Journal of Visualized Experiments

Treating surfaces with a cold atmospheric pressure plasma using the COST-Jet

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE61801R1
Full Title:	Treating surfaces with a cold atmospheric pressure plasma using the COST-Jet
Corresponding Author:	Judith Golda, Ph.D. Christian-Albrechts-Universitat zu Kiel Kiel, Schleswig-Holstein GERMANY
Corresponding Author's Institution:	Christian-Albrechts-Universitat zu Kiel
Corresponding Author E-Mail:	golda@physik.uni-kiel.de
Order of Authors:	Judith Golda, Ph.D.
	Kerstin Sgonina
	Julian Held
	Jan Benedikt
	Volker Schulz-von der Gathen
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Bochum, NRW, Germany

TITLE:
Treating surfaces with a cold atmospheric pressure plasma using the COST-Jet

AUTHORS AND AFFILIATIONS:
Judith Golda[1], Kerstin Sgonina[1], Julian Held[2], Jan Benedikt[1], Volker Schulz-von der Gathen[2]

[1]Experimental Plasma Physics, Kiel University, Kiel, Germany
[2]Experimental Physics II, Ruhr-University Bochum, Bochum, Germany

Email addresses of co-authors:
Kerstin Sgonina (sgonina@physik.uni-kiel.de)
Julian Held (julian.held@rub.de)
Volker Schulz-von der Gathen (volker.schulz-vondergathen@rub.de)
Jan Benedikt (benedikt@physik.uni-kiel.de)

Corresponding author:
Judith Golda (golda@physik.uni-kiel.de)

KEYWORDS:
Atmospheric pressure plasma, COST-Jet, plasma medicine, plasma treatment, handling protocol, liquid, surface treatment, CAP

SUMMARY:
This protocol is presented to characterize the setup, handling, and application of the COST-Jet for the treatment of diverse surfaces such as solids and liquids.

ABSTRACT:
In recent years, non-thermal atmospheric pressure plasmas have been used extensively for surface treatments, in particular, due to their potential in biological applications. However, the scientific results often suffer from reproducibility problems due to unreliable plasma conditions as well as complex treatment procedures. To address this issue and provide a stable and reproducible plasma source, the COST-Jet reference source was developed.

In this work, we propose a detailed protocol to perform reliable and reproducible surface treatments using the COST reference microplasma jet (COST-Jet). Common issues and pitfalls are discussed, as well as the peculiarities of the COST-Jet compared to other devices and its advantageous remote character. A detailed description of both solid and liquid surface treatment is provided. The described methods are versatile and can be adapted for other types of atmospheric pressure plasma devices.

INTRODUCTION:
Cold atmospheric pressure plasmas (CAPs) have attracted increased interest in recent years due to their potential for surface treatment applications. CAPs are characterized by their non-equilibrium properties, enabling complex plasma chemistry with a high density of reactive

species while maintaining a low thermal impact on treated samples. Therefore, CAPs are considered in particular for the treatment of biological tissue[1–4]. Numerous concepts and designs of CAPs are successfully used for wound disinfection and healing, blood coagulation, and cancer treatment, among other biomedical applications. A large proportion of biological tissue contains liquids. Therefore, research is also increasingly focused on investigating the effects of CAPs on liquid surfaces such as cell medium or water[5–7].

However, the scientific results often suffer from reliability and reproducibility problems[8–10]. On one hand, the treated biological substrates are subject to natural variations. On the other hand, biological mechanisms were seldom directly attributed to plasma processes (such as electric fields, UV radiation, and long- and short-lived species, etc.). Additionally, these plasma processes in turn depend strongly on the individual plasma source and the exact type of its application.

Additionally, detailed protocols of treatment procedures are rarely available. This makes it difficult to isolate the influence of a particular plasma parameter on the outcome of the treatment, which renders the obtained results non-transferable.

Therefore recently, various attempts have been made to standardize the treatment of surfaces, tissues, and liquids using cold atmospheric pressure plasmas. Here we present only some selected examples.

To simplify the direct comparison of different plasma sources, a reference source was developed. Inspired by the low-pressure plasma community, a reproducible and stable discharge design (COST-Jet) was developed in the framework of the COST action MP 1101 that can serve as a reference source for future biomedical research[11].

To enable comparability, reference protocols for individual applications were developed. To standardize the comparison of the antimicrobial properties of cold atmospheric pressure plasmas, for example, Mann et al. defined a reference protocol for microorganism treatment by normalizing the treatment time per area unit[12].

For a more flexible approach, Kogelheide et al. developed a method to investigate plasma-induced chemical modifications on macromolecules[13]. Using tracer compounds such as cysteine and or cysteine-containing glutathione (GSH) in combination with FTIR and mass spectrometry, they tried to extrapolate the chemical modifications on biological substrates. Using this method, several plasma sources such as the COST-Jet, the kinPen, and the Cinogy DBD have already been compared[14–16].

To directly compare individual plasma sources, comparable control parameters must be established. Basic plasma parameters such as electron temperature, electron density, and the flux densities of reactive species are hard to measure in atmospheric pressure plasmas since such plasmas are often transient and their dimensions are small. Instead, external control parameters such as generator power, applied voltage or ignition, and arcing points are often used as a reference, especially when comparing results to simulations[17,18]. More recently, the measured electrical energy consumption has been used as a more reliable control parameter[19–21].

Despite these efforts, comparing the results of different studies may still be impossible, simply due to the challenge of correctly applying a plasma source onto a surface. There are a vast number of prevalent pitfalls that have to be tackled when working with atmospheric pressure plasma applications such as the influence of external electric fields (compensation circuits), feedback loops between plasma and surrounding environment (shielded atmosphere), species transport (ionic wind) and control parameters (voltage, current, power).

The main objective of this work is to provide a thorough, detailed protocol on the application of the COST-Jet for surface treatments. The COST-Jet is a reliable plasma source that was developed for scientific reference purposes rather than for industrial or medical use. It provides reproducible discharge conditions and a broad database of available studies [22,23]. The COST-Jet is based on a homogeneous, capacitively coupled RF-plasma. Because the electric field is confined perpendicular to the gas flow, charged species are mostly kept in the discharge region and do not interact with the target or the surrounding atmosphere. Additionally, the laminar gas flow ensures reproducible plasma chemical conditions in the plasma effluent.

In this paper, we will address the most common challenges and introduce possible solutions that have been used in the literature. These include proper gas supply, discharge control, ambient atmosphere influence, and surface preparation. Compliance with the protocol presented here should ensure the reproducibility and comparability of the measurements.

The protocol might also serve as an example for other atmospheric pressure sources. It must be refined for other jet plasma sources according to the individual gas flow and electric field configuration. Where applicable, we will try to point out possible adjustments to the protocol. The described steps should be considered and reported on when publishing studies applying atmospheric pressure plasmas to treated samples.

PROTOCOL:

1 Feed gas supply and controlled atmosphere

1.1 Set up the gas supply consisting of all-metal gas lines, avoiding any TPFE or similar plastics tubing[24]. Keep gas supply lines as short as possible to avoid any impurities and facilitate pumping of the gas supply system.

1.2 Choose the mass flow controllers used to provide the feed gas according to the typical gas flow rates of the COST-Jet. Use working gas with a purity of at least 99.999%.

NOTE: The COST-Jet's primary working gas is helium. Operation can be realized at flow rates between 100 sccm and about 5000 sccm, with 1000 sccm being the most common value.

1.3 Realize the admixture of reactive gases by a system consisting of multiple mass flow controllers. For smaller admixtures, use a counter-mixing unit to reduce the time needed for the mixing to complete[25].

NOTE: Common admixtures are oxygen and nitrogen with a flow rate in the order of 5 sccm (0.5% of the working gas).

1.4 Add a valve between the gas supply lines and the jet to prevent moist air entering the gas supply when the device is not in use as water is the most common and most problematic impurity in atmospheric pressure plasmas, critically influencing the plasma chemistry.

1.5 Clean the gas supply lines before the surface treatment, to reduce impurities in the tubing. To do so, either simply set a moderate gas flow of about 1000 sccm helium and flush the supply lines or, preferably, repeatedly pump and refill the supply lines (about three times).

NOTE: When simply flushing the gas supply lines, multiple hours might be needed to clean the system, depending on the state of contamination.

1.6 Add a molecular sieve trap or cold trap (e.g., using liquid nitrogen) to the gas supply lines to further reduce the humidity in the feed gas.

1.7 If, instead, a controlled amount of water is desired as a reagent, add a bubbler to the system[26,27].

1.8 Consider setting up a controlled atmosphere for your experiment as changes in the composition of the ambient atmosphere might influence chemical reactions in the plasma effluent.

