to evaluate the reactive species which are more probably reacting with the cells or living tissues, FTIR measurement were performed showing the production of biologically active molecules such as NO , NO_2 , N_2O and acids, HNO_3 and HNO_2 . Moreover it is shown that the quantity of air admixture in the buffer gas influences the ratio of reactive species produced, consequently in real biomedical treatment conditions variation of their concentration are expected.

This chapter gives information about the plasma physical and chemical properties in the free-jet mode. These experiments were carried out as preliminary studies of the PG plume when impinging on a target mimicking the biological samples.

French summary of Chapter 4

Ce chapitre est dédié à l'étude du Plasma Gun, surtout en ce qui concerne la propagation et la production d'espèces réactives, quand celui-ci fonctionne en mode "jet libre" (en anglais "Free-jet"). Le "jet libre" est un jet de plasma se propageant dans l'air ambiant à potentiel flottant.

Une première partie de ce chapitre résume les travaux de doctorat effectués en parallèle par V. Sarron [1] (2009-2013) sur la génération et la propagation du plasma à l'intérieur du capillaire, via une comparaison modèle numérique et expériences. La dénomination de *colonne de plasma pulsée à pression atmosphérique* (en anglais: *Pulsed Atmospheric-pressure Plasma Stream* (PAPS)) y est expliquée ainsi que les modes de propagation *Homogène* et le long des parois du capillaire (en anglais: *Homogeneous* et *Wall-Hugging*). Les résultats permettant le contrôle de la longueur de propagation par la modification de la forme du pulse de tension sont aussi présentés.

En deuxième partie, l'étude se porte sur la propagation du jet dans l'air ambiant, en sortie de capillaire via l'imagerie rapide, l'imagerie Schlieren et des mesures spectroscopiques.

Une interaction entre le gaz porteur et le plasma y est décrite. Il se produit en effet une canalisation du gaz par le plasma, qui semble être liée à la dérive des ions produits. Cette canalisation est aussi identifiée grâce à de l'imagerie filtrée en longueur d'onde, et apparaît comme dépendante des paramètres tel que le débit de gaz, et la fréquence de répétition de l'impulsion de tension.

La spectroscopie d'émission optique, spatialement et/ou temporellement résolue, permet d'identifier les espèces excitées produites à différents stades de la propagation du plasma et d'élaborer des hypothèses sur la cinétique réactionnelle sous-jacente. Bien que toutes les espèces possèdent une partie de leur excitation par collision électronique, d'autres mécanismes de transfert d'énergie sont identifiés tel que les transferts de charges et d'ionisation par le biais des métastables d'Hélium et d'azote moléculaire.

Entre les composantes du plasma susceptibles de produire un effet biologique lors du traitement des cellules ou tissus vivants, les espèces secondaires produites par recombinaison ou désexcitation, telles que les radicaux et les molécules réactives à longue durée de vie, jouent un rôle central. Elles sont ici identifiées, dans une approche préliminaire, par mesures Infrarouge à Transformée de Fourier (FTIR). Ces mesures ont mis en évidence la production de NO, NO₂, N₂O et d'acides HNO₃ et HNO₂, dans le gaz effluent et sa sensibilité à la présence d'un ajout de quelques % d'air dans le gaz porteur.

Ce chapitre donne des informations à propos des propriétés physico-chimiques du plasma dans le mode "jet libre". Ces résultats sont une introduction pour mieux appréhender l'influence de la présence d'une cible biologique, ou d'une cible imitant la cible biologique sur les propriétés du plasma.

References

- [1] Vanessa Sarron " Étude et optimisation d'une décharge « Plasma Gun » à pression atmosphérique, pour des applications biomédicales" PhD thesis, Université d'Orléans, 2013.
- [2] Z. Xiong and M. J. Kushner, Atmospheric pressure ionization waves propagating through a flexible high aspect ratio capillary channel and impinging upon a target. *Plasma Sources Sci. Technol.* 21 (2012) 034001.
- [3] Xiong Z, Robert E, Sarron V, Pouvesle J-M and Kushner M J 2013 "Atmospheric-pressure plasma transfer across dielectric channels and tubes" *J. Phys. D: Appl. Phys.* 46 15520
- [4] Z. Xiong, E. Robert, V. Sarron, J-M. Pouvesle, and M. J. Kushner. Dynamics of ionization wave splitting and merging of atmospheric-pressure plasmas in branched dielectric tubes and channels. *Journal of Physics D : Applied Physics*, 45(27) :275201, 2012.
- [5] Teschke, M., Kedzierski, J., Finantu-Dinu, E.G., Korzec, D., Engemann, J., 2005. High-speed photographs of a dielectric barrier atmospheric pressure plasma jet. *IEEE Transactions on Plasma Science* 33, 310–311. doi:10.1109/TPS.2005.845377
- [6] Robert E, Sarron V, Riès D, Dozias S, Vandamme M and Pouvesle J M, "Characterization of pulsed atmospheric-pressure plasma streams (PAPS) generated by a plasma gun" *Plasma Sources Sci. Technol.* 21 (2012) 034017.
- [7] Rubin Ye and Wei Zheng "Temporal-spatial-resolved spectroscopic study on the formation of an atmospheric pressure microplasma jet" *APPLIED PHYSICS LETTERS* 93, 071502 2008
- [8] Robert E, Barbosa E, Dozias S, Vandamme M, Cachoncinlle C, Viladrosa R and Pouvesle J M 2009 "Experimental study of a compact nanosecond plasma gun" *Plasma Process. Polym.* 6 795–802
- [9] Kim J Y, Kim S O, Wei Y and Li J 2010 "A flexible cold microplasma jet using biocompatible dielectric tubes for cancer therapy" *Appl. Phys. Lett.* 96 203701
- [10] J. Jarrige, M. Laroussi, and E. Karakas, "Formation and dynamics of plasma bullets in a non-thermal plasma jet: influence of the high-voltage parameters on the plume characteristics," *Plasma Sources Sci. Technol.*, 19 (6), 065005, (2010).
- [11] Xiong, Z., Kushner, M.J., 2010. Surface corona-bar discharges for production of pre-ionizing UV light for pulsed high-pressure plasmas. *J. Phys. D: Appl. Phys.* 43, 505204. doi:10.1088/0022-3727/43/50/505204

- [12] Q. Xiong, X. Lu, Y. Xian, J. Liu, C. Zou, Z. Xiong, W. Gong, K. Chen, X. Pei, F. Zou, J. Hu, Z. Jiang, and Y. Pan. Experimental investigations on the propagation of the plasma jet in the open air. *Journal of Applied Physics*, 107(7) :3302, (2010).
- [13] Z. Xiong, X. Lu, Q. Xiong, Y. Xian, C. Zou, J. Hu, W. Gong, J. Liu, F. Zou, Z. Jiang, and Y. Pan. Measurements of the propagation velocity of an Atmospheric-Pressure plasma plume by various methods. *IEEE Transactions on plasma science*, 38(4) :1001–1007, (2010).
- [14] J. Shi, F. Zhong, J. Zhang, D. W. Liu, and M. G. Kong. A hypersonic plasma bullet train traveling in an atmospheric dielectric-barrier discharge jet. *Physics of plasmas*, 15 :013504, (2008).
- [15] Z. Xiong, X. Lu, Y. Xian, Z. Jiang, and Y. Pan. On the velocity variation in atmospheric pressure plasma plumes driven by positive and negative pulses. *Journal of Applied Physics*, 108(10) :103303, 2010.
- [16] N. Mericam-Bourdet, M. Laroussi, A. Begum, and E. Karakas, “Experimental investigations of plasma bullets,” *J. Phys. D. Appl. Phys.*, 42 (5), 055207, (2009).
- [17] C. Jiang, M. T. Chen, and M. a Gundersen, “Polarity-induced asymmetric effects of nanosecond pulsed plasma jets,” *J. Phys. D. Appl. Phys.*, 42 (23), 232002, (2009).
- [18] X. Lu and M. Laroussi, “Dynamics of an atmospheric pressure plasma plume generated by submicrosecond voltage pulses,” *J. Appl. Phys.*, 100 (6), 063302, (2006).
- [19] G. V. Naidis, “Modeling of helium plasma jets emerged into ambient air: Influence of applied voltage, jet radius, and helium flow velocity on plasma jet characteristics,” *J. Appl. Phys.*, 112 (10), 103304, (2012).
- [20] E. Karakas, M. Koklu, and M. Laroussi, “Correlation between helium mole fraction and plasma bullet propagation in low temperature plasma jets,” *J. Phys. D. Appl. Phys.*, 43 (15), 155202, (2010).
- [21] Jiang, N., Yang, J., He, F., Cao, Z., 2011. Interplay of discharge and gas flow in atmospheric pressure plasma jets. *Journal of Applied Physics* 109, 093305. doi:10.1063/1.3581067
- [22] Robert E, Sarron V, Darny T, Riès D, Dozias S, Fontane J, Joly L and Pouvesle J-M 2014 “Rare gas flow structuration in plasma jet experiments” *Plasma Sources Sci. Technol.* 23 b012003

- [23] Graves D B 2012 "The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology" *J. Phys. D: Appl. Phys.* **45** 263001
- [24] Z. Machala , M. Janda, K. Hensel, I. Jedlovsky, L. Lestinska, V. Foltin, V. Martisovits, M. Morvova "Emission spectroscopy of atmospheric pressure plasmas for bio-medical and environmental applications" *Journal of Molecular Spectroscopy* 243 (2007) 194–201
- [25] Lu XP, Jiang ZH, Xiong Q, Tang ZY and Pan Y. "A single electrode room-temperature plasma jet device for biomedical applications" *APPLIED PHYSICS LETTERS* **92** (2008) 151504
- [26] J. L. Walsh, J. J. Shi, and M. G. Kong "Contrasting characteristics of pulsed and sinusoidal cold atmospheric plasma jets" *APPLIED PHYSICS LETTERS* **88** (2006) 171501
- [27] G V Naidis and J L Walsh "The effects of an external electric field on the dynamics of cold plasma jets—experimental and computational studies" *J. Phys. D: Appl. Phys.* 46 (2013) 095203
- [28] Thesis C. Douat " Étude d'un micro-jet de plasma à pression atmosphérique" Université Paris-Sud, February 2014
- [29] Urabe K., Morita T., Tachibana K., Ganguly B.N., "Investigation of discharge mechanisms in helium plasma jet at atmospheric pressure by laser spectroscopic measurements" *J. Phys. D: Appl. Phys.* 43 (2010) 095201
- [30] Yukinori Sakiyama, David B. Graves, Julien Jarrige and Mounir Laroussi " Finite element analysis of ring-shaped emission profile in plasma bullet" *APPLIED PHYSICS LETTERS* **96**, 041501 _2010
- [31] Darny T, Robert E, Riès D, Dozias S, Pouvesle J-M " Unexpected plasma plume shapes produced by a microsecond plasma gun discharge" *Plasma Science IEEE Transaction on* Volume PP issue 99
- [32] Pipa, A., Ropcke, J., 2009. Analysis of the Mid-Infrared Spectrum of the Exhaust Gas From an Atmospheric Pressure Plasma Jet (APPJ) Working With an Argon-Air Mixture. *IEEE Transactions on Plasma Science* 37, 1000–1003. doi:10.1109/TPS.2009.2013865
- [33] Matthew J Pavlovich, Hung-Wen Chang, Yukinori Sakiyama, Douglas S Clark and David B Graves " Ozone correlates with antibacterial effects from indirect air dielectric barrier discharge treatment of water" *J. Phys. D: Appl. Phys.* 46(2013) 145202
- [34] Sakiyama, Y., Graves, D.B., Chang, H.-W., Shimizu, T., Morfill, G.E., 2012. Plasma chemistry model of surface microdischarge in humid air and dynamics of reactive

neutral species. J. Phys. D: Appl. Phys. 45, 425201. doi:10.1088/0022-3727/45/42/425201

Chapter 5

Influence of a target on the Plasma Gun plume

1. Introduction

In Chapter 4, we have seen how the production of reactive species is linked to PAPS propagation into the PG dielectric capillary and in the ambient air. It has been shown that parameters such as voltage amplitude, pulse repetition rate and gas flow rate can influence their spatial distribution. Moreover results shown in Chapter 3 suggest complex interaction are undergoing between plasma and the target whether dealing with *in vitro* or *in vivo* studies.

This Chapter will give insights of the influence of the nature of the target on the physical and chemical properties of the PAPS. This parameter appears to be essential as biomedical applications deal with various types of target. As a matter of fact their "nature" -humidity content as well as electrical conductivity- is varying not only from *in vitro* to *in vivo* studies but also from one organ to another. Two conditions standing for extreme cases are here taken into consideration. Dielectric targets will be first considered and then the influence of conductive targets, combined with gas flow and pulse repetition rates, gap distances and voltage amplitude modifications, will be described by means of fast and wavelength-filtered imaging, Schlieren photography, OES and FTIR.

2. Dielectric targets

Some biomedical applications deal with dielectric targets when to cite only two of them treating polymers for biocompatibility or *in vitro* studies.

An important remark can be made; when the depth of the dielectric material is changed, the coupling between the plasma and the grounded metal plate, which in our experimental setup supports the target, will be modified. Thus the capacitive effect of the dielectric will vary and will induce changes in the plasma behavior.

Figure 5.1 presents conventional camera pictures of the PG plume impinging on either distilled water surface or a gas pipe filled with Neon as well as a plasma jet plume impinging on a glass sheet, b) and c) extracted from literature [1-2] respectively. Exposure time of those conventional camera pictures are of the order of the ms, thus pictures are integrating multiples voltage pulses.

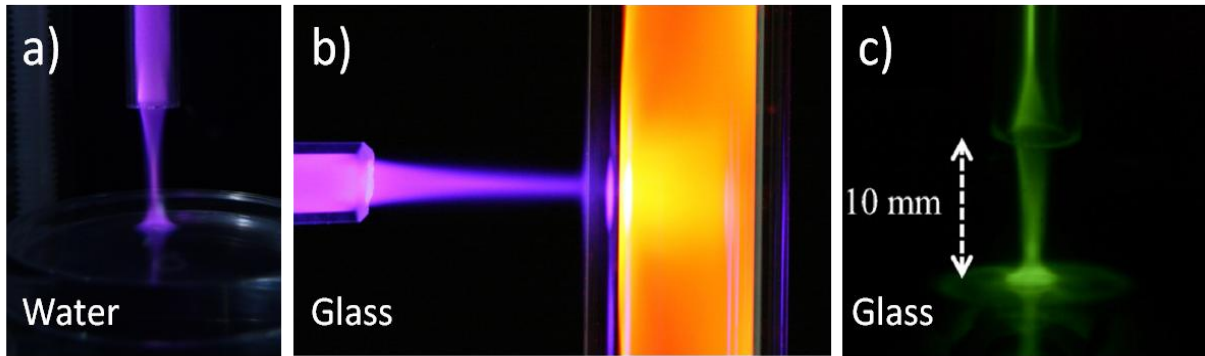


Figure 5.1: Conventional camera pictures of PG impinging on a) a distilled water surface and b) on a borosilicate gas pipe flushed with Neon, extracted from [1] c) on a glass sheet, extracted from [2] and imaged in false colors.

Pictures exhibit quite similar distribution of plasma visible emission with small differences due to the difference of gap between the tube outlet and the target surface. However for all of them, the distribution is constituted by a typical conical plume ending by a reverse cone, more or less flattened, which seems to spread on the surface. Yonemori *et al.* [2] observed that this reverse cone onto the surface (Figure 5.1c) in false color) becomes ring-shaped with increasing gas flow rate. This annular distribution on the target is due to the emission of the excited reactive species, and implies production of different ground state species in regard of those present before plasma propagation, induced by the air diffusion into the jet. Gas flow rate used changes the position where this diffusion is happening. Moreover this distribution is dependent on the distance between the tube outlet and the target. Indeed this feature would need further experiments to be fully understood, such as fast imaging to reveal the dynamic of impingement on the surface. Another remark that could be made by observing the pictures is that intensity of the visible emission seems quite homogeneous over the whole plasma plume.

Figure 5. shows a spectrum performed at the surface of a dielectric target, here a 5 cm thickness Delrin® piece positionned at 12 mm from the tube outlet.

Similarly to the case of the free-jet (see Figure 4.8), main emission is coming from N₂ SPS, Helium and oxygen while hydroxyl radical and nitric oxide emissions are barely present probably due to the position of the measurement from the tube outlet, here 12 mm compared to 6 mm in the free jet mode (Figure 4.8), as those emissions are decreasing along the propagation.

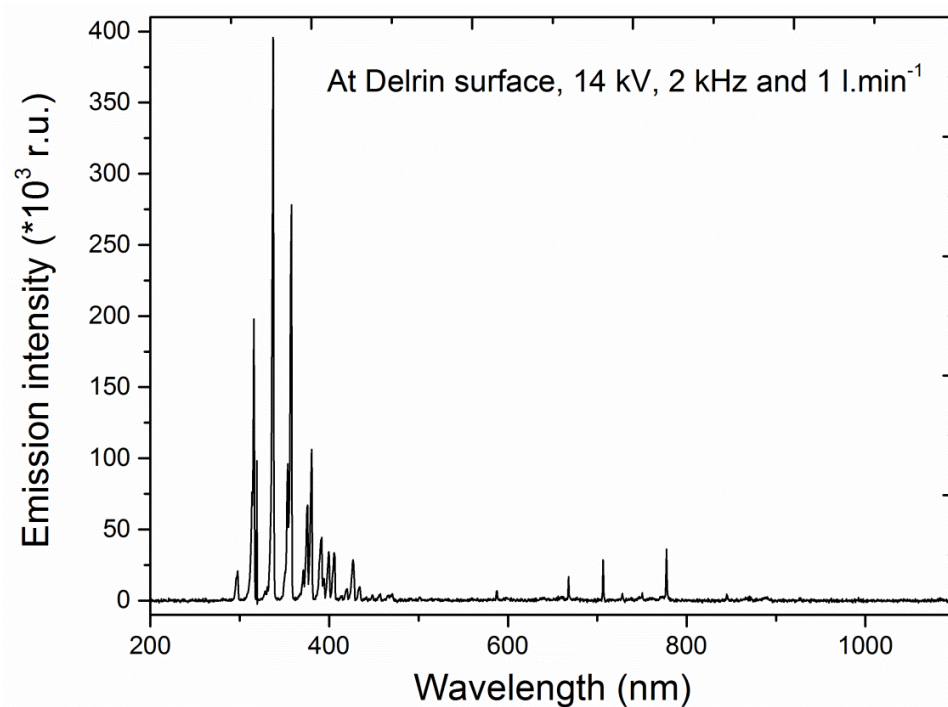


Figure 5.2: UV-visible spectra in the PG plume at 12 mm from the tube outlet when impinging on a Delrin[®] piece. Flushed with Helium 1 l.min⁻¹, 14 kV, 2 kHz and PG vertical configuration.

To determine spatial distribution of the excited reactive species, spectra are performed along the gap using a UV optical fiber and emission intensities at selected wavelengths are plotted. This experiment was performed for two different dielectric targets, a petri dish filled with distilled water and a piece of Delrin[®] of 5 cm thickness. Both targets are placed on a grounded metal plate.

Figure 5.33 shows that normalized emission intensity is decreasing along the gap for all species under interest. Comparison with Figure 4.11, case of the free jet mode, enlightens the difference of behavior of N₂^{*}(C-B) emission. In the plume operating in free jet mode (see Figure 4.11), N₂^{*}(C-B) emission was increasing when moving away from the tube outlet while here only a slower decrease can be observed. This feature may be due to the change in the hydrodynamics of the jet. While in the first case the jet was expanding freely, here an obstacle is positioned in front of the flow. It has been shown by Schlieren imaging [3] that in the case of a jet positioned horizontally, the gas flow impinging on a glass sheet is expanding on the surface before being diluted in ambient air. Indeed this behavior is gas flow rate dependant. In our experiment, the PG is positioned top-down which induces a gas flow first along the surface and then upward on each side, limiting in the mean time the penetration of ambient air inside the jet.

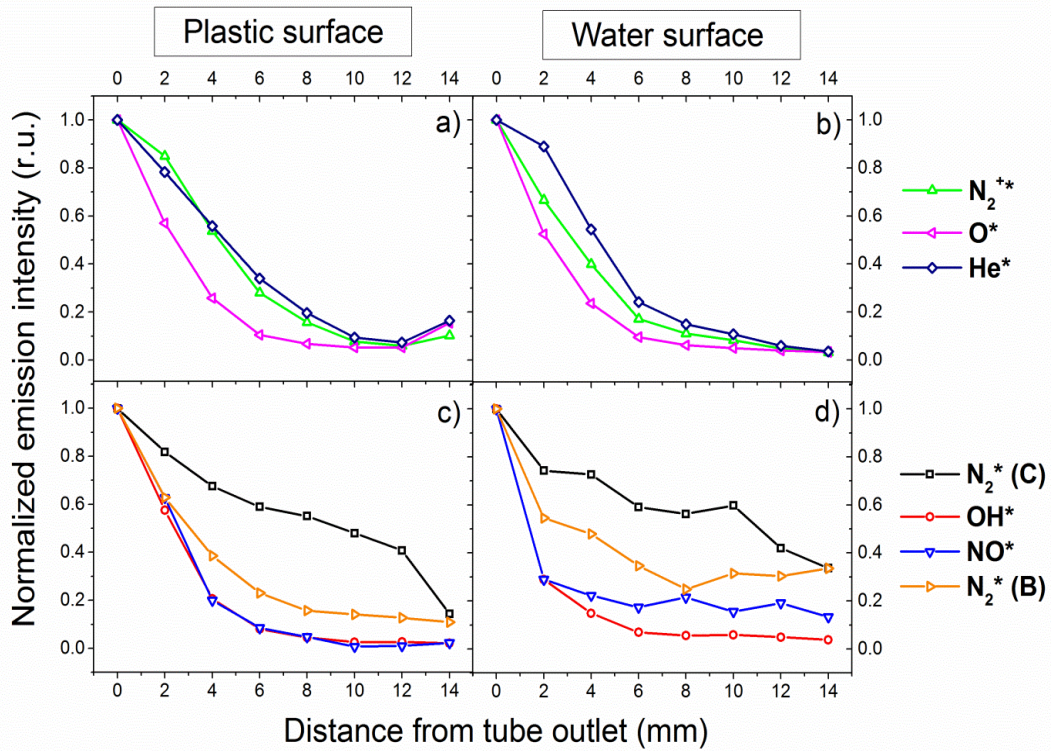


Figure 5.3: Emission intensities of plasma species along its propagation in the 15 mm gap between tube outlet (set at 0 position) to a) c) the plastic surface and to b) d) the water surface.

Wild *et al.* suggest [4] by measuring electric charges deposited on a dielectric target surface that the transferred charges from the plasma jet to the target surface lead to a deformation of the electric field which allows the formation of a counter-propagating plasma. We do not observed in our experiments the formation of such counter-propagating plasma on dielectric targets. However changes in the electric field due to charge deposition had also been proposed by Xiong *et al.* in their model which shows really good agreement with our experiments of PAPS splitting and transfers [5-6].

From conventional picture emission patterns (Figure 5.1), spatial distribution of the reactive species along the gap (Figure 5.3) as well as from the spectrum shown (Figure 5.), the influence of the presence of a dielectric target in front of the PG seems limited. Expect the spatial distribution of N₂^{*}(C-B) which could be understood in regard of the hydrodynamic of the gas jet, most of the features described earlier (see Chapter 4) for free jet mode are still valid when the PG plume impinges on a dielectric surface.

3. Conductive targets

During *in vivo* studies, plasma is directed onto the tissues to treat. Those tissues have different nature, humidity contain and electrical conductivity. It is worth noting that for *in vitro* experiments also the nature of the target can vary, per example the electrical conductivity when molecular adjuvant are present in the cell culture media, which could be solid or liquid. In our biomedical *in vivo* protocol, mice are placed on a grounded conductive plate. As the characterization of the plasma impingement is posterior to the biomedical assays developed in Chapter 3, we had no opportunities to perform *in situ* experiments. Thus to characterize plasma properties in condition as close as possible to biomedical treatment, we used either a potato positioned on the grounded conductive plate as well as the grounded conductive plate alone to mimic the mouse. Results shown in the chapter will show that to some extent conductive grounded targets of different nature have high analogies in their influence on the plasma properties.

3.1. The counter-propagating PAPS

Plasma *in-air* propagation when impinging on a metal grounded surface is shown in Figure 5.4. Fast imaging, seven 50 ns snapshots with varying delays from plasma exiting the PG capillary, allows separating the PAPS dynamics into four steps.

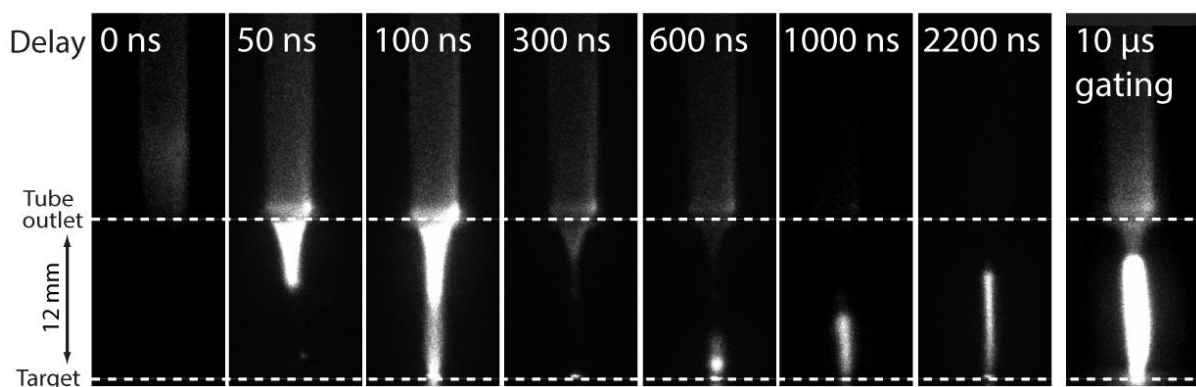


Figure 5.4: 50 ns snapshots of plasma propagation when impinging on a conductive surface at 12 mm from tube outlet in the air with labelled delays and 10 μ s time-integrated snapshot delayed by 300 ns. Time origin when PAPS reach the borosilicate capillary outlet.

First, plasma expands inside the flow of high He fraction which results in a conic shape due to energy transfers to the surrounding air ($t=50$ ns) similarly as in the free jet mode. In a second step, a "connection" between the ionization front and the target is observed ($t = 100$ ns). We have seen that in the free jet mode that the ionization front propagates until spreading in multiple channels following the gas flow ((Figure 4.6 and Figure 4.7). With the

presence of the conductive plate, instead of the spreading in multiple channels (Figure 4.6), the whole plasma emission is channeled towards the target. During the third step, between $t = 100$ and $t = 300$ ns, a quasi-extinction of the plasma emission is taking place. At $t = 300$ ns, a small volume of plasma confined on the target surface is observed which will extend upstream.

This final step (from $t = 300$ to 2200 ns) is the counter-propagation of a secondary plasma at moderate velocity, $\sim 5 \cdot 10^5 \text{ cm.s}^{-1}$ from the target toward the capillary outlet. This plasma stops at one point, in these conditions around 3 mm before the capillary outlet (see delay 2200 ns), which depends on discharge parameters such as voltage amplitude, pulse repetition rate, gas flow rate and distance between capillary and target. More information on this feature will be given through this chapter.

The maximum upstream propagation position is reached relatively quickly, at around 1800 ns after the quasi-extinction step; nevertheless the secondary plasma emission is still observable during around $8 \mu\text{s}$ before vanishing with the voltage potential. The extinction process is similar to the one happening in the capillary (see Figure 4.1), i.e. the ionization front emission intensity decreases followed by the plasma tail emission extinction.

On the right hand side of the Figure 5.4, the time-integrated, during the whole voltage pulse ($10 \mu\text{s}$), image of the secondary plasma enlightens the importance of the secondary upstream plasma in the total emission intensity. Thus the presence of a conductive target induces non-negligible increase of spontaneous emissions in the visible range along the plasma jet plume.

Figure 5.5 presents a series of 20 ns snapshots describing a more detailed propagation of the PAPS in the gap for same conditions, 2 kHz , 14 kV and Helium 1 l.min^{-1} except that the gap distance is moved to 20 mm .

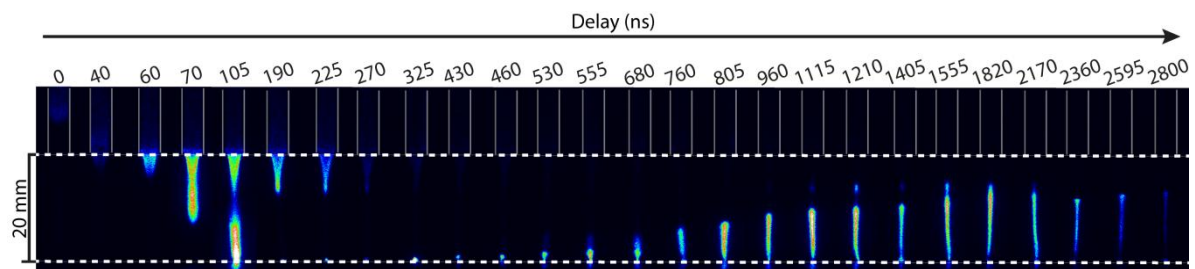


Figure 5.5: 20 ns snapshots of plasma propagation when impinging on a conductive surface at 20 mm from tube outlet in the air with labelled delays. Time origin when PAPS reach the borosilicate capillary outlet. Snapshots presented in false colors.

The propagation is similar as the one shown in Figure 5.4 however with an increased gap some other features are observable. At 70 ns delay, a drop-like shape is following the already observed conical emission at the tube outlet. This second contribution is even

clearer at 105 ns delay where the drop-like emission reaches the target surface. In this picture a lower intensity region is seen at the tip of the conical emission, at around 7 mm. During the next 130 ns, from 190 to 325 ns, the first conical emission is decreasing in intensity while a small and quite intense emission appears on the target surface. From 270 ns delay, the plasma at the surface starts to propagate upstream and continues to increase in length until 1555 ns delay. During this propagation step, a rather low and narrow emission is however always observable from tube outlet to the target.

Fast imaging has been performed when plasma plume impinged on a metal grounded target. Nonetheless Figure 5.4 showed that the counter-propagating feature could be deduced from time-integrated snapshot. Thus comparison can be made with a conventional camera picture to estimate the plasma behavior in real biomedical conditions. Figure 5.6 shows the plasma plume emission and part of the mouse lying on the grounded conductive plate. The picture has been performed during a biomedical experiment with plasma parameters as follow: 14 kV positive polarity voltage amplitude, 2 kHz pulse repetition rate, a 12 mm gap and a Helium gas flow of 1 l.min^{-1} .



Figure 5.6: Plasma Gun plume impinging on mouse skin, 14 kV, 2 kHz, gap 12 mm and 1 l.min^{-1}

Global aspect of the plasma plume observed with naked eyes when impinging on a conductive surface can be described as follow. A first conical shape and diffuse light violet plasma prolonged with a thinner channel of bright pink color. This spatial distribution is coherent with the previous descriptions of a plasma propagating downstream and upstream. Thus it can be stated that the downstream propagation emission is characterized by a violet emission while the upstream contribution is pink. A similar pink emission has been reported recently [7] in a configuration of two *in-air* colliding jets which can be related at a certain extent to our case where two plasmas counter-propagate, the downstream and upstream PAPSs. However in Douat *et al.* experiment [7], the pink emission results from the interaction of two similar discharges producing two electric fields which are interacting and producing an electron trap. The origin of the pink emission observed in Figure 5.6 will be discussed in paragraph 3.3. of this chapter.

It is worth mentioning that the same time-integrated emission is observed on a potato, mouse and grounded metal surfaces whereas no secondary upstream plasma emission contribution is seen when the plasma plume is impinging on an dielectric material such as a Delrin[®] piece, on the surface of distilled water or on a glass sheet as seen in the previous section (2. of the chapter). This suggests that the secondary plasma is generated only when the target is conductive.

Literature reports few recent studies on the impingement of a plasma jet on a conductive surface [2, 8-11]. Propagation of plasma plume when impinging on conductive surface has been studied by Nastuta *et al.* on a fingertip [8]. Figure 5.77 shows their observations after plasma propagation from left to right until impingement on the fingertip where time origin is set. The finger tip is represented by the white vertical line on the right of each figure and is placed at 15 mm from the capillary outlet.

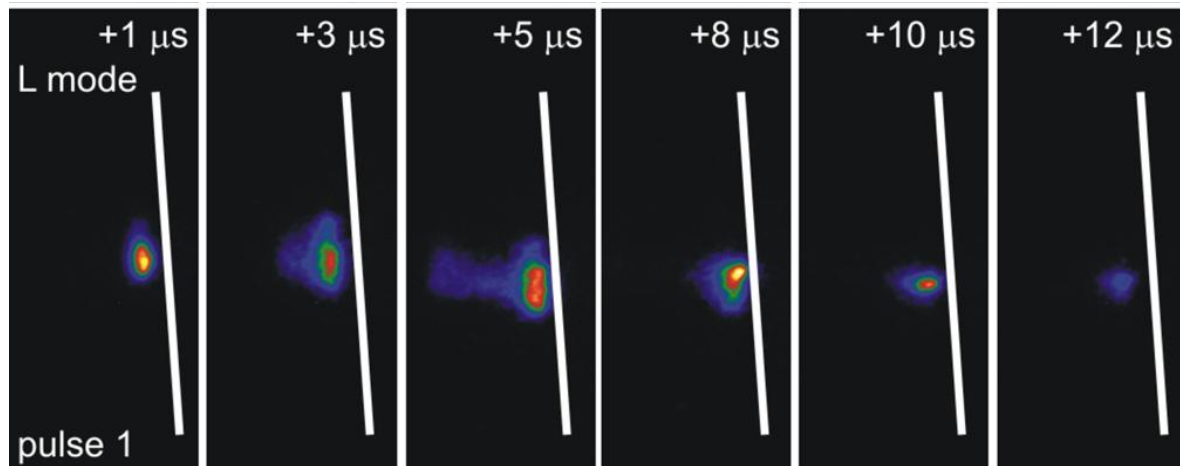


Figure 5.7: Images of plasma structure spreading on to the finger surface (30 ns exposure time), with a 15 mm gap. Time origin set when the plasma landing on fingertip. Reported from [8].

Nastuta *et al.* experiment enlightens a plasma emitting after impingement onto the surface in two steps. During the first one, the plasma extends in the gap from the finger tip towards the capillary outlet (from +1 μ s to +5 μ s) followed by the decrease of emission intensity at the target surface from +8 μ s to +12 μ s. This plasma dynamics is the closest observation to what is observed in our experiments however the full plasma propagation in the plume is not reported such as any information on the quasi-extension phase is given.

The presence of the conductive target seems to influence the PAPS properties long before its *in-air* propagation thus long before impingement on the target as shown in Figure 5..

As a matter of fact by modifying the contrast of representative snapshots (in this case the 0 and 40 ns delay of Figure 5.5) a thin emission in sub-mm scale can be observed from the ionization front to the target surface. This emission is small however clearly observable for

all pictures at small delays only when a conductive target is present in front of the PG. Before PAPS reaches the tube outlet, it seems that there is a pre-ionization phenomenon which could increase the plasma propagation velocity compared to the free-jet mode, as observed in literature [12-13].

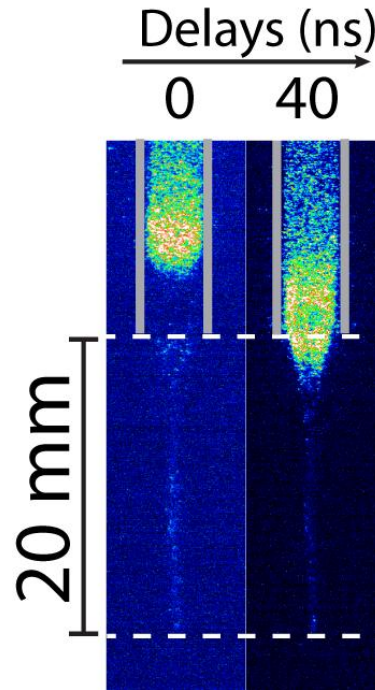


Figure 5.8: High contrast 20 ns images for early delays of plasma propagation. At 2 kHz, 14 kV, He 1 l.min⁻¹ and 20 mm gap in false colors.

3.2. Gas flux modification induced by plasma

As shown in Chapter 4, there exist an interplay between gas jet hydrodynamic and plasma propagation. The presence of a conductive surface in front of the plasma jet is another parameter that is able to induce modification in the general behavior of the plasma plume. To enlighten this feature, Schlieren diagnostic which is described in Chapter 2, is used. Same experimental setup has been used than the one presented in Figure 4.6. However in this case, the experiment has been implemented by the connection of the metal target to the ground potential (Figure 5. c)) [14]. The target is placed at 45 mm away from the tube outlet; the plasma gun is fed with 1 l/min⁻¹ Helium flow, and powered by the μ s driver at 2 kHz and 14 kV in negative polarity.

The Helium flow channeling observed when switching the power on is stronger when the target is grounded [14]. The Helium flow which was going up to 22 mm in Figure 5. b) is impinging the target at 45 mm in Figure 5. c). As can be seen on the picture, after 22 mm the

gas jet channel appears thinner and multiple oblique channels are observable. Thus only a portion of the helium flow impinges on the grounded target due to coupling between the plasma and the conductive grounded target.

