



## MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

### TREBALL FINAL DE MÀSTER

**"Study of air flow and temperature on corneal Surface using  
a model eye"**

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Terrassa, 22 de Gener de 2020

Facultat d'Òptica i Optometria de Terrassa

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## MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

### **“Study of air flow and temperature on corneal Surface using a model eye”**

#### **RESUM**

L'estesiòmetre d'aire és un instrument no invasiu que s'està desenvolupant per a millorar la tècnica de mesura de la sensibilitat corneal. L'objectiu d'aquesta investigació, la qual forma part d'un projecte més gran en el marc d'una tesi doctoral a la Universitat de Waterloo (Canada), era participar en el desenvolupament i avaluació d'un nou estesiòmetre estudiant les característiques del seu flux d'aire i els canvis de temperatura que aquest produeix. Primer, es va analitzar la força de l'aire que sortia del estesiòmetre mitjançant una microbalança. Les lectures de la balança (g) augmenten segons la intensitat de sortida d'aire de l'aparell. Després, es va estudiar la dispersió del flux d'aire que emet l'estesiòmetre mitjançant pols de licopodi. El diàmetre dels craters generats per la dispersió de la pols són més grans quan la intensitat de l'aparell augmenta i els resultats de la ploma de pols corresponent també es veuen alterats. Finalment, es mesurava la mida en diàmetre de la petjada tèrmica generada per l'aire, utilitzant la càmera tèrmica FLIR A655sc (FLIR Systems Inc., Portland, USA) i un ull model desenvolupat a la mateixa Universitat de Waterloo per l'equip de “Murphy Laboratory for Experimental Optometry (MLEO)”. En aquest cas, la petjada tèrmica és més gran a mesura que augmenta la distància entre l'estesiòmetre i l'ull model. A més, per intensitats baixes el diàmetre és més petit que per a intensitats altes. Com a conclusió, és necessària més recerca per determinar les característiques òptimes de l'estímul per les mesures *in vivo*. De moment, sembla que hi hagi relació entre la mida del diàmetre trobat al segon experiment i la petjada tèrmica del tercer experiment. També s'ha trobat en els tres experiments una relació on la durada de l'estímul no és un factor tant crític com la intensitat de la sortida d'aire de l'aparell, que es podria considerar el paràmetre més rellevant. La termografia també es mostra com una modalitat d'imatge fiable per estudiar els canvis de temperatura de la superfície corneal.

*Paraules clau:* estesiòmetre, còrnia, sensibilitat corneal, termografia.



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### **"Study of air flow and temperature on corneal Surface using a model eye"**

#### **RESUMEN**

El estesiómetro de aire es un instrumento no invasivo que se está desarrollando para mejorar la técnica de medida de la sensibilidad corneal. El objetivo de esta investigación, que forma parte de un proyecto más amplio, en el marco de una tesis doctoral en la Universidad de Waterloo (Canada), era participar en el desarrollo y evaluación de un nuevo estesiómetro estudiando los parámetros de su flujo de aire y los cambios de temperatura que este produce. Primero, se analizó la fuerza ejercida por el aire que emitía el estesiómetro a partir de una microbalanza. Las lecturas de la balanza (g) aumentan según la intensidad del flujo de aire del aparato. Después, se estudió la dispersión del flujo de aire que salía del estesiómetro mediante polvo de licopodio. El diámetro de los cráteres generados por la dispersión del polvo es más grande cuando la intensidad del aire aumenta y los resultados de la pluma de polvo correspondiente también se ven alterados. Finalmente, se media el tamaño de la huella térmica generada por el aire, utilizando la cámara térmica FLIR A655sc (FLIR Systems Inc., Portland, USA) y un ojo modelo desarrollado en la misma Universidad de Waterloo por el equipo de "Murphy Laboratory for Experimental Optometry (MLEO)". En este caso, la huella térmica es mayor a medida que aumenta la distancia entre el estesiómetro y el ojo modelo. Además, para intensidades bajas el diámetro es más pequeño que para intensidades altas. Como conclusión, se necesita más investigación para determinar las características óptimas del estímulo para las medidas *in vivo*. De momento, parece ser que hay una relación entre la medida del diámetro encontrado en el segundo experimento y la huella térmica del tercero. También se ha encontrado en los tres experimentos una relación donde el tiempo del estímulo no es un factor tan crítico como la intensidad de salida de aire del estesiómetro, que se podría considerar el parámetro más relevante. La termografía también demostró ser una modalidad de imagen fiable para estudiar los cambios de temperatura de la superficie corneal.

*Palabras clave:* estesioterapia, cornea, sensibilidad corneal, termografía.



## MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

### Study of air flow and temperature on corneal Surface using a model eye

#### ABSTRACT

The non-contact corneal aesthesiometer (NCCA) is a non-invasive instrument being developed to measure corneal sensitivity. The objective of this research, which is part of a larger project leading to a PhD thesis at the University of Waterloo (Canada), was to assist in the development and evaluation of a new NCCA by studying the air-flow parameters and the temperature changes using a model eye. First, the force exerted by the exit-jet was analyzed using a microbalance. Microbalance readings (g) increased with the intensity of the air-flow. Second, the dispersion of the air-flow exiting the aesthesiometer was studied using lycopodium powder. The diameters of the craters resulting from dispersion of the powder were larger at higher intensities and the corresponding dust plume was also altered. Finally, the thermal footprint size of the air-flow was measured using the FLIR A655sc infrared camera (FLIR Systems Inc., Portland, USA) and a model eye developed by the Murphy Laboratory for Experimental Optometry (MLEO). In this case, for longer distances between the aesthesiometer and the model eye the results of the footprint showed larger diameters. In addition, at low intensities of the air-jet the footprint size was smaller. More research is needed to determine the best characteristics of the stimulus for *in vivo* measurements but a good relationship was found between the diameter of the crater in the experiment with lycopodium powder and the thermal footprint size. The three experiments were in agreement in that the duration of the stimulus was not as critical a factor as the intensity of the air-jet, which could be considered the most relevant parameter. Thermography was also shown as a valuable imaging modality to study ocular surface temperature changes.

**Keywords:** aesthesiometer, cornea, corneal sensibility, thermography.



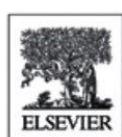
## ACKNOWLEDGEMENTS

To Genis, Paul and Melanie who gave me the chance  
and my family and friends who provided their support and unconditional love.



This project is written in an article format aiming to be sent to *Contact Lens Anterior Eye* (Elsevier). This magazine belongs to the British Contact Lens Association and occupies the place 29/59 (Q2) in the Ophthalmology category of the Journal Citation Reports, with an Impact Factor of 1.985 (2018).

Instructions for the authors of the magazine can be found in Annex 4.



FEBRUARY 2019 VOLUME 42 NUMBER 1 ISSN 1367-0484

# contactlens & ANTERIOR EYE

The Journal of the British Contact Lens Association

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Scleral Lens Special Edition

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- Visual and physiological outcomes of scleral lens wear
- Relationship of Placido corneal topography with scleral lens fitting
- Tear dynamics under scleral lenses
- Corneal oedema during sealed miniscleral CL wear
- Anterior eye surface changes following miniscleral CL wear
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Elisabet Simó Bertran

# Study of air flow and temperature on corneal Surface using a model eye

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## ABSTRACT

The non-contact corneal aesthesiometer (NCCA) is a non-invasive instrument being developed to measure corneal sensitivity. The objective of this research, which is part of a larger project leading to a PhD thesis at the University of Waterloo (Canada), was to assist in the development and evaluation of a new NCCA by studying the air-flow parameters and the temperature changes using a model eye. First, the force exerted by the exit-jet was analyzed using a microbalance. Microbalance readings (g) increased with the intensity of the air-flow. Second, the dispersion of the air-flow exiting the aesthesiometer was studied using lycopodium powder. The diameters of the craters resulting from dispersion of the powder were larger at higher intensities and the corresponding dust plume was also altered. Finally, the thermal footprint size of the air-flow was measured using the FLIR A655sc infrared camera (FLIR Systems Inc., Portland, USA) and a model eye developed by the Murphy Laboratory for Experimental Optometry (MLEO). In this case, for longer distances between the aesthesiometer and the model eye the results of the footprint showed larger diameters. In addition, at low intensities of the air-jet the footprint size was smaller. More research is needed to determine the best characteristics of the stimulus for in vivo measurements but a good relationship was found between the diameter of the crater in the experiment with lycopodium powder and the thermal footprint size. The three experiments were in agreement in that the duration of the stimulus was not as critical a factor as the intensity of the air-jet, which could be considered the most relevant parameter. Thermography was also shown as a valuable imaging modality to study ocular surface temperature changes.

**Keywords:** aesthesiometer, cornea, corneal sensibility, thermography.

## 1. Introduction

The cornea is considered to be one of the most innervated tissues of the human body. Corneal innervation plays a role in protecting the tissue from adverse factors that can be detrimental to its integrity and metabolism [1,2,3,4]. Any alteration in the sensitivity of the cornea may compromise the functional unit of the tear, thus leading to the development of dry eye disease, as well as impacting the ability to detect any foreign body that could cause significant damage to the ocular surface [5]. A regular control of corneal sensitivity is important to aid in the prevention and management of ocular surface alterations such as dry eye, keratitis, and herpes, and to assess the decreased corneal sensitivity often associated with eye surgeries, diabetes, and the use of contact lenses and therapeutic agents [6].

The cornea is innervated by the ophthalmic division of the trigeminal nerve, through the long ciliary nerves. Corneal innervation contributes to maintaining the integrity of the ocular surface through fostering cell growth and the proliferation of epithelial cells, wound healing, and repair [7,8]. Stimuli to the cornea are detected by free nerve endings, which consist of two types of axons: the first type is the myelinated A-delta fibers, which respond to mechanical stimuli, and conduct nerve impulses quickly; the second type of nerve fibers are the C-fibers, which respond to thermal, mechanical, and chemical stimuli. Unlike the A-delta fibers, the C-fibers are unmyelinated, which results in a slower conduction rate of nerve impulses. The C-fibers represent the majority of the sensory nerves reaching the cornea, and act as a polymodal nociceptors [9,10].