NOTE: This effect is likely not very pronounced for the COST-Jet[28], since the electric field configuration confines the plasma to the inside of the discharge channel but might play an important role for other CAP devices where the active plasma is partly outside the device.

2 Assembly and setup of the device

2.1 Connect the COST-Jet device to a gas supply. Directly connect the device to ¼ inch stainless steel Swagelok tubing. Use adapters for different tubing standards.

2.2 Connect the COST-Jet to the power supply using a shielded BNC cable equipped with an SMC connector.

2.3 Connect the integrated electrical probes to an oscilloscope to monitor voltage and current using a 50 Ohm resistor as termination.

2.4 Open the COST-Jet housing and connect a properly compensated commercial voltage probe to the powered copper line as well as a grounded part of the jet (e.g., the Swagelok gas tube) and the oscilloscope.

2.5 Perform a probe calibration routine: Apply a small voltage to the COST-Jet and tune the variable capacitor of the LC-circuit using a screwdriver to reach the optimum coupling (maximize measured voltage). Perform a voltage calibration by comparing the actual voltage (commercial probe) to the measured voltage (implemented probe) using linear regression and calculate a calibration constant. Remove the commercial voltage probe and close the COST-Jet housing.

2.6 Again, apply a small voltage to the COST-Jet and tune the variable capacitor of the LC-circuit using a screwdriver to reach the optimum coupling.

2.7 Ignite a plasma in the COST-Jet device: Firstly, set up a gas flow rate of approximately 1 slpm of helium using mass flow controllers (MFCs). Open the valve between the gas supply system and the COST-Jet last. Then, apply a low voltage to the electrodes and increase the amplitude until the plasma ignites.

2.8 If, upon the first ignition, the electrodes are unclean and impeding the ignition, apply a high initial voltage and quickly reduce it after ignition. Alternatively, use a spark gun to facilitate an easier first ignition.

2.9 Set the operation control parameters (gas flow, applied voltage) to the desired values.

2.10 Give the setup a little warm-up time to allow for thermal stabilization (approx. 20 minutes) to ensure stable and reproducible operation conditions.

2.11 To change the gas composition during the experiments, allow for an approximate 2 minute equilibration time depending on the gas supply setup.

NOTE: The COST-Jet is now ready for application.

3 Power measurement

3.1 Connect the oscilloscope monitoring the voltage and current applied to the COST-Jet to a computer.

3.2 Install the 'COST power monitor' software to the computer[29] which allows real-time power monitoring[11,19].

3.3 Adjust the communication between the software and oscilloscope by implementing the required commands for controlling the specific oscilloscope.

3.4 Start the COST power monitor software and switch to the **Settings** panel. Fill in the correct channels connected to the oscilloscope and the calibration constant determined in step 2.4.

NOTE: The **Find** button can be used to automatically calculate the calibration factor if the commercial voltage probe is attached to the COST-Jet.

3.5 Change to the **Sweep** panel. Take a reference phase while the plasma is still off by pressing the **Find** button. Switch off the gas flow before this measurement and apply a voltage that is in the typical range of voltages used for the actual operation of the discharge as the plasma will not ignite in air due to much higher ignition voltage compared to noble gas dominated gas mixtures. Use this measurement to automatically correct for the relative phase shift between voltage and current probes, assuming a 90° phase of the perfect capacitor here.

3.6 Press the **Start** and **Pause** button to start or pause the electrical measurements.

3.7 Operate the COST-Jet as desired. Use the actual electrical power calculated from voltage and current amplitudes as well as their phase shift, which are continuously displayed in the software for monitoring and as a control parameter.

4 (Solid) surface treatment

4.1 Set up a controlled atmosphere for your experiment.

NOTE: In the case of the COST-Jet, the controlled atmosphere is less important than for sources with active plasma chemistry outside of the confined discharge channel.

4.2 Clean the gas supply lines as described in step 1.5.

4.3 Set the desired operating parameters and wait for approximately 20 minutes until the COST-jet to reach a stable temperature.

4.4 Choose the distance between the COST-Jet and the treated surface as the distance determines the amount of reactive species impinging on the treated surface[30]. Use an xyz-stage to mount the substrate for easy manipulation.

NOTE: For the COST-Jet, the safety gap adds one extra millimeter to the distance between the plasma discharge and the treated surface.

4.5 Start the treatment time: Either simply switch on the plasma or use a mechanical shutter. Be aware of a possible voltage overshoot during the switching event leading to a constricted discharge. For better control in the ms range, use a rotatable shutter.

4.6 Treat the sample for the desired amount of time and end the treatment time by switching off the plasma or by use of a shutter.

4.7 If necessary, check the gas flow pattern in front of the target using Schlieren imaging when treating a substrate as effects of surface charging, ion drag forces, or ambient air mixing due to buoyancy can influence the amount of reactive species reaching a surface.

5 Liquid treatment

5.1 Set up a controlled atmosphere for the experiment.

5.2 Clean the gas supply lines as described in step 1.5.

5.3 Set the desired operating parameters and wait approximately 20 minutes for the COST-jet to reach a stable temperature.

5.4 Choose the distance between the COST-Jet and the treated liquid.

5.5 Pour the liquid to be treated into an adequate container. Use inert material to avoid reactions of potentially generated reactive species in the liquid with the container. Choose the size of the container according to the volume of liquid that is treated.

5.6 Consider the influence of the gas flow on the liquid surface: Depending on the gas flow rate, be aware of a concave meniscus that may form, thus changing the distance between plasma and liquid surface.

5.7 Start the treatment. Avoid pressure surges on the surface of the liquid caused by a sudden change in gas flow as this could cause liquid splashes into the discharge geometry, possibly causing a short circuit and certainly contaminating the plasma. Instead, use a mechanical shutter or slowly increase the gas flow.

5.8 Take into account mixing/stirring of the liquid due to friction between neutral gas flow and liquid surface as this influences transport processes and concentration profiles in the liquid. Additionally, depending on the treatment time, correct for the evaporation of liquid during the treatment (e.g., when calculating reaction constants). Depending on the plasma source, be aware of this evaporation possibly causing back coupling to the discharge, thus changing the plasma chemistry.

5.9 Please also consider that the reactivity with possible reagents in liquids is also affected by the surface activity of this agent. Thus, in some cases, surfactants might play an important role in the interaction between short-lived species and liquids.

REPRESENTATIVE RESULTS:

Using the methods and equipment described above, we exemplary applied the COST-Jet to different surfaces and liquids. **Figure 1** shows the experimental setup used for the treatment including the power supply, gas supply system, voltage and current probes as well as a controlled atmosphere and a mechanical shutter.

[Place **Figure 1** here]

Using the voltage and current probe implemented in the COST-Jet, the dissipated electrical power can be calculated. **Figure 2** shows the measured electrical power in a helium plasma generated in five different COST-Jet devices using a gas flow of 1 slpm. All devices show similar behavior. The deviation between the different devices originates from the uncertainty of the power measurement as well as microscopic differences in the setups such as the electrode distance. More detailed measurements of reactive species (e.g., atomic oxygen and ozone), temperature and power as well as bactericidal activity measurements have been performed by Riedel[22].

[Place **Figure 2** here]

Figure 3 shows the etch profile of an a:C-H film for a 3 min treatment with the COST-Jet using a gas flow of 1.4 slpm helium with an admixture of 0.5% oxygen measured using an imaging spectroscopic reflectometer[31]. The etch pattern shows a circular structure representing the cylindrical symmetry of the plasma effluent. Based on etch profiles in combination with numerical simulations, the surface loss probability of atomic oxygen could be estimated.

[Place **Figure 3** here]

Figure 4 shows the occurring vortices in liquid caused by the gas stream impinging on the liquid surface. A laser sheet illuminating tracer particles in the liquid makes it possible to observe the trajectory and velocity of these particles via particle image velocimetry and therefore study the fluid flow[32]. It is important to consider similar densities of the seeding particles and the fluid so that the trajectories of the particles represent the movement of fluid. With this visualization of the fluid flow measurements and numerical simulations can be compared[33]. The vortices are due to the surface friction between effluent gas flow and liquid surface. **Figure 4** also shows the occurring depression of the liquid surface underneath the gas channel of the plasma jet, the so-called meniscus. It is visualized by a blue line.

[Place **Figure 4** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Experimental setup used for the plasma treatment of surfaces and liquids using the COST-Jet. A cold trap is used to purify the feed gas. The controlled atmosphere is realized by a pumped vacuum chamber at atmospheric pressure. The mechanical shutter facilitates the time management of solid and liquid surface treatment. The flexible stage allows controlling the distance between the plasma jet and the surface.