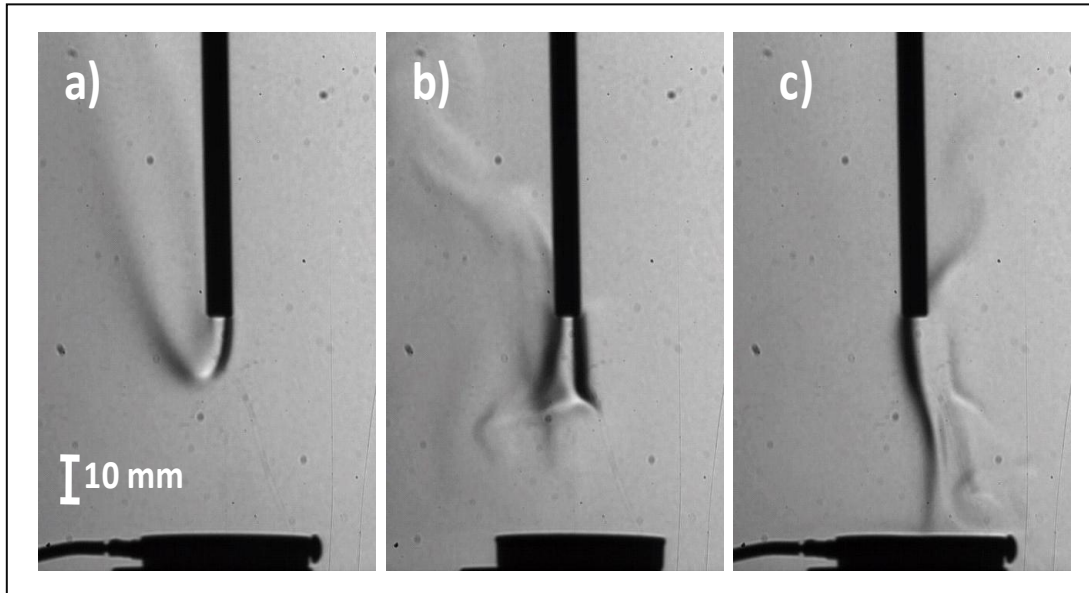


Figure 5.9: Schlieren images when helium is flushed at 1 l/min through a 15 cm long vertical capillary. The dark disk at the bottom of the images is the 40 mm in diameter metallic target. a) the PG is not powered. In b) and c) the PG is powered at 2 kHz, -14 kV with b) floating potential target and c) grounded target. Reported from [14].

The difference of channeling induced by the fact that the metal plate is grounded or not, suggests that the gas flow is not channeled by a temperature increase of the gas. As a matter of fact the gas hydrodynamics could be influenced by a change of gas temperature but in our case it has been shown that the temperature increase in the core of the plasma jet is rather low, around $320 \text{ K} \pm 10 \text{ K}$ (see Figure 2.11). Another remark concerning the hypothetical increase of temperature due to the plasma ignition is that this phenomenon would be independent of the potential of the target.

It is more probable that the channeling may result from a drift towards the target of ionic species produced in the PAPS [14]. Although the number of electronic collisions is higher than those of the ionic species, it is the latest which induce the more effective kinetic energy transfer with the neutrals due to the difference of mass ratio. In the case of a positive polarity voltage pulse, the positive ions are directed toward the grounded metal plate with a slow velocity motion, as compared to the electrons velocity. In [14] we proposed that the drift of these ions may either be induced by the fast travelling intense electric field generated at the head of the PAPS during its propagation through ionization wave processes or by the transient field imposed between the PAPS head and the target.

When the grounded conductive target is positioned at 12 mm from the tube outlet as shown in Figure 5.10, the Helium flow reaches the target only when plasma is ignited. In this case the full Helium flow is channeled straight to the target. In regards to biomedical applications, this channeling has to be taken into account. As a matter of fact when dealing with control process during *in vivo* or *in vitro* studies, gas jets alone are often used to estimate possible effect of gas flow. Those Schlieren images show that gas jets are behaving differently with the presence of a conductive target and the gap used.

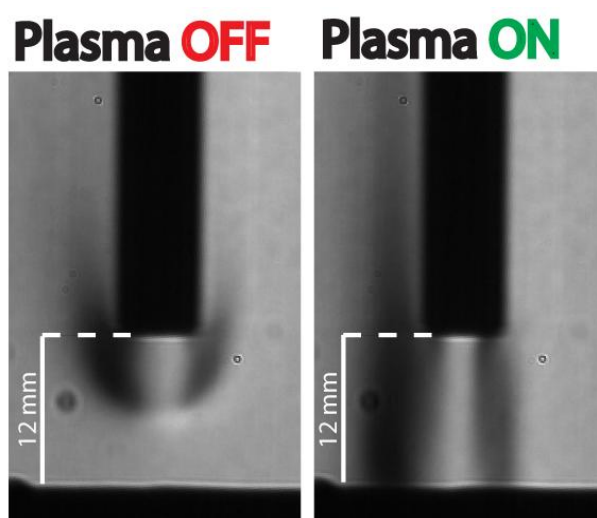


Figure 5.10: Schlieren imaging of PG impinging on a metal grounded target at 12 mm from tube outlet. PG fed with He 1 l.min⁻¹, 2 kHz and +14 kV.

Figure 5.10 shows in addition, that the PAPS propagation downstream and upstream (Figure 5.4) appears to happen in the channel mainly constituted by Helium. This rare gas channel is partially protecting PAPS from quenching due to air constituent.

3.3. Modification of the production of excited species

To identify the species responsible for the pink emission (shown in Figure 5.6), optical emission spectroscopy has been undertaken (Figure 5.11) with Maya spectrometer. Lines identification is shown on a spectrum retrieved at the middle of the gap of 12 mm between the capillary outlet and a potato surface.

Major emission is coming from the N₂^{*} (C-B) system, additional molecular emissions coming from OH^{*} (A-X), N₂⁺ (B-X) system and atomic lines from He^{*} and O^{*} are observed. Interestingly NO^{*} (γ bands) and N₂^{*} first positive system ((B-A) or FPS) are over-expressed in this spectrum as compared to Figure 4.8 and Figure 5.. The N₂^{*} (B-A) system (478-1100 nm) was even observed neither in the free jet mode configuration nor with the dielectric targets.

This observation suggests that the population of those excited species are enhanced and produced because of the presence of the conductive surface.

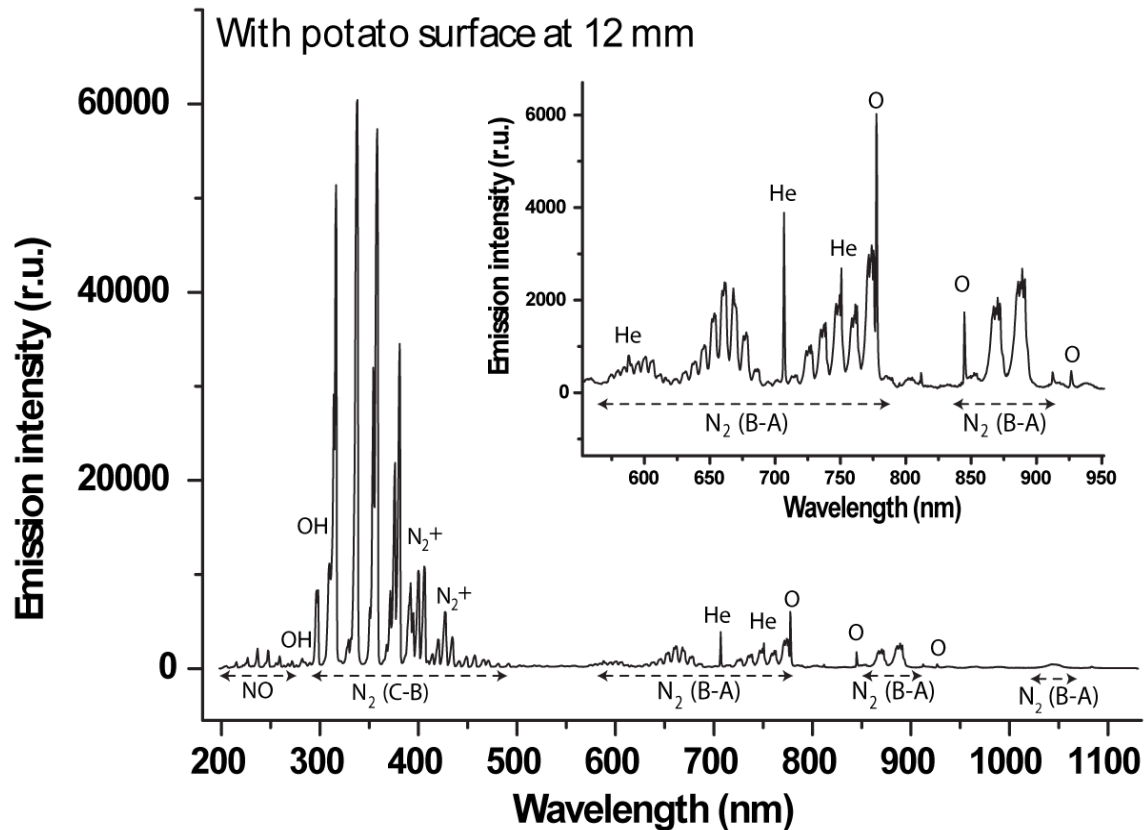


Figure 5.11: UV-visible spectrum of time-integrated PAPS (1 s exposure, 2kHz, +14 kV, He 1 l.min⁻¹). Spectrum of the emission in the middle of a 12 mm gap between tube outlet and a potato surface with identification of the lines and bands. The insert is a zoom from 550 to 950 nm.

In Figure 5. a comparison of spectra, normalized to the N₂ SPS band head at 337.13 nm, retrieved at the target surfaces, metal a) and potato b) surfaces are compared to a free jet mode c) spectrum. The targets are positioned at 12 mm from the tube outlet and equivalent voltage amplitude 14 kV, pulse repetition rate 2 kHz and He flow of 1 l.min⁻¹ are used.

For spectra achieved with targets (Figure 5.a) and b)), three reactive species systems are mainly contributing to the emission: NO γ bands, N₂ (SPS) (C-B) and N₂ (FPS) (B-A). We have seen that the counter-propagating plasma emission is contributing a lot in the whole propagation plasma emission (see Figure 5.4, Figure 5.6). These time-integrated spectra are mainly dominated by the counter-propagating plasma emission, partially due to its lower propagation velocity. Additionally a spectrum performed in the free jet mode is shown. This spectrum was taken at 6 mm from the capillary outlet due to the high decrease of emission intensity with distance in those conditions (see Figure 4.11). In this case and similarly when

PG impinges on a dielectric target (see Figure 5.), very few NO^* and no N_2^* ($B-A$) emissions are observed while He^* , O^* and N_2^{+*} are over-expressed.

The pink emission observed when the PG impinges on a conductive surface (Figure 5.6) can be explained by the combination of the N_2^* ($B-A$) emission from the red visible wavelength range (from 600 to 850 nm) and the N_2^* ($C-B$) emission from the violet visible wavelength range (from 400 to 500 nm). The fact that N_2^* ($B-A$) emission is present only when PG impinges on a conductive surface explains the presence of this pink emission only in this condition.

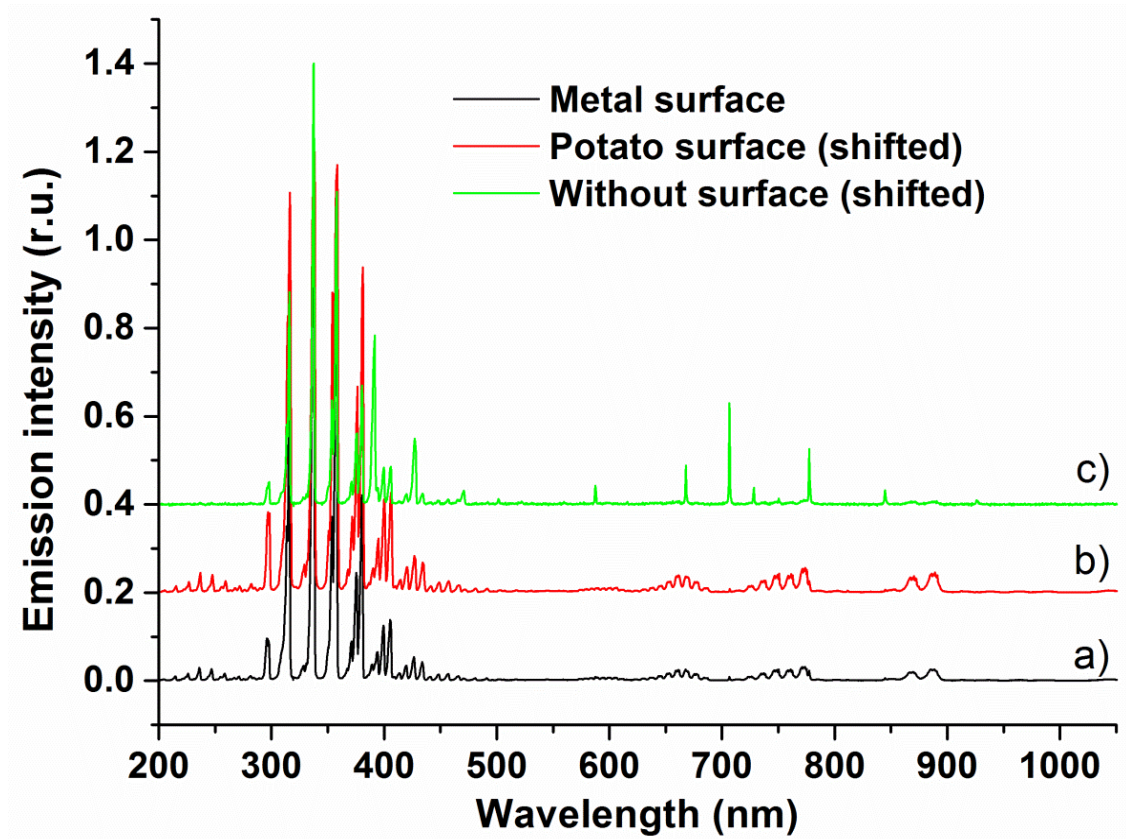


Figure 5.12: UV-visible spectra of time-integrated PAPS (1 s exposure). Spectra performed a) at the metal target surface. b) at the potato target surface with a gap of 12 mm and c) in the free jet mode at 6 mm from tube outlet.

It is clear that the plasma impingement on a conductive target induces drastic changes on the production of reactive species on the target surface. Moreover the high analogy between spectra a) and b) confirms that, in a preliminary approach, a metal grounded surface can be used to mimic biological samples.

3.4. Spatial distribution of the reactive species

3.4.1. Influence of the gap

As mentioned in 5.3.1, the distance between the tube outlet and the conductive target surface influences the plasma propagation and gas hydrodynamics features. Some teams [4, 8-9, 15] already reported on the influence of the gap distance: on charge deposition in the case of dielectric targets, or on the plasma properties such as production of ground state and excited state species and plasma propagation after impingement on a conductive target surface. Here we focus on the influence of this parameter on the production of the excited reactive species along the gap. The gap has been first set to 12 mm to confirm the modification in the excited species production, especially concerning NO^* and N_2^* (B), observed in Figure 5. thanks to spatially integrated OES.

To evaluate spatial evolution of each species within the gap, spectra have been measured from 1 mm to 11 mm, with a 2 mm step. Normalization of the emission intensities of the species is performed in regards of their emission at 1 mm after the tube outlet to clarify the description. Plotting the emission intensity of the species of interest versus the position in the gap underlines two groups of species (Figure 5.a) and b)). The first one including He^* , O^* and N_2^{+*} , shows an intensity decreasing continuously across the gap similarly to the case of the free jet and when the PG plume impinges on a dielectric target. A second consisting of N_2^* (C-B), N_2^* (B-A), OH^* and NO^* exhibits a different behavior in the case of a conductive target. Those species present a bell-shape distribution along the gap with a maximum of emission intensity peaked around 9 mm. This distribution is coherent with the emission intensity measured by spectra shown in Figure 5.11 and Figure 5. performed at a mid-gap distance and at the surface respectively.

Figure 5.b) also presents a conventional camera picture of the PG plume with the light profile of a time integrated (10 μs) ICCD image (picture already shown in Figure 5.4).

Comparison between the picture, the light profile, which is peaked around 7 mm and the spatial distribution of the reactive species confirms the high contribution of the counter-propagating PAPS in the production of the second group of reactive species.

N_2^* (B) and N_2^* (C) excitations require lower energy (6 and 11 eV, respectively see Table 1 of Chapter 1) than excitation of He^* states (around 20 eV for metastable states and around 23 eV for excited states leading to transitions in the visible range) as well as excitation of N_2^{+*} (B) state (18.8 eV). As a matter of fact, in our work the counter-propagating plasma is appearing after the PAPS propagation in the capillary and in the air. Previously reported results [10, 18]] revealed current, velocity and intensity drops linked to a decrease of the ionization source function along the propagation. Moreover, in our case, charges neutralization when impinging the target can induce additional energy losses. However

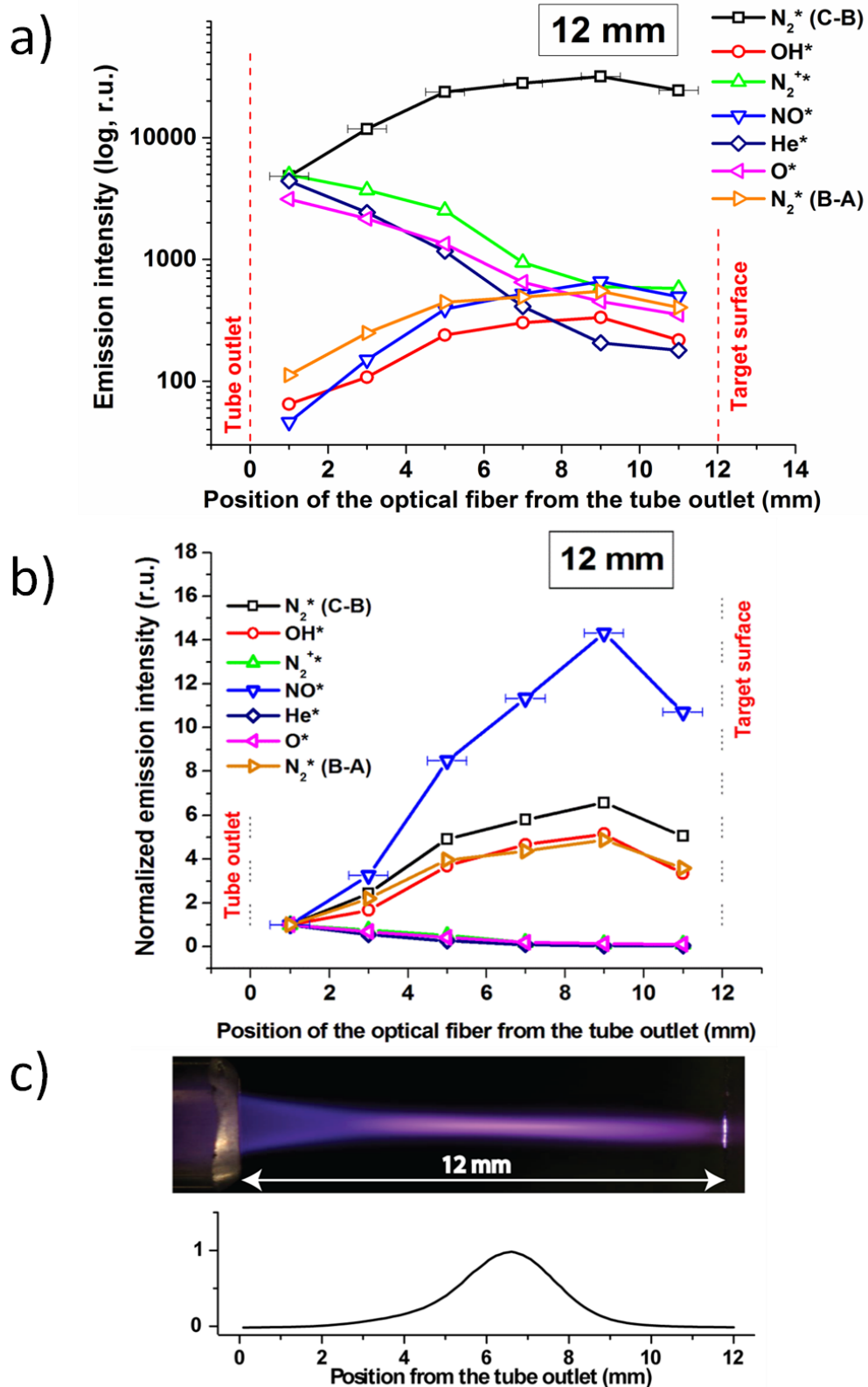


Figure 5.13: a) Emission intensity of several species in logarithmic scale b) Emission intensity of several species normalized to each species emission intensity at 1 mm from the capillary outlet. The capillary outlet is at zero position and the target at 12 mm. c) Conventional camera picture and cross section of time-integrated (10 μ s) ICCD snapshot.

those energy losses are pretty low as compared to the input energy, delivered by the power supply, still sustaining plasma through the ionized channel.

Thus, it could be deduced that the first group of species is populated first by direct excitation with high energy electrons and transfers from high energetic levels of He^* . The second group is more likely to be populated by lower energy electrons (counter-propagation) and probable successive energy transfers to lower energetic levels (see table 5.1).

When changing the gap to lower and higher distances, 8 and 20 mm respectively, the distribution of the reactive species are modified as can be seen in Figure 5.14.

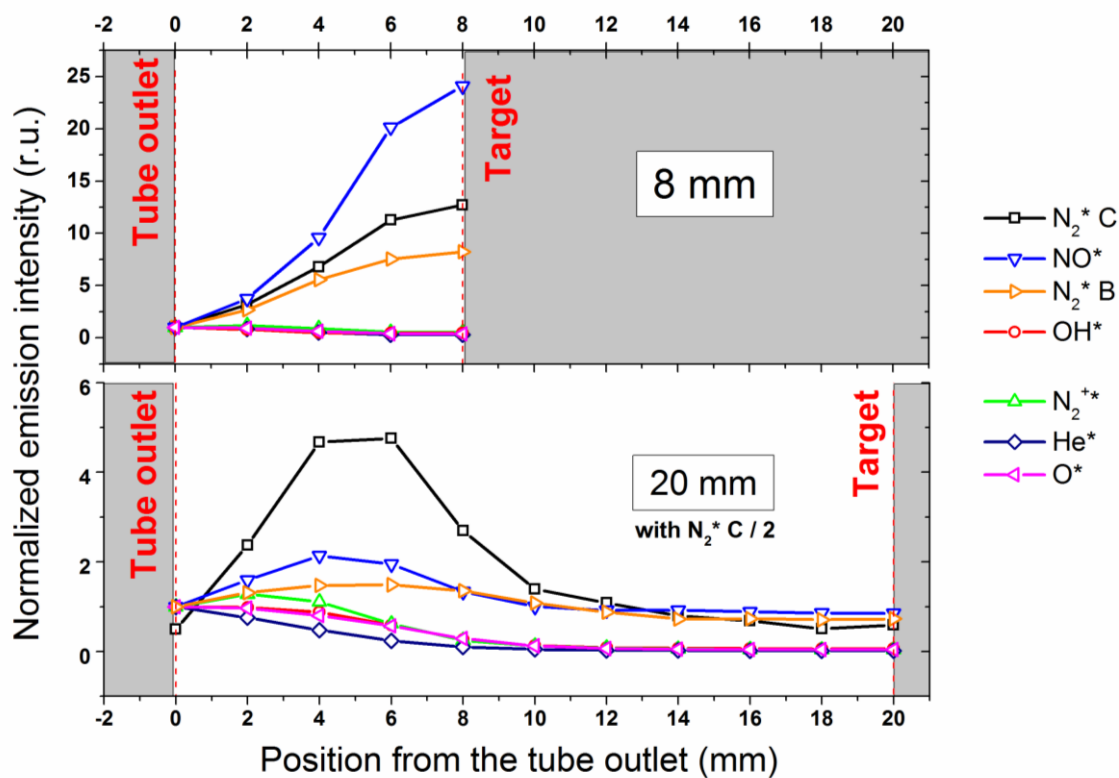


Figure 5.14: Emission intensity of several reactive species normalized to each species intensity at the capillary outlet. The capillary outlet is at zero position and the target at 8 and 20 mm respectively for top and bottom graphs.

On one hand at a 8 mm gap, the same groups can be observed, with in this case $\text{N}_2^* \text{SPS}$, $\text{N}_2^* \text{FPS}$ and NO^* increasing along the whole gap and the maximum emission intensity is observed on the surface. Interestingly in this configuration OH^* emission follows the same trend as He^* probably induced by the low diffusion of ambient air allowed by the small gap configuration.

With the 20 mm gap, N_2^* (C), NO^* and N_2^* (B) are peaked between 4 and 6 mm, O^* and OH^* have same behavior with slow decrease from 0 to 10 mm while N_2^{+*} exhibits a small increase in intensity between 2 and 4 mm before following the same trend as O^* and OH^* . He^* is as in the other configurations decreasing along the whole gap.

For the three gap distances mentioned above (Figure 5.a) and Figure 5.14), the excited species can be shared into two groups suggested to be linked with the energy of the electrons needed to populate their upper state. The emission intensity of the first group decrease along the gap while the second group exhibit a maximum further downstream; position which depends on the gap distances. As previously mentioned, ambient air is gradually diffusing into the Helium laminar flow such as the region close to the tube outlet is constituted by a high He/air ratio switching gradually to a low He/air ratio with increasing distances. As the first group of excited species, He^* , O^* and N_2^{+*} are maximized at the tube outlet, it reveals that these species are transferring their energy when air constituent become too numerous; without neglecting the fact that they are populated by high energetic electrons.

For the second group of excited species, it can be deduced that a certain amount of air diffusion in the jet is necessary to populate the upper level and consequently with increasing distances the He/air ratio become too low and they are also quenched. As the maximum of emission of the second group appears at approximately the same position for the three gaps, we can suppose that the position of the metal surface, in this range of gap distances, is not significantly modifying the ambient air diffusion into the Helium jet.

Furthermore, we have seen that the second group is mainly populated during the counter-propagating PAPS, with the presence of the representative pink emission (Figure 5.a) and Figure 5.c)). From the evolution of the emission intensities along the gap, we can suggest that the counter-propagation length is modified with the gap distance. As a matter of fact with small gap distance, the counter-propagating PAPS seems to be constrained close to the surface while at higher gap distance it extends further upward (Figure 5.14). Thus to deliver maximum excited reactive species onto the target surface, there is a major interest in optimizing the positioning of the target. Through those experiments it seems that the optimum gap is 8 mm, keeping in mind that this position is gas flow rate dependant.

Natsuta *et al.* observed [8] that when plasma is impinging on a finger tip as shown in Figure 5.7, species emission is greatly modified with the gap between the tube outlet and the finger. They distinguished between two groups of species, those which increased in emission intensity when a target is positioned close to the tube outlet and those which decreased. Their observations are coherent with the ones of this work, OH^* and N_2^* C emission intensity are more intense for a 5 mm gap as compared to a 15 mm one or even to the free jet mode, while He^* , O^* and N_2^{+*} emission intensities decreased when a target was set on. It is worth noting that they observed NO^* only when the conductive surface is in front of the

jet with higher emission for shorter gap and they did not observe $N_2^+ B$ system unlike in our experiments.

To estimate the evolution of the emission of reactive species versus gap, Figure 5.15 presents the normalized emission intensities at the tube outlet and at the surface of the target when varying the gap from 2 to 24 mm. Normalization has been performed with the emission intensity for a 2 mm gap distance. For visibility reason OH^* emission is magnified by 10.

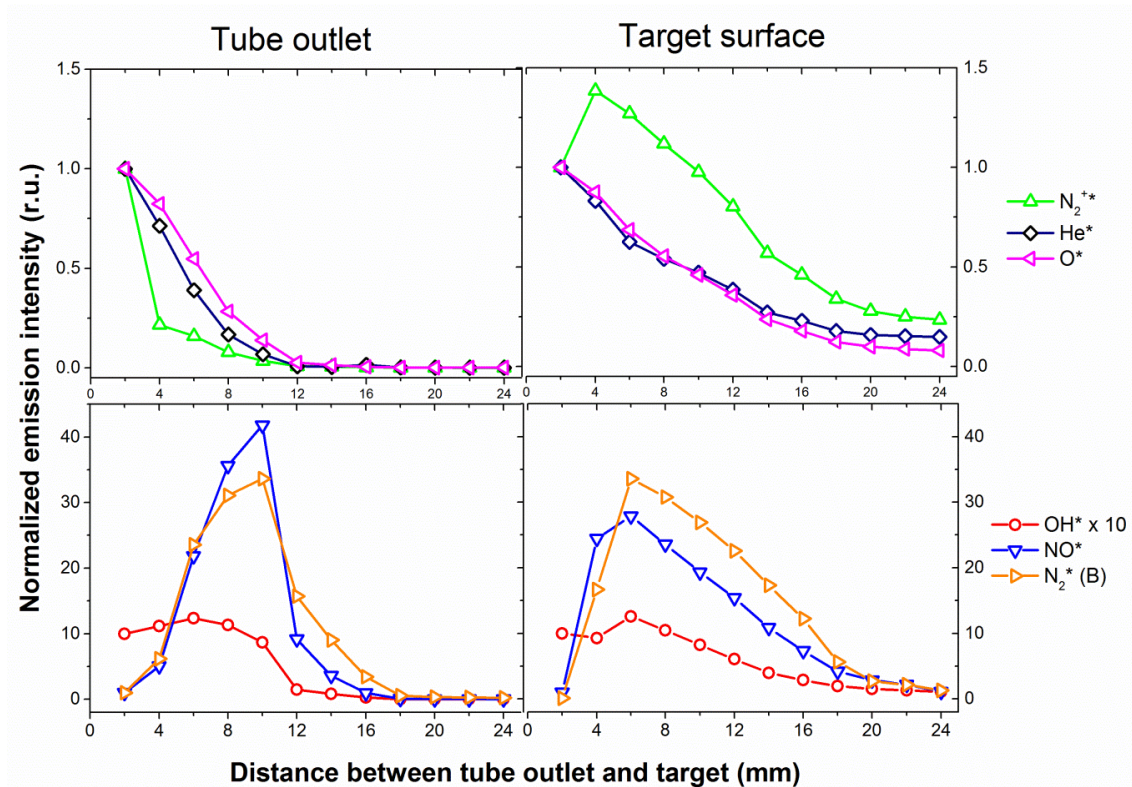


Figure 5.15: Emission intensity of several species versus gap between tube outlet and target surface, at the tube outlet and the target surface. Each species normalized to its emission at 2 mm gap. OH is magnified by 10.

By observing these graphs we can see that there exists three behaviors according to gap distance ranges. One for long distances between tube outlet and target surface, emission intensities decrease for all the excited species. Another one for short distances where the gap is mainly filled with Helium flow; in the latest, the core of the jet is dominated by emission from N_2^+ , He^* , O^* and $N_2^+(C)$, as can be deduced from Figure 5.a). It is worth mentioning that the secondary propagation has not been evidenced yet for this gap distance range.

Between these gap distances, it appears that an optimal gap exists for the production of NO^* , $N_2^+(B-A)$ and OH^* species at the tube outlet as well as at the target surface. In those conditions (2 kHz, 14 kV, 1 l.min⁻¹) the maxima of emission at the tube outlet is obtained at

10 mm while at the surface it is at 6 mm. This observation is coherent with the propagation of the counter-propagating PAPS and the suggested excitation mechanisms of those species. Indeed NO^* , $\text{N}_2(B)$ and OH^* are mainly coming from energy transfers. Some air diffusion inside the Helium channel is then needed to populate those states. When the gap is small from 2 to 4 mm, a gradual Helium-air mixing allows the increase of the second group emissions. From 6 until 10 mm, the counter-propagating PAPS length increases and maximum emission intensity is positioned closed to the tube outlet. It is highly probable that from 10 mm the maximum length of the counter-propagating PAPS is reached, in these conditions, and that its emission front is moved away from tube outlet with increasing gap.

When exceeding a 12 mm gap, plasma still impinges on the surface while facing some energy losses. RONS production decreases which may result from the increase of air penetration inside the jet due to a less efficient channeling of the Helium flow or from the decrease of the transient electric field, between the PAPS head and the target. When exceeding 20 mm emissions intensity is relatively low for all the species. The target may be too far for efficient influence of the transient electric field imposed between the PAPS head and the target onto the plasma properties, leading to a condition close to the free-jet mode. In this condition, the decrease of the emission intensity at the tube outlet and increasing air penetration into the jet especially close to the target, are expected.

To resume, this experiment identifies gap dimension between the capillary outlet and the target as another key parameter for the propagation dynamic and the production of reactive species. Figure 5.15 allows specifying the observation made thanks to the Figure 5. and Figure 5.14. It appears that, with the input plasma parameters (2 kHz, 14 kV and 1000 sccm), the optimal gap to maximize excited species on the target surface is 6 mm.

3.4.2. Influence of voltage amplitude

We have seen that the choice of the gap distance between tube outlet and target surface is influent in the production of the reactive species. As shown in 5.1 [16] as well as in [13], voltage amplitude modifies the length of propagation inside the capillary, similarly it has been shown [12, 17] that input voltage amplitude increases the length and velocity of the plasma propagation in free jet mode. Here, we attempt to identify the effect of the variation of voltage amplitude on the production of the reactive species in the PG plume when impinges on a conductive surface. The voltage amplitude is varied over the range (7 to 17 kV) for three fixed gaps of 8, 12 and 20 mm.

The following general trend of the emission intensities is expected: It is reasonable to assume that higher voltages correspond to higher power deposition into the plasma, which, in turn, leads to higher populations of the emitting levels and higher emission intensities.

In Figure 5.16, we see that the voltage amplitude increases the production of reactive species with some small variations in the behavior depending on the gap and species. For a 8 mm gap, He^* , O^* and OH^* emissions exhibit a bell-shape feature, with He^* and O^* emissions increasing up to 14 kV and presenting a plateau to 16 kV while OH^* reaches its plateau from 10 to 16 kV. For other species, the reactive nitrogen species ($\text{N}_2^*(\text{C})$, NO^* , N_2^{+*} and $\text{N}_2^*(\text{B})$), an increase over the whole tested voltage amplitude range is observed with even a increased slope from 14 kV for $\text{N}_2^*(\text{B})$. For the 12 mm gap, all emissions are increasing except He^* which decrease slightly with voltage amplitude, suggesting enhanced energy transfers on O^* and N_2^{+*} . For the 20 mm gap, an increase is also observed for all species however the slope of NO^* , $\text{N}_2^*(\text{B})$ and OH^* is smaller than the ones in the 12 mm gap and $\text{N}_2^*(\text{C})$ appears as the most intensively populated species at high voltage amplitude.

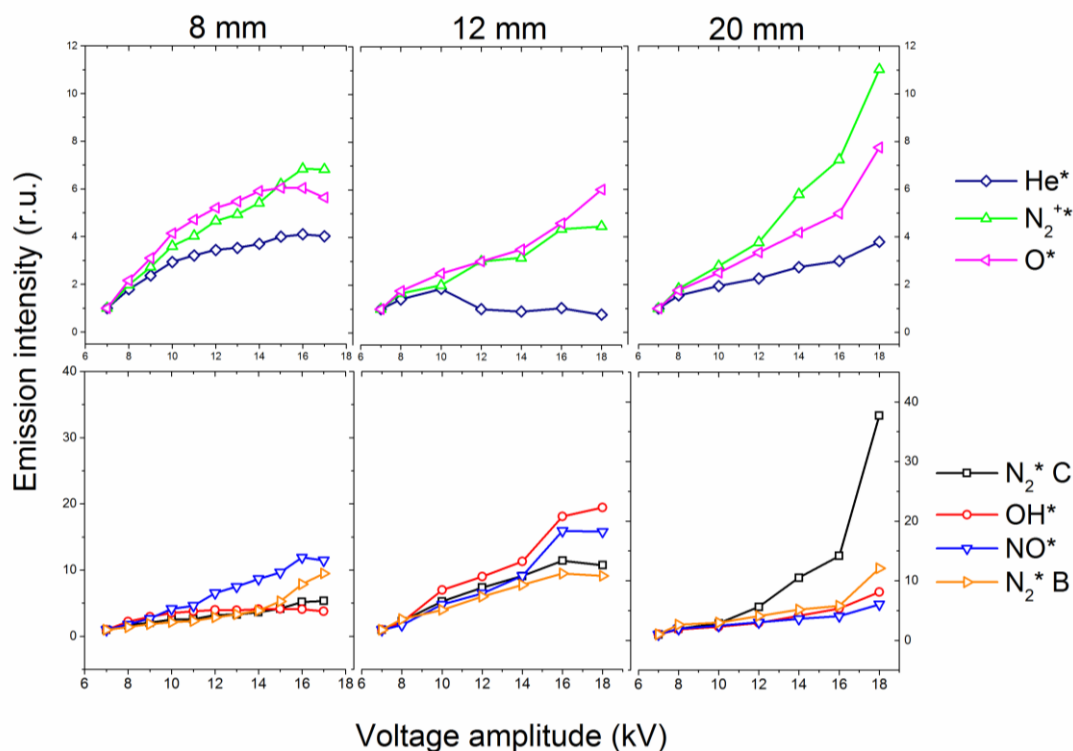


Figure 5.16: Evolution of normalized emission intensity of RONS versus voltage amplitude at the surface of the conductive target. Voltage amplitude from 7 to 17 kV, 2 kHz and He 1 l.min⁻¹.

The voltage amplitude also influences the plasma propagation as the position where the counter-propagation stops depends on the voltage amplitude. It has been described (see Figure 4.3), when PG is powered with the ns power supply, that inside the capillary the propagation length is increased with increasing pulse duration [16] because plasma is sustained until a sufficient amount of voltage is delivered to the ionization front through the plasma tail. When PG is powered by the μs power supply, the change of voltage amplitude is not changing the duration of the pulse but is changing the time where voltage is above the

limit of a few kV (~ 4 kV) where plasma vanishes [16]. Therefore when increasing the voltage amplitude, the plasma is propagating further downstream. In the case of PAPS impinging a conductive target, the counter-propagation stops at a maximum position while emission is still maintained until the delivered voltage goes below the threshold value. It was observed however that the higher the voltage amplitude the closer the plasma counter-propagates toward the tube outlet - and even upstream inside the capillary - probably due to an increase of the ionization of the PAPS tail facilitating the propagation upstream.

It would be thus interesting to follow the emission of the excited species integrated in the whole plume to draw conclusion on the influence of the voltage amplitude.

3.5. Influence of pulse repetition rate, case of nitric oxide

Another parameter that can influence the production of reactive species is the pulse repetition rate. In the above results, a 2 kHz pulse repetition rate has been used. Here we cover the range from 100 Hz to 3 kHz and focused on the spatially integrated (over the entire gap) evolution of four species: the main biologically active reactive species, NO^* and OH^* , and N_2^* (C) and N_2^{+*} as representative species of the two groups defined above. The gap between tube outlet and metal target surface is fixed at 12 mm and Helium is flushed at 500 sccm. Figure 5.17 shows the species emission intensity of one unique voltage pulse and normalized to the emission intensity measured at 100 Hz.