Corneal sensitivity is measured using an instrument known as an aesthesiometer. Sensitivity is the inverse of the threshold response to a stimulus, which can be mechanical, chemical, or thermal. Von Frey started stimulating the corneal nerves mechanically using horse hairs in 1894. This method was later modified first by Boberg-Ans in 1955, and then by Cochet-Bonnet. The Cochet-Bonnet aesthesiometer (COBO) is known as the gold standard aesthesiometer and involves stimulating the mechanosensory nerve fibers (specifically the A-delta fibers) pressing the cornea with a nylon thread. However, this method is not only very invasive, but may also be affected by many external agents, such as room temperature and humidity, maintenance of the device, technique and expertise of the examiner, and patient apprehension [1,11,12,13]. These limitations have led to the development of other aesthesiometers which allow the measurement of corneal sensitivity to mechanical, chemical, or thermal stimuli, whilst avoiding contact with the ocular surface [14].

One such example is the non-contact corneal aesthesiometer (NCCA) engineered by Murphy and associates [11]. This non-invasive instrument uses a controlled mechanical pulse of air to stimulate the cornea. As the air makes contact with the cornea, a cooling effect is produced by the localized evaporation of tear film from the ocular surface, thus stimulating the thermal receptors. However, one key limitation of the device is that there is a delay of approximately 0.2 seconds in reaching maximum stimulus intensity [15]. Other non-invasive aesthesiometers developed over the years have added other stimuli such as temperature (hot and cold air pulses), and chemical substances (irritant chemical stimuli) to assess corneal sensitivity [14,16,17]. However, with these aesthesiometers there is often an overlapping of stimuli, thus making it difficult to isolate the mechanical and thermal responses, for example [15]. To overcome the limitations of the existing aesthesiometers, the Murphy Laboratory of Experimental Optometry (MLEO) at the University of Waterloo has developed a new and improved non-invasive aesthesiometer. With new instruments it is important to understand the mechanics and the stimulus characteristics, as well as to determine the optimal parameters for testing corneal sensitivity, such as the appropriate nozzle diameter, test distance, duration, and intensity [11].

The aim of this research, which is part of a larger project leading to a PhD thesis at the University of Waterloo (Canada), was to perform several experiments to determine the optimal stimulus parameters of the air-jet of the future new aesthesiometer. The present article will describe the design and initial results of several studies, which aimed at determining: (a) the force exerted by the jet using an analytical microbalance; (b) the pattern of the air-flow using lycopodium powder and; (c) the footprint size of the air-flow stimulus using a model eye and a thermal camera.

## 2. Material and methods

The different combinations of the parameters used in this study were based on the results of previous work by Murphy and their co-workers [11,13,15]. A brass nozzle adapted on the exit-jet of the blower [Fig. 1] was used with the new device in order to improve performance. The exit-jet of the microblower has a diameter of 0.75 mm, which is reduced to 0.50 mm with the addition of the brass nozzle. The units used for the intensity of this new aesthesiometer are still not properly normalized, so for the moment measurements shall be labelled as “unit”.



**Fig. 1.** Blower of the new aesthesiometer without the brass nozzle (left), brass nozzle and adaptor (centre) and brass nozzle adapted on the micro-blower (right).

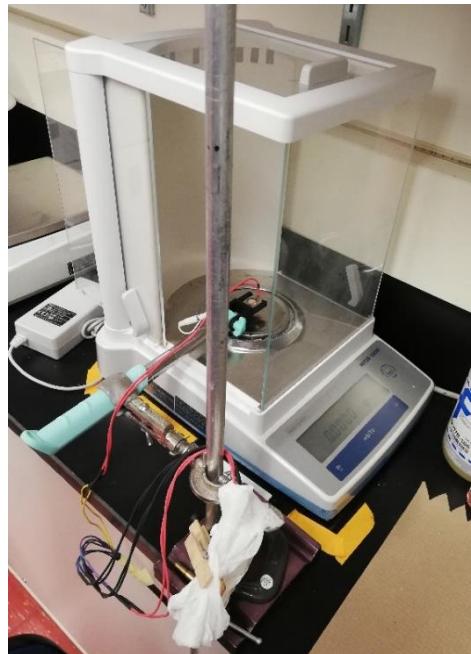
### 2.1. Force exerted by the jet analysed with a microbalance

The force exerted by the new air jet was measured with an analytical microbalance. The micro-blower device was held in parallel to the microbalance with a metallic rod of 30 cm with a modified support capable of housing the aesthesiometer device. The rod was held horizontally with a metallic clamp and a support bar [Fig. 2]. This allowed for easy fine-tuning of the different distances required for the analysis.

The microbalance was calibrated before starting the experiment using a range of standard weighs from 1 mg to 100 g.

The experiment was carried out at four different distances between the microbalance and the air jet: 0.5 cm, 1 cm, 1.5 cm and 3 cm. The air pressure intensities were selected by the control unit of the air-flow and set at four different values: 250 unit, 210 unit, 140 unit and 70 unit. In addition, five different durations of air-flow were tested: 10 s, 5 s, 1.5 s, 1 s and 0.5 s.