Figure 2: Dissipated power as a function of applied voltage in a helium plasma. The data represents five identical COST-Jet devices[34]**.** The small deviations at high voltages are due to uncertainties of the measurement as well as small deviations in the gas discharge channel geometry[22].

Figure 3: Etch profile of a plasma-treated a:C-H film. The dip in the film was etched using a gas

mixture of 1.4slm helium with an admixture of 0.6% oxygen at a voltage of 230 V_{rms} and a treatment time of 3 min.[31]

Figure 4: Photograph of illuminated cornstarch particles in 3 ml of water stirred by the gas flow. The vortices are due to the surface friction between effluent gas flow and liquid surface.

Figure 5: Schlieren images of the COST-Jet with and without applied voltage for two different gas flow rates. During plasma operation, the gas flow pattern exactly resembles the pattern with only the gas flow.

DISCUSSION:

Here, we demonstrate the use of an atmospheric pressure plasma jet for surface treatments of different materials. The experimental setup for an atmospheric pressure plasma jet can have a tremendous effect on the plasma parameters, chemistry, and performance and consequently influences the outcome of plasma treatments and is a critical step in the protocol.

As an example, the gas supply lines play an important role regarding the most common impurity in the feed gas of the plasma which is humidity. In particular, the production of reactive nitrogen species in the plasma is reduced while the reactive oxygen species production is favored, due to the low ionization energy of oxygen compared to water molecules and nitrogen[35]. Winter[24] found out that feed gas humidity originating from water molecules on the surface of the inner tube is an order of magnitude higher using polymeric tubes compared to metal tubes due to the higher porosity and storage capacity. It can be reduced by flushing the lines with feed gas. However, drying the line by flushing takes a couple of hours. Therefore, polymeric tubing should be avoided or at least kept as short as possible. These findings are underlined by studies from Große-Kreul[25]. They compared the effect of polyamide and stainless steel tubing on the plasma chemistry using mass spectrometry. Their measurements confirm water cluster ion formation in the plasma due to water outgassing from polymeric tubes and faster drying times with metal tubes. Additionally, they investigated the effect of gas purification methods such as a molecular sieve trap and a liquid nitrogen cold trap on the plasma chemistry which helped to reduce the amount of impurities by about two orders of magnitude.

Instead of trying to purify the feed gas, there is also the approach of adding a controlled amount of humidity. As this intentional impurity then dominates over the natural impurities and thus controls the plasma chemistry, reproducible conditions are ensured as long as the amount of added humidity is precisely known.

For the ignition of the discharge, the applied voltage to the electrodes can usually simply be increased until the point of breakdown. However, depending on the surface conditions of the electrodes, sometimes a high voltage is necessary. To facilitate ignition, a high voltage spark gun can be used. This can also be useful when trying to ignite an argon discharge in the COST-Jet.

Before applying the COST-Jet to any surfaces, sufficient time should be allocated for the device to equilibrate. When set to the desired control parameters, the COST-Jet needs approximately

20 minutes to reach stable conditions[11]. During this time, the temperature of the device, the gas temperature as well as the plasma chemistry are reaching a steady state.

For comparison of scientific results, comparable plasma control parameters are necessary. For measuring the electrical input power, the COST power monitor can be used[29]. The software is open-source and compatible with a range of different types of oscilloscopes. The software operates according to the principle described by Golda[19].

In addition to the effect of feed gas humidity on the plasma chemistry, the transport of reactive species from the plasma to the substrate plays an important role in the effluent composition and is another critical step in the protocol. The surrounding atmosphere can influence the species created in the plasma on their way to the substrate. To minimize this influence, two different concepts are used: (i) Firstly, a controlled atmosphere can be set up that consists of the feed gas. Thus, the composition of the surrounding atmosphere can be kept constant. Depending on the purity level required for the treatment, the controlled atmosphere can be realized via protective housings equipped with a one-way valve to prevent overpressure. For higher purity levels, a vacuum chamber with a pump can be used. (ii) Secondly, a controlled atmosphere can be created by using a shielding gas curtain around the plasma effluent[36,37]. Usually, it consists of an inert gas, but it can also be varied according to the needs of the application.

Fortunately, for the COST-Jet, the influence of the surrounding atmosphere is comparably low. Using isotopic labeling, Gorbanev have shown that for a parallel-field configuration plasma jet, the reactive oxygen and nitrogen species reaching a liquid surface were formed in the plasma gas phase as well as in the region between the plasma nozzle and the sample[38,39]. In contrast, using the same technique for the COST-Jet, they found out that RONS almost exclusively originated from the plasma phase instead of the surrounding environment[28]. This is probably due to the electric field being confined to the plasma channel of the COST-Jet discharge. This makes the plasma discharge largely independent of its environment and gives it a certain remote character.

For a longitudinal electric field plasma jet, Darny et al.[40] have shown that the polarity of the electric field modifies the gas flow pattern and thus also on the reactive species that reach a target due to ionic wind. The dependence of the reactive species density on the environment was confirmed by measurements by Stancampiano et al.[7]. They reported on the difference of the number of reactive species created in treated water depending on the electrical characteristics. To compensate for these differences, they had to create a compensating electrical circuit. This behavior is different for the COST-Jet: **Figure 5** compares Schlieren images of the COST-Jet without an applied voltage and during operation for two different gas flow rates. The images were taken using a single mirror inline alignment as described by Kelly[41]. They show how the horizontally aligned COST-Jet effluent hits a flat glass substrate. Both images show the exact same gas flow pattern. This results from the lack of ionic wind due to the absence of charged species in the plasma effluent.

Additionally, the COST-Jet exhibits a very laminar flow pattern. Kelly[41] showed Schlieren images similar to the ones presented in **Figure 5**, for various gas flow rates. Even at comparably high gas flow rates of 2 slpm, the plasma effluent shows no signs of turbulence. At very low gas flow rates of 0.25 slpm and below, the buoyancy of the helium effluent begins to play a role. However, up to 4 – 5 mm distance from the nozzle, the ambient atmosphere does not influence the gas composition reaching the surface as demonstrated by Ellerweg using mass spectrometry[17].

All of the above-mentioned characteristics add to the remote character of the COST-Jet. This makes it an ideal candidate for the controlled, comparable treatment of surfaces.

[Place **Figure 5** here]

Depending on the desired effect on the treated sample, the control parameters gas flow mixture, applied electrical power, and distance between plasma source and surface can be adjusted accordingly. For the COST-Jet, a broad literature database of studies investigating reactive species in the effluent exists. As an example, Willems[30] measured the atomic oxygen density using mass spectrometry whereas Schneider[42] measured atomic nitrogen densities in the effluent.

The treatment of liquids with atmospheric pressure plasma can cause a variety of possible reaction mechanisms driven by reactive species, ions, photons, or electric fields. Due to the previously described characteristics of the COST-Jet, the effect of the electric field, ions, and photons are negligible compared to plasma sources where the plasma is in direct contact with liquids. Therefore, for studying the effect of short-lived reactive species like atomic oxygen on a phenol solution the COST-jet was used by Hefny[43] and Benedikt[44]. Furthermore, the COST-Jet provides a convenient possibility to compare experiments and numerical simulations of liquid treatment[28]. As the interaction between plasma and liquid is dominated by the gas flow of reactive species from plasma to the liquid surface, the model complexity can be reduced.

The gas flow induced stirring of the liquid increases the reaction rate between plasma generated reactive species and the liquid. In contrast to surface treatments of solids, the convection of the liquid constantly changes the local concentration of reactants. Additionally, the reaction rates between plasma generated species with reactants in liquid are also affected by the surface activity of these reactants. With increasing surface activity, the concentration of the reactant at the liquid surface increases. These surfactants might play an important role in the reactivity of short-lived species generated by the plasma.

Next to stirring the gas flow impinging on the liquid surface also induces evaporation which must be considered. Using the COST-Jet with short treatment times the evaporation might play a minor role, although still has to be considered for calculating correct reaction rates. The discharge of the COST-Jet is not affected by the evaporation and therefore the plasma chemistry is also not affected. For different plasma sources, where e.g. the plasma is in direct contact with liquid, the plasma chemistry is changing significantly with evaporation as shown by Tian and Kushner[45] for a dielectric barrier discharge. Also, for the kINPen, an effect of evaporations was determined[46]. Besides these mentioned differences in plasma chemistry that need to be considered for different plasma sources, also the topology of the meniscus induced by gas stream on liquid surface changes. The depth of this meniscus is usually depending on the gas velocity. For plasma sources where the electrode configuration induces a significant electrical field reaching the liquid or even with a plasma in contact with the liquid, this meniscus can be elevated[47,48]. As shown, several effects need to be considered according to the used plasma source.