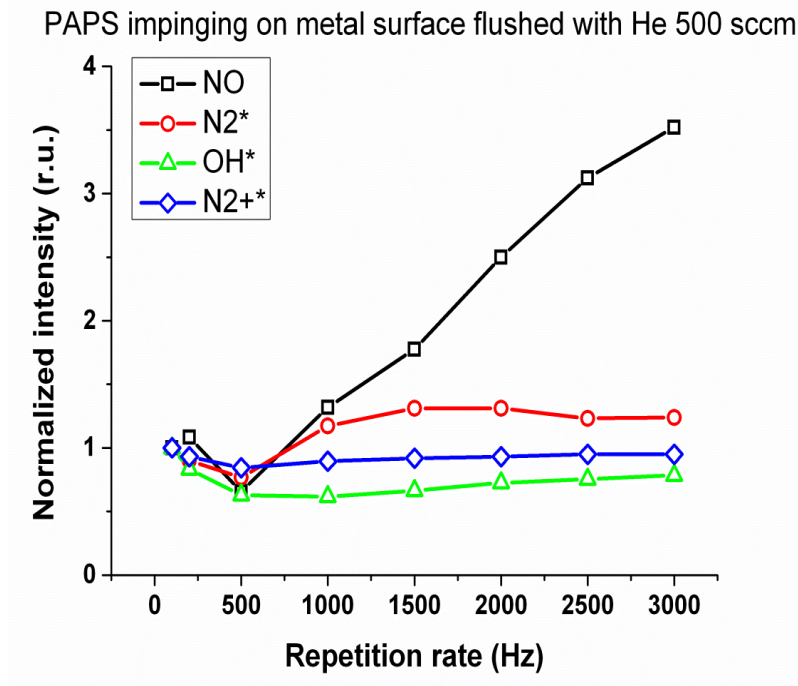


Figure 5.17: Evolution of normalized emission intensity per voltage pulse versus pulse repetition rate from 10 to 3000 Hz. Gap of 12 mm, 14 kV and 500 sccm He flow.

It can be seen that all reactive species except NO^* are quasi-stable over the repetition rate while NO^* increase drastically with pulse repetition rate. The first hypothesis was that some accumulation of the NO ground state was happening from one pulse to another, and that NO^* was repopulated more easily from ground state. Another idea was that some specific chemical reactions could happen on the metal target surface. To test these assumptions same experiment was performed with three different gas flow rates and using two different targets, the metal grounded plate and a potato positioned on this metal target.

Figure 5.18 shows the evolution of the NO^* emission for He gas flow rates equal to 200, 500 and 1000 sccm.

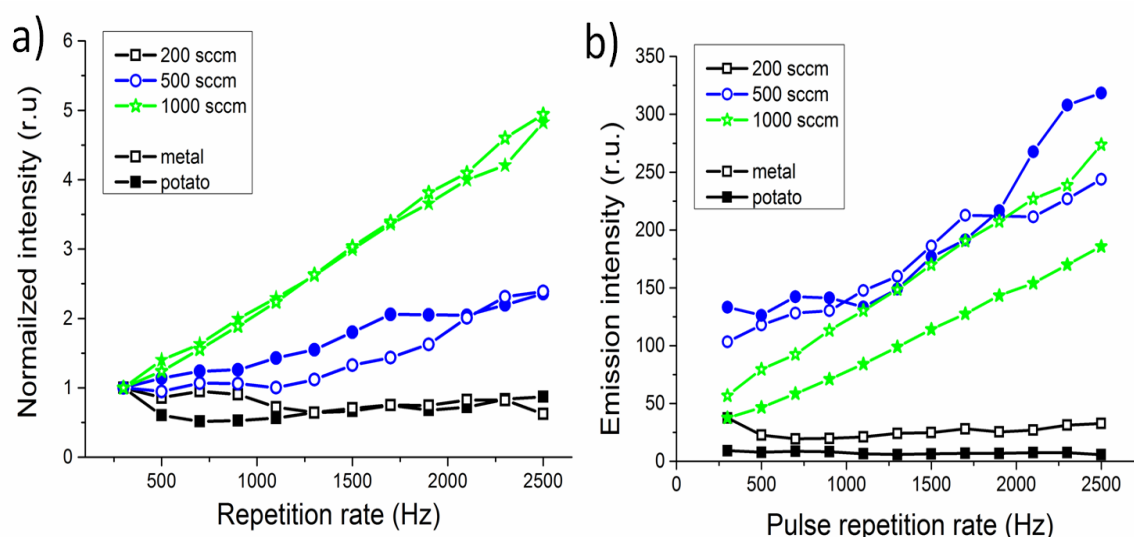


Figure 5.18: Evolution of emission intensity of NO^* per voltage pulse versus pulse repetition rate from 100 to 3000 Hz. a) Normalized and b) raw data. Gap of 12 mm, 14 kV and 500 sccm He flow.

It can be seen that NO^* emission versus pulse repetition rate trend is changing with gas flow rate. At 200 sccm, its emission is stable over the whole tested pulse repetition rate range while for higher gas flow rates, 500 and 1000 sccm an increase in the emission can be observed. The three slopes (Figure 5.18a)) of the NO^* emission intensity versus pulse repetition rate, increase with the gas flow rates. This result discredits the hypothesis of the NO ground state accumulation, as NO^* emission would have been decreasing with increasing gas flow rates. More than the increase observed versus pulse repetition rate, the Figure 5.18b) inform us that emission intensity is equivalent for 500 and 1000 sccm gas flow rates suggesting that there exists a lower limit where NO^* is not dependant on the pulse repetition rate.

Another point of this experiment is the similarities between the two targets, NO^* behaves the same way on a potato and on a metal target which suggest that the humidity content

and roughness of the target has rather low influence on the production of NO^* . For the excited species characterization, similarities observed in Figure 5.18 between the potato and the metal targets give another argument on the use of the metal target for PG plume characterization.

The two hypotheses mentioned above are invalidated by this experiment (Figure 5.18). The non-straight-forward NO^* emission behavior with the gas flow and pulse repetition rates leads to perform wavelength-filtered imaging.

3.6. Wavelength-filtered imaging

Figure 5.19 shows wavelength-filtered imaging of the Plasma Gun plume when impinging on a grounded conductive target positioned at 20 mm from the tube outlet. Similarly to Figures 4.12 and 4.13, images present emissions of the two species N_2^* and NO^* plus the emission with $\lambda > 515$ nm filter dominated by He^* emission, for gas flow rates from 200 sccm to 1000 sccm at 2 kHz and 14 kV. Exposure time is 10 μs and He^* and N_2^* images are averaged on 10 pulses and NO^* on 50 pulses due to its lower emission intensity.

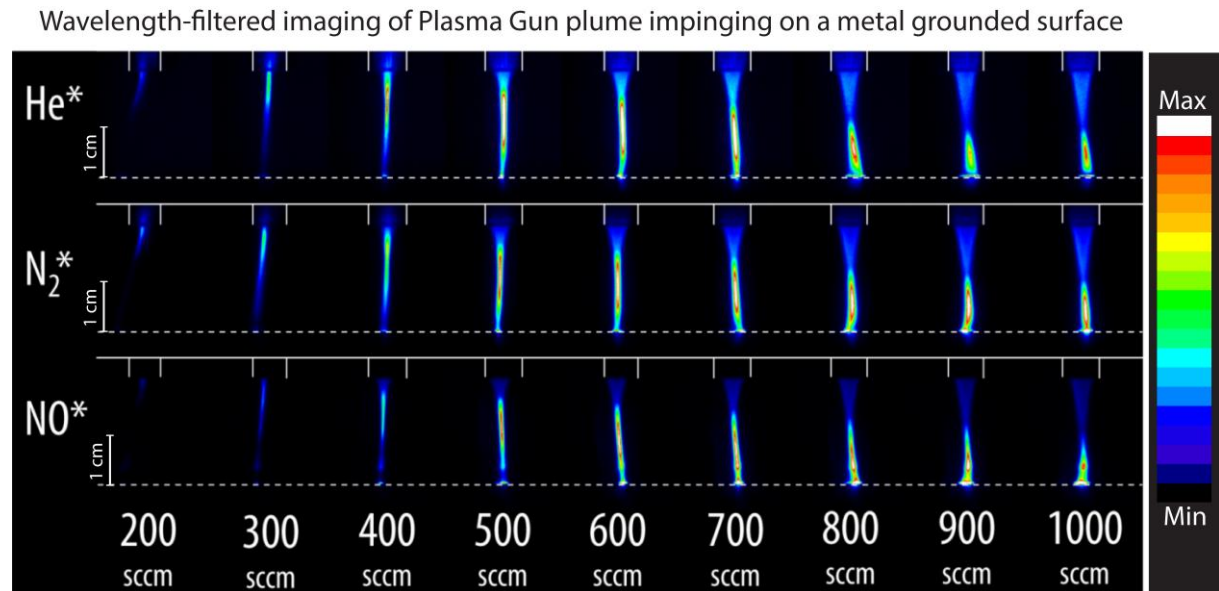


Figure 5.19: Wavelength-filtered imaging versus gas flow rate from 200 to 1000 sccm with a gap of 20 mm, PG operating at 2 kHz and 14kV. Color scale in false colors.

First observation can be made when comparing Figure 4.12 to the above images. When a target is placed at 15 mm of the PG outlet, the three scanned species exhibit similar emission distributions unlike the free-jet mode emission distributions.

At 200 sccm a needle-like emission is observed for the three excited species with lower intensity for NO*. This emission is going from the tube outlet to the target surface. Its emission intensity is non-homogeneous, with a zone of relatively high intensity from the tube outlet to ~ 7 mm and a second zone from ~ 7 mm to the target where emission barely observable. Moreover we can notice that the emission channel is bent and that its diameter is around 1 mm inside and outside the capillary. Above 300 sccm, the emission intensity is increased allowing observation of a more vertical channel through the entire gap. From 300 to 500 sccm, the diameter of the emission channel is increasing from the needle-like feature (~ 1 mm in diameter) to a cylindrical channel of about 2 mm in diameter. In the mean time the emission inside the capillary is also enlarged to fill at 500 sccm the whole capillary (4 mm in diameter).

Simultaneously the emission intensity maximum is shifted from the tube outlet toward the target. This shift is continuing when increasing the gas flow rate, reaching the target surface at 800 sccm.

At 500 sccm, a conical-like emission shape starts to be observed in the first mm of the gap followed by a cylindrical high emission intensity contribution. These two contributions become even clearer when increasing the gas flow rate, with the increase in length of the conical feature. The first contribution, the conical-like emission, can be linked to the gradual mix of air to the Helium flow while the second, the cylindrical emission, is linked to the counter-propagating PAPS, its high emission intensity resulting from the long time exposure used in Figure 5.19. It is interesting to notice that when increasing the gas flow rate, the counter-propagation is more and more confined in the vicinity of the target surface, region following the increased length of the Helium channel as already described in Figure 4.12. Thus the position where the counter-propagating PAPS stops is highly influenced by the gas flow rate such as the higher the gas flow rate the shorter the counter-propagation is.

Same experiments presented in Figure 5.20 have been run for the influence of pulse repetition rate from 10 to 3000 Hz with a gas flow rate of 500 sccm and a gap of 20 mm.

As can be seen in the figure, the increase of the pulse repetition rate brings the plasma plume emission on the central axis which is associated to a stabilization effect. This is due to the hydrodynamics of the jet which has been described in [14]. Schlieren imaging presented above (Figure 5., Figure 5.10) are time-integrated in tens of ms, thus events happening in the ms time scale are not observable. It was shown in [14] by using time-resolved Schlieren imaging that the channeling of the gas flow requires several pulses, after first plasma ignition, to be completed; this event is in the tens of ms time scale. However even when the channel is built, some variations may happen during the lapse of time between one voltage pulse to the next one, depending on the pulse repetition rate. A transition in the emission channeling can be observed between 500 and 1000 Hz. As a matter of fact below 500 Hz the emission is not channeled through the whole gap while above the channeling is happening.

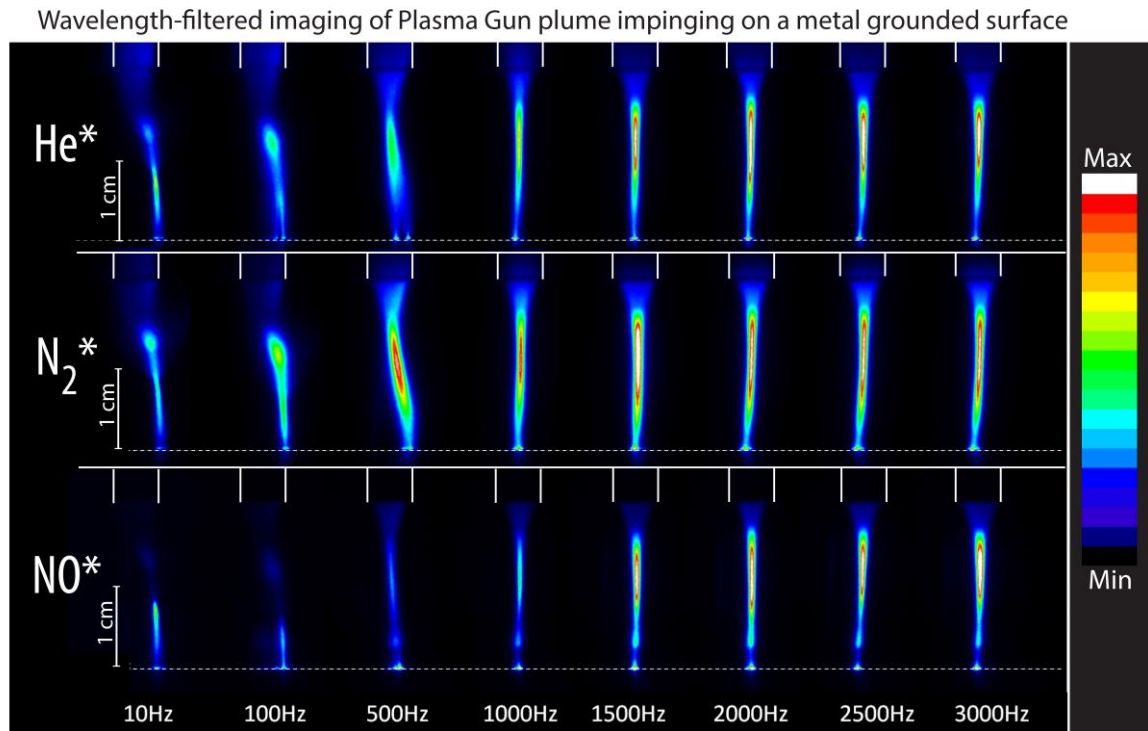


Figure 5.20: Wavelength-filtered imaging versus pulse repetition rate from 10 Hz to 3000 Hz with a gap of 20 mm, PG operating at 500 sccm and 14kV. Color scale in false colors.

Focusing on the pulse repetition rate range from 10 to 500 Hz, the general emission distribution of the three reactive species is constituted of 3 zones. First a blue conical emission corresponding to the low emission intensity can be observed from 0 to around 6 mm, second a spherical zone with diffuse emission from ~6 to ~9 mm and finally a narrow channel from ~9 to the metal target at 15 mm. When comparing this distribution to the case of a free jet (see Figure 4.12), the spherical emission zone can be associated to the bending of the Helium flow due to buoyancy effects, where the gas jet would flow upward if no plasma was ignited (in the presented conditions at around 7 mm from tube outlet) see Figure 5.a). This observation leads to suggest that the channeling is repetition rate dependant such as the process is directly linked to the time between pulses. This mechanism is under study and further experiments would be needed to draw conclusions. However recent experiments using Schlieren imaging confirmed that under a certain repetition rate the Helium flow cannot be channeled. This phenomenon is under study by T. Darny (PhD).

The third zone can be described as one or multiple supplementary narrow channels from the "bending zone" to the metal surface. As suggested in Figure 5.b) and c), where multiple gas channels are observed, those might be induced by a reduced energy in the PAPS not allowing for complete gas flow channeling. However plasma creates narrower paths, forcing a small portion of the Helium flow through the ambient air to reach the target. It can be seen

that this contribution is increasing in diameter with the pulse repetition rate; the multiple channels become one simultaneously with the Helium channel stabilization.

Looking now at the intensity of the emission patterns, the three species exhibits different features; He^* and N_2^* appears different from NO^* . While the first ones exhibit their maximum emission intensity from the second to the third zone, NO^* emission is concentrated in the third zone. This third zone is associated with electrons of lower energy as mentioned above as with the spatial distribution shown in 3.4. However to produced NO^* prior high energetic electrons are needed to dissociate O_2 and N_2 molecules. The extended fan-like shape in the free jet mode (Figure 4.12 and Figure 4.13) as well as the intense emission of NO^* in the third zone (Figure 5.20) suggests that NO^* needs relatively high air/Helium ratio and lower energetic electrons.

The gap between the tube outlet and the conductive target is also influencing the emission distribution has already discussed earlier. Figure 5.21 shows the NO^* emission versus pulse repetition rate for a 20 mm gap for a gas flow rate of 1 l.min^{-1} .

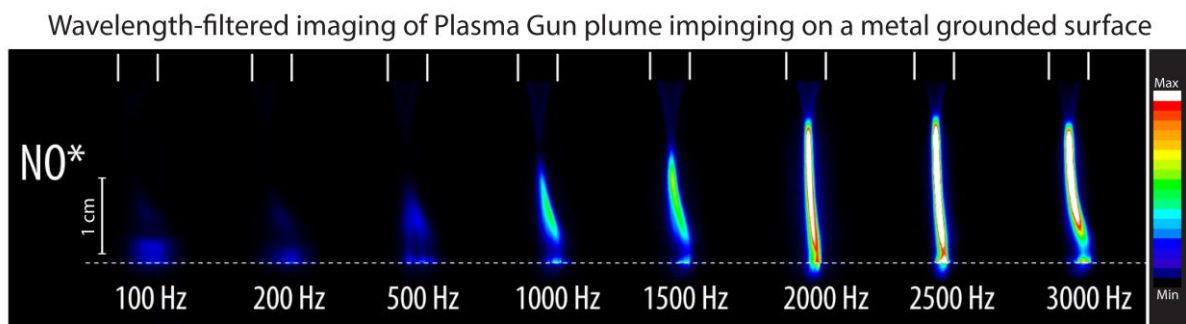


Figure 5.21: NO^* distribution by wavelength-filtered imaging versus pulse repetition rate from 100 Hz to 3000 Hz with a gap of 20 mm, PG operating at 1000 sccm and 14kV. Color scale in false colors.

This figure shows clearly that NO^* production is increased along the gap with pulse repetition rate. In this case, with a higher gas flow rate, the second contribution of the emission is closer to the surface and multiple emission channels are observable on the last 5 mm. When increasing the pulse repetition rate, the helium flow is channeled and PAPS upstream propagation is homogenizing the emission up to 8 mm from the target surface.

It is worth noting that emission visible below the target (Figure 5.19, Figure 5.20 and Figure 5.21) are coming from reflections of the plasma on the metal target and do not stand for any physical phenomenon.

To sum up, the presence of the conductive target in front of the PG modified drastically the emission distribution of the reactive species. It is shown that gas flow rate as well as pulse repetition rate influences their distributions and by the same way the hydrodynamics of the jet and the counter-propagation of the PAPS. These images allow us to draw a conclusion about the NO^* behavior presented in Figure 5.17 . As a matter of fact, when the gas flow

rate is 200 sccm, the Helium channel is really narrow and is barely reaching the target surface (see Figure 5.19). NO* production in this case is not really influenced by the pulse repetition rate. However when increasing the gas flow rate (Figure 5.20), the Helium flow is already approaching the target surface and is stabilized and structured with increasing pulse repetition rate allowing greater production of NO*. Interestingly He* and N₂* (C) temporally and spatially integrated emissions were observed to be quasi stable. Indeed pulse repetition rate induces an increase of their emission intensity (see Figure 5.20) however when normalizing it, this increase appears to be negligible compared to the increase of NO* emission.

3.7. Temporal evolution of excited species

Temporal evolutions integrated over the whole gap are shown in Figure 5.22 for O* (777 nm), He* (706.5 nm), two nitrogen bands of N₂* (B-A) and (C-B) at 675 nm and 337.1 nm respectively, N₂⁺* (391.4 nm), OH* (306,8 nm) and NO* (247,7 nm). It is important to note that the N₂* (B-A) system emission (710-780 nm) may overlap with the O* emission and influence its temporal evolution. The PG is powered by the μ s power supply at 14 kV, 2 kHz and flushed with Helium 1 l.min⁻¹.

During the first hundreds of ns, observations made for the free jet mode (see Figure 4.14) can be also made when the plasma plume impinges on a surface at 12 mm from the capillary outlet (Figure 5.22). As a matter of fact an early emission characterized by an intense population and a fast decay can be observed for almost all the excited reactive species. Nevertheless a second contribution, called "late emission", lasting for more than 2000 ns and with maxima shifted by around 1000 ns can be observed for He*, N₂* (C-B) and N₂* (B-A). The weak repopulation of He* induces no late emission of N₂⁺*, which is probably due to the very small amount of He metastable states. N₂* (C-B) and N₂* (B-A) exhibit a strong late emission peaked around 1200 ns, stronger for the latest. Only a small repopulation of O* can be observed, together with a small break in the OH* emission decay (~1500 ns). NO* is the main species to be excited during the late emission with relative emission intensity higher than the early excitation.

As expected with the use of the μ s power supply, the excited species emissions vanish 4500 ns after the ionization front emission thus when the voltage pulse amplitude reach the critical value of few kV. However NO* emission vanishes after 16000 ns, far after the voltage extinction. This longer emission suggests that for the late emission, NO* is partially produced following the PAPS ionization front propagation. Successive energy transfer processes may explains the slow decay of the NO* emission after the power is switched off, part of them might be linked to the N₂ (A) metastable state as suggested in [19].

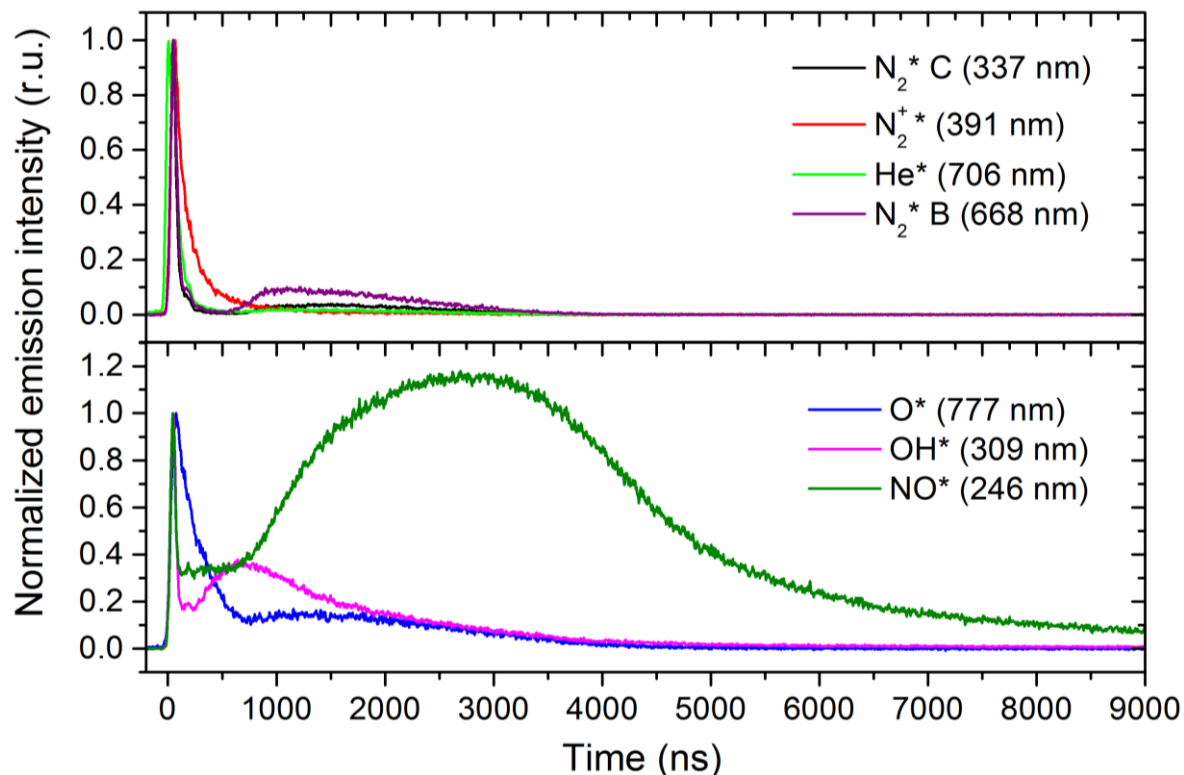


Figure 5.22: Temporal evolution of the normalized intensity of different emissions of ROS and RNS integrated over the whole gap. PG parameters: 14 kV, 2 kHz, 1 l.min⁻¹ Helium gas flow rate and gap 12 mm.

As shown by the late emission contribution in Figure 5.22, the counter-propagating plasma is mainly efficient for the excitation of NO*, N₂* (B-A) and N₂* (C-B) although O* and OH* are also weakly repopulated during the counter-propagation. The small O* and OH* late emissions induced by the presence of the conductive surface, confirms that part of the population of these excited species are coming from energy transfers as mentioned for Figure 4.1. It is worth noting that these emissions are lasting during thousand of ns which is much longer than the 12 mm propagation of PAPS (velocity of the downstream PAPS in the order of few 10⁷ cm.s⁻¹) while still shorter than the voltage pulse duration (10 μs).

The global emission measured with a PMT over the whole gap (Figure 5.) appears to be mainly due to the emission of N₂* positive systems as suggested by the time where maximum emission is reached (~1500 ns) and by its high contribution on optical emission spectra (see Figure 5.11). However the shape of the late emission and the intensity ratio between the early and the late ones, clearly show that the other excited species are contributing in the whole emission.

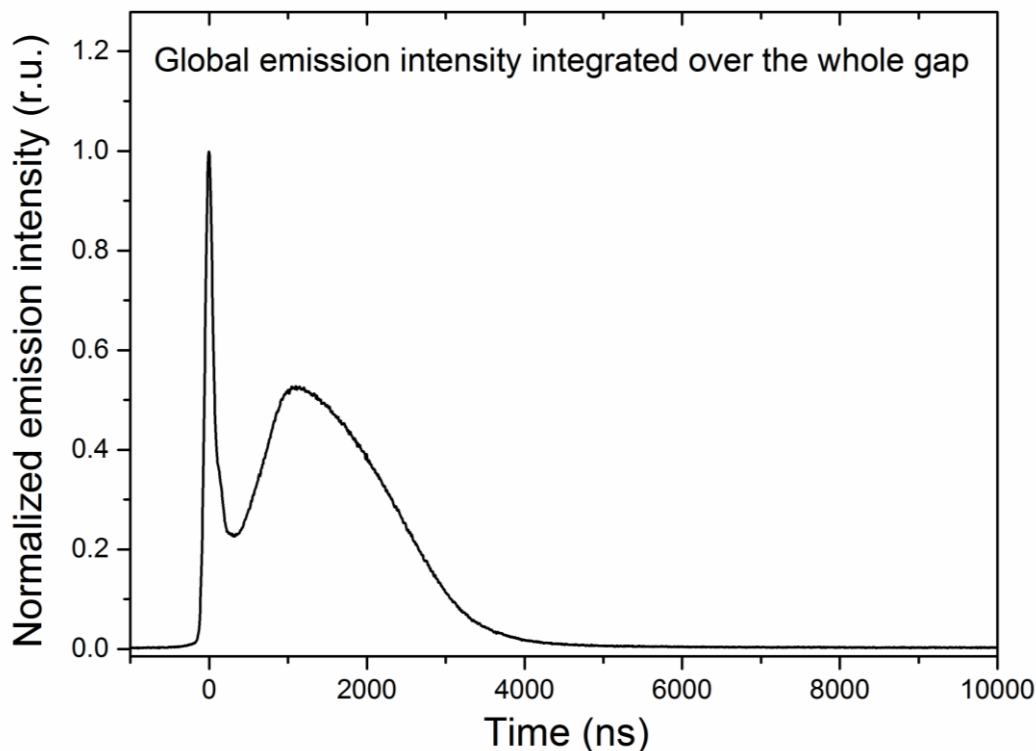


Figure 5.23: Normalized temporal evolution of spatially integrated plasma plume emission. PG parameters: 14 kV, 2 kHz, 1 l.min⁻¹ Helium gas flow rate and gap 12 mm.

The presented temporal evolutions are spatially integrated over the whole gap and represent the averaging of the events during the whole propagation, the downstream and upstream one. It has been already describe that two groups of excited species are evolving differently in the gap. As a matter of fact He^* , O^* N_2^{+*} are mainly emitting during downstream propagation or at the capillary outlet (as seen in Figure 5.14, Figure 5.15 and Figure 5.16) while $\text{N}_2^*(\text{C})$, NO^* , OH^* and $\text{N}_2^*(\text{B})$ are mainly emitting from the target surface to the end of the upstream propagation. Considering emission is spatially non homogeneous, some processes happening specifically at the target surface are hidden by the spatial averaging due to the overlapping of the processes.

Temporal evolutions of the reactive species at the target surface (see Figure 5.24) are measured with the help of band-pass filters and a high-pass filter to differentiate between the whole averaged emissions and those specific at the target surface. The setup used to measure the surface is the same as presented in Chapter 2 (Figure 2.9) although the filters have been positioned between the plasma plume and the UV optical fiber.

N_2^*C has a large late emission coherent with the spatially integrated one while OH^* which in Figure 5.22 presented only a slight change of slope is here clearly repopulated. The OH^* late emissions is delayed of around 300 ns from the N_2^*C one until vanishing simultaneously around 4000 ns. This delay is coherent with the previously suggested phenomenon where OH^* would be linked to the transfers from O^* states.

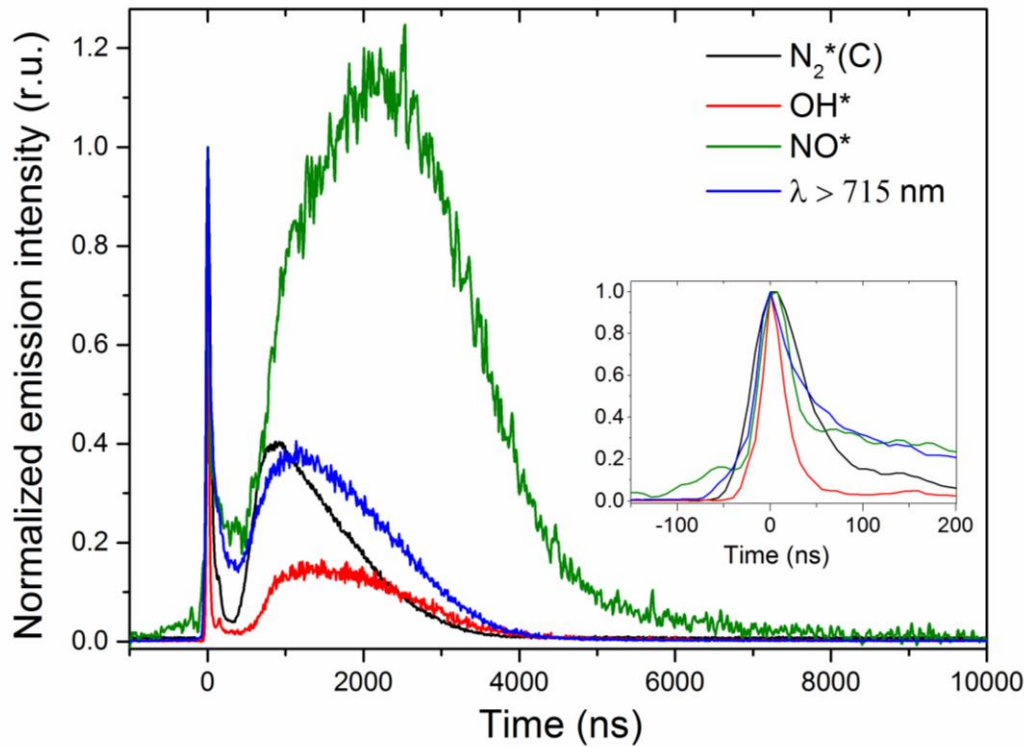


Figure 5.24: Temporal evolution of the normalized intensity of different emissions of ROS and RNS at the surface of the metal target. PG parameters: 14 kV, 2 kHz, 1 l.min⁻¹ Helium gas flow rate and gap 12 mm.

The measurement performed with the high-pass filter, $\lambda > 715$ nm integrates the N_2^* B system and O^* lines emissions. However OES informed us (Figure 5.) that O^* lines emissions are really weak at the surface of the target thus the use of the $\lambda > 715$ nm filter mainly measure the $N_2^*(B)$ emission. It can be seen that $N_2^*(B)$ is relatively efficiently repopulated at the metal surface, observation which is coherent with the spectra shown in Figure 5.a) as well as spatial distributions from Figure 5. and Figure 5.14.

NO^* is peaked around 2200 ns, ~ 800 ns earlier than for the spatially integrated measurement and vanishes around 8000 ns which is half the time measured in Figure 5.22. This difference in the late emission duration suggests that NO^* production is following the counter-propagating PAPS ionization front. For such long excited species emission, the relatively low velocity of plasma counter-propagation implies an important broadening of the temporal evolution of late emission.

This experiment confirms that the chemical kinetics is spatially evolving along the plasma plume axis. Indeed some variations happened as well in the radial direction. Multiple gradients, such as air penetration modifying the quenching rate and electron energy which is reduced along the propagation should be kept in mind when characterizing the plasma plume. However when dealing with biological trials, it is expected that the living target is responding to an average of the processes undergoing at or slightly above the target surface.

3.8. Measurements of long living species by FTIR

In addition to the FTIR experiments presented in 4.4., other experiments were performed in INP Greiswald by François Riberi, supervised by Eric Robert and Stephan Reuter. This experimental setup is described in Chapter 2 and briefly, has the characteristic to be constituted of two cells, one at atmospheric pressure for the collection of PG effluent and a second at 100 mbar for FTIR measurement. Difference of pressure in the measurement cell can indeed modified the plasma kinetics in the plasma effluent as was presented in the case of a DBD by Pipa *et al.* [20]. It is worth reminding that comparison between FTIR in Figure 4.16 and in Figure 5.25 is impossible. These spectra are shown to estimate the influence of the presence of a conductive target in front of the PG in regards of the long living species. The PG was powered by the μ s power supply and operating at 2 kHz and 1 l.min⁻¹ of Helium flow with a gap of 15 mm between the tube outlet and the metal grounded surface. The identification of the peaks has been performed in comparison with a mid-infrared absorption spectrum established by A.V Pipa *et al.* in 2009 [21].

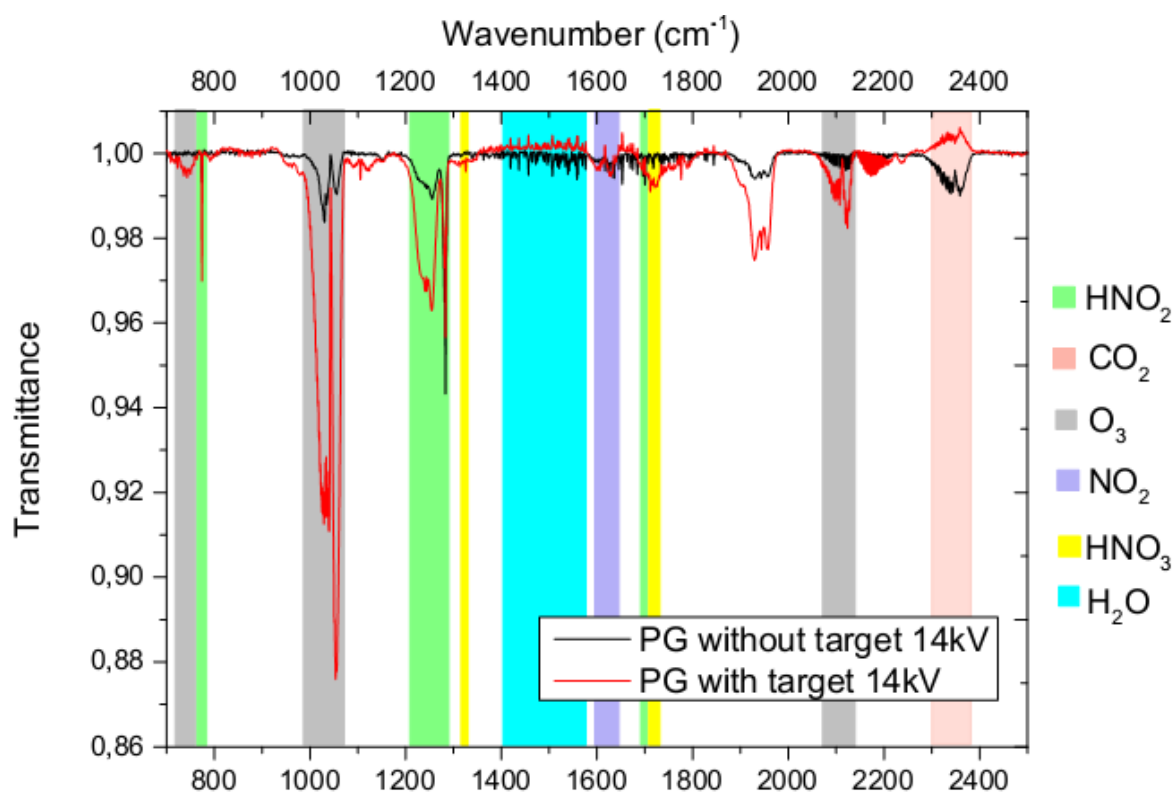


Figure 5.25: Infrared Absorption spectrum performed by FTIR of the Plasma Gun with and without a metallic target with gap distance 15 mm and 14 kV

The previously identified chemicals, HNO_2 , HNO_3 and NO_2 , produced when using the spark ns power supply at atmospheric pressure (see Figure 4.16) are also observed here with the μs PG one at low pressure. However in this case, O_3 is significantly present and N_2O and NO are not clearly identified in this case. The reason of those differences can stand in the difference of pressure which induces modification of recombination mechanisms. More interestingly than the presence or not of the observed species, Figure 5.25 shows that the presence of the target in front of the Plasma Gun increases all produced reactive species with a maximum difference for O_3 .

The increased production of the excited reactive species due to the counter-propagating PAPS leads to a higher production of subsequent molecules HNO_2 , HNO_3 acids, and O_3 . We expect that at atmospheric pressure NO_2 , N_2O and NO will be as well increased by the presence of the target. This observation confirms one more time that the presence of the targeted surface is mandatory to characterize the Plasma jets developed for biomedical applications.