For each combination of these parameters, ten consecutive measurements were conducted to determine the repeatability of the procedure. Results were recorded as grams.



**Fig. 2.** Set up of microbalance and aesthesiometer.

## 2.2. Pattern of the air-flow

The study of the dispersion of the air-flow exiting the aesthesiometer was first performed by Boberg-Ans (1952), and repeated by Murphy (1996) to further the understanding of these phenomena. The aim of this part of the study was to repeat those experiments with the new prototype of aesthesiometer.

A rectangular (28 x 14.7 cm) paper frame was used as a limiting surface over which a thin layer of lycopodium powder was spread. An adaptable support was employed to hold the air jet parallel to the surface, with the nozzle directed to the powder. This set up permitted placing the micro-blower exit-jet at four different distances from the surface with powder: 0.5 cm, 1 cm, 1.5 cm and 3 cm. The aesthesiometer control unit was adjusted for a continuous air-flow at room temperature of 22°C and three different durations: 0.5 s, 0.9 s and 1.5 s. The stimulus intensity of the air-flow was set at four different values: 250 unit, 210 unit, 140 unit and 70 unit. These parameters were selected according to published literature for the evaluation of air exit volume and the determination of the resulting diameters [5,11,15].

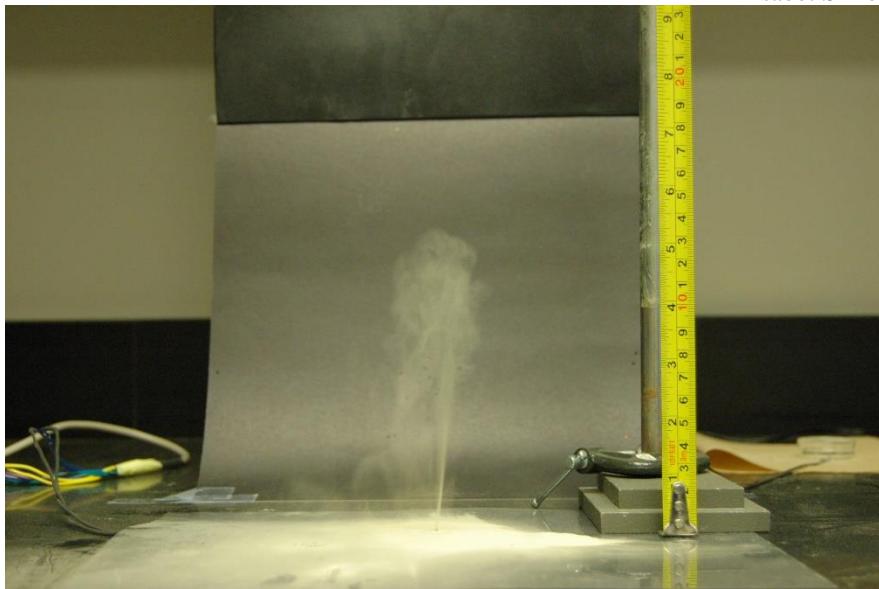
Each measurement was repeated two times for each predetermined distance so eight craters were registered on the powder surface. A ruler was attached next to the rectangular card as a reference measure to determine the diameter of the craters during posterior image analysis with the freely available software ImageJ v.151 (National Institutes of Health, US) [Fig. 3]. Measurements were conducted without and with the brass nozzle attached to the aesthesiometer.



**Fig. 3.** Trial at 1.0 cm distance and with an air-flow duration of 0.9 s without (left) and with (right) the brass nozzle adapted on the exit-jet.

A similar set up was used to visualize the air-flow laterally. The rectangular limiting base was covered with a plastic surface with a small hole in the middle. In this case, the micro-blower was placed under the plastic base with the exit-jet pointing upwards and emerging from the hole. That protected the micro-blower from the lycopodium powder spread above.

A ruler was held perpendicular to the basis using a metallic clamp and a black cardboard was employed as background to obtain a better view of the dust plume generated by the air-flow [Fig. 4]. The aesthesiometer control unit was adjusted for a continuous flow of air of two different durations: 1 s and 10 s. Four different stimulus intensities of the air-flow were investigated: 250 unit, 210 unit, 140 unit and 70 unit.



**Fig. 4.** Dust plume resulting from an intensity of 250 unit and air-flow duration of 10 s.

The measurements were repeated five times with all the possible combinations of the parameters. Video recordings were obtained for each measurement, and posterior frame-by-frame analysis was conducted to determine the height of the dust plume.