In the future, this protocol can be used to conduct and describe surface and liquid treatments using the COST-Jet. It is a stable, reproducible plasma source exhibiting a unique remote character amongst the plethora of different plasma jet designs. The same methods are not limited to the COST-Jet source only and can be modified and adapted to use with any cold atmospheric pressure plasma source.

ACKNOWLEDGMENTS:
The authors thank Volker Rohwer (Institute of Experimental and Applied Physics, Kiel University) for help with the equipment. The work was supported by the DFG within CRC 1316 *Transient Atmospheric Plasmas*, in the project *Cold atmospheric plasmas for the study of fundamental interaction mechanisms with biological substrates* (project-ID BE 4349/5-1), and in the project *Plasma-generated nitric oxide in wound healing* (project-ID SCHU 2353/9-1) .

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:
1. Morfill, G.E., Kong, M.G., Zimmermann, J.L. Focus on Plasma Medicine. *New Journal of Physics.* **11** (11), 115011 (2009).
2. Schlegel, J., Köritzer, J., Boxhammer, V. Plasma in cancer treatment. *Clinical Plasma Medicine.* **1** (2), 2–7 (2013).
3. Weltmann, K.-D., Woedtke, T. von Plasma medicine—current state of research and medical application. *Plasma Physics and Controlled Fusion.* **59** (1), 14031 (2017).
4. Graves, D.B. Low temperature plasma biomedicine: A tutorial review. *Physics of Plasmas.* **21** (8), 80901 (2014).
5. Bruggeman, P.J. et al. Plasma–liquid interactions: A review and roadmap. *Plasma Sources Science and Technology.* **25** (5), 53002 (2016).
6. Simoncelli, E., Stancampiano, A., Boselli, M., Gherardi, M., Colombo, V. Experimental Investigation on the Influence of Target Physical Properties on an Impinging Plasma Jet. *Plasma.* **2** (3), 369–379 (2019).
7. Stancampiano, A. et al. Mimicking of human body electrical characteristic for easier translation of plasma biomedical studies to clinical applications. *IEEE Transactions on Radiation and Plasma Medical Sciences*. **1** (2019).
8. Nature Editorial. Reality check on reproducibility. *Nature.* **533** (7604), 437 (2016).
9. Baker, M. Is there a reproducibility crisis? *Nature.* **533**, 452-454 (2016).
10. Begley, C.G., Ioannidis, J.P.A. Reproducibility in science: Improving the standard for basic and preclinical research. *Circulation research.* **116** (1), 116–126 (2015).
11. Golda, J. et al. Concepts and characteristics of the 'COST Reference Microplasma Jet'. *Journal of Physics D: Applied Physics.* **49** (8), 84003 (2016).
12. Mann, M.S., Schnabel, U., Weihe, T., Weltmann, K.-D., Woedtke, T. von A Reference

Technique to Compare the Antimicrobial Properties of Atmospheric Pressure Plasma Sources. *Plasma Medicine.* **5** (1), 27–47 (2015).

13. Kogelheide, F. et al. FTIR spectroscopy of cysteine as a ready-to-use method for the investigation of plasma-induced chemical modifications of macromolecules. *Journal of Physics D: Applied Physics.* **49** (8), 84004 (2016).
14. Lackmann, J.-W. et al. Chemical fingerprints of cold physical plasmas - an experimental and computational study using cysteine as tracer compound. *Scientific Reports.* **8** (1), 7736 (2018).
15. Lackmann, J.-W. et al. Nitrosylation vs. oxidation - How to modulate cold physical plasmas for biological applications. *PloS one.* **14** (5), e0216606 (2019).
16. Ranieri, P. et al. GSH Modification as a Marker for Plasma Source and Biological Response Comparison to Plasma Treatment. *Applied Sciences.* **10** (6), 2025 (2020).
17. Ellerweg, D., Keudell, A. von, Benedikt, J. Unexpected O and O_3 production in the effluent of He/O_2 microplasma jets emanating into ambient air. *Plasma Sources Science and Technology.* **21** (3), 34019 (2012).
18. Waskoenig, J. et al. Atomic oxygen formation in a radio-frequency driven micro-atmospheric pressure plasma jet. *Plasma Sources Science and Technology.* **19** (4), 45018 (2010).
19. Golda, J., Kogelheide, F., Awakowicz, P., Schulz-von der Gathen, V. Dissipated electrical power and electron density in an RF atmospheric pressure helium plasma jet. *Plasma Sources Science and Technology.* **28** (9), 95023 (2019).
20. Golda, J., Held, J., Gathen, V.S.-v.d. Comparison of electron heating and energy loss mechanisms in an RF plasma jet operated in argon and helium. *Plasma Sources Science and Technology.* **29** (2), 25014 (2020).
21. Beijer, P.A.C., Sobota, A., van Veldhuizen, E.M., Kroesen, G.M.W. Multiplying probe for accurate power measurements on an RF driven atmospheric pressure plasma jet applied to the COST reference microplasma jet. *Journal of Physics D: Applied Physics.* **49** (10), 104001 (2016).
22. Riedel, F. et al. Reproducibility of 'COST reference microplasma jets'. *Plasma Sources Science and Technology* (2020).
23. COST Reference Microplasma Jet Homepage, www.cost-jet.eu.
24. Winter, J. et al. Feed gas humidity: a vital parameter affecting a cold atmospheric-pressure plasma jet and plasma-treated human skin cells. *Journal of Physics D: Applied Physics.* **46** (29), 295401 (2013).
25. Große-Kreul, S., Hübner, S., Schneider, S., Keudell, A. von, Benedikt, J. Methods of gas purification and effect on the ion composition in an RF atmospheric pressure plasma jet investigated by mass spectrometry. *EPJ Techniques and Instrumentation.* **3** (1), 6 (2016).
26. Benedikt, J. et al. Absolute OH and O radical densities in effluent of a He/H$_2$O micro-scaled atmospheric pressure plasma jet. *Plasma Sources Science and Technology.* **25** (4), 45013 (2016).
27. Willems, G., Benedikt, J., Keudell, A. von Absolutely calibrated mass spectrometry measurement of reactive and stable plasma chemistry products in the effluent of a He/H 2 O atmospheric plasma. *Journal of Physics D: Applied Physics.* **50** (33), 335204 (2017).
28. Gorbanev, Y. et al. Combining experimental and modelling approaches to study the sources of reactive species induced in water by the COST RF plasma jet. *Physical chemistry chemical physics : PCCP.* **20** (4), 2797–2808 (2018).

29. Held, J. mimurrayy/COST-power-monitor v0.9.2 (Version v0.9.2): Zenodo (2019).

30. Willems, G. et al. Corrigendum: Characterization of the effluent of a He/O 2 micro-scaled atmospheric pressure plasma jet by quantitative molecular beam mass spectrometry (2010 New J. Phys.12 013021). *New Journal of Physics.* **21** (5), 59501 (2019).

31. Mokhtar Hefny, M., Nečas, D., Zajíčková, L., Benedikt, J. The transport and surface reactivity of O atoms during the atmospheric plasma etching of hydrogenated amorphous carbon films. *Plasma Sources Science and Technology.* **28** (3), 35010 (2019).

32. Grant, I. Particle image velocimetry: A review. *Proceedings of the Institution of Mechanical Engineers, Part C: Journal of Mechanical Engineering Science.* **211** (1), 55–76 (2016).

33. Semenov, I.L., Weltmann, K.-D., Loffhagen, D. Modelling of the transport phenomena for an atmospheric-pressure plasma jet in contact with liquid. *Journal of Physics D: Applied Physics.* **52** (31), 315203 (2019).

34. Golda, J. Cross-correlating discharge physics, excitation mechanisms and plasma chemistry to describe the stability of an RF-excited atmospheric pressure argon plasma jet. Dissertation. Ruhr-Universität Bochum. Bochum (2017).

35. Lietz, A.M., Kushner, M.J. Molecular admixtures and impurities in atmospheric pressure plasma jets. *Journal of Applied Physics.* **124** (15), 153303 (2018).

36. Reuter, S. et al. Controlling the Ambient Air Affected Reactive Species Composition in the Effluent of an Argon Plasma Jet. *IEEE Transactions on Plasma Science.* **40** (11), 2788–2794 (2012).

37. Reuter, S. et al. From RONS to ROS: Tailoring Plasma Jet Treatment of Skin Cells. *IEEE Transactions on Plasma Science.* **40** (11), 2986–2993 (2012).

38. Gorbanev, Y., O'Connell, D., Chechik, V. Non-Thermal Plasma in Contact with Water: The Origin of Species. *Chemistry (Weinheim an der Bergstrasse, Germany).* **22** (10), 3496–3505 (2016).