The voltage amplitude influence has been also considered in the range of 12 to 16 kV and is presented in Figure 5.26.

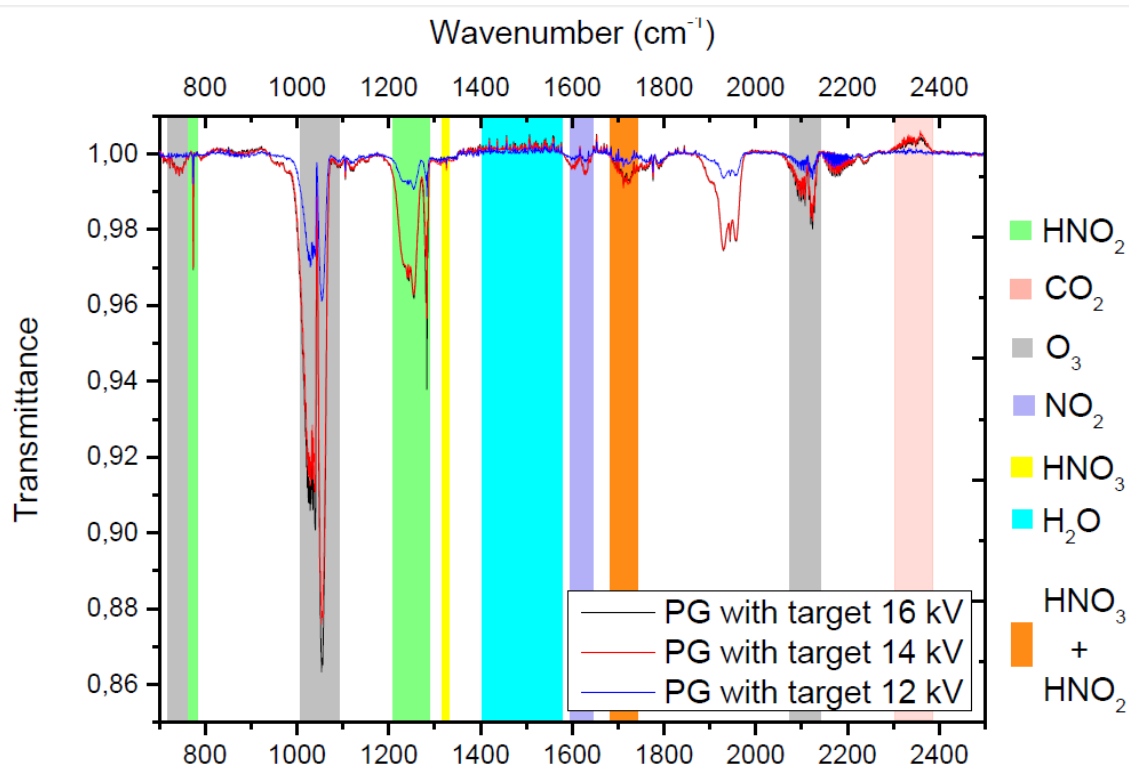


Figure 5.26: Infrared Absorption spectrum performed by FTIR of the Plasma Gun with gap distance 15 mm in front of the exit of the dielectric tube, at 12, 14 and 16 kV

Voltage amplitude increase from 12 to 14 kV results in a highly significant increase production of all the molecules while from 14 to 16 kV only small increase can be seen for O_3 . We can suggest that in those conditions of pressure and when PG impinges on a target, a plateau of species production is obtained when reaching ~ 14 kV. The proposed trend for ground state species may be due to a wide range of chemical reactions that would need

further experiments to be understood. FTIR experiments when PG impinges on a conductive target would need to be performed at atmospheric pressure to be compared to the results presented in this chapter and to draw conclusions on the chemical mechanisms undergoing.

4. Conclusion

In this Chapter, the analysis of diverse parameters influence on the plasma properties has been carried out when the PG impinges on a target surface. We have seen that the presence of a dielectric target is slightly changing the global visible emission as compared to the free jet condition. This modification is induced by the obstacle on the gas jet resulting in a spreading onto the surface. However on the axis of propagation the emission distribution of the excited reactive species is almost similar as the ones in the free jet mode.

On the contrary the presence of a conductive target has shown to have drastic effect on plasma properties. The propagation is implemented by a counter-propagating plasma which leads to important changes in RONS production and distribution. This counter-propagating PAPS has a lower velocity than the downstream one but conserves some similarities such as the connection through the PAPS tail to the powered electrode and extinction with the voltage amplitude decrease. The gas flow hydrodynamics is also modified by the presence of a grounded target that induces a drift of ionic species channeling the Helium flow toward the surface. In this Helium channel, energy losses are decreased compared to Helium-air mixing zone which could partially explain why the plasma can follow its propagation upstream. This plasma has a characteristic pink emission linked to the transition of N_2^* (B-A) system. It has been seen that NO^* γ bands emission is also drastically enhanced by the presence of the conductive target. Parametric studies focusing on how reactive species evolves with the gap distance, the voltage amplitude, the gas flow and pulse repetition rates had been performed by OES and wavelength-filtered imaging. It appears that the gas hydrodynamics and plasma counter-propagation behaviors are influenced by combination of all the parameters above leading to differences in the production of reactive species. Almost all the excited species are repopulated during the counter-PAPS propagation, except N_2^{+*} . The ratio between the early emission and the late emission in time-resolved emission measurements as well as the spatial distribution along the gap allowed sharing the reactive species into two groups. First group mainly excited by electron impact of high energy, He^* , O^* and N_2^{+*} and the second group by those of lower energy N_2^* (C), N_2^* (B), OH^* and NO^* . The influence of the conductive surface has also been shown by FTIR measurements where long living species production is increased as compared to the free jet mode.

All those results presented in this chapter lead us to emphasize on the need to consider the target and its nature as a part of the system to study. It is clear now that the characterization

of the plasma in the free jet mode is not relevant to estimate the plasma properties during biomedical applications. Another remark that can be made is that multi-diagnostics approach is the only way to retrieve coherent information about plasma jet plume characteristics.

However, due to high inter-connection of all the parameters, this chapter is mainly descriptive. Obtaining keys for the full understanding of the observed features will only be possible thanks to a numerical model. This model would need to include, together with the plasma dynamics, gas jet hydrodynamic mechanisms.

French summary of Chapter 5

Dans ce chapitre, l'influence de divers paramètres a été étudiée dans le cas spécifique de la plume plasma atteignant une cible. En effet l'étude des propriétés du plasma dans ces conditions est d'une grande nécessité pour les applications biologiques. Il a été suggéré au Chapitre 3 une interaction entre le plasma et la cible, variant suivant la nature de celle-ci telle que sa contenance en eau et sa conductivité électrique. Deux cas ont été étudiés, représentant deux situations extrêmes, l'utilisation d'une cible diélectrique et l'autre conductrice.

Pour les cibles diélectriques choisies, une boîte de Petri rempli d'eau distillée ou bien pour le morceau de Delrin, peu de variations dans les comportements du plasma ont été observés. Ces différences sont la résultante de l'ajout de la cible en face de la plume plasma qui perturbe la propagation du jet d'Hélium.

Contrairement aux cibles diélectriques, les cibles conductrices ont une influence majeure sur les propriétés physico-chimiques du plasma. Il est observé qu'un canal lumineux, depuis le front d'ionisation jusqu'à la plaque en métal, précède la propagation du PAPS. De plus après avoir atteint la cible, le plasma se contre-propage dans ce dit canal en direction opposée jusqu'à extinction avec la diminution de la tension. Cette contre-propagation amène des changements important dans la production des espèces réactives et dans leur distribution spatiale. L'émission rose de ce plasma se contre-propageant vient de l'émission simultanée des raies d'Hélium (He^*) avec l'apparition du système N_2^* (B-A). L'émission du système γ de NO^* apparait comme fortement augmenté par la présence de la cible conductrice. Le comportement du jet de gaz est aussi grandement modifié par la présence d'une cible conductrice qui semble renforcer la canalisation du jet d'Hélium. Ce phénomène paraît résulter d'une augmentation de la dérive des ions. Une étude paramétrique faisant varier la distance entre la sortie du tube et la cible, le débit de gaz, la fréquence de répétition de l'impulsion de tension ainsi que l'amplitude de tension a montré que ces paramètres influencent conjointement l'hydrodynamique du jet et la contre propagation du plasma. En conséquence, la production des espèces réactives excitées se trouve modifiée. Toutes les espèces excitées, excepté N_2^+ , sont repeuplées durant la contre-propagation. Deux groupes d'espèces ont été déterminé grâce à l'observation des ratios d'émissions précoce et tardive de chaque espèce ainsi que leur distribution spatiale de la sortie du tube à la cible. Le premier groupe est constitué de He^* , O^* et N_2^{+*} . Il est majoritairement présent en sortie de capillaire et résulte d'excitation dominé par les collisions électroniques à hautes énergies. Alors que le second, constitué de N_2^* (C), N_2^* (B), OH^* and NO^* , serait majoritairement excité par collisions électroniques à basses énergie ainsi que des transferts d'énergies avec

des métastables. L'influence de la cible conductrice a aussi été montrée par des analyses FTIR, impliquant une augmentation de la concentration des espèces à long durée de vie en comparaison avec le "jet libre".

Ces résultats montrent combien il est important d'étudier le jet de plasma dans des conditions aussi proche que possible de l'application. En comparant les résultats présentés dans ce chapitre avec ceux du chapitre 4, il apparaît clair que l'étude du "jet libre" n'est pas pertinente en vue d'estimer les propriétés du plasma lors de l'application, surtout lorsque celle-ci est de nature biomédicale. Il est important de noter aussi que l'approche multi-diagnostique permet d'acquérir une vision cohérente du système. Malgré ces observations, seule la confrontation avec un modèle numérique intégrant la dynamique du plasma ainsi que l'hydrodynamique du jet de gaz, permettra la compréhension des mécanismes physico-chimiques sous-jacents.

References

- [1] Vanessa Sarron " Étude et optimisation d'une décharge « Plasma Gun » à pression atmosphérique, pour des applications biomédicales" PhD thesis, Université d'Orléans, 2013.
- [2] Yonemori, S., Ono, R., 2014. Flux of OH and O radicals onto a surface by an atmospheric-pressure helium plasma jet measured by laser-induced fluorescence. *J. Phys. D: Appl. Phys.* **47**, 125401. doi:10.1088/0022-3727/47/12/125401
- [3] Oh, J.-S., Olabanji, O.T., Hale, C., Mariani, R., Kontis, K., Bradley, J.W., 2011. Imaging gas and plasma interactions in the surface-chemical modification of polymers using micro-plasma jets. *J. Phys. D: Appl. Phys.* **44**, 155206. doi:10.1088/0022-3727/44/15/155206
- [4] Wild R., Gerling T., Bussiahn R., Weltmann K-D. and Stollenwerk L." Phase-resolved measurement of electric charge deposited by an atmospheric pressure plasma jet on a dielectric surface" *J. Phys. D: Appl. Phys.* **47** (2014) 042001
- [5] Xiong, Z., Robert, E., Sarron, V., Pouvesle, J.-M., Kushner, M.J., 2013. Atmospheric-pressure plasma transfer across dielectric channels and tubes. *J. Phys. D: Appl. Phys.* **46**, 155203. doi:10.1088/0022-3727/46/15/155203
- [6] Xiong, Z., Robert, E., Sarron, V., Pouvesle, J.-M., Kushner, M.J., 2012. Dynamics of ionization wave splitting and merging of atmospheric-pressure plasmas in branched dielectric tubes and channels. *J. Phys. D: Appl. Phys.* **45**, 275201. doi:10.1088/0022-3727/45/27/275201
- [7] Douat, C., Bauville, G., Fleury, M., Laroussi, M., Puech, V., 2012. "Dynamics of colliding microplasma jets." *Plasma Sources Sci. Technol.* **21**, 034010. doi:10.1088/0963-0252/21/3/034010
- [8] Nastuta A. V., Pohoata V. and Topala I. " Atmospheric pressure plasma jet—Living tissue interface: Electrical, optical, and spectral characterization" *J. Appl. Phys.* **113** (2013) 183302
- [9] Schröder, D., Bahre, H., Knake, N., Winter, J., Arcos, T. de los, Gathen, V.S. der, 2012. Influence of target surfaces on the atomic oxygen distribution in the effluent of a micro-scaled atmospheric pressure plasma jet. *Plasma Sources Sci. Technol.* **21**, 024007. doi:10.1088/0963-0252/21/2/024007
- [10] Gerling, T., Nastuta, A.V., Bussiahn, R., Kindel, E., Weltmann, K.-D., 2012. Back and forth directed plasma bullets in a helium atmospheric pressure needle-to-plane discharge

- with oxygen admixtures. *Plasma Sources Sci. Technol.* 21, 034012. doi:10.1088/0963-0252/21/3/034012
- [11] B. Niermann, "The role of metastable atoms in radio-frequency micro-plasma jet discharges operated at atmospheric pressure," PhD thesis, Ruhr Universität Bochum, Germany (2012).
- [12] Naidis, G.V., Walsh, J.L., 2013. The effects of an external electric field on the dynamics of cold plasma jets—experimental and computational studies. *J. Phys. D: Appl. Phys.* 46, 095203. doi:10.1088/0022-3727/46/9/095203
- [13] M Mussard, M.D.V.S., Guaitella, O., Rousseau, A., 2013. Propagation of plasma bullets in helium within a dielectric capillary—influence of the interaction with surfaces. *J. Phys. D: Appl. Phys.* 46, 302001. doi:10.1088/0022-3727/46/30/302001
- [14] Robert, E., Sarron, V., Darny, T., Riès, D., Dozias, S., Fontane, J., Joly, L., Pouvesle, J.-M., 2014. Rare gas flow structuration in plasma jet experiments. *Plasma Sources Sci. Technol.* 23, 012003. doi:10.1088/0963-0252/23/1/012003
- [15] Brandenburg, R., Ehlbeck, J., Stieber, M., v. Woedtke, T., Zeymer, J., Schlüter, O., Weltmann, K.-D., 2007. Antimicrobial Treatment of Heat Sensitive Materials by Means of Atmospheric Pressure Rf-Driven Plasma Jet. *Contrib. Plasma Phys.* 47, 72–79. doi:10.1002/ctpp.200710011
- [16] Robert, E., Sarron, V., Riès, D., Dozias, S., Vandamme, M., Pouvesle, J.-M., 2012. Characterization of pulsed atmospheric-pressure plasma streams (PAPS) generated by a plasma gun. *Plasma Sources Sci. Technol.* 21, 034017. doi:10.1088/0963-0252/21/3/034017
- [17] Mericam-Bourdet, N., Laroussi, M., Begum, A., Karakas, E., 2009. Experimental investigations of plasma bullets. *J. Phys. D: Appl. Phys.* 42, 055207. doi:10.1088/0022-3727/42/5/055207
- [18] Xiong, Z., Kushner, M.J., 2012. Atmospheric pressure ionization waves propagating through a flexible high aspect ratio capillary channel and impinging upon a target. *Plasma Sources Sci. Technol.* 21, 034001. doi:10.1088/0963-0252/21/3/034001
- [19] Abraham Franciscus Hubertus van Gessel "Laser diagnostics on atmospheric pressure plasma jets" PhD thesis, Eindhoven University, 2013.
- [20] Pipa, A.V., Schmidt, M., Becker, K., 2007. Ultraviolet (UV) emissions from a unipolar submicrosecond pulsed dielectric barrier discharge (DBD) in He-Air mixtures. *J. Phys.: Conf. Ser.* 86, 012014. doi:10.1088/1742-6596/86/1/012014

- [21] Pipa A.V. and Ropcke J. 2009 Analysis of the mid-infrared spectrum of the exhaust gas from an atmospheric pressure plasma jet (APPJ) working with an argon–air mixture Plasma Science, IEEE Transactions on 37 1000-3

Chapter 6

In situ diagnostic of hydroxyl radical by laser induced fluorescence

1. Objective and state of the art

In chapter 4 and 5, the influence of the presence of a conductive target in front of the PG was enlightened. It has been shown that such plasmas are highly influenced by the modification of the external electric field due to the presence of targets [1-4] leading to various phenomena such as production of a counter-propagating PAPS after impingement on the target surface, to modification of the production of reactive species as well as to modification of the gas flow dynamics. Thanks to the new understanding of the target induced modifications on plasma properties, it appears clearly that the whole system, target plus Plasma Gun should be taken into account in all characterization.

While a lot of efforts have been carried out to characterize reactive species produced by plasma jets [5-8] and the plasma induced biological effects on treated samples [9-18], only few experimental studies report *in situ* spectroscopic diagnostics [19-21]. For biomedical applications, then, the challenge is to understand the influence of real experimental conditions on plasma properties and reactive species production.

Furthermore to settle future clinical treatments there is a need for quantification of reactive species produced during treatments. To start with quantification, focus is made on the OH radical as one of the main biologically active specie [22] produced by non-thermal atmospheric pressure plasma jets. There have been quite extensive studies on OH detection by LIF. Measurements have been reported in pulsed corona discharges [23-26], also in contact with liquids [27], in dielectric barrier discharges [28-30], in pin-to-pin single filament discharges [31-32], in ns discharges for plasma-assisted combustion [33-34], and in various kinds of plasma jets [35-39].

As previously described the in-air expending jets are spatially non-homogeneous systems due to the progressive penetration of humid air into the effluent He/Ne/Ar plasma jet. LIF measurements are greatly affected by the collision processes of the laser-excited electronic state ($\text{OH}^* (\text{A}^2\Sigma^+)$ in our case). These processes depend strongly on the gas mixture composition so that, in a plasma-jet, strong spatial gradients of humid air must be taken into account in the measurement interpretation. On the other hand, since collision quenching rate coefficients are well known, the measurement of the quenching rate from the LIF pulse decay can be used to infer the local amount of humid air. This can be done assuming a constant water content in the fraction of humid air that penetrates the effluent jet, as in [36]. When the jet impinges on a liquid surface, however, this assumption is no longer valid, i.e. evaporation, and a way to evaluate air and water concentrations independently is mandatory.

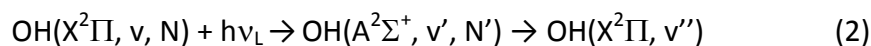
To this end experiments have been performed in IMIP Bari under Dr Giorgio Dilecce supervision in the frame of a Short Term Scientific Mission (STSM) of the COST action MP1101. The method developed by Dilecce *et al.* and proposed in [40] has been applied. Briefly it consists in the measurement of both electronic quenching and vibrational relaxation in the OH ($\text{A}^2\Sigma^+$) $v=0, 1$ manifold which will be detailed later. This work is the first of this kind and has been recently reported in [41].

This Chapter is composed of a brief description of the LIF theory main principles, then the experimental setup and procedure used in this work including the OH model will be explained before moving to the obtained results. Some insights in temporal evolution of OH radicals will be given to allow understanding of OH, Air and H_2O spatial distributions in various experimental conditions.

2. LIF method

Laser induced fluorescence (LIF) is a spectroscopic technique where a laser is used to specifically excite a molecular or atomic specie. When the particle relaxes to a lower energy it emits a photon, the fluorescence. LIF has the advantage to be specific of a particle, atom or molecule, transition thanks to the tuning of the laser, usually chosen to be different from the fluorescence wavelength, avoiding interference between laser excitation wavelength and detected fluorescence.

In this work, the ground state of the hydroxyl radical is measured by LIF. The excitation-detection scheme, shown in Figure 6.12 is the same as that used in [29-30], involving transitions of the 306,4 nm OH(A-X) system:



with $v=0, v'=v''=1, N=N'=1$.

The ground state of OH X state of vibrational number $v = 0$ is excited by the laser beam at 282 nm to reach the first electronically excited state, A state $v' = 1$, which relax by emitting its vibrational band head at 312.2 nm. Some of the latest excited states will undergo vibrational energy transfer to A state $v' = 0$, that emits the (0,0) vibrational band (band head at 306.4 nm).

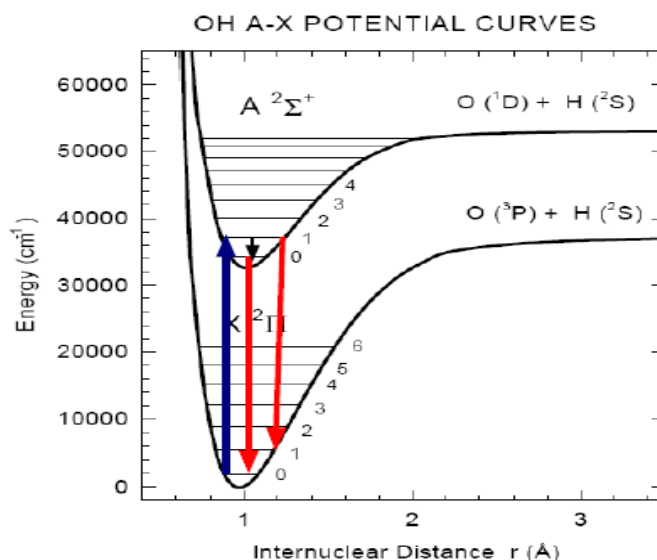


Figure 6.12 OH A-X Potential curves with arrows representing the laser transition excitation (blue), radiative de-excitations (red) and vibration energy transfer in $A^2\Sigma^+$, (black) from $v = 1$ to $v = 0$.

Transitions are presented below:

Transition of the (1,1) vibrational band: $OH(A^2\Sigma^+, 1, N') \rightarrow OH(X^2\Pi, 1, N')$

Vibrational energy transfer: $OH(A^2\Sigma^+, 1, N') \rightarrow OH(A^2\Sigma^+, 0, N')$

And transition of the (0,0) vibrational band: $OH(A^2\Sigma^+, 0, N') \rightarrow OH(X^2\Pi, 0, N')$

3. Experimental setup and procedure

3.1. Experimental setup

A general scheme of the experimental setup is shown in Figure 6.13. The laser is a pulsed Nd-YAG pumped dye laser, with Rhodamine 560 dye and BBO second harmonic generator. The energy/pulse at 282 nm is about 2 mJ and the laser pulse duration is 10 ns FWHM. The beam energy is modulated by a variable attenuator (rotatable half-wave plate + polarizer), after which only a reflection from a quartz plate is focalized into the Plasma Gun plume. In this way up to a factor of 10^3 attenuation can be achieved. The beam is then spatially cleaned through a 1 mm diaphragm and focused by a 20 cm focal length lens.

Laser shots were triggered by the voltage pulse of the μ s power supply to synchronize OH fluorescence and detection with the discharge. The beam energy is measured by a pyroelectric detector connected to an oscilloscope LeCroy WaveRunner 6030. The detection section is composed of a 1:1 telescope with two 30 cm fl lenses of 5 cm in diameter, one 500 mm fl monochromator (SPEX 500M with 600 gr/mm grating) with two selectable output ports. One port is equipped with a 3 mm width slit and a Hamamatsu R2949 photomultiplier (PMT) connected to a HP 54545B digitizing oscilloscope. The other port is fitted to an Andor DH5H7-18F-01 gated intensified CCD (ICCD) connected to a computer. The input slit of the monochromator is set at 100 μ m.

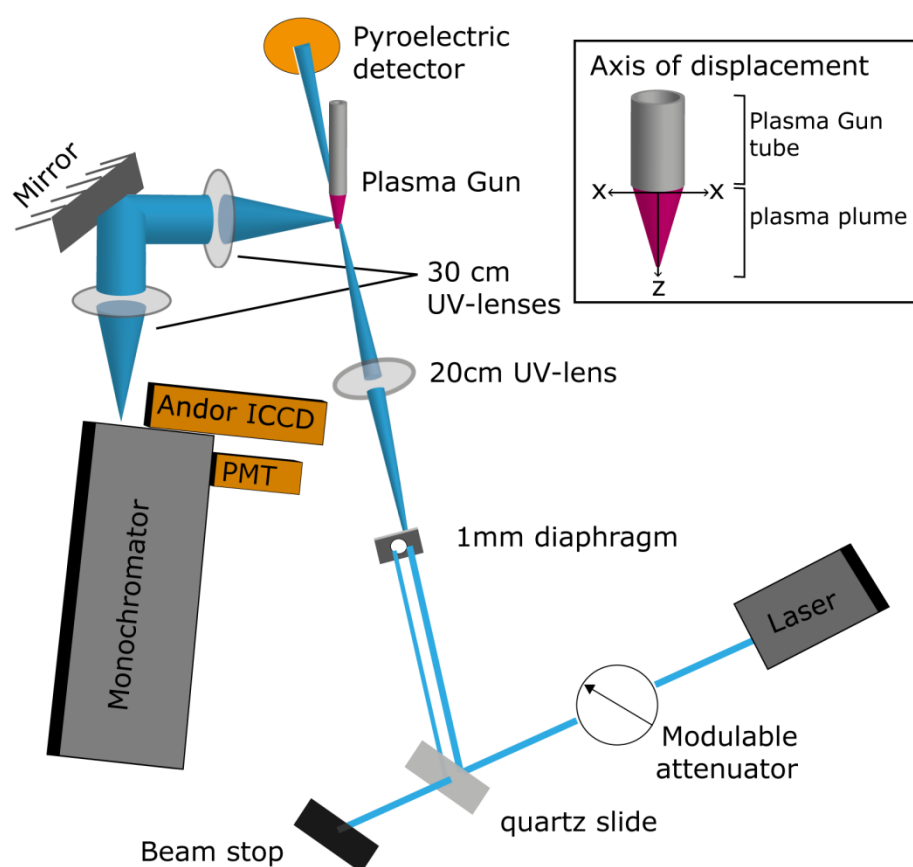


Figure 6.13: Scheme of the experimental setup for relative LIF measurements.

The spot surface is observed by positioning a photographic paper at the LIF measurement point and visualizing its trace thanks to a fluorescence microscope. Fluorescence imaging and optical photography of the beam spot are shown in Figure 6.14.

On the Figure 6.14a), the photosensitive paper is altered by a two-spot overlapping impact as an example of an unadjusted focus. Such beam focus would decrease strongly the

accuracy of the volume of LIF measurement. Thus to reach the optimal focus the phase matching angle of the BBO crystal have been accurately adjusted resulting in a roughly elliptical spot as can be seen in the Figure 6.14b). In Figure 6.14c), it can be observed that the spot area determined by the fluorescence ($\sim 120 \times 100 \mu\text{m}^2$) is underestimating the surface impinged by the laser. As a matter of fact, a granularity is present on the paper fibers. It is estimated that the the photosensitive paper is altered on a elliptical spot with major and minor axes of the order of 150 and 100 μm respectively.

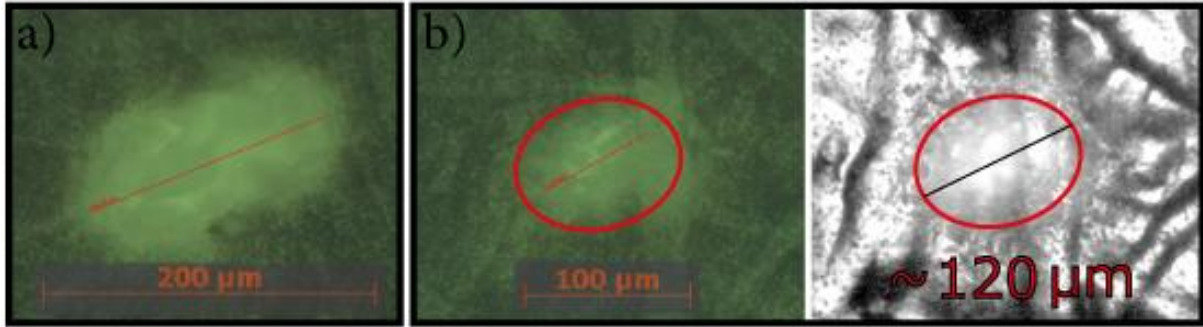


Figure 6.14: Microscopy on a photosensitive paper after ~100 laser beam shots. a) beam spot before adjusting phase matching, b) best focus obtained. Fluorescence imaging a) and b) (left) and (right) an optical photography.

The LIF sampled volume is determined by the width of the monochromator input slit which selects a part of the image of the laser focal spot image (1:1 imaged by the telescope). It is approximated as a cylinder of volume V :

$$V = \pi \cdot r^2 \cdot h$$

With r = radius of the beam spot = 50 μm and h = width of the monochromator slit = 100 μm . Thus the volume of detection considered in our experiments is equal to $8 \cdot 10^{-4} \text{ mm}^3$.

The system PG + target, is mounted on two-dimensional computer-controlled motorized platform in order to allow for high resolution spatial movements while keeping the laser optical path and light detection system fixed. The measurement steps have been set, in general, to 1 mm in vertical and radial directions, information are given for each experiment in the captions. This method implies that the target should be positioned perfectly horizontally. Control of the inclination of the target has been performed with help of a HeNe laser aligned to the monochromator optical axis (HeNe 05-LHR-121, Melles Griot).

During the displacement of the platform, either the solid angle of detection or the laser beam can be modified by the presence of the target and the PG tube, respectively. In the case of perturbations coming from the borosilicate PG tube, an additional fluorescence signal is observed. In most cases, measurements were limited to area without this perturbation, however in some figures data have been corrected by subtracting the

fluorescence signal when plasma was Off to the signal when plasma was On (Figure 6.26, Figure 6.27 and Figure 6.30).

For measurements close to the target surface, distances less than 1 mm from the target are omitted because scattered-laser light from the target would have to be considered.

The scattered laser light from the target is, for the rest of the scanned zone, negligible because of the highly spatially selective optical collection system. As a matter of fact, the plasma region imaged onto the slit of the monochromator is a rectangle of 100 μm width and 2 mm height which theoretically never collects the light touching the target surface. Moreover the target-reflected light comes with a large angle with respect to the optical axis so that it cannot reach the monochromator entrance slit.

For the measurements performed at 1 mm from the target surface, a correction factor has to be considered. The factor estimation is based on the geometry shown in Figure 6.15.

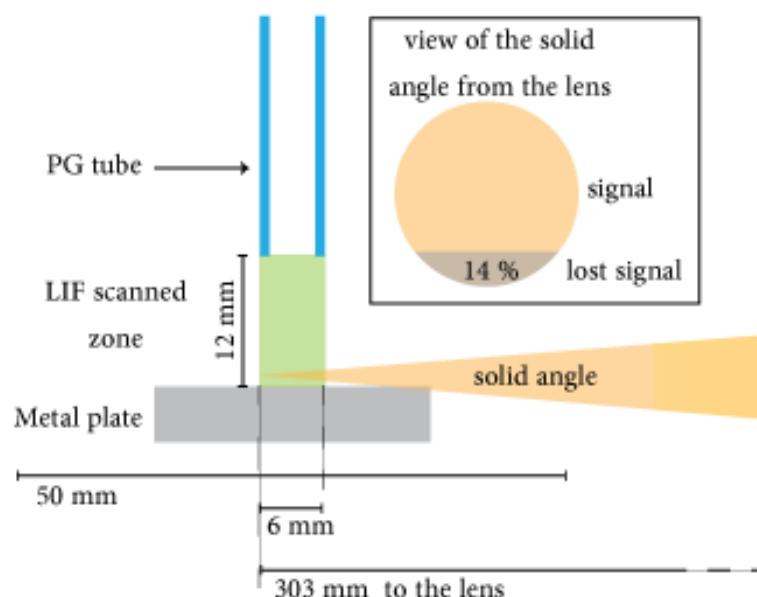


Figure 6.15: Scheme of the detection solid angle for measurements close to the target

This correction factor has been calculated for all the targets used, Petri dish and metal plate. The larger target being the metallic one with a diameter of 25 mm, the higher correction factor had been calculated in this case. The solid angle of detection defines a cone with height $H = 300$ mm, the distance between the laser beam and the lens (equal to the lens focal length), and the diameter of the base $B = 50$ mm, the lens diameter. When considering the extreme case of measurement position ($x = 3$ mm opposite radial direction from the lens and $z = 1$ mm from the target), the solid angle is reduced by a factor of $\sim 14\%$. This loss of signal is then added to the values at $z = 1$ mm obtained by the OH model which is described

in the next paragraph. Such correction has been used for those figures: Figure 6.25, Figure 6.27, Figure 6.28, Figure 6.29 and Figure 6.30.

3.2. Numerical model for LIF measurements

The laser, tuned at 2819.14 Å with a bandwidth of about 0.06 Å (FWHM), excites simultaneously the Q₁(1) (2819.13 Å) and Q₁₂(1) (2819.16 Å) ro-vibrational transitions of the OH(X) (v'=1, v''=0) manifold (Figure 6.16).

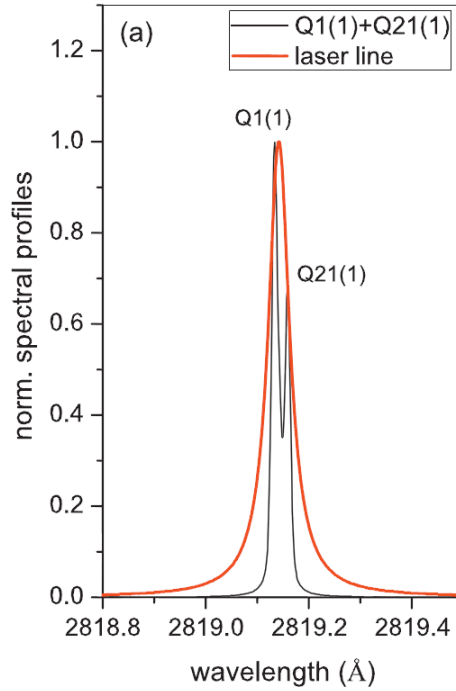


Figure 6.16: Calculation of OH density from LIF measurements: overlap of the laser line with the broadened Q1(1) and Q21(1) lines. Reported from [30]

Due to fast collision-induced vibrational relaxation from v'=1 to v'=0 in the OH(A) manifold, fluorescence from both vibrational levels is observed. In particular the (0,0) and (1,1) bands are detected simultaneously in our scheme, both by the PMT and the ICCD [30].

The model description of the LIF signal is based on the rate equations describing the time evolution of the population of levels OH(X, v=0) - P_X, OH(A, v=0) - P_{A0} and OH(A, v=1) - P_{A1}:

$$\begin{aligned}
 \frac{dP_X}{dt} &= -B\mathcal{E}_L(t)\psi(P_X - P_{A1}) \\
 \frac{dP_{A1}}{dt} &= B\mathcal{E}_L(t)\psi(P_X - P_{A1}) - Q_{A1}P_{A1} \\
 \frac{dP_{A0}}{dt} &= R_{(1 \rightarrow 0)} - Q_{A0}P_{A0}
 \end{aligned} \tag{2}$$

with initial conditions $P_{A0}(t = 0) = P_{A1}(t = 0) = 0$ and $P_X(t = 0) = F[\text{OH}]V_s$. $P_X(t = 0)$ is the initial population of OH(X, $v = 0, N = 1$), given by the product of the rotational population factor F , the OH density and the sampled volume V_s . $F = 0.2353$ at $T_{\text{rot}} = 320$ K, the temperature considered for all the measurements presented here (see later Figure 6.18 and Figure 6.19). B is the Einstein coefficient of the transition, ψ the spectral overlap integral of the laser line and the pressure/doppler broadened $Q_1(1)$ and $Q_{12}(1)$. $\mathcal{E}_L(t)$ is the radiant energy density of the laser that is a function of time, given by $W(t)/cS$ with c the velocity of light, S the laser beam section and $W(t)$ the laser power. Dilecce *et al.* [29] calculated $W(t)$ using a measured laser time profile, normalized such that the time integrated $W(t)$ is equal to the measured laser pulse energy. Q_{A0} and Q_{A1} are the electronic collision quenching rates of OH(A) levels $v'=0,1$ respectively, and $R_{(1 \rightarrow 0)}$ is the vibrational collision relaxation rate. These rates are strongly dependent on the gas mixture composition. A collection of selected collision rate coefficients relevant to He + humid air mixtures, i.e. with He, N₂, O₂ and H₂O colliders, can be found in Table 1 [from [42] and [29] (the latter without those by O₂)].

Table 6.5: Total collision quenching (electronic + vibrational relaxation) and vibrational relaxation rate coefficients for OH with various colliders ($\times 10^{-11}$ (cm⁻³ s⁻¹)). Reported from [42, 29].

Colliders	$v = 0$	$v = 1$	$1 \rightarrow 0$	References
N ₂	2.8 ± 1.2 2.7 ± 1.0	23.6 ± 1.5	23.3 ± 0.04	[43]
				[44]
		24.1 ± 1.3	21.1 ± 2.1	[45]
		19 ± 1.5	24 ± 3	[46]
		19 ± 1.5	14	[47]
O ₂	9.6 ± 1.2 9.2 ± 1.5	20.6 ± 1.5	2.1 ± 0.2	[43]
				[44]
		20.2 ± 1.6	3.3 ± 0.7	[45]
		17	1.5	[47]
H ₂ O	68.0 ± 6.1 69.1 ± 5.0	66 ± 4		[48]
			≤ 12	[49]
			7.3 ± 0.5	[46]
				[43]
He		0.004 ± 0.0015	0.002	[29]
			0.13 ± 0.04	[50]

Two detectors, the PMT and the ICCD, are needed to obtain the full set of variables. We measure by the PMT the fluorescence pulse $S(t)$, sum of the two bands:

$$S(t) = S_1(t) + S_0(t) = C(A_{(1,1)}P_{A1} + A_{(0,0)}P_{A0}) \quad (3)$$

and, by the ICCD, the time integrated spectrally dispersed fluorescence $\mathcal{I}_{LIF}(\lambda)$:

$$\mathcal{I}_{LIF}(\lambda) = \Psi_{(1,1)}(\lambda) \int_0^{T_G} S_1(t) dt + \Psi_{(0,0)}(\lambda) \int_0^{T_G} S_0(t) dt \quad \text{with} \quad \int_0^\infty \Psi(\lambda) d\lambda = 1 \quad (4)$$

Here $A_{(1,1)}$ and $A_{(0,0)}$ are the radiative rates of (1,1) and (0,0) bands, C is a constant including the sampled volume and the transfer function of the optical collection plus measuring apparatus (see [29-30] for details), the two $\Psi(\lambda)$ are the normalized emission spectra of

(1,1) and (0,0) bands. It is worth noting that T_G , the ICCD time gate, is always larger than the fluorescence lifetime to avoid possible loss of fluorescence.