### 2.3. Footprint size of the air-flow stimulus examined with a model eye and a thermal camera

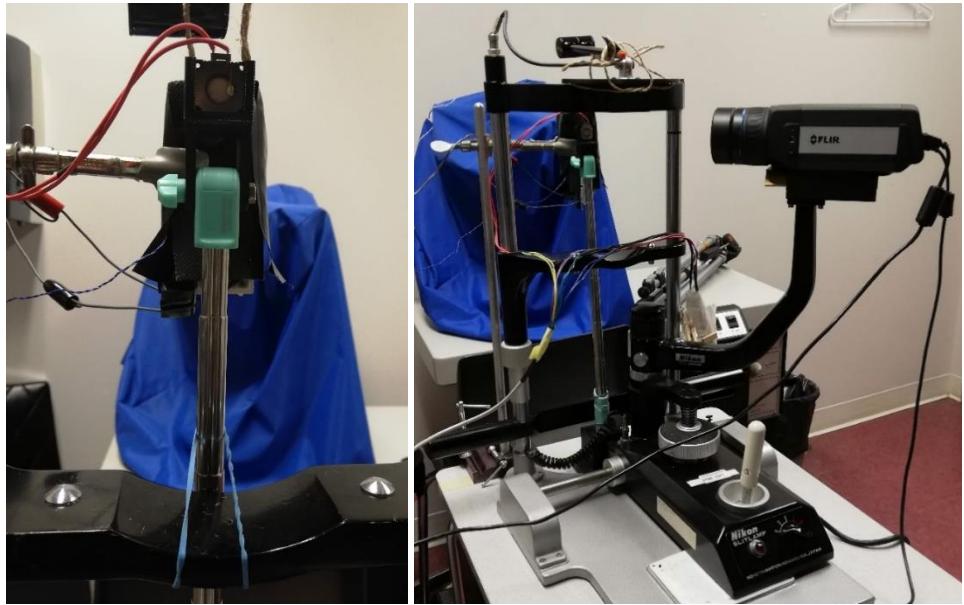
The thermal footprint size of the airflow stimulus generated by the new aesthesiometer was measured on a model eye using the FLIR A655sc infrared camera (FLIR Systems Inc., Portland, USA). The model eye design was developed by the Murphy Laboratory for Experimental Optometry (MLEO). Prior to any measurement, and to ensure similitude with the human ocular surface, the model eye was warmed at 32°C during 45 minutes. The model eye was placed in front of the thermal camera using a metallic clamp and a vertical holder simulating the corneal surface of a real eye [Fig. 5].

The camera employed the FLIR ResearchIR Max Version 4.30.1.70 software, which permits viewing, capturing and recording for posterior analysis detailed thermal images (FLIR Systems, Inc., 2014; FLIR Systems, Inc., 2018). The thermal camera records infrared radiation, whereupon temperature readings may be obtained through the Stefan–Boltzmann law, which describes the power radiated from a black body in terms of its temperature.

The exit-jet was placed in front of the model eye using a modified metallic rod of 50 cm [Fig. 6]. The thermal camera was fixed onto an adjustable jaws flex clamp mounted with a gooseneck extension tripod standing at a measurable angle slightly less than 90° to the eye.

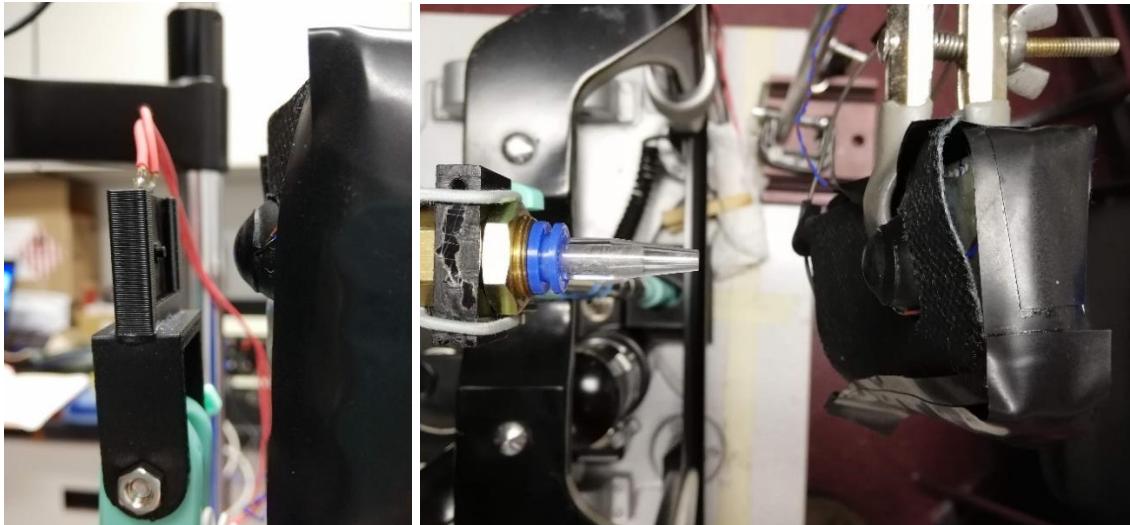
The separation between the nozzle of the blower and the centre of the model cornea was controlled by a pupilometer. Four different distances were chosen: 0.5 cm, 1 cm, 1.5 cm and 3 cm. A continuous flow of air at room temperature (22°C) and three different durations was selected using the aesthesiometer control unit: 0.5 s, 1 s and 1.5 s. The stimulus intensity of the air-flow was configured at four different values: 250 unit, 210 unit, 140 unit and 70 unit. These parameters were selected according to aesthesiometry literature [5,11,15]. The footprint size is defined as the diameter in which less than 0.5°C or 1°C temperature gradient was measured.