39. Gorbanev, Y., Soriano, R., O'Connell, D., Chechik, V. An Atmospheric Pressure Plasma Setup to Investigate the Reactive Species Formation. *Journal of visualized experiments.* (117), 54765 (2016).

40. Darny, T. et al. Plasma action on helium flow in cold atmospheric pressure plasma jet experiments. *Plasma Sources Science and Technology.* **26** (10), 105001 (2017).

41. Kelly, S., Golda, J., Turner, M.M., Schulz-von der Gathen, V. Gas and heat dynamics of a micro-scaled atmospheric pressure plasma reference jet. *Journal of Physics D: Applied Physics.* **48** (44), 444002 (2015).

42. Schneider, S., Dünnbier, M., Hübner, S., Reuter, S., Benedikt, J. Atomic nitrogen: A parameter study of a micro-scale atmospheric pressure plasma jet by means of molecular beam mass spectrometry. *Journal of Physics D: Applied Physics.* **47** (50), 505203 (2014).

43. Hefny, M.M., Pattyn, C., Lukes, P., Benedikt, J. Atmospheric plasma generates oxygen atoms as oxidizing species in aqueous solutions. *Journal of Physics D: Applied Physics.* **49** (40), 404002 (2016).

44. Benedikt, J. et al. The fate of plasma-generated oxygen atoms in aqueous solutions: Non-equilibrium atmospheric pressure plasmas as an efficient source of atomic O(aq). *Physical Chemistry Chemical Physics.* **20** (17), 12037–12042 (2018).

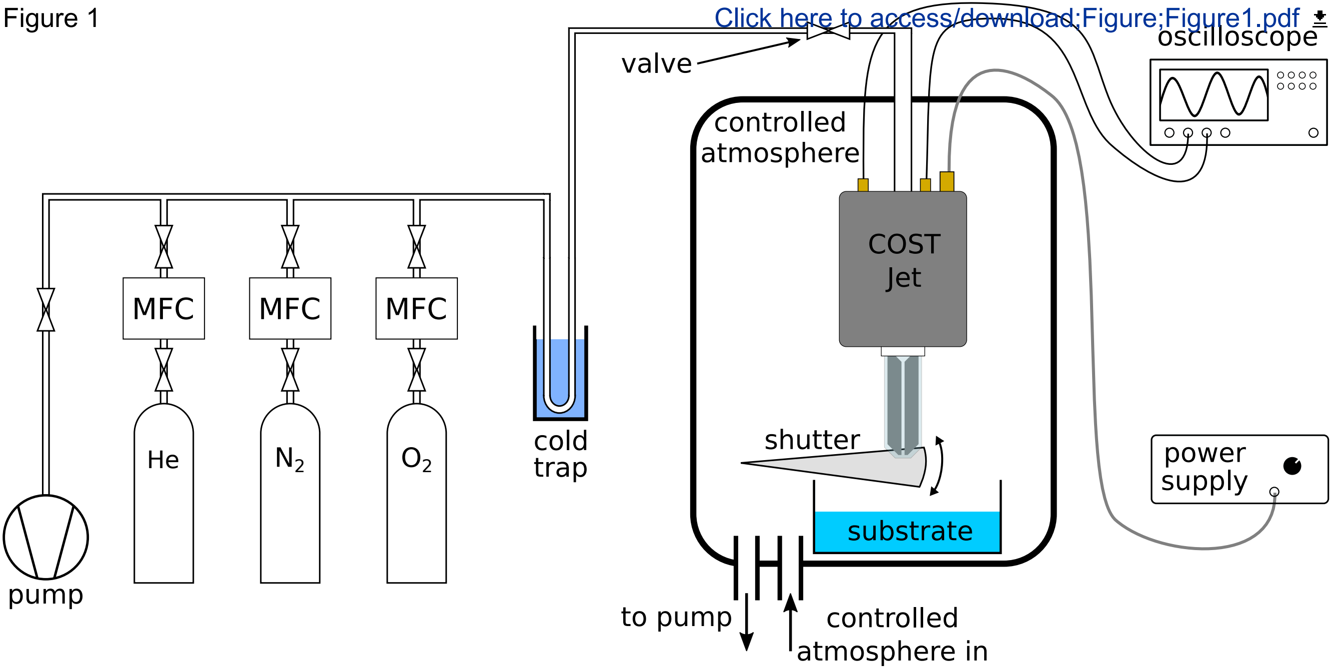
45. Tian, W., Kushner, M.J. Atmospheric pressure dielectric barrier discharges interacting with liquid covered tissue. *Journal of Physics D: Applied Physics.* **47** (16), 165201 (2014).

46. Hansen, L. et al. Influence of a liquid surface on the NO x production of a cold atmospheric pressure plasma jet. *Journal of Physics D: Applied Physics.* **51** (47), 474002 (2018).

47. van Rens, J.F.M. et al. Induced Liquid Phase Flow by RF Ar Cold Atmospheric Pressure Plasma Jet. *IEEE Transactions on Plasma Science.* **42** (10), 2622–2623 (2014).

48. Bruggeman, P., Graham, L., Degroote, J., Vierendeels, J., Leys, C. Water surface deformation in strong electrical fields and its influence on electrical breakdown in a metal pin–water electrode system. *Journal of Physics D: Applied Physics.* **40** (16), 4779–4786 (2007).

Figure 1



oscilloscope
valve
controlled atmosphere
COST Jet
shutter
substrate
power supply
MFC
MFC
MFC
He
N_2
O_2
cold trap
pump
to pump
controlled atmosphere in