3.3. Procedure

The plasma jet expanding into ambient humid air and hitting a solid/liquid surface is a highly inhomogeneous medium with strong gradients of gas mixture composition. As previously mentioned in the last chapters, gas mixture is not only changing spatially but also temporally. Although OH is highly dependent on the colliders' densities, a correct LIF measurement would require high spatial resolution together with detailed knowledge of the local mixture composition. This knowledge is taken from the LIF measurements through the model detailed above.

Appropriate spatial resolution is achieved by focusing the laser beam (Figure 6.14). In order to work in the linear regime of laser light absorption in the focus region, the beam energy must be greatly reduced. The measured LIF saturation curve in Figure 6.17 shows that at laser pulse energies lower than 6 μJ a pseudo-linear regime is operative, i.e. a straight line is a good approximation in the determined energy range. This pseudo-linear regime, which has a slope lower than that of the pure linear regime, is due to the non-homogeneous spatial profile of the laser beam, with eventual presence of hot spots and smooth borders, and to the spectral overlap of the laser line with the absorption lines, that configure the presence of spatial and spectral regions with different saturation characteristics. We estimate that in order to be in the pure linear regime the radiant energy density must be reduced at least by a further factor of ten. This could be done either by enlarging the spot size, at the expense of the spatial resolution, or by reducing the beam energy, at the expense of the signal. The main reason why it is advisable to work in the linear regime is that in this case the ground rotational level (P_x) is not significantly depleted by light absorption. On the contrary at saturation, the depletion is large and could be partially refilled by fast rotational redistribution. The rotational refilling would alter the LIF outcome and would be dependent on the gas mixture composition and temperature. In our case we estimate that such an effect would be present only at the borders of the plasma jet, where significant air penetration occurs, and it would anyway be mitigated by the pseudo-linear regime condition [51]. Our choice is then to work with the laser focused with energy close to 4.5 μJ /pulse since high spatial resolution and good signal are far more important than the risk of an increased uncertainty in a region where, as we will see, the measurement error is anyway large.

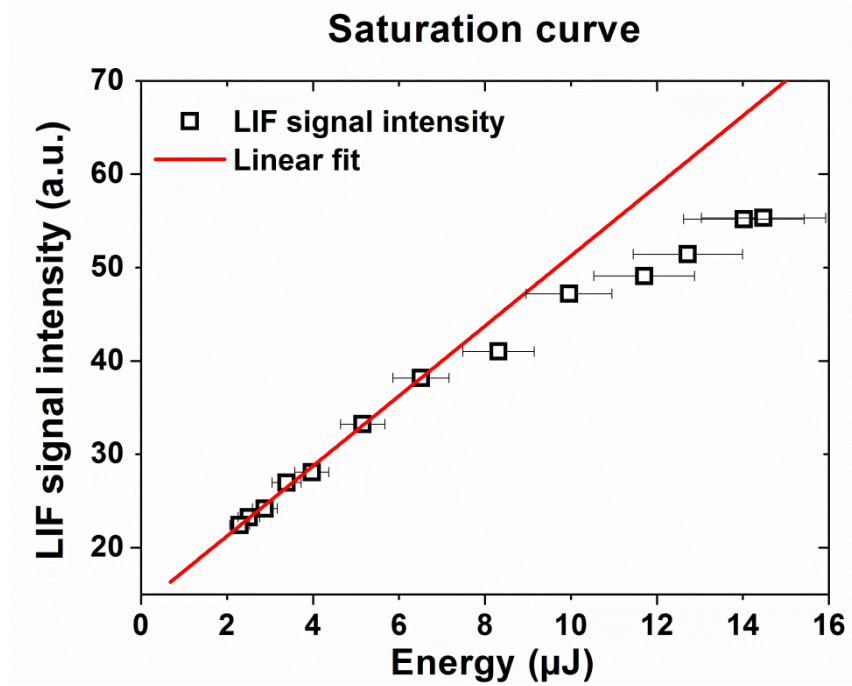


Figure 6.17: LIF saturation curve showing experimental LIF signal intensity versus laser beam energy (empty square) with a linear fit (red line)

In He + humid air mixtures the gas composition can be measured, and then accounted for in the LIF outcome, on the basis of the collision quenching and vibrational relaxation. It was demonstrated in [40] that a unique He + air + H₂O mixture composition corresponds to a given couple of electronic quenching and vibrational relaxation rates values. Here we implement this idea by measuring at each spatial position both $S(t)$ and $\mathcal{I}_{\text{LIF}}(\lambda)$ and fitting them by equation (2) of the model using the concentrations of air and water vapour and P_x as parameters. For model calculation of $\mathcal{I}_{\text{LIF}}(\lambda)$, we use synthetic $\Psi_{(1,1)}(\lambda)$ and $\Psi_{(0,0)}(\lambda)$ calculated as the convolution of the measured spectral transfer function of the monochromator with spectra simulated by LIFBASE [52]. Examples of measured and simulated $S(t)$ and $\mathcal{I}_{\text{LIF}}(\lambda)$ are shown in Figure 6.18. They correspond to two measurements standing for extreme cases of quencher densities. In Figure 6.18a) the measurement has been performed in the core of the jet close to the tube outlet (position $x = 0$ mm and $z = 2$ mm). At this position, the OH density is $\sim 2 \cdot 10^{13} \text{ cm}^{-3}$ and the partial pressures are $< 1\%$. In Figure 6.18b), the measurement is corresponding to the border of the jet (position $x = 2$ mm and $z = 8$ mm) with an OH density of $\sim 4 \cdot 10^{13} \text{ cm}^{-3}$ while $P_{\text{air}} = 36\%$ and $P_{\text{H}_2\text{O}} = 8.6\%$.

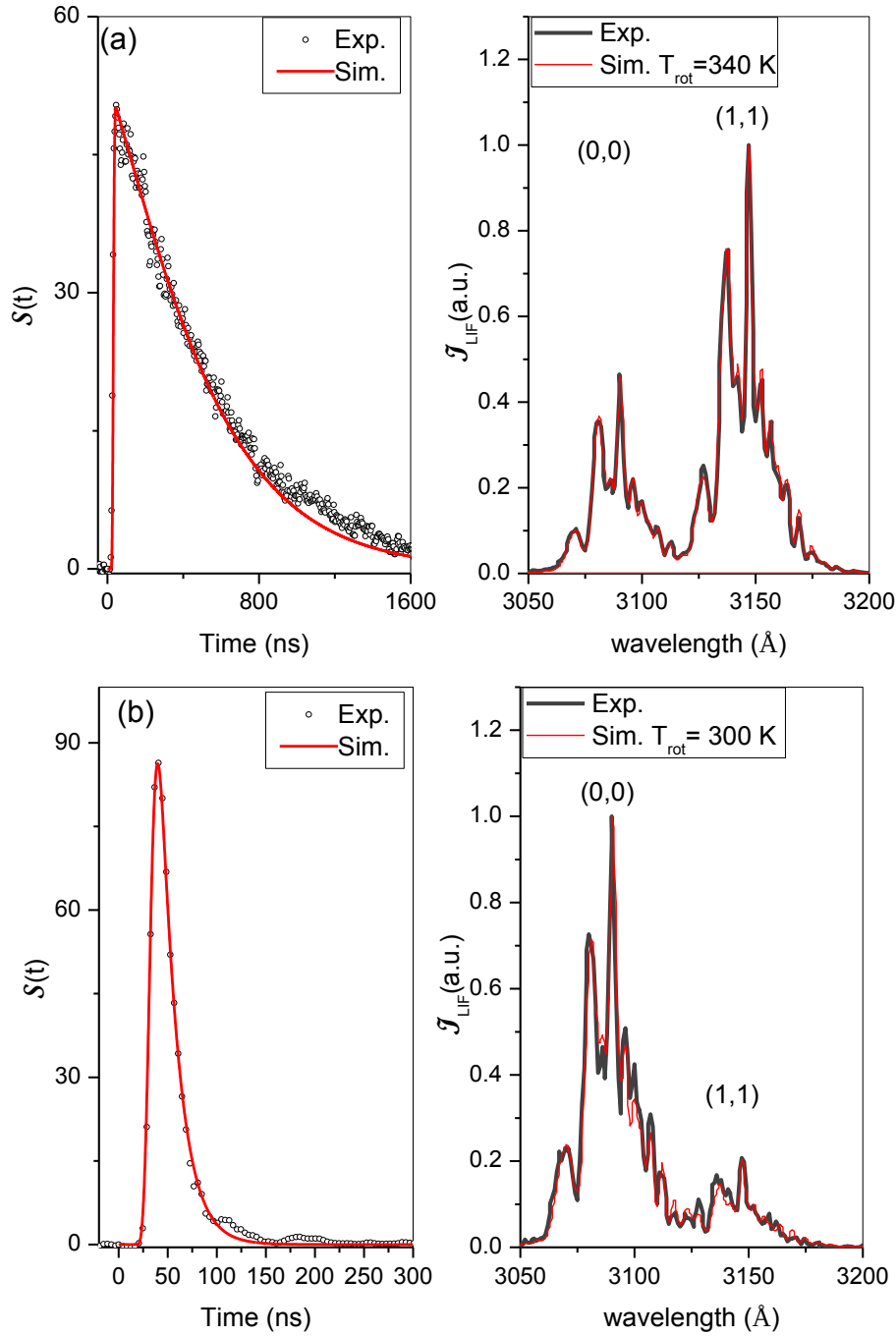


Figure 6.18: LIF pulse and spectra with model (2) fits: (a) Core, Position: X = 0 mm, Z = 2 mm and parameters: $[OH] \sim 2 \cdot 10^{13} \text{ cm}^{-3}$, $Pair = 0.2\%$ and $PH_2O = 0.5\%$ (b) border, Position: X = 2 mm, Z = 8 mm and parameters: $[OH] \sim 4 \cdot 10^{13} \text{ cm}^{-3}$, $Pair = 36\%$ and $PH_2O = 8.6\%$.

The excitation scheme involves absorption by the first rotational level, whose population depends on the rotational temperature that, in the ground state, is certainly equal to the gas temperature. Temperature gradients should then be taken into account. The temperature in the jet has been estimated from fluorescence spectra by comparison with LIFBASE simulated spectra. Since in the core the gas is mainly He, the OH(A) lifetime is large, and the number of rotational collisions in the lifetime is about 200, which allows to assume local thermodynamic equilibrium (see Figure 6.18). In our case the gradients are not a problem

since the temperature changes from about 340 K in the core of the jet (Figure 6.18a)) to ambient temperature, 300 K at the borders (Figure 6.18b)).

The rotational population factor of level 1 changes with temperature; in particular the fractional population of level 1 is 0.25 at 300 K, 0.215 at 350 K and 0.19 at 400 K. If the temperature changes in space and time by few tens of K around 320 K, then neglecting temperature variations has an effect of the order of 10 % on the spatial/temporal maps of OH density. It is clear that in cases of larger temperature gradients, the temperature would imply large modification on the fraction population and would have to be taken into account. Any attempt to measure the temperature and correct the LIF outcome accordingly would produce a comparable error. Moreover the comparison with LIFBASE simulated spectra would not allow for precise rotational temperature determination. Figure 6.19 shows synthetic spectra at different rotational temperatures from 300 to 400 K computed with LIFBASE compared to the experimental spectrum shown in Figure 6.18a).

Small variations of the relative intensity of the peaks can be observed allowing for determining a temperature of $340 \text{ K} \pm 50 \text{ K}$. This method is far less precise than the model developed by N. Sadeghi and E. Eslami [53-54] presented in Chapter 2 where the uncertainty was determined to be of the order of $\pm 10 \text{ K}$. According to this observation, an average rotational temperature of 320 K has been considered for the whole scanned region.

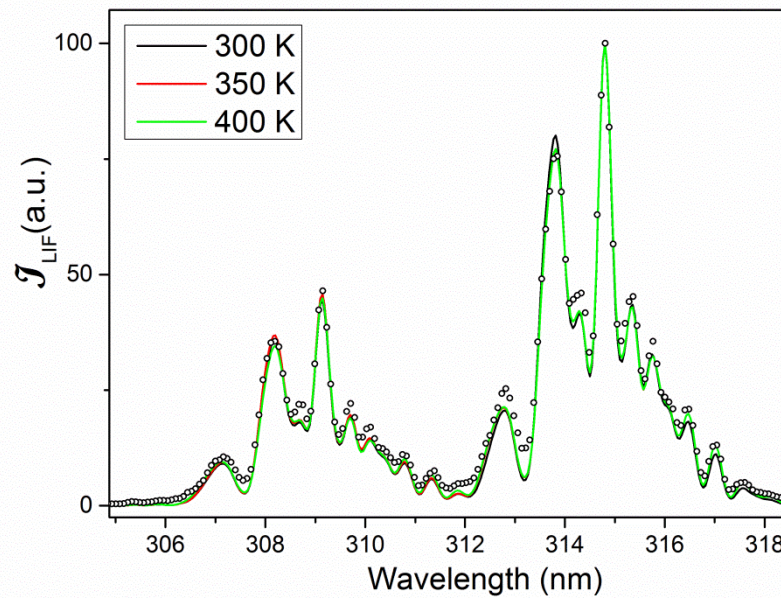


Figure 6.19: Comparison of OH(A-X) synthetic spectra at various rotational temperature. Experimental spectrum corresponding to the jet core, Position: $X = 0 \text{ mm}$, $Z = 2 \text{ mm}$ and parameters: $[\text{OH}] \sim 2 \cdot 10^{13} \text{ cm}^{-3}$, $\text{Pair} = 0.2\%$ and $\text{PH}_2\text{O} = 0.5\%$.

We find the parameters, [OH] and partial pressures of air and H₂O, by a “visual” procedure in the LabVIEW interface of the model. This “visual” procedure introduces errors that can only be reasonably estimated although developing a mathematical fitting algorithm would be a truly hard task, given that two functions with three parameters are involved, plus uncertainties in baselines, spectral transfer function and gas temperature. Estimated uncertainties for the three parameters are reported in Figure 6.20. They are obtained by identifying for each data point extreme combinations of the three parameters which enable an acceptable fit. Average noise observed on both pulse and spectrum files is also considered and its influence on densities is estimated by subtracting the corresponding value from the best fit. The interpolations curves have been plotted for visualization purposes and do not stand for any physical meaning.

Uncertainties are highly linked to the strength of LIF signal. In the case of OH density determination, the uncertainty increases with decreasing density which is linked to the proportionality of $S(t)$ with P_{A1} and P_{A0} (see eq (3)) and to the increased error due to noise. In the case of air and H₂O partial pressures, uncertainty tends to increase with increasing densities, due to an overall decrease of the signal with increasing electronic quenching. However, the high non-homogeneity of the gas mixture together with the error dependence on the strength of the LIF signal give a highly dispersed data set.

Air and H₂O partial pressures have been converted to percentage of respective concentrations in the surrounding atmosphere to better appreciate gas mixture processes with Helium flow. In the conditions of this study, a temperature of 26° C and an average humidity of 40 % have been measured and regulated in the laboratory by means of air conditioning. The saturation vapor pressure^h at 26 °C is 25.2 Torr, thus an ambient humidity of 40 % counts for 10 Torr of the total pressure in the room. As a first example, specifying that 15 % of H₂O is found at one measurement point means that only 6 % of the ambient humidity is present. Similarly, 110 % of H₂O represents an ambient humidity of 44 %.

It is worth noting that uncertainties are here over-estimated by the manual technique used for their determinations. As will be shown in the following figures, the interpolation of discrete measurement allows obtaining density distributions in good agreement with previously obtained results (by fast-imaging, wavelength-filtered imaging and/or Schlieren imaging). This *ad equation* could not be retrieved if the uncertainties would be as high as determined manually.

The averaging process, linked to the number of accumulation of the ICCD measurement, can also induce some errors of interpretation. As a matter of fact if the distribution of the OH production is undergoing high variation from one pulse to the other, the averaging could lead to a decrease of the obtained density.

^h See Annex A

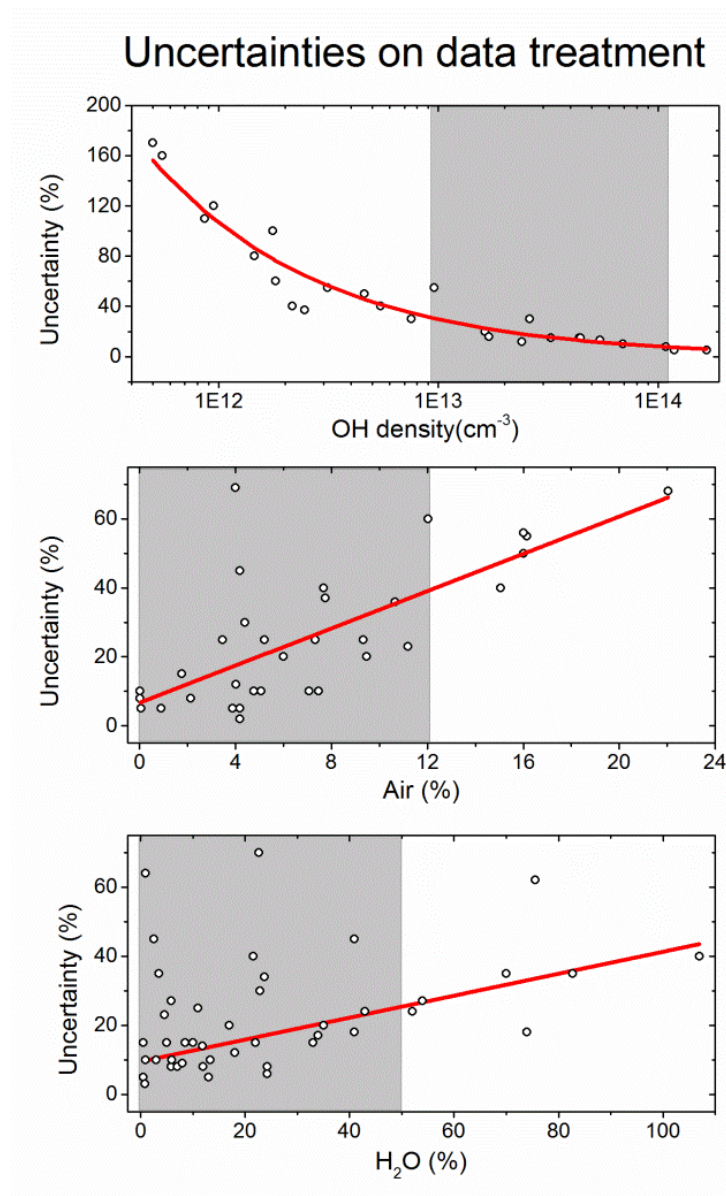


Figure 6.20: Percentage of uncertainty versus densities, OH (top), Air (middle) and H₂O (bottom).

Iséni *et al.* [55] recently described that the averaging of multiple shots could alter the instantaneous distribution of their Planar-LIF (PLIF) measurements. As air and Helium mixture can be modified from one pulse to the other by hydrodynamics processes (i.e. Chapter 5), especially at the border of the plasma plume, the fact that the spectra are averaged over 500 measurements leads to an average of the OH distribution at a given time (200 μ s) when the PG is operating in a continuous mode. It has been seen in Figure 6.22, that at a given point ($x = 0$ and $z = 10$ mm) the OH density is decreasing following a smooth exponential decay. This tends to support that, in our conditions, such alteration of the instantaneous distribution due to the averaging does not raise a significant error on the measurements. Moreover, as our main concern deals with the OH delivery on the biological targets, such averaged OH distribution is more probable to be related to what is actually deposited on the target.

Another source of error comes from the fact that P_x is equal to the actual OH density provided the right values of $\mathcal{E}_L(t)$ and $\mathcal{C}$ are used. Reasonable values can be calculated as in [30], but with a focused laser beam the difficulty is increased, mainly due to the uncertain definition of the section in the focus region and of the difficulty in defining $\mathcal{E}_L(t)$ that we have already discussed. $[\text{OH}]$ is anyway proportional to P_x , $[\text{OH}] = KP_x$, through a constant K that must be determined by a calibration method. To this end we have used as a calibrated OH source, the He-H₂O dielectric barrier discharge in which the OH density has been measured by an absolute absorption technique [56]. The discharge has been mounted in the same position as that of the plasma jet without altering the LIF configuration. The LIF outcome in a condition of known OH density is then analyzed by model (2) finding the constant K with the 25% error of the absorption method.

In practice, the methodology can be summarized as follow. The laser shot excites the OH(X) state giving rise to the fluorescence of the OH (A) (0,0) and (1,1) simultaneously. Fluorescence pulses and spectra are recorded at each spatial position by the PMT and ICCD. The scan step is in general of the order of the 1 mm. The fluorescence pulses and spectra are pretreated with Origin software, such as correction of the baseline shift, subtractions of the fluorescence coming from the borosilicate tube, etc. Then the signals are uploaded onto the LabVIEW interface [57] of the numerical model described above, allowing for building synthetic temporal LIF signals and spectra.

A set of variables, listed above, are determined into the model. Variables which are experiment-dependent are energy of the laser beam, volume of measurement, the rotational temperature, partial pressures of Air and H₂O (P_{air} and P_{H_2O} , respectively) and OH density. First three variables are determined during calibration experiments, the energy of the laser beam is kept under 4.5 $\mu\text{J}/\text{pulse}$, the volume of measurement is $8 \cdot 10^{-4} \text{ mm}^3$ and the rotational temperature is set at 320 K. The three last variables are the values of interest in those experiments, namely the OH density and the partial pressures of Air and H₂O.

For each condition, three matricesⁱ are built up in the Origin software with the obtained data covering the spatial scanned zone. These matrices are made of about 60 points. Contour plots are performed for each of them, meaning that the values of each x-z are plotted as curves with interpolated data between them (100 color levels in this chapter and 9 in the Annex B^j which are more likely to represent the measurement considering the uncertainties). The positions of the measurements are enlightened by the crossing points of a grid superimposed on the contour plots. The interpolation procedure can induce artifacts, observable as diamonds shapes. Thus the presented data in this chapter are a combination of several discrete measurements along x, the distance from the revolution axis of the PG capillary and z, the distance from the tube outlet. Theses contour plots will be called "maps" in the rest of the text for readability purposes.

ⁱ See Annex B
^j See Annex C

The experimental setup allows also for time-resolved measurement of OH density and partial pressures at the fixed position. In this case, the fluorescence pulses are scanned temporally with an automated LabVIEW programme with chosen steps up to μs resolution. Then spectra are recorded for corresponding delays.

4. Temporal evolution of OH radicals

Figure 6.21 shows examples of temporal evolution of OH fluorescence between two voltage pulses when the PG operates at 2 kHz for three gas flow rates 0.5, 1 and 1.5 l.min^{-1} . In this figure, the position of the measurement was fixed on the revolution axis of the PG tube and at 2 mm from the PG tube outlet. As gas composition is considered to be homogeneous at this position (2 mm from the capillary outlet), LIF intensity collected by the PMT reflects the OH density in relative unit.

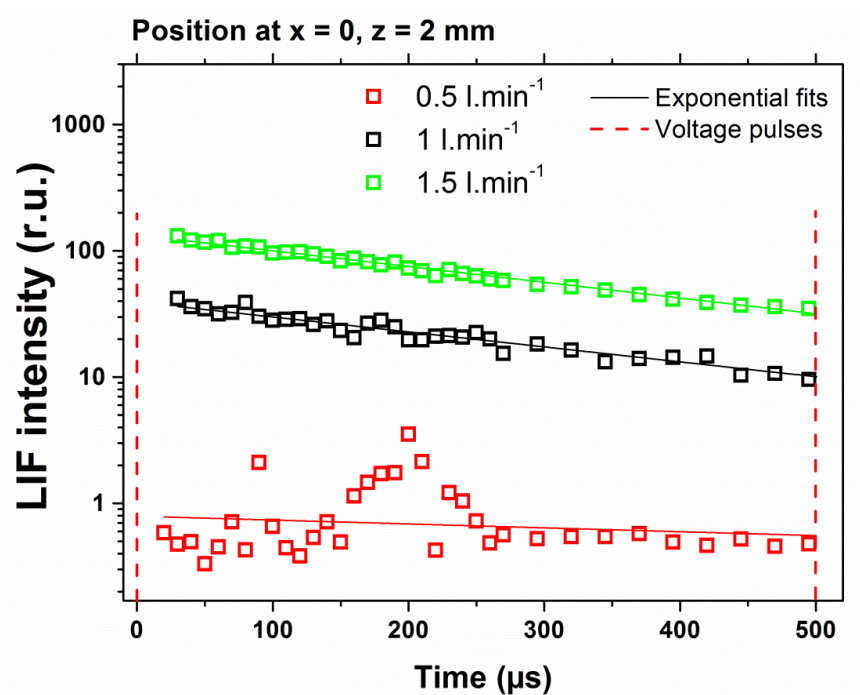


Figure 6.21: Temporal evolution of OH LIF intensity between two voltage pulses for various gas flow rates, 0.5, 1 and 1.5 l.min^{-1} . Position of the measurements: $x = 0$ and $z = 2$ mm. 2 kHz, 14 kV and target (metal, gap = 12 mm).

At 2 kHz, OH density is decreasing slightly between two voltage pulses. The faster decay rates are observed for higher gas flow rates such as at 1.5 and 1 l.min^{-1} the decay rates are $2.9 \cdot 10^3 \text{ s}^{-1}$ while at 0.5 l.min^{-1} it is $7 \cdot 10^2 \text{ s}^{-1}$. A high OH density induces a higher probability for recombination. The higher densities are obtained when increasing gas flow rate from 500 sccm to 1500 sccm. It is worth noting that for 500 sccm, the dispersion on measurements

from linear fit is relatively high due to low LIF signal (see Figure 6.20) while for higher gas flow rates the decays are well represented by exponential fits.

The gas flow velocity has to be taken into account in the interpretation of these LIF signal evolution. As a matter of fact, for a 1500 sccm gas flow rate the gas is moving of around 600 μm during the 500 μs between the pulses, which means that in this time range the OH signal measured is coming from upstream. In the case of 500 sccm however the gas is moving slowly, only 200 μm during the 500 μs , which lead to measure OH in the laser focal point overlapping with upstream OH. In addition to the gas flow velocity, one can notice that we are at a fixed repetition rate, 2 kHz. Thus the gas at 500 sccm will have undergone 3 times more discharge pulses than the gas at 1500 sccm. This consideration explain the low decay rate of the measurement at 500 sccm as compared to 1000 and 1500 sccm ones.

Temporal scans were also performed at $x = 0$, $z = 10$ mm for 0.5, 1 and 1.5 $\text{l}\cdot\text{min}^{-1}$. At such position in the gap however the amount of quenchers are not considered to be constant (see chapter 5) and LIF intensity have then been corrected to obtain OH absolute density. For 500 sccm, LIF signal/noise was too low and no data are shown.

As observed at $x = 0$, $z = 2$ mm, the densities close to the target ($x = 0$, $z = 10$ mm) are decreasing versus time with decay rates ($2.2\cdot 10^3$ and $3.5\cdot 10^3$ s^{-1} for 1 and 1.5 $\text{l}\cdot\text{min}^{-1}$, respectively). However in this case, the maximum OH density is obtained for 1 $\text{l}\cdot\text{min}^{-1}$ and not for the higher gas flow rate. This decrease of OH density with gas flow when exceeding a certain rate may result from the minimization of the air diffusion close to the target surface. A parallel can be made with the tendency of the excited species emission to be further constrained on the target surface with increasing gas flow rate as shown by wavelength-filtered imaging (Figure 5.19). This behavior was induced by a combination of the gas flow channeling and plasma counter-propagation. As will be developed further with spatial distributions of air and H_2O , OH density distribution is following influences from similar phenomena.

As expected from the results presented in Chapter 4 and 5, there exists a difference of behavior in OH decay close to the target surface as compared to the tube outlet. OH production and disappearance evolve temporally and spatially.

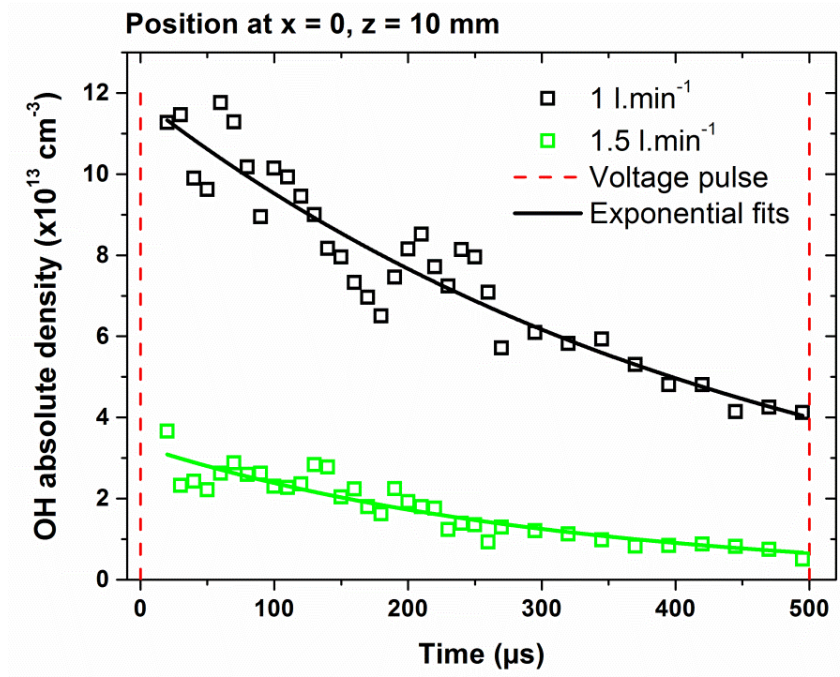


Figure 6.22: Temporal evolution of OH absolute density between two voltage pulses for two gas flow rates, 1 and 1.5 l.min⁻¹. Position of the measurements: x = 0 and z = 10 mm. 2 kHz, 14 kV and target (metal, gap = 12 mm).

For the spatial maps that will be shown in this chapter, the LIF measurements were made at a pulse repetition rate of 2 kHz and a 200 μs delay was settled. This time position gives access to approximately the time-averaged value of OH density, similarly for x = 0 mm and z = 2 or 10 mm (Figure 6.21 and Figure 6.22) although OH density decay and absolute density varied with position and gas flow rates. Another advantage of the 200 μs delay is to avoid overlapping with the discharge emission, lasting for the main part the voltage pulse duration, 10 μs (see Figure 5.22).

To evaluate its mean life time, OH has been scanned (Figure 6.23) during a period including a series of voltage pulse delivery (grey area) and a plasma extinction phase (blue area). This measurement has been performed for standard conditions of plasma delivery, 14 kV, 2 kHz, 1 l.min⁻¹ and for a gap of 12 mm between the tube outlet and the metal target, at x = 0 and z = 2 mm.

As can be seen in Figure 6.23, OH density in these conditions is at a plateau around $2 \cdot 10^{13} \text{ cm}^{-3}$ when steady state is reached. After turning Off the power supply, OH follows a two-slope decay, one fast in the order of the decay rate found between two pulses (Figure 6.21 and Figure 6.22) and one slow of $\sim 1.7 \cdot 10^2 \text{ s}^{-1}$. The lower detection limit is reached as long as 20 ms after extinction. In this condition, the gas flow displacement has to be taken into account, as 20 ms represent a displacement of 2.6 cm. This implies that the second decay rate is more likely to be linked to the OH decay inside the capillary where almost no quenchers are present.

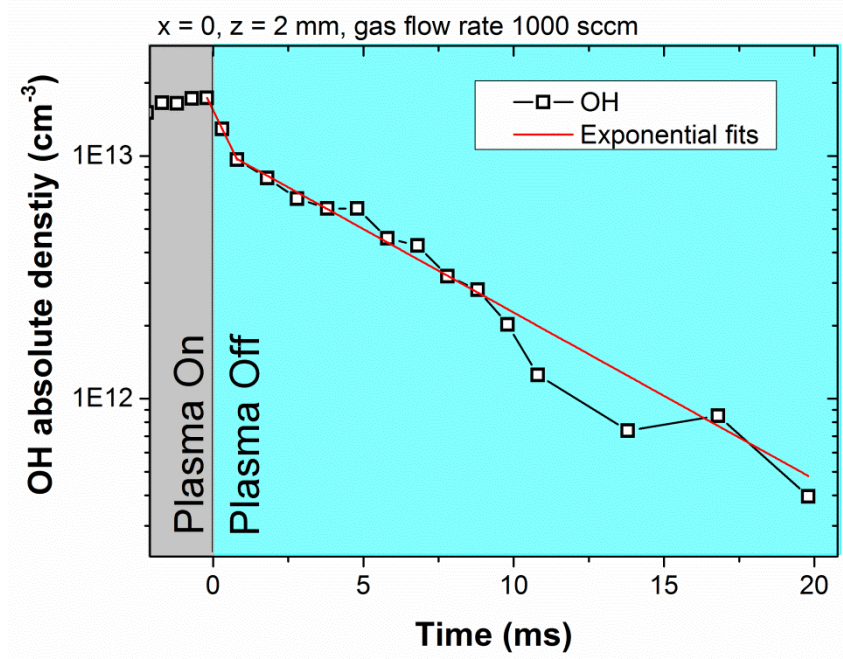


Figure 6.23: Temporal evolution of OH absolute density during and after pulse ignition. Position of the measurements: $x = 0$ and $z = 2$ mm. 2 kHz, 14 kV, 1 l.min^{-1} and target (metal, gap = 12 mm).

When small quantity of quenchers is present, the maximum duration of the LIF pulse in the plasma plume (see Figure 6.18) is of 2 μs . In this extreme condition, the gas displacement can be approximated at 3 μm during this lapse of time, movement that can be neglected considering the scanning steps of the discrete measurements. The distributions presented in this work are thus considered as instantaneous snapshots at 200 μs delay regarding the pulse voltage. This is true in conditions of moderate gas flow rate (1 l.min^{-1}), relatively larger inner diameter of the capillary (4 mm) and small beam spot ($150 \times 100 \mu\text{m}^2$). However when moving to larger gas flow rates and/or lower jet diameters, the displacement driven by the gas flow can no longer be neglected. In these cases the LIF signal decay will certainly be affected by a combination of OH^* ($A^2\Sigma^+$) relaxation and progressive disappearance of the OH radicals from the scanned zone.

5. OH, Air and H₂O spatial distributions

The majority of the maps shown in this section will be compared to a chosen "standard" condition, which has been studied by means of diverse diagnostics in Chapter 5. This standard condition has been chosen with the parameters that follow: a voltage amplitude of 14 kV, a pulse repetition rate of 2 kHz, a Helium (Aphagaz1) flow rate of 1 l.min^{-1} and a gap of 12 mm between tube outlet and a metal grounded target.

5.1. Influence of a conductive target

In the scheme of these LIF measurements focus has been set on studying OH distribution along the PG plume and humid air penetration into the gas jet for set ups close to those involved for biomedical applications. To evaluate the influence of the presence of the conductive target as well as for comparison with previous work on OH distribution in plasma jets [36, 39], Figure 6.24 presents the results concerning the PG plume in free jet mode compared to the standard condition.

Figure 6.24a) in the free jet mode shows that OH is distributed on the PAPS propagation path with density being maximum on the jet centerline, $[\text{OH}]_{\text{max}} = 2.1 \cdot 10^{13} \text{ cm}^{-3}$, and decreasing gradually to reach $2.0 \cdot 10^{12} \text{ cm}^{-3}$ in the 2 mm surrounding the production region, in radial direction. Comparison with the He* emission distribution captured by means of wavelength-filtered imaging (see Figure 4.12) give keys of understanding of this spatial distribution.

The He* emission, with the same PG parameters, can be separated into two regions. A first zone of emission situated in the laminar Helium channel linked to the gradual mixing of air to Helium. At tube outlet, the flow is already a mix of He and air, the last one being mainly at the jet borders and gradually diffusing more deeply inside the jet leading to a conical-like emission. A second zone can be observed downstream (from $z \sim 8$ to ~ 13 mm), where a priori the air proportion is much higher, leading to much less intense radiation resulting from mainly He* but also from weak O* contribution. It has been seen thanks to Schlieren imaging that this region is linked to extension of the Helium flow due to the plasma channeling. As a reminder, the plasma channeling in this region is competing with the buoyancy forces pushing the gas flow upward.

Comparison of He* distribution with the OH ground state map in the free jet mode (Figure 6.24a)) reveals that maximum OH density is obtained when Helium mixes with ambient air between 7 and 9 mm from the tube outlet suggesting that there exists an optimum proportion of water vapor into the PAPS path to produce OH radical. The good correlation between He* emission distribution and Air and H₂O distributions in Figure 6.24b) and c) respectively supports this observation. No air and H₂O could be detected in the zone of strong He* emission while proportions increase when moving to the borders of the maps. Furthermore, relatively low humid air penetration is observed in the region of high OH density and low diffuse He* emission. However the low air and water concentration zone is enlarged in regards of the He* emission distribution, which suggests that He* is highly sensitive to the presence of few % of air.