The thermal camera recorded infrared video thermography at 24 Hz for 5 seconds. For each combination of the parameters described above, measurements were repeated three times.



**Fig. 5.** View from the back of the micro-blower (left) and general view of the thermal camera and model eye experiment set up (right).

For this part of the study, measurements were conducted with and without the brass nozzle and were repeated using the Non-contact corneal aesthesiometer (NCCA) [Fig. 7]. The NCCA used for this comparison was developed by Murphy and co-workers in 1996 and is described in the literature [11]. Briefly, the main differences between the NCCA and the current aesthesiometer design resided in the exit-jet and control unit.



**Fig. 6.** Micro-blower in front of the model eye. **Fig. 7.** NCCA exit-jet in front of the model eye seen from above.

### 3. Results

#### 3.1. Force exerted by the jet analysed with a microbalance

Table 1 presents a summary of the results obtained with different intensities of the air-flow, with a duration of 10 s and at distances of 3 and 1.5 cm (the results obtained with the combination of all parameters under study are provided in Annex 1). It may be observed that the standard error of the mean (SEM) for these measurements with the brass nozzle had a small value, in comparison with the value of the mean, thus evidencing the good repeatability of the procedure. On average, microbalance readings (g) increased with the intensity of the air-flow, and when the air-flow was applied at a distance

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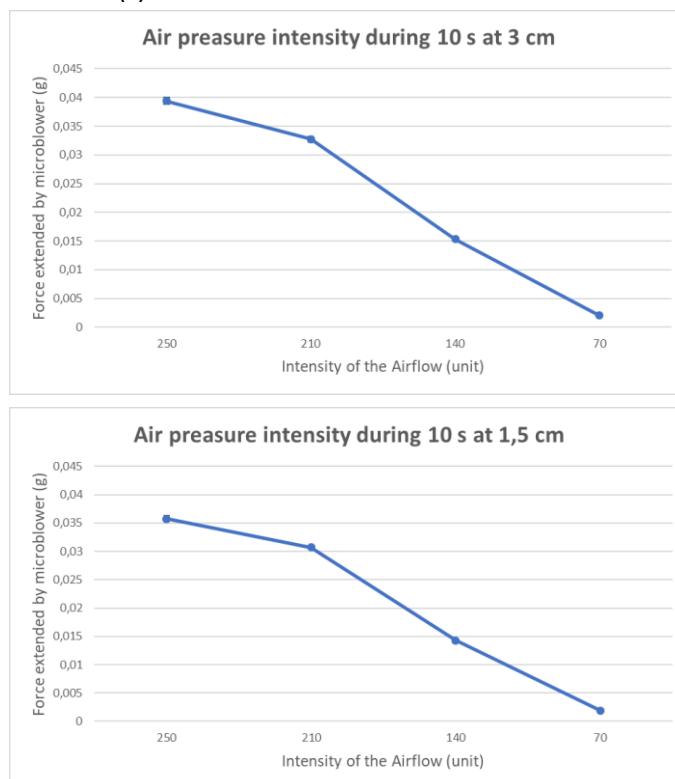
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of 3 cm results were very similar, or slightly higher, than when distance was of 1.5 cm. These results are also shown in Fig. 8.

Intensity (unit)	Average	SEM	Intensity (unit)	Average	SEM
<b>250</b>	0.03576	±5.27E-04	<b>250</b>	0.03939	±5.99E-04
<b>210</b>	0.03069	±1.68E-04	<b>210</b>	0.03278	±4.78E-04
<b>140</b>	0.01428	±5.12E-05	<b>140</b>	0.01532	±1.40E-04
<b>70</b>	0.00188	±3.89E-05	<b>70</b>	0.00205	±2.69E-05

**Table 1.** Results of the microbalance readings (g) exerted by the air at each intensity tested during 10 seconds at 3 cm (left) and during 10 seconds at 1.5 cm (right).

Plotted in the same graph, the microbalance readings (g) for intensities 250 unit, 210 unit and 140 unit, showed a plateau after obtaining a maximum value, as seen in Fig. 9. It was not possible to know exactly the time (s) when the results reached the maximum value because the recording software did not have