Figure 2

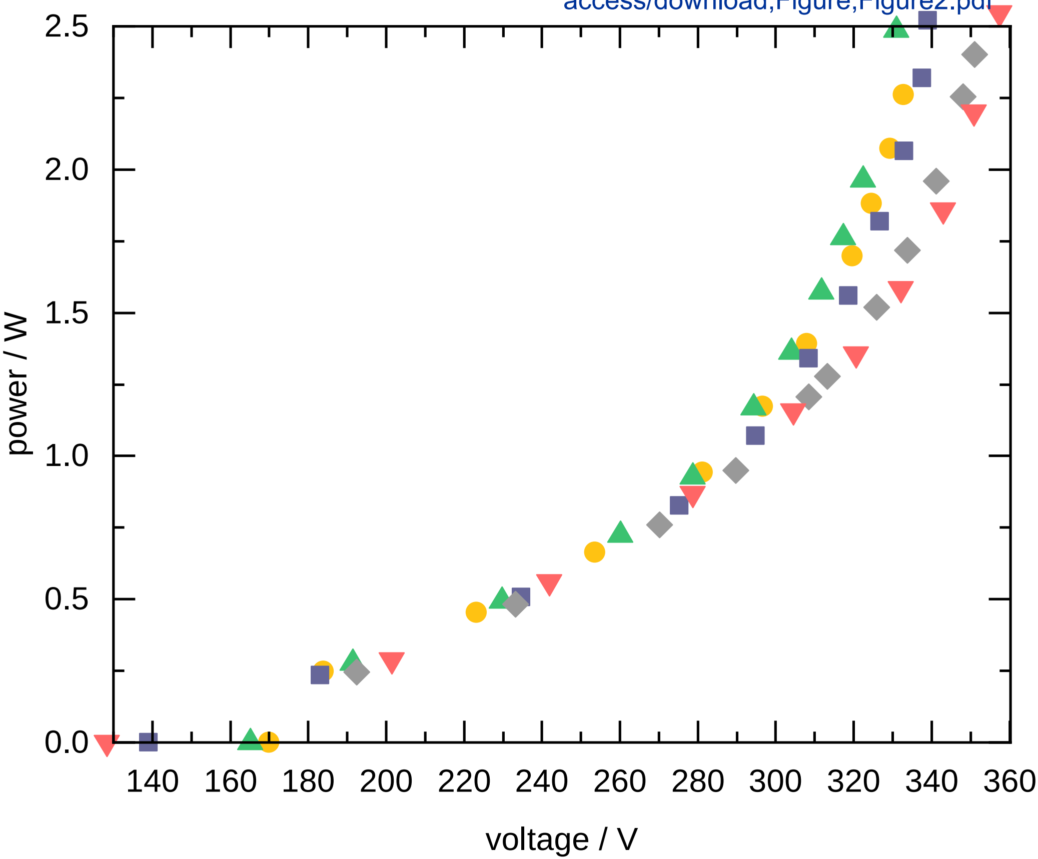
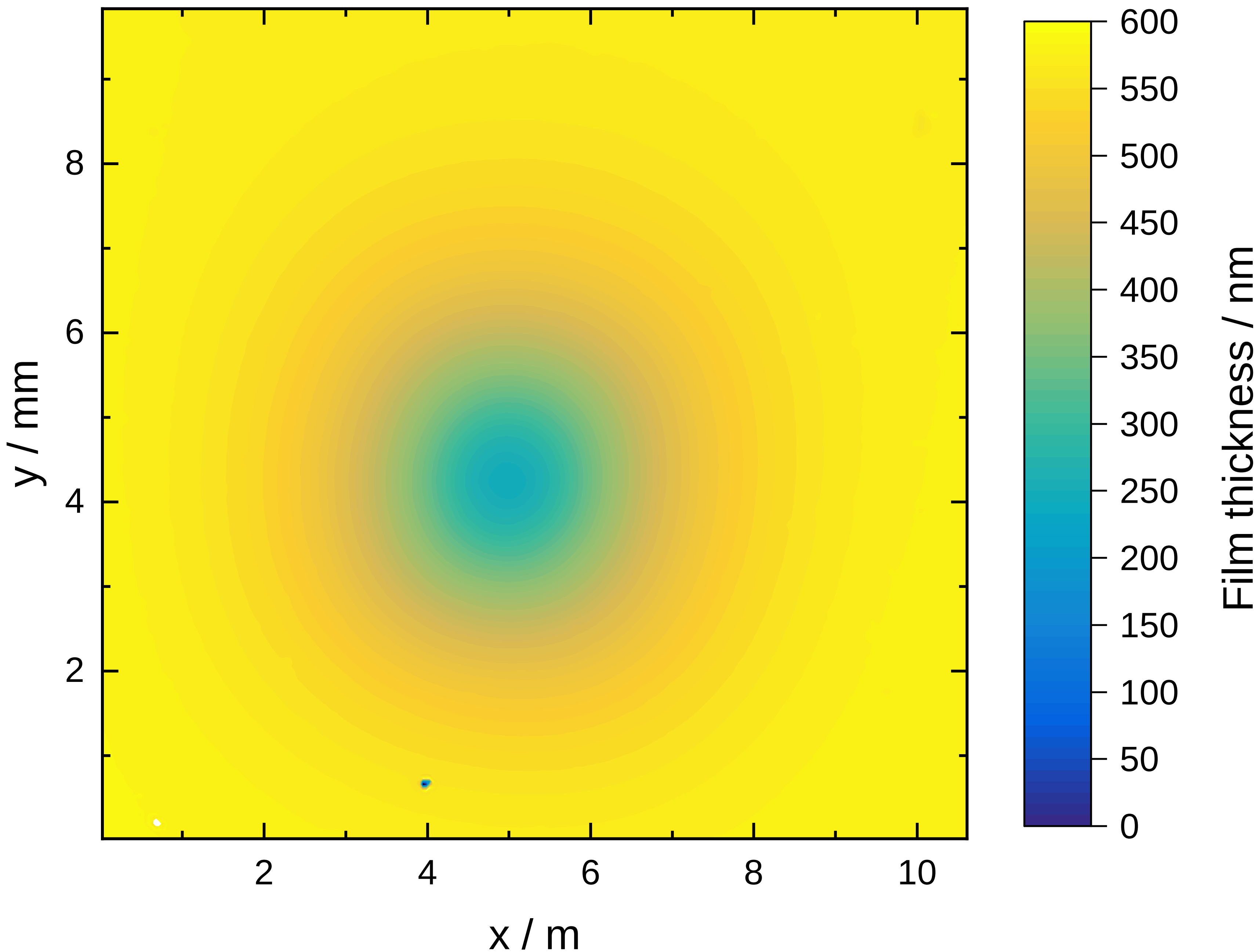

power / W
2.5
2.0
1.5
1.0
0.5
0.0
140
160
180
200
220
240
260
280
300
320
340
360
voltage / V

Figure 3



8
6
4
2
y / mm
2
4
6
8
10
x / m
600
550
500
450
400
350
300
250
200
150
100
50
0
Film thickness / nm

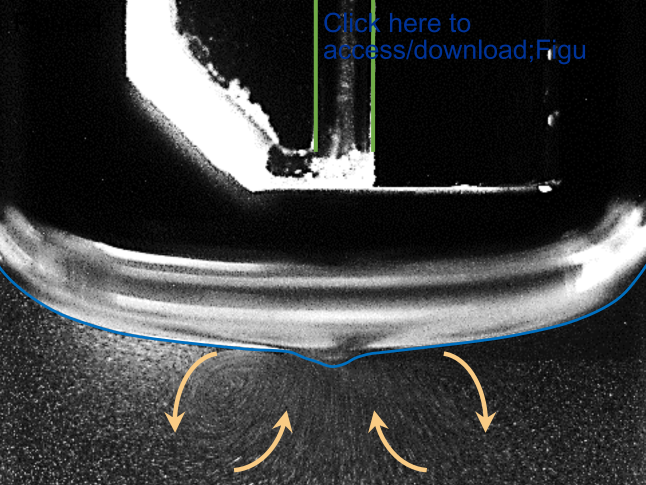


Figure 5

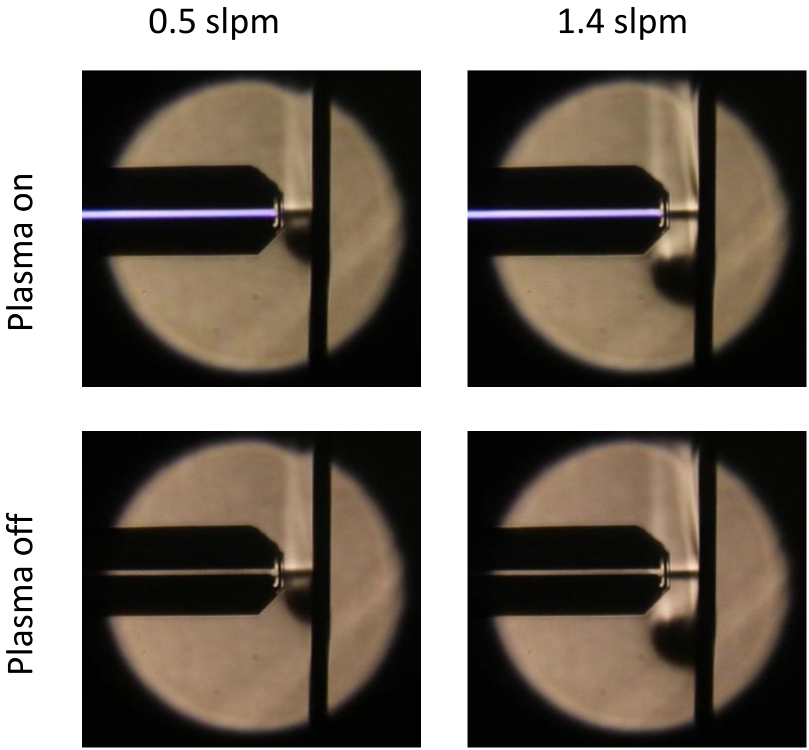


Figure1

Click here to access/download
Video or Animated Figure
Figure1.svg

Figure2

Click here to access/download
Video or Animated Figure
Figure2.svg

Figure3

Click here to access/download

Video or Animated Figure

Figure3.svg

Figure4

Click here to access/download

Video or Animated Figure

Figure4.svg

Figure5

Click here to access/download
Video or Animated Figure
Figure5.svg

Name of Material/Equipment	Company	Catalog Number	Comments/Description
COST power monitor software	home-built		according to www.cost-jet.eu and J Golda et al 2
COST-Jet (including matching circuit)	home-built		according to www.cost-jet.eu and J Golda et al 2
current probe	home-built		integrated into the COST-Jet
gas supply system	Swagelok		stainless steel
helium	Air Liquide		99.999 % purity
mass flow controller (MFC)	Analyt-MTC	series 358	5000 sccm
MFC	Analyt-MTC		50 sccm
oscilloscope	Agilent Technologies	DSO7104B	bandwidth 1 GHz, resolution 4 Gsa/s
oxygen	Air Liquide		99.9999 % purity
power supply	home-built		according to www.cost-jet.eu and J Golda et al 2
voltage probe	Tektronix	P5100A	
xyz-stage	Zaber	ZAB-X-XAZ-LSM0100A-K0059-SQ3	

016 J. Phys. D: Appl. Phys. 49 084003

016 J. Phys. D: Appl. Phys. 49 084003

016 J. Phys. D: Appl. Phys. 49 084003

08/18/2020

Article reference: JoVE61801

Manuscript title: Treating surfaces with a cold atmospheric pressure plasma using the COST-Jet

Authors: Golda, Judith; Held, Julian; Schulz-von der Gathen, Volker

Dear Editor,

Thank you for your email dated July 22nd, 2020. We are pleased to know that our manuscript was rated as potentially acceptable for publication in Journal of Visualized Experiments by all referees, subject to adequate revision and response to the comments raised by the reviewers.