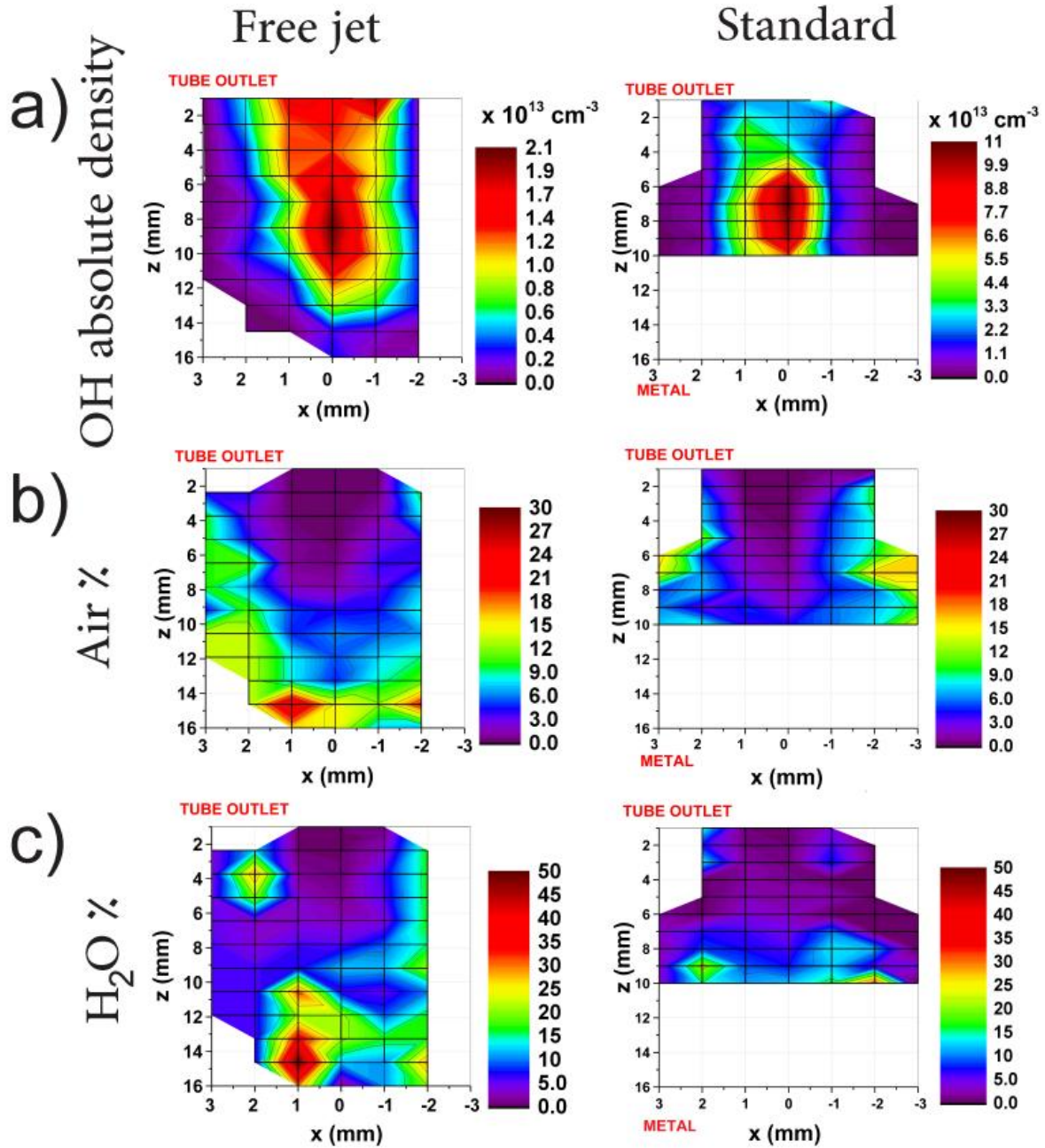


Figure 6.24: Case of a free jet (left) compared to the standard configuration. LIF maps showing distributions of a) OH density, b) air and c) H₂O percentages. Scanning steps 1.5 mm and 1 mm for vertical and radial directions respectively for the free jet case and 1 mm for vertical and radial directions for the standard configuration.

For same PG operating parameters, except the presence of a metal target 12 mm away from the tube outlet, Figure 6.24 reveals drastic changes in OH density amplitude. OH density is still around $1.10^{13} \text{ cm}^{-3}$ at the tube outlet and raises to about 10^{14} cm^{-3} around 7 to 10 mm downstream. Maximum OH density is positioned on the axis from 6 to 9 mm and decreases when close to the target. Its distribution pattern is linked to the presence of the counter-propagating PAPS as described in Chapter 5. Similarities in shape and intensity to visible emission detected by a standard camera (Figure 5.13c)) shows that the counter-propagating

PAPS induces an increase in OH density, approximately 5 times rise. It suggests that OH is produced during downstream and upstream PAPS propagation. A high correlation is observed between plasma emission (either the 10 μs ICCD snapshot of Figure 5.4 or the 300 ms camera picture from Figure 5.13) and OH ground state distribution in the standard condition. The similarities between the excited species and the OH ground state distribution indicate that OH after relaxation may not encountering major modifications, in spatial distribution and density, between two voltage pulses. It is worth mentioning that such hypothesis is valuable only when plasma is working in a continuous mode. Moreover we are using a short laser pulse, 10 ns FWHM focalized in a spot of $100 \times 150 \mu\text{m}^2$ and a moderate gas flow rate. During the laser excitation duration, the gas flow can induced an OH displacement of 26 μm and 13 μm for gas flow rate of 2 l.min^{-1} and 1 l.min^{-1} , respectively. Those displacements are negligible compared to the volume of measurement. This observation leads to the conclusion that LIF measurements show steady state of OH spatial distribution.

Investigation of Air distribution when the PG impinges on the metal plate, namely in the standard condition, shows a pronounced channeling of the Helium flow as compared to the free jet mode, indicated by the sharpening of the air-less zone. It is worth reminding that Helium flow channeling results in higher stability of the plasma plume as confirmed by naked eyes observation, Schlieren imaging (Figure 5.9) and wavelength filtered imaging described in Chapter 5 and 6. The maximum air percentage, found in all the experiments presented of this chapter, is observed for the free jet mode and is equal to 27 % of the surrounding ambient air. When air reaches this concentration the LIF measurement is at the limit of detection with OH of the order of 10^{11} cm^{-3} . This means that the plasma jet evolves in a gas channel mainly constituted of Helium, observation coherent with what have been previously determined in Chapter 5 and 6. Even a 10 % diffusion of humid ambient air in the Helium jet reduces considerably the OH density.

The influence of the conductive target on the plasma plume appears one more time as drastic. The LIF experiment confirms what have been already reported regarding of the gas flow modification by plasma (Chapter 5 and in [4]) and on the production of reactive oxygen and nitrogen species (Chapter 4 and 5 and in [8]).

5.2. Parametric study on OH distribution and humid air penetration inside the Helium jet

In order to evaluate the influences of the plasma parameters on OH production, the voltage amplitude and the gas flow rate have been decreased, from 14 to 10 kV and from 1000 to 500 sccm, respectively. These measurements allow estimating the modification of the gas flow dynamics and the plasma propagation.

5.2.1. Influence of voltage amplitude

Figure 6.25 shows OH absolute density and air and water % distribution when plasma impinges on a conductive target with same operating parameters as in the standard condition, except the voltage amplitude which has been modified from 14 kV to 10 kV.

Here OH distribution and density are similar to maps retrieved with 14 kV, but a spatial shift of OH maximum density can be observed toward the target which confirms that the higher density region is linked to the PAPS counter-propagation. As already suggested (Chapter 5) the PAPS counter-propagates until the voltage become too low to ensure the ionization front propagation similarly to the PAPS propagation inside the capillary [58]. We have seen in Chapter 4 that by decreasing the voltage amplitude the plasma propagation becomes shorter. The counter-propagating PAPS follows the same behavior vanishing with the voltage pulse as observed by fast ICCD imaging (Figure 5.4 and 5.5). Thus the modulation of the maximum OH density position by the voltage amplitude confirms the link between the OH production and the PAPS counter-propagation.

Moreover Air and water distribution shows a higher penetration into the central axis suggesting a smaller plasma guiding effect of the Helium flow as compared to the standard condition. As for previously described results (Chapter 5), it is hard to differentiate between the causes and the consequences of such phenomena. We propose two hypotheses:

On one hand, the lower voltage amplitude may induce a reduction of the ionization front transient electric field which modifies consequently the electric field between PAPS head and the target. This hypothesis can explain why the gas channeling in this condition would be less efficient. Based on this hypothesis, the over-penetration of air in the Helium jet could induce increased energy losses.

A consequence of increased energy losses may be a limit of PAPS counter-propagation. If sufficient energy is present to produce the counter-propagation PAPS but too many quenchers are present in the channel, the PAPS would be contained in the zone close to the production, thus to the grounded conductive target. It appears that in this zone, the H₂O% is optimal for OH production, leading to a high OH production close to the target.

On the other hand, it is possible that the lower voltage amplitude induces a shorter PAPS counter-propagation length. Because the PAPS counter-propagation length and duration is reduced, the production of ionic species is consequently decreased, which might cause a decrease of the gas channeling and an increase of air diffusion inside the gas jet.

As the experimental results cannot distinguish between the causes and the consequences, only hypothesis can be made and further experiments are needed to clarify the physics driving this phenomenon.

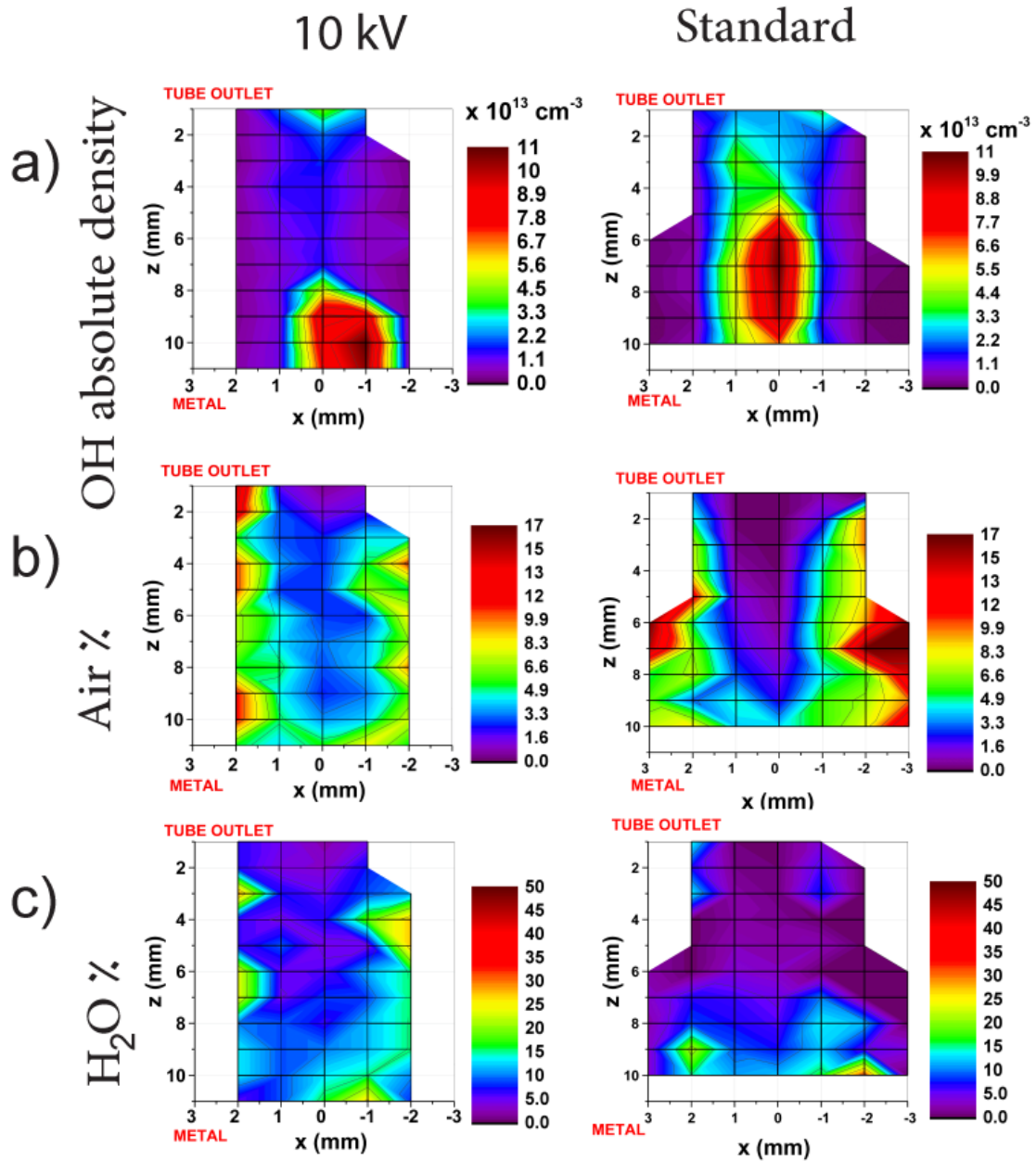


Figure 6.25: Case with a grounded metal target at 10 kV (left) compared to the standard configuration (right)., 2 kHz and He 1 l.min⁻¹. LIF maps showing distributions of a) OH density b) air and c) H₂O percentages. Scanning steps of 1 mm for vertical and radial directions.

5.2.2. Influence of the gas flow rate

To clarify the influence of the gas flow rate on the OH density production and distribution, an experiment performed at 500 sccm is compared to the standard condition as shown in Figure 6.26.

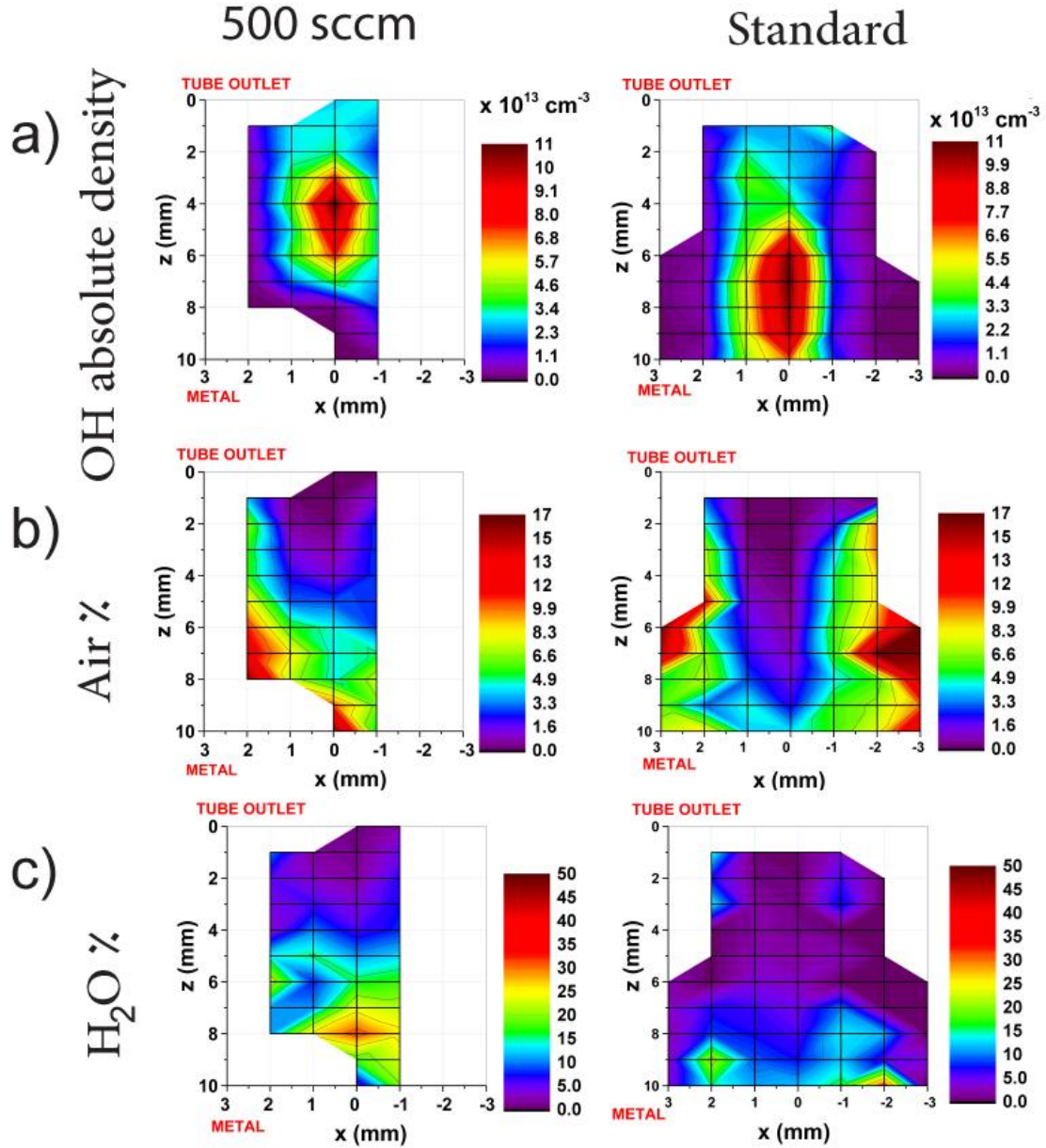


Figure 6.26: Case with a grounded metal target at 500 sccm (left) compared to the standard configuration (right), 2 kHz and 14 kV. LIF maps showing distributions of a) OH density b) air and c) H₂O percentages. Scanning steps of 1 mm for vertical and radial directions for the standard condition.

At 500 sccm, the OH density is of same order than at $1 \text{ l} \cdot \text{min}^{-1}$. However when looking at its distribution it differs from the standard condition, OH does not seem to be present on the target. The maximum OH density is situated at this gas flow rate between $z = 3$ to $z = 6$ which has to be compared to the wavelength-filtered imaging presented in Figure 4.12 in the free jet mode condition. As a matter of fact wavelength-filtered imaging informed us that at 500 sccm the emissions pattern of $\text{N}_2^*(\text{C})$ and NO^* are mainly situated around and downward the He^* emission in the relatively low Helium/air ratio zone. We can also compare with Figure 5.21 where the target surface is placed at 20 mm from the tube outlet. In this case

excited species emission patterns are covering the whole gap and exhibit their maximum in the middle-gap. Even with a smaller gap, here 12 mm, the LIF measurements show higher similarities with the free-jet mode condition. However a ratio of 5 can be observed between OH density at $z = 0$ mm and $[\text{OH}]_{\text{max}}$ which differs from the observation made in Figure 6.24a) (free-jet mode). This OH density ratio suggests that even with this small gas flow rate, the metal target as an influence on the OH production.

By comparing Air and H₂O maps to the ones at 1 l.min⁻¹, it can be pointed out that humid air is penetrating more deeply inside the helium jet, around $z = 5$ mm $z = 10$ mm.

It is worth noting that this feature does not agree with previously presented experiments performed with Schlieren imaging (Figure 5.10) and with wavelength filtered imaging (Figure 5.21) where gas flow channel as well as He*, N₂* and NO* emissions respectively reach the target surface. Knowing that the time integrated emission in the visible range is coherent with what is observed by LIF measurement, one hypothesis that could be made is that the instantaneous snapshot performed at 200 μ s after the pulse voltage is in this condition evolving in time and the averaging process as well as the reduced scanning region do not allow visualization of the thin channel of OH production to the target surface. At this specific time of measurement air and water penetration is too high which leads to OH ground state recombination.

The high penetration of humid air together with the increased OH density in the region where the Helium flow is supposed to bend show that even when no counter-propagating feature is observed, the conductive target influences the production of the OH radical.

5.3. Influence of target electrical conductivity

Since biological applications deal with samples of various water content and conductivity, OH distribution has been studied when PG plume impinges on aqueous targets of various electrical conductivities.

Plasma Gun plume impinges on an aqueous solution of 10 mm depth contained in a Petri dish placed on the same grounded metal surface as previously used, and the gap between the tube outlet and the target surface has been kept constant at 12 mm. Figure 6.27 shows comparison between conventional camera pictures and OH density distributions.

In Figure 6.27a) a solution of distilled water has been used which corresponds to an electrical conductivity of 1.5 μ S/cm while b) and c) correspond to physiological water, bought in drugstore 9 g/l of NaCl, and to a home-made saline solution, 20 g/l of NaCl, with electrical conductivities of 15.56 mS/cm and 34.7 mS/cm respectively; finally Figure 6.27d) shows the picture and OH distribution for the standard condition.

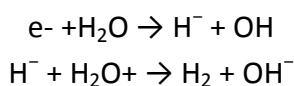
A correlation between the shape of the photographs and OH distribution in the maps can be observed, however emission intensities, dominated by N₂* (SPS and FNS), are far from

revealing information on OH radical densities. As it can be seen in Figure 6.27a), a high peaked OH density of $1.7 \times 10^{14} \text{ cm}^{-3}$ corresponds to the diffuse conic shape emission close to the target surface. On the contrary, with higher conductivity solutions, a larger volume of OH radicals localized close to the target can be observed (see Figure 6.27b) and c)), with specific features informing about the presence of a PAPS counter-propagation, in both photographs and LIF maps. It is worth noting the presence of diamond shapes in Figure 6.27b) which are, as previously mentioned, artifacts induced by interpolation between data. Despite these artifacts, maximum OH density data points are positioned in the same zone as in Figure 6.27c), which suggest that same OH distribution pattern is expected.

On one hand, comparison of OH distributions and maximum densities, $[\text{OH}]_{\text{max}} = 11 \times 10^{13} \text{ cm}^{-3}$, $9 \times 10^{13} \text{ cm}^{-3}$ and $7 \times 10^{13} \text{ cm}^{-3}$ found for metal target, saline and physiological solutions respectively suggests that the higher the target conductivity the stronger the production of PAPS counter-propagation and consequently the OH production rate. Comparison between saline and distilled water targets on the other hand, does not allow for straightforward conclusion about the role of the target conductivity.

However it can be suggest that two phenomena are undergoing. First point, distilled water which has a high dielectric constant can, under plasma irradiation, behaves as a leaky capacitor as suggested by Foster *et al.* [59]. They assumed that water can support a larger charge transfer in comparison with a solid dielectric since the charge buildup must compete with dissociative attachment and diffusion processes. The high density peaked at the surface of the distilled water will certainly not be observed for a solid dielectric surface. As a matter of fact dissociative attachment can occur from electron impact that may be facilitated by the presence of water vapor due to evaporation induced by the gas flow (as will be shown in Figure 6.28).

Dissociative attachment follows the reactions below:



Second point, the distilled water target is a dielectric or has a low electrical conductivity (see first point above) thus the OH production is confined at the water surface where charge deposition [60] and dissociative attachment [59] presumably occur. It implies that the electric field is neutralized by the charges at the surface and that no plasma counter-propagation is observed. A consequently rather low OH production is observed in the gap between the tube outlet and the target, except at the surface.

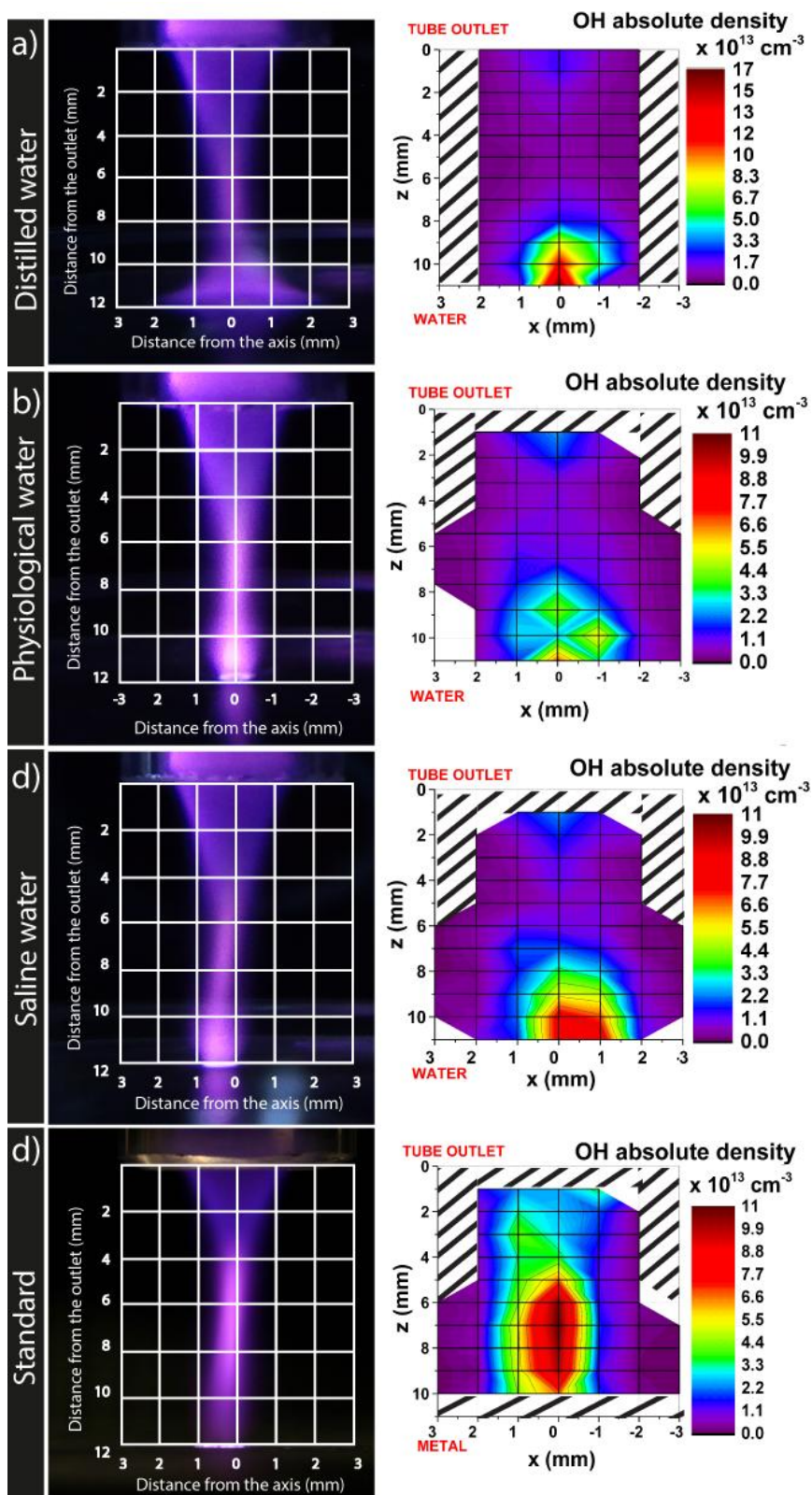


Figure 6.27: Influence of the electrical conductivity of aqueous targets on OH density distribution. Plasma parameters: 2 kHz, 14 kV, He 1 l.min⁻¹. Photographs (left side) on the same spatial scale than OH distribution maps (right side) a) Distilled water b) Physiological water c) Saline water and d) Standard condition. Scanning steps of 1 mm for vertical and radial directions.

Analysis of H_2O distributions (see Figure 6.28) allows clarification of the interplay between plasma and target surface for different conductivity. For distilled water, H_2O is not penetrating the propagation channel except at the vicinity of the surface where around 25 % is present which can be compared to the free jet configuration. As in the previous described case the maximum of OH density is situated in the region where humid air is mixing with the Helium flow. For higher solution conductivities however, some H_2O can be observed inside the channel up to 7 mm from the tube outlet. More interestingly surfaces appear disturbed at the impinging PAPS zone with high density of H_2O pushed apart from this point. The maximum H_2O percentage, $\sim 110\%$, implies that water vapor is also coming from the target.

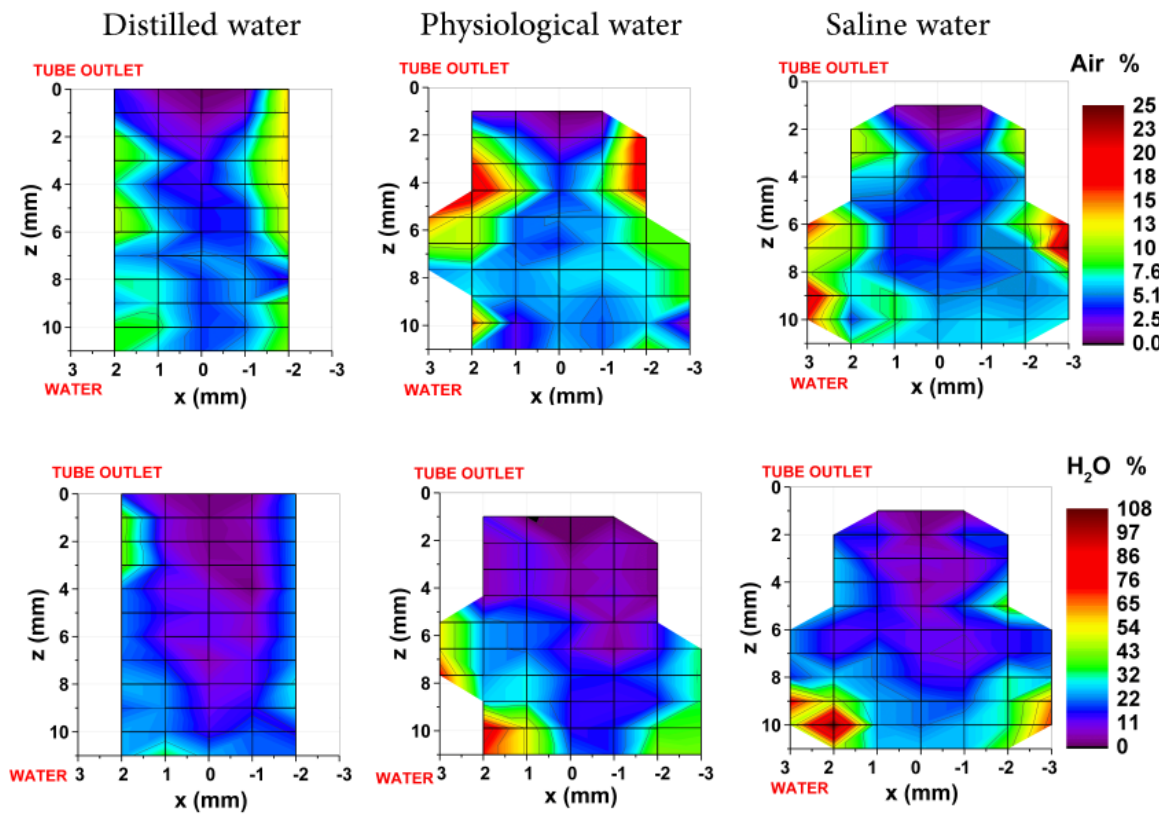


Figure 6.28: Influence of the conductivity of aqueous targets on air and H_2O distribution. Left: distilled water, middle: physiological water and right: saline solution. Scanning steps of 1 mm for vertical and radial directions.

By comparing the H_2O distribution patterns to the Air distribution ones, we observe that for the distilled water surface, the maximum of air is found around the plasma jet from the tube outlet to the target surface. This spatial air distribution is similar to the H_2O one, thus indicating that H_2O is coming from humid air. In the case of the conductive solutions, Air is mainly observed in the upper part of the gap (from $z = 2$ mm to $z = 8$ mm) while H_2O is mainly distributed close to the target surface. This confirms that H_2O in the vicinity of the target is coming from evaporation and not humid air. It is worth emphasizing that the discrimination between the quenchers (air and water) is particularly important when dealing

with complex environment such as the plasma plume expanding in the air and interacting with a liquid surface.

Regarding *in vitro* applications, these observations indicate that the type of culture media used modify the interaction between plasma and the surface, resulting in specific evaporation rate and RONS production.

5.4. Interplay between gas flow and OH production

5.4.1. Case of aqueous target: 2 l.min⁻¹

In order to increase OH density in the vicinity of the target surface, in addition to adapt delivered voltage amplitude, one may try to control H₂O uptake from the aqueous solution to reach optimal proportion between Helium and H₂O. One possibility to achieve this control is to change the gas flow rate. This has been tested and is presented in Figure 6.29 when PG plume impinges on distilled water at 2 l.min⁻¹ gas flow rate instead of 1 l.min⁻¹ in the previous reported experiment.

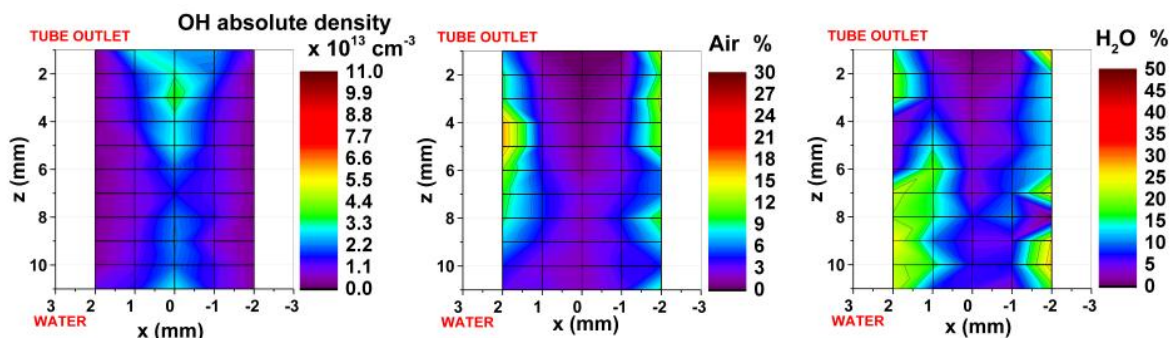


Figure 6.29: Case of distilled water with 2 l.min⁻¹ gas flow rate, 2 kHz and 14 kV. Distribution of OH density (left), air (middle) and H₂O (right). Scanning steps of 1 mm for vertical and radial directions.

In these conditions, air and water vapor do not penetrate into the Helium channel and mainly impurities contained in the buffer gas could lead to OH radical production. As seen in Figure 6.29b) and c), the Helium flow pushes everything away from the propagation axis with air proportion between 0.5 to 4% and H₂O between 1 to 10%. The maximum of 10% of H₂O, around 1400 ppm compared to 3 ppm in the buffer gas, which is measured at x = 0 mm along the gap implies that some water is coming from gas pipes and PG capillary, however the resulting jet is still a dry Helium flow. This low air and water penetration in the jet results in a more uniform OH distribution as compared to the case of 1 l.min⁻¹ He flow from z = 2 to 12 mm (see Figure 6.27a) and Figure 6.29a)). The production of OH radical in the vicinity of

the target - $[\text{OH}]_{\text{max}} = 3.7 \cdot 10^{13} \text{ cm}^{-3}$ - is much lower than at $1 \text{ l} \cdot \text{min}^{-1}$ gas flow rate $[\text{OH}]_{\text{max}} = 1.7 \cdot 10^{14} \text{ cm}^{-3}$ probably due to the fact that H_2O optimal proportion is not reached.

From the biomedical point of view, in which the local OH density on the target surface is of interest, these results suggest that a too much high gas flow may not be appropriated. However a high gas flow rate allows a control of OH distribution along the propagation path by lowering the influence of ambient air variation. Then the controlled admixture of molecular gases in the gas flow could be used as a mean for increasing the OH radical production rate.

Yonemori *et al.* reported [61] LIF and TALIF experiments on the influence of targets of various humidity contain on production of OH and O radicals. They observed that OH is influenced by the type of target especially considering their humidity, from dry to wet, as well as the flushed gas flow rate from 1.5 to $5 \text{ l} \cdot \text{min}^{-1}$. It is worth noting that their targets were always dielectrics. They end up with complementary observations in pretty good agreement with the distribution found in this work when the PG plume impinges on the distilled water surface (Figure 6.27, Figure 6.29). Although their experiments are not taking into account H_2O amount, gas flow modification and the target conductivity, they also suggested that OH production is following the plasma propagation and need some humid air impurities to be sustained. They performed experiments at different gas flow rate and observed that OH distribution become annular on the dielectric target with increasing Helium flow which pushes apart humid air. This annular shape has been also observed on dielectric targets with conventional pictures of the PG plume (Figure 5.1).

5.4.2. Influence of the gas impurities

As some impurities are already present in the Helium alphagaz1 used in all the above experiments, it is of certain interest to determine the influence of those in the distribution and density of OH radicals. For this purpose, experiment has been run with a high purity of Helium, the alphagaz2 of AirLiquid (99.9999% Helium) to be compared with the standard condition.

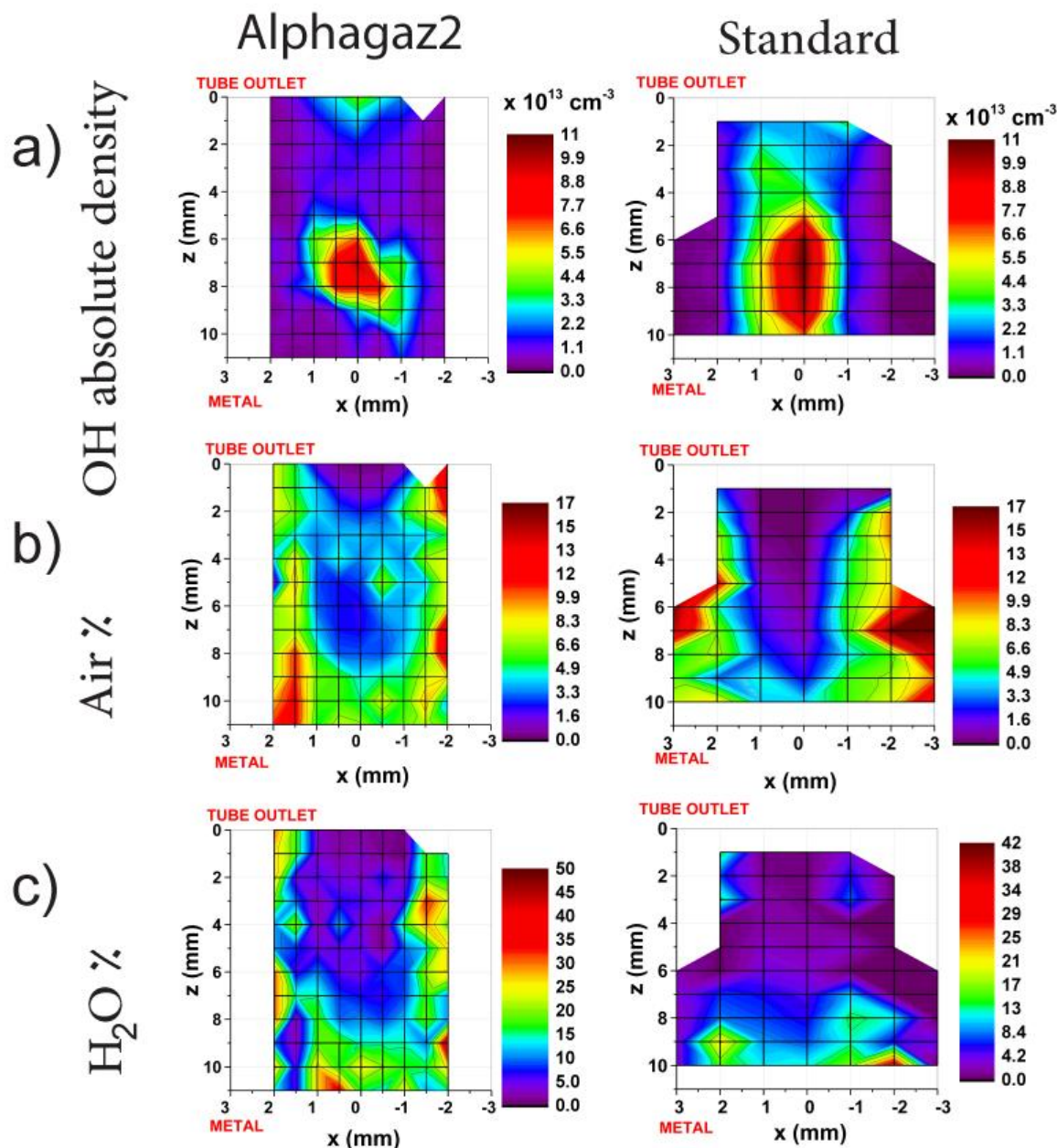


Figure 6.30: Case with a grounded metal target with He Alphagaz2 ($1 \text{ l} \cdot \text{min}^{-1}$) (left) compared to the standard configuration (right) at 2 kHz and 14 kV. LIF maps showing distributions of a) OH density b) air and c) H₂O percentages. Scanning steps of 0.5 mm and 1 mm for vertical and radial directions for the He alphagaz2 and standard conditions, respectively.