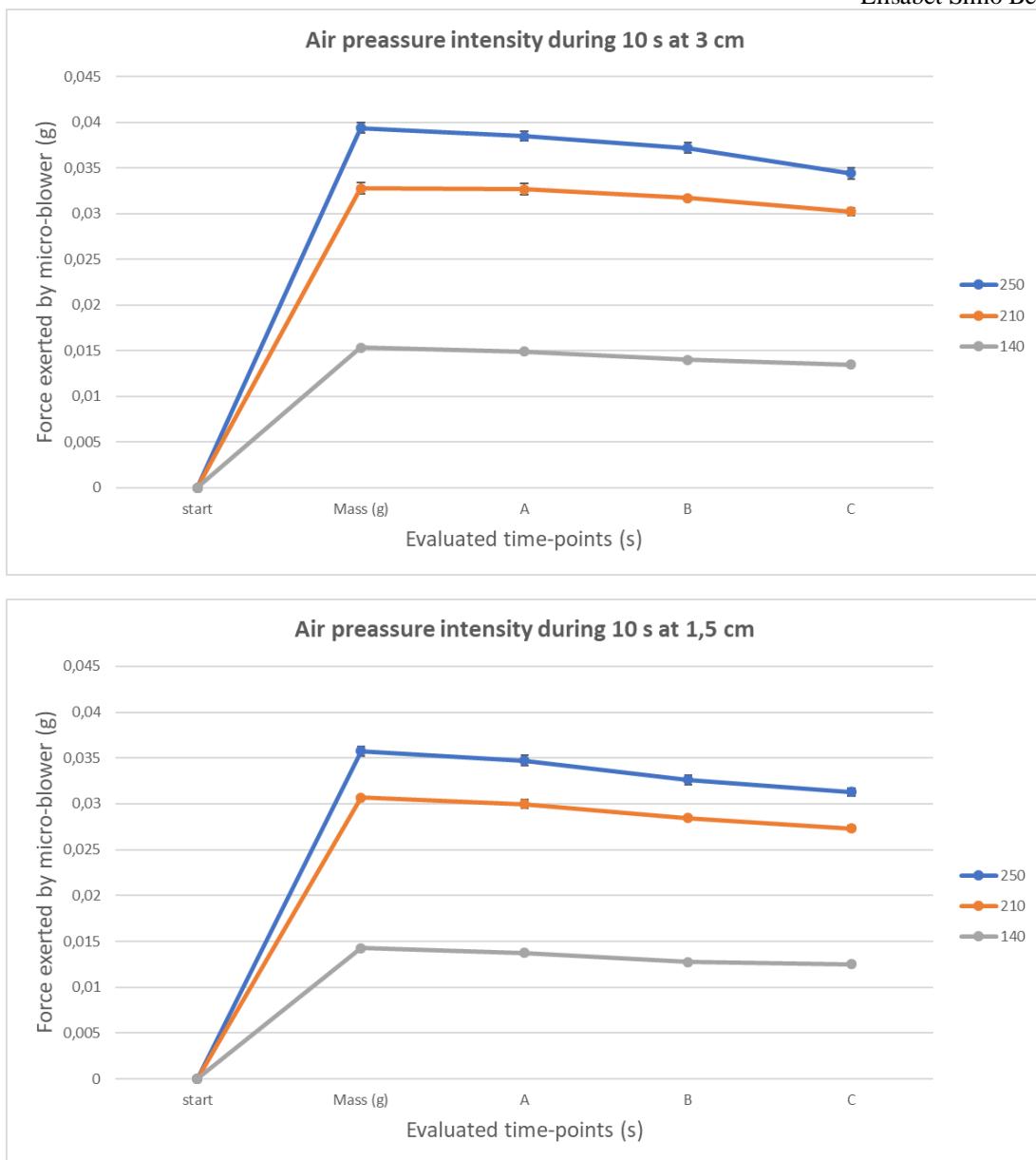
**Fig. 8.** Results obtained at the different intensities tested for the distance of 3 cm during 10s (top) and for the distance of 1.5 cm during 10 s (bottom).

observed. For measurement times shorter than 5 seconds, the resulting graphics showed a parabolic behaviour and the microbalance readings (g) were slightly smaller. The microbalance readings (g) exerted by the micro-blower without the nozzle were higher than with the brass nozzle adapted, as the exit diameters were different.

All the results not shown in the graphics may be accessed in Annex 1.

enough time resolution. Therefore, to plot the results, three reference times were chosen: A corresponds to one second after obtaining the highest value, B after 5 seconds and C after 10 seconds. The results obtained for intensities of 250 unit and 210 unit were similar but the plateau showed greater stability for an intensity of 210 unit, as may be observed in Fig. 9. With the three intensities at a distance of 3 cm from the microbalance, the maximum microbalance reading (g) was larger and the difference between that maximum value and the value, once stability was reached, was more significant than when measurements were conducted at 1.5 cm. In contrast, results at 1.5 cm and below were more stable.