We would like to thank all referees for their time and effort that they have put into assessing the previous version of our manuscript and for their valuable comments.

After carefully considering the comments made in the referees' report, we hereby submit a revised version of our manuscript for your consideration.

Please find attached to this letter a detailed point-by-point response to the referees' reports. In addition, we included a document ("Golda_JoVE_diff.docx") showing the difference between the original manuscript and the current submission.

We look forward to hearing from you regarding our submission and to respond to any further questions and comments you may have.

Yours sincerely,

Dr. Judith Golda

Institute of Experimental and Applied Physics
Experimental Plasma Physics
Kiel University
Leibnizstraße 17, 24118 Kiel
Tel.: +49 (0) 431 880 3871
Fax: +49 (0) 431 880 3809

golda@physik.uni-kiel.de

Referee Response: Dissipated electrical power and electron density in an RF atmospheric pressure helium plasma jet

This document details changes to manuscript JoVE61801 submitted on August, 19th 2020 following receipt of referees' reports. A detailed point-by-point response for editorial comments and recommendations of referee 1, 2, 3 and 4 is given (shown in blue font). This is followed by a section outlining additional changes made to this updated manuscript. An attachment " Golda_JoVE_diff.docx " enclosed with this submission shows the difference between the original manuscript and the current submission. This has been prepared using Microsoft Word. It is hoped this will clearly detail all amendments to the original article.

Point-by-Point response

Editorial comments:

You will find Editorial comments and Peer-Review comments listed below. Please read this entire email before making edits to your manuscript.

NOTE: Please include a line-by-line response to each of the editorial and reviewer comments in the form of a letter along with the resubmission.

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

As suggested, the manuscript has been proofred and checked for errors.

• Please list a minimum of 6 keywords/phrases.

The keywords are listed in the manuscript lines 26-27: Atmospheric pressure plasma, COST-Jet, plasma medicine, plasma treatment, handling protocol, liquid, surface treatment, CAP.

• **Textual Overlap:** Significant portions show significant overlap with previously published work. Please re-write lines 50-52, 56-58 to avoid this overlap.

Lines 50-52, 56-58 have been re-written to avoid overlap with previously published work.

• **Introduction:** Remove the numbered list.

Amended as suggested. The numbered list has been replaced by a key point list.

• **Protocol Language:** Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

1) Some examples NOT in the imperative: 1.1, 1.2, 1.3, 1.4, 1.5–1.7, etc.

Text in the protocol section has been changed to imperative tense.

2) Split up long steps (e.g, 2.4, 2.6, etc).

Long steps have been split up.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please ensure that all specific details (e.g. button clicks for software actions, numerical values for settings, etc) have been added to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

More details have been added to the steps, e.g. button clicks for software actions.

• **Protocol Numbering:** Please add a one-line space after each protocol step.

Amended as suggested.

• **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.
1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting.

We highlighted the steps of the protocol to be visualized in yellow.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

We addressed all of the above-mentioned categories in the discussion. Due to the complex nature of the plasma chemical treatment, we have sorted the categories by topic. To increase the focus of the discussion, we shortened part of the text. Additionally, we inserted keywords such as „critical steps“ etc. into the discussion to improve orientation.

• **Table of Materials:**
1) Please sort in alphabetical order.
Amended as suggested.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to republish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

We do not re-use figures that have been published previously.

Reviewer #1:

The Authors present a well written and perfectly described set of actions for using the COST reference plasma jet in the treatment of materials. The papaer is certainly fit for JoVE and deserves to be published. Some revisions are anyhow needed to have it published.

- First of all, the title should be changed from "Treating surfaces with a cold atmospheric pressure plasma using the COST-Jet" to something more coherent with the paper content. Results are presented only for a liquid interface and the word "solid" is used only two times besides title and abstract.

We use the word "surface" for both solids and liquids, which also constitute a surface. We changed the heading of section 4 to "(Solid) surface treatment" to clarify that this section is dedicated to the treatment of solids while section 5 is dedicated to the treatment of liquid surfaces. The summary was also amended to make this clearer, now stating: "This protocol is presented to characterize the setup, handling and application of the COST-Jet for treatment of diverse surfaces such as solids and liquids.

- The Authors might give more details on the relative importance for the reproducibility of results of using metal pipes and clean the gas supply lines given ad mandatory while the only suggest setting up a controlled atmosphere for the experiments (point 1.8).

We have already addressed both issues in the discussion of the previous manuscript: Firstly, as the purity of the feed gas and thus the tightness of the gas supply system determines the plasma chemistry, this point is relevant of all plasma sources including the COST-Jet. Therefore, it is included in step 1.1 in the protocol: “Set up the gas supply consisting of all-metal gas lines, avoiding any TPFE or similar plastics tubing (Winter[24])“.

Secondly, the surrounding atmosphere of an atmospheric pressure plasma can potentially influence the reactive species reaching a surface, as well. However, Gorbanev *et al.* have shown that for the COST-Jet this is not the case (Gorbanev, Y. *et al.* Combining experimental and modelling approaches to study the sources of reactive species induced in water by the COST RF plasma jet. *Physical chemistry chemical physics: PCCP.* **20** (4), 2797–2808, 10.1039/C7CP07616A (2018).). Therefore, we only suggest setting up a controlled atmosphere for the COST-Jet.

- The Authors should consider the opportunity of giving more information on the details of figure 4. They should explain if the meniscus is affecting the whole surface of the liquid substrate and why there's no evident dimple under the jet; I might see a dimple, but the Authors do not put that into evidence. Is the meniscus due to the geometry of the experimental area and to the liquid characteristics? Or is it given by the local fluidynamics of the experiment? It would be interesting to understand the reasons for the versus of the flow as shown by the arrows added to the raw picture. Also if it's correct that the jet axis doesn't seem to be centered in the middle of the liquid substrate area. It's particularly strange that the Authors did not mention some recent works where jets (other

than COST reference) impinging on liquid and solid targets are considered and the influence on results of the substrate properities:
A. Stancampiano, E. Simoncelli, M. Boselli, V. Colombo, M. Gherardi, Experimental investigation on the interaction of a nanopulsed plasma jet with a liquid target, Plasma Sources Science and Technology (2018) DOI:10.1088/1361-6595/aae9d0
E. Simoncelli, A. Stancampiano, M. Boselli, M. Gherardi, V. Colombo, Experimental investigation on the influence of target physical properties on an impinging plasma jet, Plasma, Special Issue "Low Temperature Plasma Jets: Physics, Diagnostics and Applications" DOI: 10.3390/plasma2030029

Thanks for pointing out that Figure 4 is not as explicit as we thought it would be. For clarity, we added some more schematic lines to address this issue. The liquid surface on the picture is defined by the blue line showing a tiny dimple directly below the COST-Jet's effluent. The visible edge above this line is created by the capillary action of the liquid inside of the cuvette. We conducted some fluid simulations to understand the movement of the liquid dependent on the impinging gas flow. The direction of the vortices is defined by the surface friction between the liquid and the effluent gas flow streaming above the liquid surface. We would really like to go into more detail regarding the details. However, we were asked to keep the description short here by the editorial comments. There will be a follow-up publication on this topic concentrating on the liquid treatment.

To underline the symmetry of the picture and the centered gas flow, we also added schematic lines to emphasize the COST-Jet's discharge channel contours in the picture.

We added the references to the introduction of the publication.

- Can the Authors comment on any evaporation of the liquid during the experiments?

Evaporation is an issue when treating liquids using cold atmospheric pressure jet sources. In particular for long treatments times and small liquid volumes, the effect on the concentration of species should be considered. Additionally, evaporating liquids might influence the plasma chemistry. In our experiments, we observed evaporation rates in the range of 0,03 ml/min.

- The Authors should describe the experimental configuration of Figure 5: Schlieren images of the COST-Jet with and without applied voltage for two different gas flow rates. Nowhere in the text the reader can understand which is the substrate for those images.
- Also, no information is given on the type of Schlieren imaging setup used by the Authors, even though Schlieren imaging is one of the paper keywords.
- To give the reader a better understanding of the Schlieren imaging techiques used to investigate the interation with surfaces the Authors might reference the recent "review" paper:
E. Traldi, M. Boselli, E. Simocelli, A. Stancampiano, M. Gherardi, V. Colombo, G. S. Settles, Schlieren imaging: a powerful tool for atmospheric plasma diagnostic, EPJ Techniques and Instrumentation: Thematic Series on Novel Plasma Diagnostics (2018), DOI:10.1140/epjti/s40485-018-0045-1

The Schlieren images were taken using a single mirror inline arrangement. As Schlieren imaging is not the main topic in this publication, we did not included a detailed description of the setup in the text but added a reference that shows a schematic and a description of the setup. Additionally we added the following sentence to the text: "They show how the horizontally aligned COST-Jet effluent hits a flat glass substrate." Additionally, we removed "Schlieren imaging" from the keywords.