Comparison of the distributions using the two gases of different purity enlightens differences in the distribution of OH radicals as well as air and H₂O penetration in the gap. In the case of the Helium alphagaz2, the channeling is less efficient and some air and H₂O are penetrating around 4 mm after the tube outlet and from 9 mm to the target surface. The zone without humid air is reduced to a small cone which stops at 2 mm from the tube outlet. From 5 to 9 mm a zone of optimal water concentration is found which leads to a quasi-spherical OH distribution. The resulting OH density is slightly decreased as compared to the standard condition but is of same order of magnitude. This observation is yet not fully

understood and some complementary experiments using Schlieren imaging, wavelength-filtered and fast imaging would be needed to link those changes to physical and chemical phenomena. Nevertheless the OH distribution is coherent with the optimal H₂O% established previously, the zone with high OH density correspond to the region of H₂O% from 10 to 15.

It is hard to differentiate the causes and the consequences of such differences because, as it has been shown in this work, all parameters influence each other. However some suggestions can be made. The few ppm of N₂ present in the Helium alphagaz1 may allow a higher channeling of the Helium flow. We can suggest that energy transfers are partially happening with impurities upstream in the capillary, achieving an equilibrium before reaching the tube outlet.

Another point is that the small quantity of N₂ present in the gas is a good charge carrier which leads to higher ionic drift responsible of the high Helium channeling. Similarly it has been reported [62] that small amount of N₂ in the buffer gas increase PAPS propagation length together with changes in ionization front pattern and velocity.

6. Conclusion

Quantitative Laser Induced Fluorescence has been performed to estimate OH density and air and water penetration in a complex system. The numerical model developed at IMIP, Bari by G. Dilecce *et al.* was for the first time applied to the plume of a plasma jet. Efforts were made to characterize PG plume in conditions as close as possible to biomedical applications by using various targets standing for mimicking living animals, humid environments or culture cells media. The targets chosen were a metallic plate or a Petri dish filled with aqueous solutions. By the use of aqueous target, the numerical model proved to allow discrimination between the different quenchers, Air and H₂O. Parameters such as gas flow rate, and voltage amplitude as well as conductivity of the aqueous targets have been modified to get an overview of the influence of those parameters on the production of OH radical ground state.

Similarly to the results obtained with other diagnostics in Chapter 4 and 5, the presence of a target appears to have a drastic influence on OH density distribution and humid air penetration inside the plasma plume. OH distribution captured when impinging on a conductive target shows specific feature of the PAPS counter-propagation which suggests that OH is produced during the whole PAPS propagation, upstream and downstream.

We have enlightened that OH density amplitude and distribution might be controlled on the vicinity of the target by modifying critical parameters such as voltage amplitude and gas flow rate. Moreover air spatial distributions which are consistent with previously reported Schlieren imaging, and water spatial distribution obtained here by LIF, have confirmed the

strong influence of target conductivity on the mutual-interaction between plasma and gas flow. This gas guiding potentiality of PAPS especially when interacting with a conductive surface is indeed important for biomedical applications but also could lead to new application of the PG.

Additional experiments were performed on the influence of the conductivity of humid target revealing an enhancement of OH production near the target for low conductivity water sample or when only a thin layer of water vapor is surrounding a conductive aqueous solution. It has been observed that a optimal H₂O% of 10-15%, enhances OH production. LIF experiments lead also to supplementary information about the influence of the N₂ contain in the buffer gas in the gas channeling by plasma.

Multi-diagnostics approach appears to be mandatory to obtain comprehensive interpretation of OH spatial distribution and density in the plasma jet's plume. As a matter of fact, understanding often comes from the comparison of LIF measurements to OES measurements, Schlieren and wavelength-filtered imaging experiments. Finally these experiments show how much care should be taken in establishment of set ups similar to biomedical treatment conditions for plasma jet *in-situ* diagnostics. These observations are of major importance for *in vivo* experiments when dealing with inner humid organs or with skin perspiration.

French summary of Chapter 6

Dans les chapitres 4 et 5, l'influence de la présence d'une cible conductrice en face de la plume plasma fut commentée. Il a été montré que le plasma produit par le Plasma Gun est fortement influencé par la modification du champ électrique consécutive à la présence de la cible. Des phénomènes tels que la production d'une contre-propagation du PAPS, la modification de la production des espèces excitées et la canalisation du gaz sont induits. Il apparaît que le système à étudier doit tenir compte de la cible pour toute caractérisation du plasma. De plus, pour envisager des futurs essais cliniques, il y a un besoin de quantifier les espèces produites par les jets de plasma. Le radical OH fut ciblé en tant que l'une des molécules les plus biologiquement actives.

Ainsi une étude quantitative de la distribution du radical OH dans la plume a été effectuée par fluorescence induite par laser (LIF), mettant l'accent sur la présence de cibles les plus similaires possible aux cibles biologiques. Les cibles choisies furent une plaque de métal et des boîtes de Petri remplies de solutions aqueuses de diverses conductivités électriques.

Cette étude fut effectuée à l'IMIP de Bari en collaboration avec Giorgio Dilecce et son équipe grâce à leur modèle numérique développé pour estimer non seulement la densité de OH mais aussi, et simultanément, la présence d'air et d'eau à chaque point de mesures. L'étude par LIF de la plume plasma est particulièrement difficile compte tenu de son inhomogénéité spatiale et temporelle. Ce modèle numérique fut appliqué pour la première fois, lors de cette étude, au système complexe d'un jet de plasma se propageant dans l'air ambiant. De plus lors de l'utilisation des cibles aqueuses, le modèle numérique a prouvé être en mesure de permettre la discrimination entre l'influence de l'air et de l'eau. L'étude LIF a permis de confirmer l'influence drastique de la présence d'une cible qui fut observée par le biais de l'OES sur les espèces excitées et par imagerie Schlieren sur la canalisation du gaz.

La distribution de OH a montré les caractéristiques spécifiques de la contre-propagation du PAPS ce qui suggère que OH est produit pendant toute la propagation, que ce soit "downstream" ou "upstream". L'étude de l'influence de paramètres tels que le débit du gaz et l'amplitude de tension révéla que la densité du radical ainsi que sa distribution spatiale peuvent être optimisées, en vue d'un dépôt sur la surface de la cible à traiter, en jouant sur ces paramètres critiques.

La distribution de l'air, qui est cohérente avec l'imagerie Schlieren et de l'eau, estimée ici par LIF, a aussi confirmée l'influence de la cible dans la canalisation du gaz par le plasma. Des expériences complémentaires ont été effectuées pour approfondir l'influence de la conductivité de la cible. Il a été suggéré qu'il existe un optimum de concentration de H₂O, 10-15% de l'humidité ambiante, pour un maximum de production de OH. Une solution aqueuse de faible conductivité électrique produit un pic de production de OH proche cible

alors qu'avec l'augmentation de la conductivité électrique, le motif caractéristique de la contre-propagation peut être observé ainsi que la perturbation de la surface liquide. Une étude, quoique préliminaire, permet de suggérer l'influence des impuretés présentes dans le gaz, et plus particulièrement de N_2 , dans sa canalisation. D'autres études complémentaires sont actuellement en cours par OES.

Il apparaît dans ce chapitre, que l'approche multi-diagnostique est fortement souhaitée pour obtenir une interprétation cohérente de la distribution spatiale et de la densité de OH dans la plume plasma. En effet, la compréhension des résultats obtenus par LIF émerge majoritairement de la comparaison avec les mesures OES et l'imagerie filtrée en longueur d'onde et/ou Schlieren. De plus, nos observations appuient le fait de considérer la cible comme partie intégrante du système à étudier et évoquent l'importance de son influence lors des expériences *in vivo* où les cibles varient en conductivité électrique et en concentration d'humidité.

References

- [1] Xiong, Z., Robert, E., Sarron, V., Pouvesle, J.-M., Kushner, M.J., 2012. Dynamics of ionization wave splitting and merging of atmospheric-pressure plasmas in branched dielectric tubes and channels. *J. Phys. D: Appl. Phys.* 45, 275201. doi:10.1088/0022-3727/45/27/275201 [2] J L Walsh, P Olszewski and J W Bradley *Plasma Sources Sci. Technol.***21** (2012) 034007
- [3] Naidis, G.V., Walsh, J.L., 2013. The effects of an external electric field on the dynamics of cold plasma jets—experimental and computational studies. *J. Phys. D: Appl. Phys.* 46, 095203. doi:10.1088/0022-3727/46/9/095203
- [4] Robert, E., Sarron, V., Darny, T., Riès, D., Dozias, S., Fontane, J., Joly, L., Pouvesle, J.-M., 2014. Rare gas flow structuration in plasma jet experiments. *Plasma Sources Sci. Technol.* 23, 012003. doi:10.1088/0963-0252/23/1/012003 [5] Kong M G *et al* 2009 “Plasma medicine: an introductory review”, *New J. Phys.***11** 115012
- [6] Gessel, A.F.H. van, Hrycak, B., Jasiński, M., Mizeraczyk, J., Mullen, J.J.A.M. van der, Bruggeman, P.J., 2013. Temperature and NO density measurements by LIF and OES on an atmospheric pressure plasma jet. *J. Phys. D: Appl. Phys.* 46, 095201. doi:10.1088/0022-3727/46/9/095201
- [7] Douat, C., Bauville, G., Fleury, M., Laroussi, M., Puech, V., 2012. Dynamics of colliding microplasma jets. *Plasma Sources Sci. Technol.* 21, 034010. doi:10.1088/0963-0252/21/3/034010
- [8] Riès, D., Robert, E., Sarron, V., Darny, T., Dozias, S., Pouvesle, J.-M., 2013. On the influence of a target on ROS and RNS production using a μ s driven Plasma Gun for biomedical applications. 31st Int. Conf. on Phenomena in Ionized Gases, ICPIG (Granada, Spain, 14–19 July 2013)
- [9] Vandamme, M., 2012. Les plasmas froids, nouvelle stratégie thérapeutique en cancérologie. Orléans.
- [10] Vandamme, M., Robert, E., Lerondel, S., Sarron, V., Riès, D., Dozias, S., Sobilo, J., Gosset, D., Kieda, C., Legrain, B., Pouvesle, J.-M., Pape, A.L., 2012. ROS implication in a new antitumor strategy based on non-thermal plasma. *Int. J. Cancer* 130, 2185–2194. doi:10.1002/ijc.26252
- [11] Brullé, L., Vandamme, M., Riès, D., Martel, E., Robert, E., Lerondel, S., Trichet, V., Richard, S., Pouvesle, J.-M., Le Pape, A., 2012. Effects of a Non Thermal Plasma Treatment Alone or in Combination with Gemcitabine in a MIA PaCa2-luc Orthotopic Pancreatic Carcinoma Model. *PLoS ONE* 7, e52653. doi:10.1371/journal.pone.0052653

- [12] Isbary, G., Zimmermann, J.L., Shimizu, T., Li, Y.-F., Morfill, G.E., Thomas, H.M., Steffes, B., Heinlin, J., Karrer, S., Stolz, W., 2013. Non-thermal plasma—More than five years of clinical experience. *Clinical Plasma Medicine* 1, 19–23. doi:10.1016/j.cpme.2012.11.001
- [13] Daeschlein, G., Scholz, S., Lutze, S., Arnold, A., von Podewils, S., Kiefer, T., Tueting, T., Hardt, O., Haase, H., Grisk, O., Langner, S., Ritter, C., von Woedtke, T., Jünger, M., 2013. Comparison between cold plasma, electrochemotherapy and combined therapy in a melanoma mouse model. *Exp Dermatol* 22, 582–586. doi:10.1111/exd.12201
- [14] Keidar, M., Walk, R., Shashurin, A., Srinivasan, P., Sandler, A., Dasgupta, S., Ravi, R., Guerrero-Preston, R., Trink, B., 2011. Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy. *Br J Cancer* 105, 1295–1301. doi:10.1038/bjc.2011.386
- [15] Fridman, G., Friedman, G., Gutsol, A., Shekhter, A.B., Vasilets, V.N., Fridman, A., 2008. Applied Plasma Medicine. *Plasma Processes Polym.* 5, 503–533. doi:10.1002/ppap.200700154
- [16] Ninomiya, K., Ishijima, T., Imamura, M., Yamahara, T., Enomoto, H., Takahashi, K., Tanaka, Y., Uesugi, Y., Shimizu, N., 2013. Evaluation of extra- and intracellular OH radical generation, cancer cell injury, and apoptosis induced by a non-thermal atmospheric-pressure plasma jet. *Journal of Physics D: Applied Physics* 46, 425401. doi:10.1088/0022-3727/46/42/425401
- [17] Zdenko Machala, B.T., 2013. Formation of ROS and RNS in Water Electro-Sprayed through Transient Spark Discharge in Air and their Bactericidal Effects. *Plasma Processes and Polymers*. doi:10.1002/ppap.201200113
- [18] Boekema, B.K.H.L., Hofmann, S., Ham, B.J.T. van, Bruggeman, P.J., Middelkoop, E., 2013. Antibacterial plasma at safe levels for skin cells. *J. Phys. D: Appl. Phys.* 46, 422001. doi:10.1088/0022-3727/46/42/422001
- [19] Sobota, A., Guaitella, O., Garcia-Caurel, E., 2013. Experimentally obtained values of electric field of an atmospheric pressure plasma jet impinging on a dielectric surface. *J. Phys. D: Appl. Phys.* 46, 372001. doi:10.1088/0022-3727/46/37/372001
- [20] Topala, I., Nastuta, A.V., Jijie, R., Pohoata, V., Dumitrascu, N., 2012. Temporal kinetics of light emission from plasma at the interface with animal tissues. 4th International Conference on Plasma Medicine, ICMP4 (Orléans, France, 7-21 June 2012).
- [21] Xiong, Q., Nikiforov, A.Y., González, M.Á., Leys, C., Lu, X.P., 2013. Characterization of an atmospheric helium plasma jet by relative and absolute optical emission spectroscopy. *Plasma Sources Sci. Technol.* 22, 015011. doi:10.1088/0963-0252/22/1/015011

- [22] Graves, D.B., 2012. The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology. *J. Phys. D: Appl. Phys.* 45, 263001. doi:10.1088/0022-3727/45/26/263001
- [23] Ono, R., Oda, T., 2002. Dynamics and density estimation of hydroxyl radicals in a pulsed corona discharge. *J. Phys. D: Appl. Phys.* 35, 2133. doi:10.1088/0022-3727/35/17/309
- [24] Ono, R., Oda, T., 2003. Dynamics of ozone and OH radicals generated by pulsed corona discharge in humid-air flow reactor measured by laser spectroscopy. *Journal of Applied Physics* 93, 5876–5882. doi:10.1063/1.1567796
- [25] Kanazawa, S., Tanaka, H., Kajiwara, A., Ohkubo, T., Nomoto, Y., Kocik, M., Mizeraczyk, J., Chang, J.-S., 2007. LIF imaging of OH radicals in DC positive streamer coronas. *Thin Solid Films, SPSM 18 - The 18th Symposium on Plasma Science for Materials SPSM 18* 515, 4266–4271. doi:10.1016/j.tsf.2006.02.046
- [26] Nakagawa, Y., Ono, R., Oda, T., 2011. Density and temperature measurement of OH radicals in atmospheric-pressure pulsed corona discharge in humid air. *Journal of Applied Physics* 110, 073304. doi:10.1063/1.3638457
- [27] Kanazawa, S., Kawano, H., Watanabe, S., Furuki, T., Akamine, S., Ichiki, R., Ohkubo, T., Kocik, M., Mizeraczyk, J., 2011. Observation of OH radicals produced by pulsed discharges on the surface of a liquid. *Plasma Sources Sci. Technol.* 20, 034010. doi:10.1088/0963-0252/20/3/034010
- [28] Magne, L., Pasquiers, S., Blin-Simiand, N., Postel, C., 2007. Production and reactivity of the hydroxyl radical in homogeneous high pressure plasmas of atmospheric gases containing traces of light olefins. *J. Phys. D: Appl. Phys.* 40, 3112. doi:10.1088/0022-3727/40/10/015
- [29] Dilecce, G., Benedictis, S.D., 2011. Laser diagnostics of high-pressure discharges: laser induced fluorescence detection of OH in He/Ar–H₂O dielectric barrier discharges. *Plasma Phys. Control. Fusion* 53, 124006. doi:10.1088/0741-3335/53/12/124006
- [30] Dilecce, G., Ambrico, P.F., Simek, M., De Benedictis, S., 2012. LIF diagnostics of hydroxyl radical in atmospheric pressure He–H₂O dielectric barrier discharges. *Chemical Physics, Chemical Physics of Low-Temperature Plasmas (in honour of Prof Mario Capitelli)* 398, 142–147. doi:10.1016/j.chemphys.2011.03.012
- [31] Verreycken, T., Horst, R.M. van der, Baede, A.H.F.M., Veldhuizen, E.M.V., Bruggeman, P.J., 2012. Time and spatially resolved LIF of OH in a plasma filament in atmospheric pressure He–H₂O. *J. Phys. D: Appl. Phys.* 45, 045205. doi:10.1088/0022-3727/45/4/045205

- [32] Verreycken, T., Bruggeman, P.J., 2014. OH density measurements in nanosecond pulsed discharges in atmospheric pressure N₂–H₂O mixtures. *Plasma Sources Sci. Technol.* 23, 015009. doi:10.1088/0963-0252/23/1/015009
- [33] Choi, I., Yin, Z., Adamovich, I.V., Lempert, W.R., 2011. Hydroxyl Radical Kinetics in Repetitively Pulsed Hydrogen–Air Nanosecond Plasmas. *IEEE Transactions on Plasma Science* 39, 3288–3299. doi:10.1109/TPS.2011.2163736
- [34] Lacoste, D.A., Xu, D.A., Moeck, J.P., Laux, C.O., 2013. Dynamic response of a weakly turbulent lean-premixed flame to nanosecond repetitively pulsed discharges. *Proceedings of the Combustion Institute* 34, 3259–3266. doi:10.1016/j.proci.2012.07.017
- [35] Xiong, Q., Nikiforov, A.Y., Li, L., Vanraes, P., Britun, N., Snyders, R., Lu, X.P., Leys, C., 2012. Absolute OH density determination by laser induced fluorescence spectroscopy in an atmospheric pressure RF plasma jet. *Eur. Phys. J. D* 66, 1–8. doi:10.1140/epjd/e2012-30474-8
- [36] Yonemori, S., Nakagawa, Y., Ono, R., Oda, T., 2012. Measurement of OH density and air–helium mixture ratio in an atmospheric-pressure helium plasma jet. *J. Phys. D: Appl. Phys.* 45, 225202. doi:10.1088/0022-3727/45/22/225202
- [37] Voráč, J., Dvořák, P., Procházka, V., Ehlbeck, J., Reuter, S., 2013. Measurement of hydroxyl radical (OH) concentration in an argon RF plasma jet by laser-induced fluorescence. *Plasma Sources Sci. Technol.* 22, 025016. doi:10.1088/0963-0252/22/2/025016
- [38] XPei, X., Lu, Y., Wu, S., Xiong, Q., Lu, X., 2013. A study on the temporally and spatially resolved OH radical distribution of a room-temperature atmospheric-pressure plasma jet by laser-induced fluorescence imaging. *Plasma Sources Sci. Technol.* 22, 025023. doi:10.1088/0963-0252/22/2/025023
- [39] Verreycken, T., Mensink, R., Horst, R. van der, Sadeghi, N., Bruggeman, P.J., 2013. Absolute OH density measurements in the effluent of a cold atmospheric-pressure Ar–H₂O RF plasma jet in air. *Plasma Sources Sci. Technol.* 22, 055014. doi:10.1088/0963-0252/22/5/055014
- [40] Dilecce, G., 2014. Optical spectroscopy diagnostics of discharges at atmospheric pressure. *Plasma Sources Sci. Technol.* 23, 015011. doi:10.1088/0963-0252/23/1/015011
- [41] Riès, D., Dilecce, G., Robert, E., Ambrico, P.F., Dozias, S., Pouvesle, J.-M., 2014. LIF and fast imaging plasma jet characterization relevant for NTP biomedical applications. *J. Phys. D: Appl. Phys.* 47, 275401. doi:10.1088/0022-3727/47/27/275401

- [42] De Benedictis, S., Dilecce, G., 2013. Low Temperature Plasma Technology: Methods and Applications. Chu, P.K., Lu, X.P., chapter 9, 261-284 CRC Press.
- [43] Williams, L.R., Crosley, D.R., 1996. Collisional vibrational energy transfer of OH ($A\ 2\Sigma^+$, $v'=1$). The Journal of Chemical Physics 104, 6507–6514. doi:10.1063/1.471371
- [44] Copeland, R.A., Dyer, M.J., Crosley, D.R., 1985. Rotational-level-dependent quenching of $A\ 2\Sigma^+$ OH and OD. The Journal of Chemical Physics 82, 4022–4032. doi:10.1063/1.448843
- [45] Burris, J., Butler, J.J., Mcgee, T.J., Heaps, W.S., 1988. Collisional deactivation rates for $A\ 2\Sigma^+$ ($v' = 1$) state of OH. Chemical Physics 124, 251–258. doi:10.1016/0301-0104(88)87154-4
- [46] Copeland, R.A., Wise, M.L., Crosley, D.R., 1988. Vibrational energy transfer and quenching of OH($A2\Sigma^+, v'=1$). Journal of physical chemistry 92, 5710–5715.
- [47] German, K.R., 1976. Collision and quenching cross sections in the $A\ 2\Sigma^+$ state of OH and OD. The Journal of Chemical Physics 64, 4065–4068. doi:10.1063/1.432015
- [48] Wysong, I.J., Jeffries, J.B., Crosley, D.R., 1990. Quenching of $A\ 2\Sigma^+$ OH at 300 K by several colliders. The Journal of Chemical Physics 92, 5218–5222. doi:10.1063/1.458558
- [49] Bailey, A.E., Heard, D.E., Henderson, D.A., Paul, P.H., 1999. Collisional quenching of OH($A\ 2\Sigma^+$, $v'=0$) by H₂O between 211 and 294 K and the development of a unified model for quenching. Chemical Physics Letters 302, 132–138. doi:10.1016/S0009-2614(99)00076-7
- [50] Lengel, R.K., Crosley, D.R., 1978. Energy transfer in $A\ 2\Sigma^+$ OH. II. Vibrational. The Journal of Chemical Physics 68, 5309–5324. doi:10.1063/1.435707
- [51] Cathey, C., Cain, J., Wang, H., Gundersen, M.A., Carter, C., Ryan, M., 2008. OH production by transient plasma and mechanism of flame ignition and propagation in quiescent methane–air mixtures. Combustion and Flame 154, 715–727. doi:10.1016/j.combustflame.2008.03.025
- [52] Luque, J., and Crosley, D., 1999. International report p99-009 Technical report SRI
- [53] Belostotskiy, S.G., Ouk, T., Donnelly, V.M., Economou, D.J., Sadeghi, N., 2010. Gas temperature and electron density profiles in an argon dc microdischarge measured by optical emission spectroscopy. Journal of Applied Physics 107, 053305–053305–7. doi:10.1063/1.3318498

- [54] Eslami, E., 2005. Etude des mécanismes de formation de la zone d'ionisation secondaire en post-décharge d'un plasma micro-onde d'azote. PhD thesis, Grenoble I, (2005).
- [55] Iseni, S., Reuter, S., Schmidt-Bleker, A., Weltmann, K.-D., 2014. Flow and Discharge Development in an Argon Atmospheric Pressure Plasma Jet Observed by ICCD and PLIF Imaging. IEEE Transactions on Plasma Science PP, 1–1. doi:10.1109/TPS.2014.2321226
- [56] Dilecce, G., Ambrico, P.F., Simek, M., Benedictis, S.D., 2012. OH density measurement by time-resolved broad band absorption spectroscopy in an Ar–H₂O dielectric barrier discharge. J. Phys. D: Appl. Phys. 45, 125203. doi:10.1088/0022-3727/45/12/125203
- [57] LabVIEW (National Instruments Corporation, Austin, Texas)
- [58] Robert, E., Sarron, V., Riès, D., Dozias, S., Vandamme, M., Pouvesle, J.-M., 2012. Characterization of pulsed atmospheric-pressure plasma streams (PAPS) generated by a plasma gun. Plasma Sources Sci. Technol. 21, 034017. doi:10.1088/0963-0252/21/3/034017
- [59] Foster, J., Sommers, B., Weatherford, B., Yee, B., Gupta, M., 2011. Characterization of the evolution of underwater DBD plasma jet. Plasma Sources Sci. Technol. 20, 034018. doi:10.1088/0963-0252/20/3/034018
- [60] Xiong, Z., Robert, E., Sarron, V., Pouvesle, J.-M., Kushner, M.J., 2013. Atmospheric-pressure plasma transfer across dielectric channels and tubes. J. Phys. D: Appl. Phys. 46, 155203. doi:10.1088/0022-3727/46/15/155203
- [61] Yonemori, S., Ono, R., 2014. Flux of OH and O radicals onto a surface by an atmospheric-pressure helium plasma jet measured by laser-induced fluorescence. J. Phys. D: Appl. Phys. 47, 125401. doi:10.1088/0022-3727/47/12/125401
- [62] Darny, T., Robert, E., Dozias, S., Pouvesle, E., 2014. Modulation of ionization front propagation velocity in a μ s plasma gun helium discharge with nitrogen admixture. GD2014, Orléans, France

Conclusion and perspectives

Since the development of first non-thermal plasma jets at atmospheric pressure few years ago, the study of their physics and chemical properties has been reported in a continuously growing number of publications worldwide. The apparent disconnection of the "plasma bullet" to the power source together with the close-to-room temperature of such plasma; were especially attractive among the plasma community. These plasma sources quickly appeared well suited for biomedical applications such as a new applications-directed research field emerged, Plasma Medicine. At GREMI one APR and one ANR project, PLASMED and PAMPA, were directed to the optimization of the Plasma Gun and understanding of both the physics behind the plasma and the plasma-induced biological mechanisms in cancerology. In this frame, the main aim of this work was to estimate qualitatively the influence of input parameters on the production of reactive species in order to optimize biomedical applications.

To this extend multiple diagnostics had been carried out for the plasma characterization as well as for the biomedical application.

Prior to the characterization of the plasma, the antitumor potential of the Plasma Gun was investigated through *in vitro* and *in vivo* experiments on Mia PaCa cell line corresponding to human pancreatic carcinoma. Parametric study *in vitro* was carried out to estimate if the voltage waveform or the pulse repetition rate could influence the survival rate of the cells. It appeared that the ns rise time pulses were more efficient than μ s ones however this power source was pulse-repetition-rate limited. The cell response *in vitro* is linearly dependant on the number of delivered pulses thus by increasing the pulse repetition rate the treatment efficiency can be improved. However tolerance studies showed that living tissues could be damaged when exceeding 3 kHz, limiting the treatment to 2 kHz pulse repetition rate.

The *in vivo* studies showed that whatever the rising front and pulse duration as well as the pulse repetition rate and the nature of the buffer gas, the tumor activity was reduced. This result tends to support that the plasma is acting as a stimulus inducing biological processes inside the organism. During a comparison between the reference chemotherapy, gemcitabine, and the plasma, the plasma treatment has proven higher efficiency in reducing the tumor volume and activity. Moreover the combination of the two treatments appeared as significantly more efficient than the gemcitabine or plasma treatments alone. This study was, to the best of our knowledge, the first *in vivo* demonstration of non-thermal plasma jet antitumor activity on orthotopic tumors. It would indeed be needed to further extend the assays to estimate if the plasma action has a synergic effect with the gemcitabine. Although the results presented in this work are promising, long-term *in vivo* experiments should be

first conducted on animal models, to estimate a potential plasma antitumor efficiency on humans.

As the Plasma Gun demonstrated antitumor action on cells and tumors, the characterization of the plasma produced by the Plasma Gun was then under investigation. We first focus on the characterization of the Plasma Gun when freely expending in the ambient air, so called the "free-jet" mode.

Experiments from V. Sarron parallel PhD thesis, focused on the understanding of the mechanisms governing the ignition and propagation of the plasma inside the Plasma Gun capillary. It is described why the denomination Pulsed Atmospheric-pressure Plasma Stream (PAPS) is more adapted than the historically "plasma bullet" terminology. As a matter of fact, the control of the ionization front propagation length through the voltage pulse duration experimentally indicates the key role of the plasma tail in sustaining its propagation. Comparison with numerical model from Xiong and Kushner supports those observations.

In this PhD study, the Plasma Gun has been positioned top-down to limit the differences with biomedical applications conditions. The characterization of the PG plume was undertaken thanks to imaging systems and optical emission spectroscopy. A mutual interaction between the gas flow and the plasma, leading to channeling of the gas flow, is evidenced through Schlieren photography. The gas jet channeling by the plasma, which exhibits a dependency to pulse repetition and gas flow rates, is confirmed through wavelength-filtered imaging and fast-imaging diagnostics. Spatial and time resolved OES allowed identifying the excited reactive species produced by the PG, and to elaborate hypothesis concerning the kinetics undergoing. In this configuration, a two-step process can be enlightened. The first main excitation mechanism comes from electronic collisions with the neutral species as well as direct dissociation of water and penning and charge transfers from He excited atoms and molecular ions to N_2 ; N_2 and H_2O being present as impurities in the buffer gas. Then transfers to the surrounding air component are occurring. The produced excited species are recombining and relaxing, forming radicals and long living reactive species which are identified in the gas effluent through preliminary Fourier Transform InfraRed spectroscopy (FTIR) measurements. Molecules such as NO , NO_2 , N_2O and acids HNO_2 and HNO_3 were evidenced through those measurements. These reactive molecules are well-known from the biologists to induce oxidative stresses to the living organisms.

We then focused on the analysis of diverse parameters influence on the plasma properties when the PG impinges on a target surface, first on dielectric targets and then on conductive targets. We have seen that the presence of a dielectric target is only slightly changing the RONS production distribution as compared to the free-jet condition. This modification is induced by the obstacle placed on the gas flow.

On the contrary the presence of a conductive target has shown to have drastic effect on plasma properties. The plasma propagation as well as the gas flow is modified prior to the

impingement as a result of the modification of the potential distribution induced by the presence of the grounded conductive target. We observe PAPS counter-propagation after the impingement. This secondary plasma propagation leads to important changes in RONS production and distribution. This counter-propagation conserves some similarities with the downstream propagation such as the connection through the PAPS tail to the powered electrode and extinction with the voltage amplitude decrease, however its velocity is much lower. Further experiments and probably numerical models would be needed to better understand the physics producing the counter-propagating PAPS.

Another important modification observed is the Helium flow channeling toward the grounded surface which appeared much more efficient in the presence of a grounded target. The hypothesis of an increased drift of ionic species is made, induced by the difference of potential between the ionization front and the grounded metal plate together with the ionization front induced electric field. Energy losses are greatly decreased in the Helium channel as compared to Helium-air mixing zone which could partially explain why the plasma may then experience an upstream propagation. This plasma has a characteristic pink emission mainly linked to the transition of N_2^* (B-A) system observable only in this condition. Moreover NO^* γ bands emission is also drastically enhanced by the presence of the conductive target.

The parametric studies undertaken indicates that the gas hydrodynamics and the plasma counter-propagation behaviors are dependent of a combination of many parameters such as the gap distance, the voltage amplitude, the gas flow and pulse repetition rates. Indeed the modification of the He channeling and the plasma propagation with the parameters leads to differences in the production of the excited reactive species.

It has been shown that the excited species are repopulated during the counter-PAPS propagation, except N_2^{+*} . Those species have been shared into two groups in regards of their early and late emission ratio in time-resolved emission measurements as well as their spatial distribution along the gap. First group is thought to be mainly excited by electron impact of high energy, He^* , O^* and N_2^{+*} and the second group by those of lower energy and subsequent energy transfers, N_2^* (C), N_2^* (B), OH^* and NO^* . Performing simultaneous time-resolved Schlieren and wavelength-filtered imaging would allow to validate some hypothesis and to lead to a better understanding of the system.

The influence of the conductive surface has also been shown by FTIR measurements where long living species production is increased as compared to the free jet mode. Further experiments would be needed aiming at quantifying the produced species in the different operating modes and as a function of the input plasma parameters by FTIR. Chemical water titrations and mass spectrometry could also be interesting diagnostics to overlap information and gain insights in the long term kinetics.

In the last section, we focused on the estimation of OH density together with air and water penetration by quantitative Laser Induced Fluorescence. Those experiments were performed at IMIP, Bari in collaboration with G. Dilecce and his group. They allowed first validation of the numerical method developed by his team onto a plasma plume expending in the air and impinging on targets. We used various targets standing for mimicking living animals, humid environments or culture cells media. The targets chosen were a metallic plate or a Petri dish filled with aqueous solutions of different electrical conductivity.

Similarly to the results obtained with other diagnostics in Chapter 4 and 5, the presence of a target appears to have a drastic influence not only on OH density distribution but also on humid air penetration inside the plasma plume. On one hand, OH distribution pattern showed specific feature of the PAPS counter-propagation when impinging on a conductive target. This suggests that OH is produced during the whole PAPS propagation, upstream and downstream. Thanks to those results, the optimization of OH density distribution in the vicinity of the target might be reached by modifying the critical parameters such as voltage amplitude and gas flow rate.

On the other hand, air and water distribution confirmed the strong influence of target conductivity on the inter-play between plasma and gas flow. A more detailed study on the plasma induced channeling is now going on. This phenomenon is indeed important for biomedical applications but also could lead to new application of the PG such as in the plasma actuators field.

To enlarge the study on the influence of the target conductivities, OH density distribution has been plotted when plasma impinged on different aqueous solutions. It revealed an enhancement of OH production near the target for low conductivity water sample or when water vapor is surrounding a conductive aqueous solution. It appeared that an optimal H₂O% of 10-15% enhances OH production. Another point linked to the impurity contain of the buffer gas has enlightened the influence of the N₂ in the gas channeling by plasma. Complementary experiments on this phenomenon are now undertaken by means of OES.

To improve knowledge on kinetics undergoing in the Plasma Gun plume, it would be interesting to performed spatially and temporally resolved LIF and/or absorption spectroscopy to scan other species such as metastable states of He, N₂ and O₂ as well as radicals such as NO and O.

Main results obtained during this work could be resumed in few points as follow:

1. Antitumor activity of PG has been observed *in vitro* and *in vivo*;
2. Plasma propagation channels the gas flow;

3. The presence of a conductive surface has drastic effects on both plasma propagation and production of reactive species;
4. Quantification of OH radical has been performed by space and time resolved LIF.

Main perspectives are listed below:

1. Perform *in situ* quantitative diagnostic on other biologically active compounds in ground state;
2. Compare with a numerical model including plasma and gas dynamics, to gain insights on the counter-propagation feature;
3. Perform long-term *in vivo* experiments, with optimized PG parameters.

All those results presented in this manuscript lead us to emphasize on the need to consider the target and its nature as a part of the system to study, especially for *in vivo* experiments when dealing with inner humid organs or with skin perspiration at different electrical conductivities. It is clear now that the characterization of the plasma in the free jet mode is not relevant to estimate the plasma properties during biomedical applications. A further stage would be to perform *in situ* characterization during biological treatment and thus replace the metal grounded plate by a living organ. Indeed the obtained information could lead to optimized biological experiments *in vivo*. It would be now possible to approach the best position of the target for an optimal reactive species deposition onto the target.

Another remark that can be made is that multi-diagnostics approach is the only way to retrieve coherent information about plasma jet plume characteristics. To go beyond the descriptive mode caused by the high inter-connection of all the parameters, comparison with numerical models is surely needed. Furthermore efforts should be made to perform experiments in given protocols to help developing those numerical models which is one key for understanding the physics and chemistry of plasma jet plume for the Plasma Medicine field. It is important that the model includes the plasma dynamics together with gas jet hydrodynamic mechanisms well as the chemical kinetics.

French summary of the conclusion

Depuis le développement des premiers jets de plasma à pression atmosphérique, l'étude de leurs propriétés physico-chimiques ne cesse de croître dans le monde entier. L'intérêt majeur pour cette nouvelle source plasma vient de l'apparente déconnexion de la "balle de plasma" à la source de tension ainsi que sa température proche de l'ambiante. Les premières études montrèrent que les jets de plasma étaient potentiellement utilisables dans le cas d'application biomédicale. Des lors un nouveau domaine de recherche, Plasma-Médecine, naquit. Au GREMI, la recherche pour l'optimisation de la source Plasma Gun et la compréhension des phénomènes physiques sous-jacents à la génération, propagation et production d'espèces réactives du plasma s'est effectuée dans le cadre d'un ANR, PAMPA. Simultanément l'optimisation des traitements des cellules et tissus vivant ainsi que l'étude des effets anti-tumoraux induit par le plasma ont été effectués dans le cadre d'un APR PLASMED. C'est dans ce contexte que mon travail de recherche a débuté. L'objectif principal étant d'estimer l'influence des paramètres appliqués au plasma, conjointement aux paramètres découlant des conditions expérimentales, dans le but d'optimiser les applications biomédicales.

Dans ce but, plusieurs moyens de caractérisation ont été mis en œuvre tant pour les études concernant le plasma que pour les études biologiques.

Dans un premier temps, les potentialités anti-tumorales du plasma ont été examinées par des études *in vitro* et *in vivo* sur une lignée cellulaire de carcinome pancréatique, Mia PaCa. Une étude paramétrique *in vitro* a été effectuée pour estimer l'influence de la forme de l'impulsion de tension ainsi que sa fréquence de répétition sur le taux de survie des cellules cancéreuses. Il apparut que les impulsions avec un front de montée ns étaient plus efficaces que celle générée en μ s par l'Abiopulse. Néanmoins l'étude suivant la fréquence de répétition a montré que son augmentation améliorait l'efficacité du traitement quoi qu'étant limité à 3 kHz par les études de tolérance. Le générateur au front de montée en ns étant limité en fréquence de répétition, l'Abiopulse fut privilégié pour les applications biomédicales.

Les études *in vivo* ont montrées que quel que soit le front de montée de l'impulsion de tension, sa durée et le gaz utilisé, le traitement par plasma induit une réduction de l'activité tumoral. Ce résultat tend à suggérer que le plasma agit tel un stimulus au niveau des organismes induisant des cascades de processus biologiques. Une comparaison de l'activité tumoral durant des traitements plasma et avec la chimiothérapie de référence, la gemcitabine, il fut observé que le plasma induisait une réponse nettement plus élevée que la chimiothérapie. De plus la combinaison des deux traitements s'avéra plus efficace encore que les deux traitements séparés. Cette étude fut, à notre connaissance, la première à

démontrer l'activité anti-tumorale in vivo d'un jet de plasma sur des tumeurs orthotopique. Il serait intéressant de compléter cette étude pour déterminer si l'action du plasma et de la gemcitabine est synergique. Ces résultats étant prometteurs il serait nécessaire d'envisager des études à long terme sur des animaux de tailles moyennes pour mieux estimer le potentiel du traitement plasma pour des applications sur les humains.

Comme il fut démontré que le Plasma Gun a un fort potentiel anti-tumoral, la caractérisation du plasma fut par la suite au cœur du travail. Dans une première étape nous avons décrit le plasma quand il s'épand dans l'air de manière libre, ce qui fut appelé le mode "jet-libre" (Free-jet en anglais).

Tout d'abord quelques rappels du travail effectué en parallèle de ces travaux pendant la thèse de V. Sarron sont présentés. Ces expériences ont ciblés la compréhension des mécanismes de génération et de propagation du plasma au sein du capillaire du Plasma Gun. Il y est décrit pourquoi la dénomination "Colonne de plasma pulsée à pression atmosphérique" (Pulsed Atmospheric-pressure Plasma Stream" en anglais) ou PAPS, est plus adapté que la terminologie historique de balle de plasma. En effet, le contrôle de la longueur de propagation du front d'ionisation par la modification de la forme de la tension, a explicité le rôle central de la colonne plasma reliant le front à la source de tension. Une comparaison avec un model numérique développé par Xiong et Kushner a appuyé ces observations.

La suite des expériences présentées dans le manuscrit, le Plasma Gun a été positionné verticalement, la sortie vers le bas, pour limiter les différences avec les conditions d'application biomédicale. La caractérisation de la plume plasma a été entreprit par diverses techniques d'imagerie et par spectroscopie d'émission optique (OES). Une interaction mutuelle entre le flux de gaz et le plasma fut observé par imagerie Schlieren, résultant en une canalisation du gaz par le plasma. Cette canalisation du gaz est dépendante de la fréquence de répétition de l'impulsion et du débit du gaz, comme confirmé par l'imagerie filtrée en longueur d'onde et par imagerie rapide. L'OES résolue en temps et spatialement ont permit d'identifier les espèces générées par le plasma et de formuler des hypothèses quant à la cinétique réactionnelle. Dans cette configuration, un processus en deux-étapes a été identifié. Premièrement, au sein du capillaire, le principal mécanisme d'excitation apparait être la collision électronique inélastique avec les neutres. Toutefois des processus tel que la dissociation de l'eau, l'ionisation Penning et les transferts de charges des atomes et ions moléculaire d'He sur N₂ sont responsable de la production de OH et des différentes espèces excitées de l'azote, ces derniers provenant des impuretés présentes dans le gaz. Dans une seconde étape, des transferts avec l'air ambiant sont identifiés. Les espèces excitées produites recombinent ou se dé-excitent, produisant des radicaux et molécules actives à longue durée de vie. Ces espèces ont été identifiés grâce à des mesures préliminaires par spectroscopie infrarouge à transformée de Fourier dans les effluents. NO, NO₂, N₂O et les acides tels que HNO₂ et HNO₃ qui ont été identifiés par ces mesures, sont bien connus des biologistes pour induire des stress oxydatifs au sein des organismes vivant.

Puis nous nous sommes intéressés à l'analyse de l'influence des paramètres lorsqu'une cible est positionnée en face de la plume plasma. Les premières cibles étudiées furent diélectrique et montrèrent que peu d'influence dans la production des espèces excitées ainsi que dans la propagation du gaz et du plasma. Il fut déterminé que l'influence de la cible diélectrique vient principalement de l'obstacle qu'elle génère dans le flux de gaz.

Nous avons ensuite étudié le comportement du plasma lorsque les cibles sont conductrices et nous avons observés des changements drastiques. La propagation du plasma ainsi que le flux de gaz sont modifiés résultant de la différence de potentiel imposé par la présence de la cible conductrice reliée à la masse. Il semble d'après les observations que le plasma se contre-propage après avoir atteint la cible. Cette contre-propagation entraîne des changements importants dans la production et la distribution des espèces réactives. Quoique la vitesse du plasma se contre-propageant est amoindrie, il conserve des similarités avec le PAPS telles que la connexion par la colonne plasma à la source de tension et son extinction lorsque la tension atteint un seuil critique. D'autres expériences sont à prévoir ainsi que la comparaison avec des modèles numériques pour mieux comprendre les phénomènes physiques sous-jacents à cette contre-propagation.

Une autre modification importante concerne le flux de gaz. La canalisation du flux par le plasma s'avère beaucoup plus efficace avec la cible conductrice. Ce résultat permet l'hypothèse d'un accroissement de la dérive des ions, qui serait induit par d'une part la différence de potentiel entre le front d'ionisation et la cible à la masse ainsi que d'autre part la modification du champ électrique induit au niveau du front d'ionisation. Dans ce canal d'Hélium, les pertes d'énergie sont grandement diminuées comparé aux pertes induites par la pénétration d'air dans le jet d'Hélium. Cette hypothèse peut partiellement expliquer le fait de la contre-propagation. Ce plasma a pour caractéristique une émission de couleur rose identifiée par OES comme résultante des transitions du système N_2^* (B-A). De plus l'émission de NO^* est grandement augmentée quand il y a présence de la cible conductrice.

L'étude paramétrique effectuée a permis de conclure que l'hydrodynamique du jet de gaz et de la contre-propagation du PAPS sont dépendant d'une combinaison de nombreux paramètres dont la distance séparant la sortie du tube à la cible conductrice, l'amplitude de tension, le débit de gaz et la fréquence de répétition du pulse. La modification de la canalisation du jet de gaz et de la contre-propagation du plasma induit des différences dans la production des espèces excitées réactives.

Il a été montré que toutes les espèces excitées, hormis N_2^{+*} , sont ré-excitées durant la contre-propagation du plasma. Ces espèces peuvent être séparées en deux groupes grâce à l'analyse de leur ratio d'intensité précoces et tardives et à leur distribution spatiale. Le premier groupe résulte principalement de l'excitation par collision électronique avec des électrons de grande énergie, He^* , O^* et N_2^{+*} alors que le deuxième groupe seraient plutôt produit par des collisions électroniques avec des électrons de faible énergie aussi bien que par des transferts d'énergies ultérieurs, N_2^* (C), N_2^* (B), OH^* et NO^* . Il serait utile

d'effectuer simultanément de l'imagerie Schlieren et de l'imagerie filtrée en longueur d'onde pour valider nos hypothèses et mieux comprendre la cinétique du système.

L'influence de la cible conductrice a aussi été observé par mesure FTIR. En effet les espèces à longue durée de vie sont augmentées par rapport au mode jet libre. D'autres expériences, dans le but de quantifier les espèces produites par rapport aux nombreux paramètres, sont nécessaires. Il serait intéressant d'effectuer des expériences de titrage chimique dans l'eau ou des mesures avec un spectromètre de masse pour donner des informations complémentaires qui comparés permettra de mieux comprendre la cinétique de long terme des espèces chimiques produites ainsi que leur possible interaction avec les cellules ou tissus vivants.

Dans la dernière partie du manuscrit nous nous sommes intéressés à l'estimation de la densité du radical OH dans son état fondamental ainsi que la distribution d'air et d'eau en effectuant des mesures quantitative par fluorescence induite par laser (LIF). Ces expériences ont été menées à IMIP à Bari en collaboration avec Giorgio Dilecce et son groupe. Ces expériences ont permis premièrement de valider le modèle numérique développé par l'équipe à Bari dans le cas d'un jet de plasma se propageant dans l'air et rencontrant un cible. Nous avons utilisés plusieurs cibles pour représenter les différentes conditions rencontrés lors des traitements biologiques. Les cibles étaient une plaque de métal et une boîte de Pétri contenant des solutions aqueuses de différentes conductivités électriques.

Comme les résultats obtenus les Chapitres 4 et 5, avec les autres diagnostics, la LIF a mis en évidence l'effet de la cible conductrice reliée à la masse sur la distribution de la densité de OH mais aussi sur la pénétration de l'air dans la plume plasma. La distribution d'OH montre les caractéristiques de la contre-propagation du PAPS, ce qui suggère qu'OH est produit pendant toute la propagation que ce soit "downstream" ou "upstream". Grâce à ces résultats, l'optimisation de la déposition du radical OH dans la zone proche de la cible apparaît facilitée, et pourrait être atteinte en jouant sur les paramètres tels que l'amplitude de tension et/ou le débit de gaz.

De plus les distributions d'air et d'eau ont confirmés l'influence majeure de la conductivité électrique de la cible dans l'interaction plasma-flux de gaz. Une étude approfondie sur la canalisation du gaz par le plasma est en cours. Ce phénomène est évidemment d'une grande importance pour les applications biomédicales mais aussi pourrait ouvrir à de nouveaux domaines d'applications du Plasma Gun, tel que les actionneurs plasma.

Pour élargir l'étude sur l'influence de la conductivité de la cible, la distribution du radical OH a été tracée quand la plume plasma rencontre différentes solutions aqueuses. Cette expérience a montré une augmentation de la production de OH proche de la cible dans le cas des basses conductivités électriques mais aussi dans la vapeur d'eau recouvrant les cibles de plus hautes conductivités, avec un pourcentage d'eau optimum de 10-15% pour la production de OH. Un autre point lié à la concentration d'impureté dans le gaz porteur a

montré que la présence de N_2 influé dans la canalisation du gaz. Des expériences complémentaires sur ce phénomène sont en cours par l'utilisation d'OES.

Il serait intéressant d'effectuer des mesures spatialement et temporellement résolues par LIF et/ou par spectroscopie d'absorption ciblées sur d'autres espèces que OH. Les espèces telles que les métastables d'He, N_2 et O_2 ainsi que les radicaux NO et O pourrait améliorer la compréhension de la cinétique réactionnelle dans la plume plasma.

Les principaux résultats obtenus durant ce travail peuvent être résumés en quels points comme suit:

1. L'activité antitumoral du PG a été observé *in vitro* et *in vivo*;
2. La propagation du plasma canalise le flux de gaz;
3. La présence d'une surface conductrice produit des effets drastiques sur la propagation du plasma et la production des espèces réactives;
4. La quantification du radical OH a été effectuée par LIF résolu en temps et spatialement.

Les majeures perspectives sont énoncées ci-dessous:

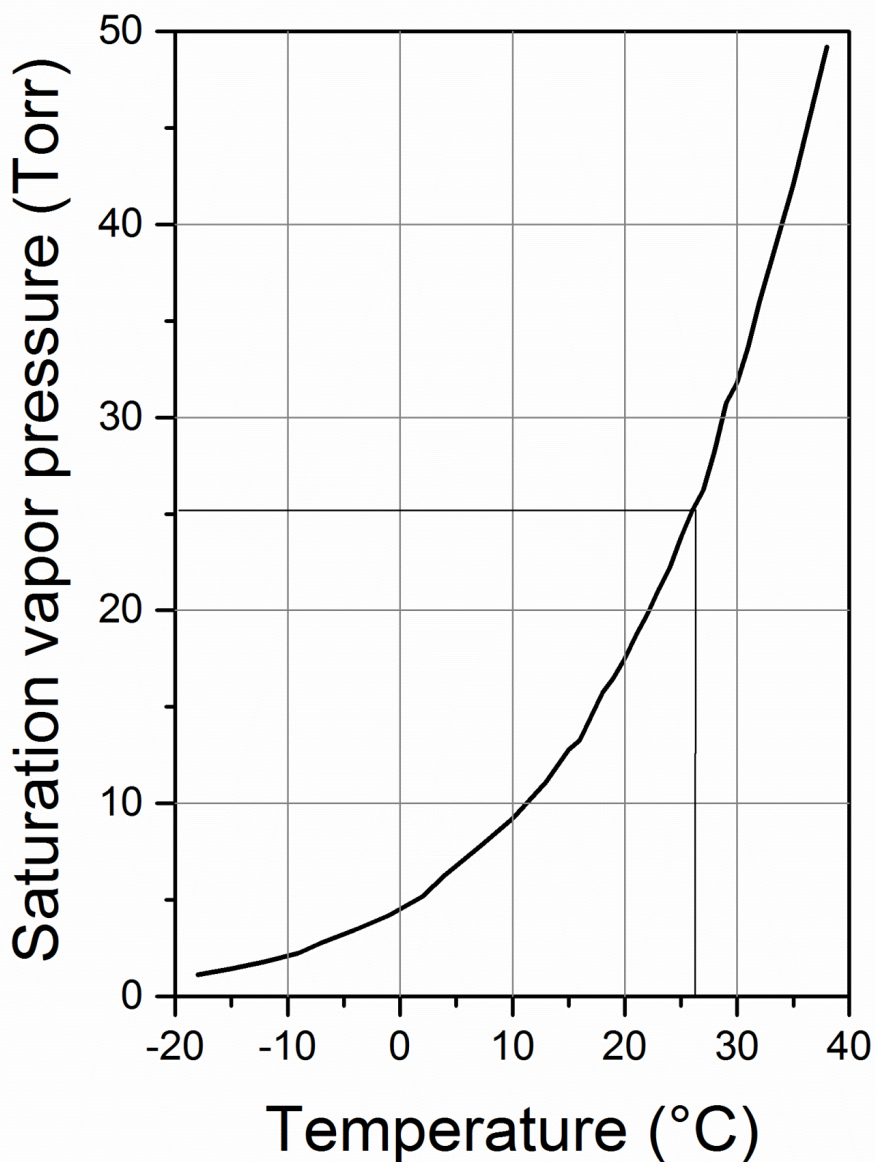
1. Effectuer des mesures quantitatives *in situ* pour d'autres composés biologiquement actifs dans leur état fondamentale;
2. Comparer les résultats obtenus avec un modèle numérique intégrant la dynamique du plasma et du flux de gaz, pour mieux comprendre le phénomène de contre-propagation;
3. Effectuer des expériences *in vivo* de long terme avec des paramètres optimisés du Plasma Gun.

Tous les résultats présentés dans ce manuscrit nous amènent à insister sur le fait de considérer la cible et sa nature comme une partie inhérente du système à étudier. En effet, il apparaît comme primordial de considérer la cible lorsque le but est d'optimiser des traitements plasma *in vivo*. En effet lors de traitement *in vivo*, la nature de la cible peut varier, comme dans les cas de la peau recouverte de transpiration ou des organes internes humides, ayant chacune des conductivités électriques et un taux d'humidité différents. Il est certain maintenant que la caractérisation de la plume plasma dans le mode jet-libre n'est pas pertinente pour estimer les propriétés du plasma lors des applications biomédicales. Une prochaine étape serait d'effectuer une caractérisation *in situ* pendant un traitement biologique, ainsi remplacer la plate de métal par un organe vivant. Les informations obtenues permettent une optimisation des traitements biologiques *in vivo*, en approchant par exemple la distance optimale entre la sortie du tube et la cible à traiter pour une déposition optimale des espèces réactives sur la surface.

Une autre remarque qui émerge de ce travail est l'utilisation d'une approche multi-diagnostiques comme unique voie pour obtenir des informations cohérente sur la plume plasma. Pour dépasser le mode descriptif induit par la corrélation de tous les paramètres, une comparaison avec des modèles numériques serait essentielle. D'un point de vue expérimental, des efforts doivent être fait pour effectuer les expériences dans des conditions normées pour aider au développement de ces dits-modèles. Pour que les modèles numériques soient la clé de la compréhension des phénomènes physiques et chimiques en jeu dans les jets de plasma, ils devront inclure la dynamique des plasmas, l'hydrodynamique des jets de gaz ainsi que la cinétique réactionnelle.

Annexes:

A. Saturation vapor pressure versus temperature



B. Examples of raw data matrices of LIF measurements

Table A.6: OH density in cm^{-3} for the PG impinging on the metal plate. Parameters: 1 l.min⁻¹, 14 kV, 2 kHz.

z/x	3	2	1	0	-1	-2	-3
10	5.54E+11	4.89E+12	4.45E+13	6.73E+13	1.82E+13	1.82E+12	5.87E+11
9	5.01E+11	2.16E+12	5.10E+13	1.00E+14	1.27E+13	1.04E+12	5.75E+11
8	9.48E+11	4.60E+12	5.32E+13	1.04E+14	1.63E+13	1.21E+12	5.84E+11
7	1.13E+12	4.48E+12	5.45E+13	1.10E+14	1.77E+13	1.76E+12	8.64E+11
6	1.49E+12	3.15E+12	4.38E+13	1.07E+14	1.39E+13	3.12E+12	--
5	--	4.70E+12	3.77E+13	6.62E+13	1.20E+13	1.23E+12	--
4	--	5.61E+12	3.34E+13	3.55E+13	1.35E+13	1.25E+12	--
3	--	8.76E+12	4.06E+13	2.78E+13	1.70E+13	1.26E+12	--
2	--	7.39E+12	3.25E+13	2.61E+13	1.95E+13	1.45E+12	--
1	--	1.39E+13	2.41E+13	2.46E+13	3.54E+13	--	--

Table A.7: H₂O % for the PG impinging on the metal plate. Parameters: 1 l.min⁻¹, 14 kV, 2 kHz.

z/x	3	2	1	0	-1	-2	-3
10	3	14.9	11	11.8	16.2	32.5	0.2
9	3	21.6	9	7.3	12	5.5	4
8	2	8.4	7.1	5.6	13.3	11.5	0.2
7	3	6.8	6	4.6	10	0.2	0.2
6	2	0.2	3.5	2.7	0.2	0.2	0.5
5	--	0.2	2.5	1.8	2.5	0.2	--
4	--	0.2	1.6	1.3	0.3	0.2	--
3	--	13.4	1.8	0.7	7.9	0.2	--
2	--	7	1	0.5	5.9	0.2	--
1	--	14	0.5	0.4	3.5	--	--

Table A.8: Air % for the PG impinging on the metal plate. Parameters: 1 l.min⁻¹, 14 kV, 2 kHz.

z/x	3	2	1	0	-1	-2	-3
10	7.33333	8.26667	4.02667	4.17867	5.77067	7.73867	13.996
9	6	3.12	4.69333	2.13333	5.77067	6.93867	9.86267
8	5.06667	7.068	2.93333	1.52	4.404	5.73867	13.996
7	14.66667	6.54267	2.13333	0.892	5.524	16.13867	16
6	16	4.94267	1.77333	0.52	5.79067	9.46267	16
5	--	12	1.32	0.328	5.03333	8.66267	--
4	--	7.476	0.62667	0.13733	4.95333	8.66267	--
3	--	7.2	0.27733	0.04267	3.99333	9.86267	--
2	--	4.8	0.08933	0.01467	1.85333	9.86267	--
1	--	3.06667	0.024	0.01067	0.65067	0	--

Table A.9: OH density in cm^{-3} for the PG impinging on physiological water. Parameters: 1 l.min^{-1} , 14 kV, 2 kHz.

z/x	3	2	1	0	-1	-2	-3
10	--	5.04E+12	1.76E+13	6.91E+13	3.20E+13	8.46E+12	2.37E+12
9	--	3.28E+12	2.81E+13	2.75E+13	5.50E+13	1.01E+13	8.88E+11
8	--	1.73E+12	2.42E+13	4.42E+13	2.35E+13	4.71E+12	1.03E+12
7	9.66E+11	2.79E+12	1.64E+13	2.79E+13	1.26E+13	4.01E+12	7.33E+11
6	1.05E+12	2.71E+12	1.33E+13	1.14E+13	9.60E+12	3.89E+12	7.57E+11
5	1.45E+12	3.60E+12	9.83E+12	8.11E+12	7.27E+12	2.85E+12	7.57E+11
4	--	1.56E+12	6.38E+12	8.58E+12	4.91E+12	9.95E+11	--
3	--	2.49E+12	6.26E+12	7.69E+12	4.81E+12	1.10E+12	--
2	--	1.43E+12	8.82E+12	1.85E+13	8.76E+12	2.52E+12	--
1	--	3.75E+12	1.55E+13	2.39E+13	1.04E+13	--	--

Table A.10: OH density in cm^{-3} for the PG impinging on distilled water. Parameters: 2 l.min^{-1} , 14 kV, 2 kHz.

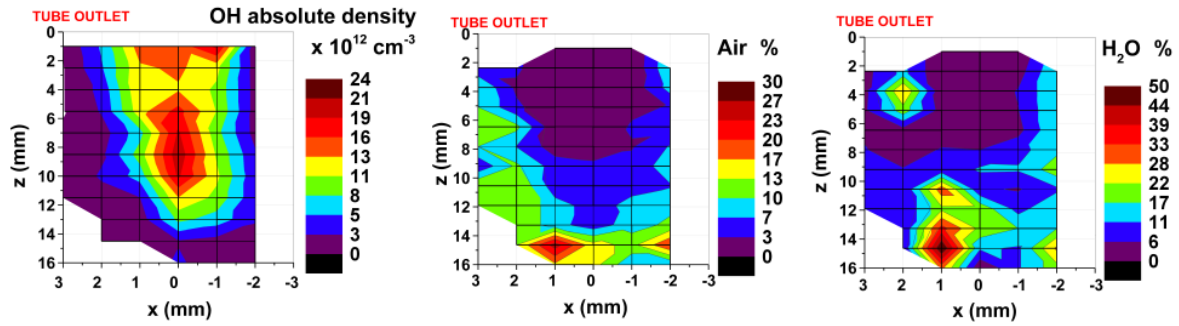
z/x	2	1	0	-1	-2
10	1.10E+13	1.38E+13	3.04E+13	1.26E+13	3.82E+12
9	3.52E+12	1.17E+13	2.12E+13	1.31E+13	3.30E+12
8	3.30E+12	1.04E+13	2.25E+13	8.28E+12	3.52E+12
7	2.70E+12	8.46E+12	2.19E+13	1.35E+13	1.87E+12
6	2.63E+12	8.34E+12	1.59E+13	1.18E+13	3.42E+12
5	1.73E+12	1.01E+13	2.08E+13	1.24E+13	1.97E+12
4	3.22E+12	7.51E+12	3.10E+13	1.16E+13	2.38E+12
3	2.79E+12	1.38E+13	2.96E+13	1.25E+13	2.64E+12
2	3.35E+12	1.48E+13	3.74E+13	1.45E+13	3.50E+12
1	2.12E+12	1.69E+13	3.23E+13	2.03E+13	1.64E+12
0	2.45E+12	3.11E+13	2.46E+13	2.38E+13	8.05E+12

C. LIF maps with lower interpolations curves

The number of color intervals in the contour plots has been reduced to 9 while the presented maps in chapter 6 used 100 intervals.

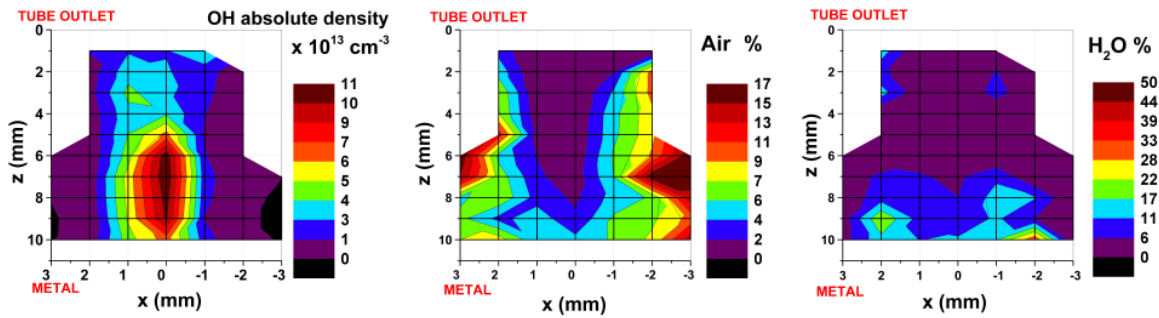
Free-jet conditions:

Plasma parameters: 2 kHz, 14 kV, 1 l.min⁻¹



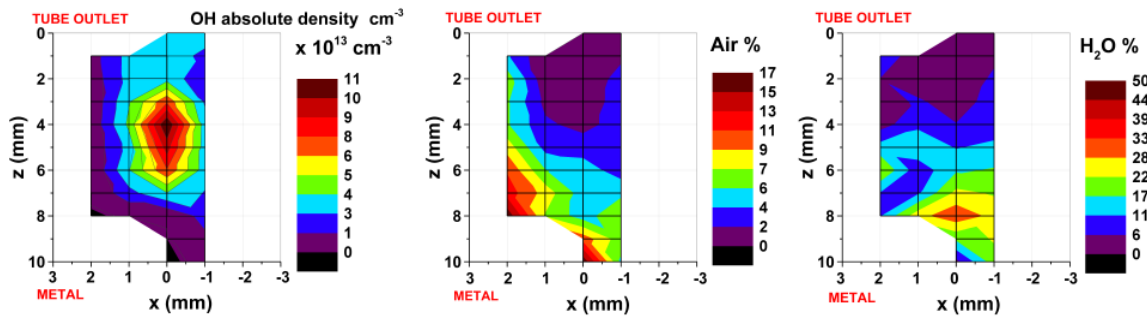
Standard conditions:

Plasma parameters: 2 kHz, 14 kV, 1 l.min⁻¹, gap 12 mm with metal plate



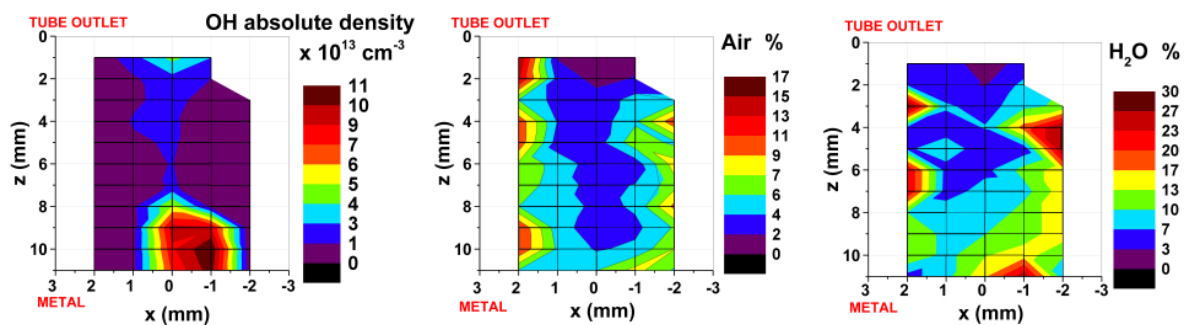
500 sccm:

Plasma parameters: 2 kHz, 14 kV, 500 sccm, gap 12 mm with metal plate

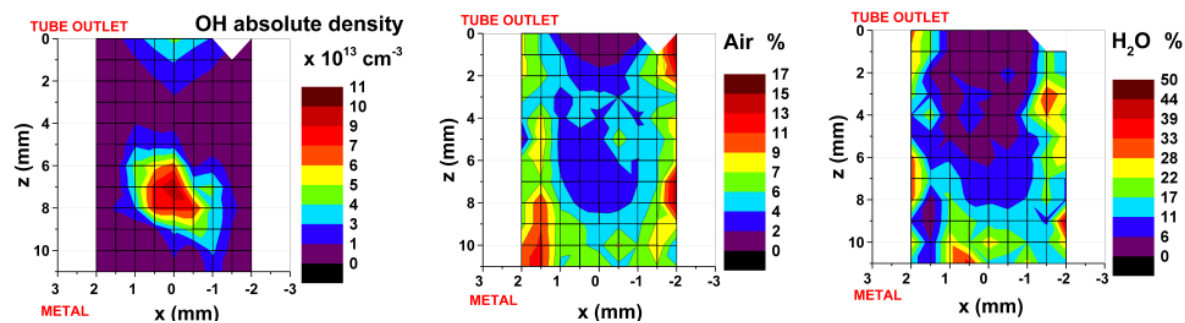


10 kV:

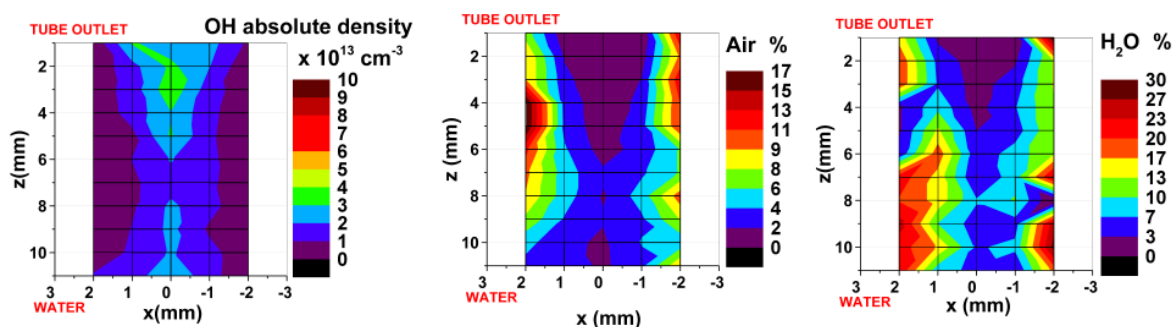
Plasma parameters: 2 kHz, 10 kV, 1 l.min⁻¹, gap 12 mm with metal plate

**He Alphagaz2:**

Plasma parameters: 2 kHz, 14 kV, 1 l.min⁻¹, gap 12 mm with metal plate

**2 l.min⁻¹:**

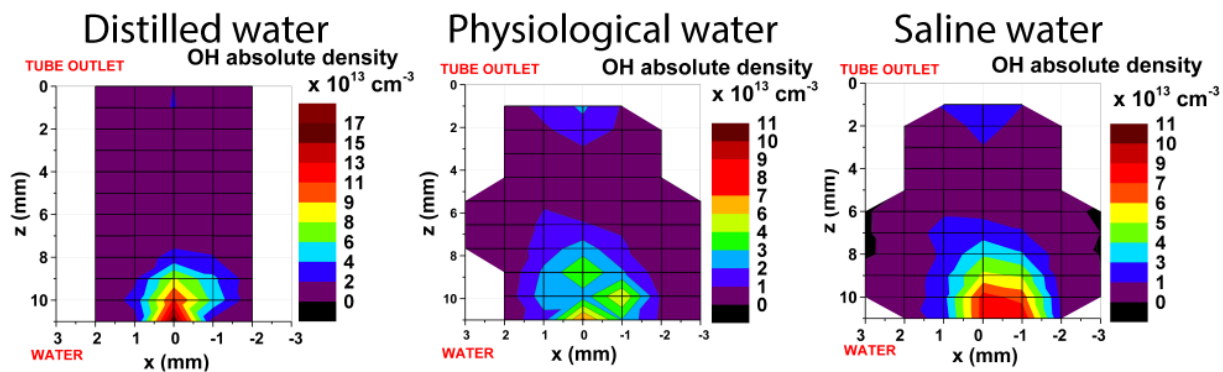
Plasma parameters: 2 kHz, 14 kV, 2 l.min⁻¹, gap 12 mm with distilled water



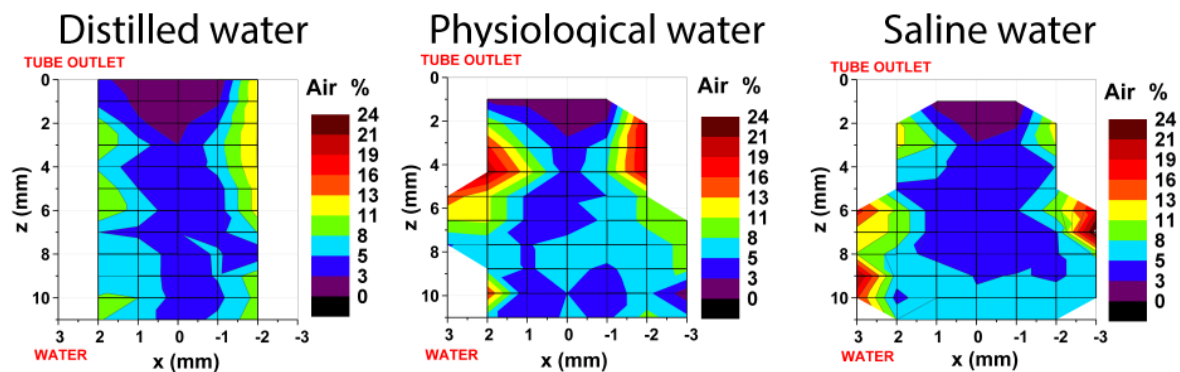
PG impinging on distilled, physiological and saline waters:

Plasma parameters: 2 kHz, 14 kV, 1 l.min⁻¹, gap 12 mm with water surfaces

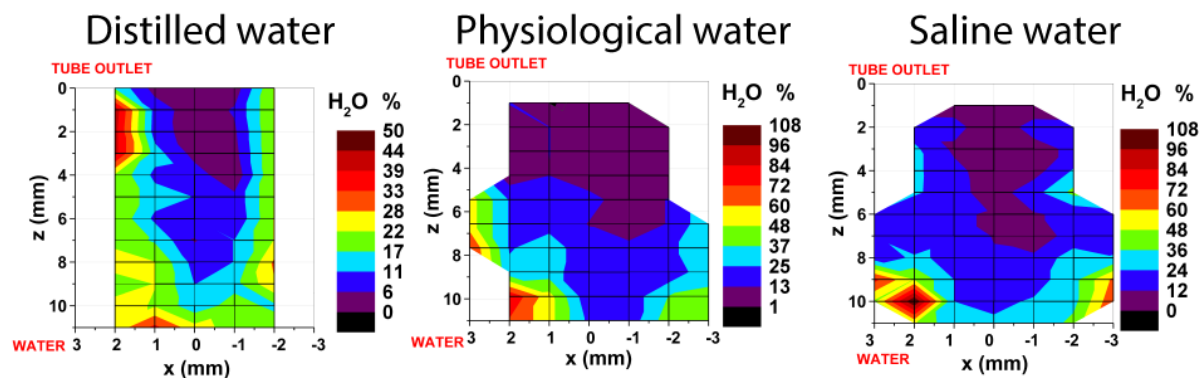
OH density:



Air %:



H₂O %:



Etude d'une décharge hors équilibre à pression atmosphérique pour des applications biomédicales. Physique de la décharge, cinétique de la production des espèces réactives lors de l'interaction avec des cellules et des tissus vivants.

Résumé :

Durant la dernière décennie, un nouveau type de décharge hors équilibre thermodynamique à pression atmosphérique a suscité un engouement croissant compte tenu de sa capacité de produire un plasma s'étendant dans l'air ambiant à une température proche de l'ambiante. Ces jets de plasma, souvent basés sur un réacteur de type décharge à barrière diélectrique, sont intéressants du point de vue de leurs propriétés physico-chimiques. De plus, ces jets de plasmas ont l'avantage de permettre des applications des matériaux thermosensibles, ouvrant ainsi un nouveau domaine de recherche, Plasma Médecine. Au GREMI le Plasma Gun, a été développé tant pour l'étude de la physique des jets de plasma que pour les applications biomédicales notamment dans le domaine de la cancérologie. Dans une première étape, des traitements par Plasma Gun *in vitro* et *in vivo*, dans le cadre d'un modèle murin du carcinome pancréatique, ont été effectués. L'action anti-tumorale du plasma a été démontrée ainsi que la combinaison bénéfique avec un traitement chimiothérapique. Fondée sur ces résultats encourageants, l'objectif principal de cette étude porte sur l'influence drastique de la cible de l'application sur les propriétés du plasma (propagation et production des espèces réactives) ainsi que l'interaction du gaz et du plasma. Des diagnostics tels que l'imagerie rapide et filtrée en longueur d'onde, la spectroscopie d'émission optique, l'imagerie Schlieren ainsi que la spectroscopie infrarouge à transformée de Fourier ont été utilisés pour caractériser le jet de plasma. Une étude quantitative de la distribution spatiale et temporelle du radical hydroxyle (densité comprise entre 5.10^{11} et $1.10^{14} \text{ cm}^{-3}$) a été réalisée par fluorescence induite par laser. L'étude de l'OH en combinaison avec un modèle numérique a permis une meilleure compréhension de la pénétration de l'air dans le jet de gaz et de l'interaction avec les surfaces humides. L'interaction complexe entre le comportement du gaz, du plasma et la nature de la cible est mise en avant en vue d'optimiser les applications biomédicales.

Mots clés : Plasma hors équilibre, pression atmosphérique, application biomédicale, espèces réactives

Non-equilibrium atmospheric pressure discharge for biomedical applications. Discharge physics, kinetics of reactive species production during the interaction with living cells and tissues.

Abstract :

Over the past decade, a new type of non-equilibrium discharge at atmospheric pressure has attracted growing interest, given the ability to produce a plasma extending in ambient air close to room temperatures. These plasma jets, often based on a dielectric barrier discharge type of reactor, are interesting on their physicochemical property perspectives. In addition, these cold plasma jets have the advantage of allowing applications to heat sensitive materials, creating a new field of research, Plasma Medicine. At GREMI the Plasma Gun, has been developed for both the study of the physics of plasma jets and for biomedical applications particularly in the field of cancerology. In a first step, *in vitro* and *in vivo* were performed, within a rodent model of pancreatic carcinoma. The anti-tumor action of the plasma has been demonstrated as well as its benefic combination with a chemotherapeutic treatment. Based on these encouraging biomedical results, the main focus of this study is to report on the drastic influence of the application target on the plasma properties (propagation and production of reactive species) and on the strong coupling between gas jet and plasma discharge. Diagnostics such as fast, wavelength-filtered and Schlieren imaging, optical emission spectroscopy as well as Fourier transform infrared spectroscopy were used to characterize the plasma. A quantitative study on spatial and temporal distribution of hydroxyl radicals (OH density ranging between 5.10^{11} and $1.10^{14} \text{ cm}^{-3}$) was performed by laser-induced fluorescence. The study of the OH in combination with a numerical model allowed a better understanding of the moist air penetration into the gas jet and the interaction with wet surfaces. This PhD work enlightened the complex interaction between the gas flow, the plasma and the nature of the target which has to be taken into account for further optimization of biomedical applications.

Keywords : Non-equilibrium plasma, Atmospheric pressure, biomedical application, reactive species