- Why it is true that some of the methods proposed in the paper might be usefully in common use also for other plasma jet sources, to avoid what might be read as an inflated claim ("It is a stable, reproducible plasma source exhibiting a unique remote character amongst the plethora of different plasma jet designs"), the Authors should also say something on the possible limits of this source, if existent, in really treating solids and liquids for materials of biomedical purposes besides laboratory experiments.

We added a sentence in the introduction to underline the scientific purpose of the COST-Jet: “The COST-Jet is a reliable plasma source that was developed for scientific reference purposes rather than for industrial or medical use”.

- The text of reference 15 must be corrected.

Thanks for pointing this out, we corrected the typo.

Reviewer #2:
Manuscript Summary:
The manuscript is written smoothly. Although there is not nay novelty in the manuscript, the authors have tried to explain the protocols to perform reliable and reproducible surface treatments using the COST-Jet.

Major Concerns:
There are few comments that needs to be addressed. This has been suggested in the manuscript. E.g, how did you calculate the power? The phase angle has not been mentioned. Can you please include the formula used for the estimation of power? as there are many formula for estimating the power?

For power calculation, we used the “COST power monitor” software as described in the manuscript it is based on the formula $P = U * I * \cos(\varphi)$ as described in previous publications (J Golda *et al* 2016 *J. Phys. D: Appl. Phys.* **49** 084003, J Golda *et al* 2019 *Plasma Sources Sci. Technol.* **28** 095023). We added these references to the protocol.

It will be better if you could calculate the variation of electron density with the change in voltage, flow-rate.

We agree that this is an important and interesting measurement. Therefore, we did this in a previous publication (J Golda *et al* 2019 *Plasma Sources Sci. Technol.* **28** 095023).

Is the 50Ω current measuring resistor part of experimental setup, or is it used only during the analyses of plasma? If latter, does the resistor influence the plasma properties?
The author has should explain their apparatus/ experimental setup in detail.

The experimental setup is described in detail in previous publications (J Golda *et al* 2016 *J. Phys. D: Appl. Phys.* **49** 084003, J Golda *et al* 2019 *Plasma Sources Sci. Technol.* **28** 095023). As the 50 Ω resistors are part of the oscilloscope, it is not explicitly drawn in the schematics. The resistors are always used when measuring current or voltage. They do not influence the plasma properties as they are only part of the measuring circuit for electrical diagnostics. As advised by the editors of JoVE, we kept the description of the setup as concise as possible and instead added references to previous publications (e.g. J Golda *et al* 2019 *Plasma Sources Sci. Technol.* **28** 095023) instead.

Minor Concerns:
There are minor grammatical mistakes in the manuscript that needs to be addressed. Further, there are repetition of the sentences in the 2.8 and 4.3. Make it short and sweet

We proof-read the manuscript once again and hope to have found all of the grammatical mistakes and typos. The repetitions are due to the protocol that requires a strict description of the required actions.

Reviewer #3:
Manuscript Number: JoVE61801
The manuscript entitled: Treating surfaces with a cold atmospheric pressure plasma using the COST-Jet has been revised.

My comments; It is an interesting and valuable piece of work and worth the publishing after considering the following comments:
- There is no sufficient statement in the introduction to highlight the necessity and importance of the present work.

Due to the the complex conditions in biomedical plasma treatments, standardized sources and protocols might increase the reproducibility of scientific results. Therefore, we decided to make a detailed description available of how to apply the COST-Jet to surfaces. This is underlined in the introduction using the sentence "However, the scientific results often suffer from reliability and reproducibility problems" which cites two articles in *Nature* depicting the challenge. To make this even more clear, we added another sentence in the introduction: "Compliance with the protocol presented here should ensure the reproducibility and comparability of the measurements."

Reviewer #4:
Manuscript Summary:
Authors demonstrate a protocol for the COST-Jet, 1 mm plasma exit for surface/liquid treatment.

Major Concerns:
Instead of the voltage to power plot (figure 2), should authors provide reactive species to power plot as a representative result to fulfil the surface/liquid treatment (figure3,4). The COST-Jet aims to stand for He-O jet plasma source, then the O-atom dose that reach the surface(s) might mostly be informed in the protocol.

We also think that the oxygen density in dependence of the applied power and target distance is an important and interesting measurement. Therefore, these measurements have already been performed and published in previous papers (e.g. Gert Willems *et al* 2019 *New J. Phys.* **21** 059501 or Frederik Riedel et al 2020 Plasma Sources Sci. Technol. in press https://doi.org/10.1088/1361-6595/abad0) which are also referenced in the text. We added a sentence to the representative results section: "More detailed measurements of reactive species (e.g. atomic oxygen and ozone), temperature and power as well as bactericidal activity measurements have been performed by Riedel et al.[23].".

Additional corrections and amendments

We additionally exchanged single words and corrected misspellings for better readability. For details, please see attached 'Golda_JoVE_diff.docx'.

Judith Golda received a Master of Science degree in physics from the Ruhr University Bochum, Bochum, Germany, in 2014 and the Ph.D. in plasma physics in 2018 for the study of excitation mechanisms in an atmospheric pressure argon plasma jet by electrical as well as optical emission and absorption diagnostics. She is currently a Postdoc in the Experimental Plasma Physics Group of the *Institute of Experimental and Applied Physics* at Kiel University, Kiel, Germany. Her research focuses on the fundamental physics of atmospheric pressure plasmas and plasma-surface interaction.

Kerstin Sgonina received the Master of Science degree in physics from the Ruhr University Bochum, Bochum, Germany, in 2017. She is currently a Ph.D. student in the Experimental Plasma Physics Group of the *Institute of Experimental and Applied Physics* at Kiel University, Kiel, Germany. Her investigations focus on the interaction between plasma generated reactive species and liquids by combining experimental and modelling studies.

Julian Held received a Master of Science degree in physics at the Ruhr University Bochum in Germany. Since 2017, he is employed as a Ph.D. candidate at the Chair of Experimental Physics II at the Ruhr University Bochum. His work is primarily on the plasma physics of high power impulse magnetron discharges.
Mr Held also works on plasma diagnostics of atmospheric pressure plasmas. He has been involved with the COST-Jet project since 2015 and has assembled more than 20 devices.

Jan Benedikt received the Diploma degree at the University of West Bohemia in Czechia and Ph.D. degree in Physics at the Eindhoven University of Technology in the Netherlands in 2000 and 2004, respectively. He was between 2004 and 2010 a research assistant and between 2010 and 2017 a junior professor at the Faculty of Physics and Astronomy of the Ruhr University Bochum in Germany. He was appointed a full professor at the Kiel University, group Experimental Plasma Physics, in 2017. His research focus are plasma-chemical processes in reactive low and atmospheric pressure plasmas with focus on molecular beam mass spectrometry, on the plasma-surface interaction, and on the generation of nanostructured material. He has received Hans-Werner-Osthoff Plasma Physics Prize in the year 2009. He is a Board member of the International Plasma Chemistry Society (IPCS) and member of the German Physical Society (DPG) and the Union of Czech mathematicians and physicists.

Volker Schulz-von der Gathen received Diploma degree in physics from the University of Duisburg-Essen, Essen, Germany in 1978 and the Dr. rer. nat. (‚Dr rerum naturalis‘, Ph.D.) in 1990 for the investigation of a contracted hydrogen arc by means of a combination of diagnostics.
Since 1983 he worked as researcher at the Institute for Laser and Plasma Physics on a variety of low and atmospheric pressure discharges, as contracted arcs, capacitively coupled rf discharges and finally atmospheric pressure rf discharges. During this time he supervised the practical training for advanced students.
In 2006 he moved to the Chair of Experimental Physics II (Physics of reactive plasmas) at the Ruhr University Bochum. The focus of his research and his group lies on - in particular - optical diagnostics of reactive species in atmospheric pressure plasmas as jets or microplasma array devices e.g. for plasma medicine and surface interactions. Other activities are directed towards dielectric barrier and low pressure HIPIMS discharges. The projects are funded from various agencies.
Mr. Schulz-von der Gathen is a member of the German Physical Society (DPG), the German Society on Plasma Technology, and the International Society for Plasma Chemistry.