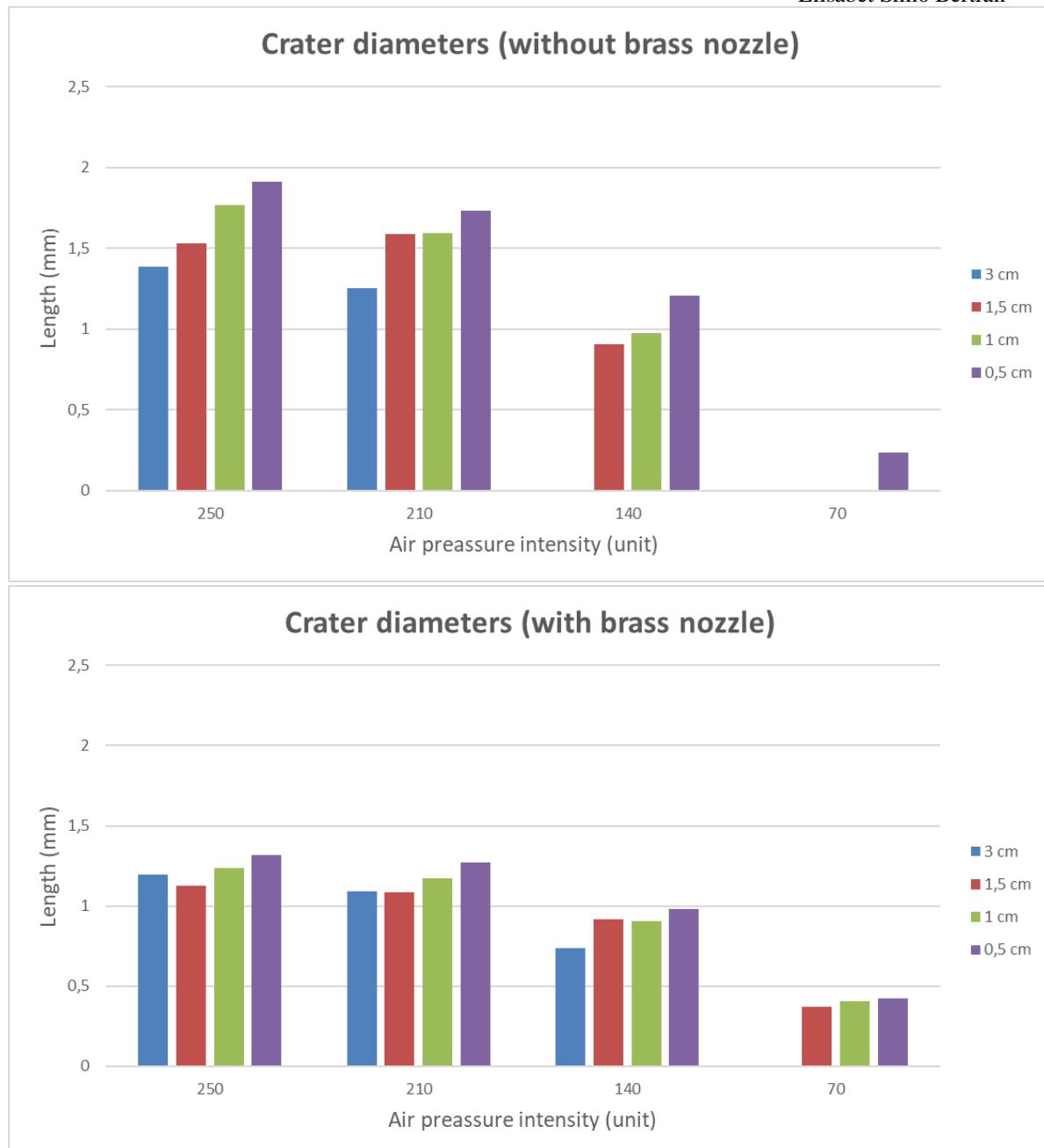
The experiment could not be repeated at 0.5 cm without the brass nozzle due to the sensibility of the microbalance and the set up of the aesthesiometer. Similarly, the results below 140 unit were not reliable as no noticeable measurement maximum was



**Fig. 9.** Comparison of the stability of the microbalance readings (g) exerted by the micro-blower with intensities 250 unit, 210 unit and 140 unit during 10 s at 3 cm trial (top) and 1.5 cm trial (bottom).

### 3.2. Pattern of the air-flow

The mean of the diameters measured for each intensity and distances during 1.5 s are shown in Fig. 10. The SEM for these measurements were always between  $\pm 0.01$  and  $\pm 0.13$ . It may be observed that the diameter of the craters is larger without the brass nozzle adapted on the exit-jet. For both experiments, with and without the brass nozzle, the diameter increased when the intensity was higher and when the distance was shorter between the air jet and the powder. This pattern was only partially reversed at the experimental condition with brass nozzle and 250 unit pressure. It must be noted that not all stimulus pressures caused displacement of the powder, particularly at the lower pressures, and measurements were taken only when a definite crater was seen by the examiner.



**Fig. 10.** Diameter of the craters drawn on the lycopodium powder by the air-flow during 1.5 s and various distances, without the adapted brass nozzle (top) and with the adapted brass nozzle (bottom).

The rest of the results are summarized in Annex 2. It may be observed that, in average, the measured diameters for an air-flow time of 0.9 s were slightly smaller than the results showed above but the error margins were larger (between  $\pm 0.01$  and  $\pm 0.36$ ) and the whole results were more unstable.

Regarding the air-flow visualization experiment, it was found that the length of the dust plume increased with the distance of the flow, as it may be seen in table 2. Three marks were defined to measure the diameter of the plume by the division of the selected length in 3 parts. At high air-flow intensities and times of 10 seconds, the dust plume reached a lower altitude than at 140 unit, but they had larger diameters, that is, dust spread more. The maximum diameters with not significant changes were obtained at high intensities and different stimulus durations. In contrast, at 140 unit results were more different.

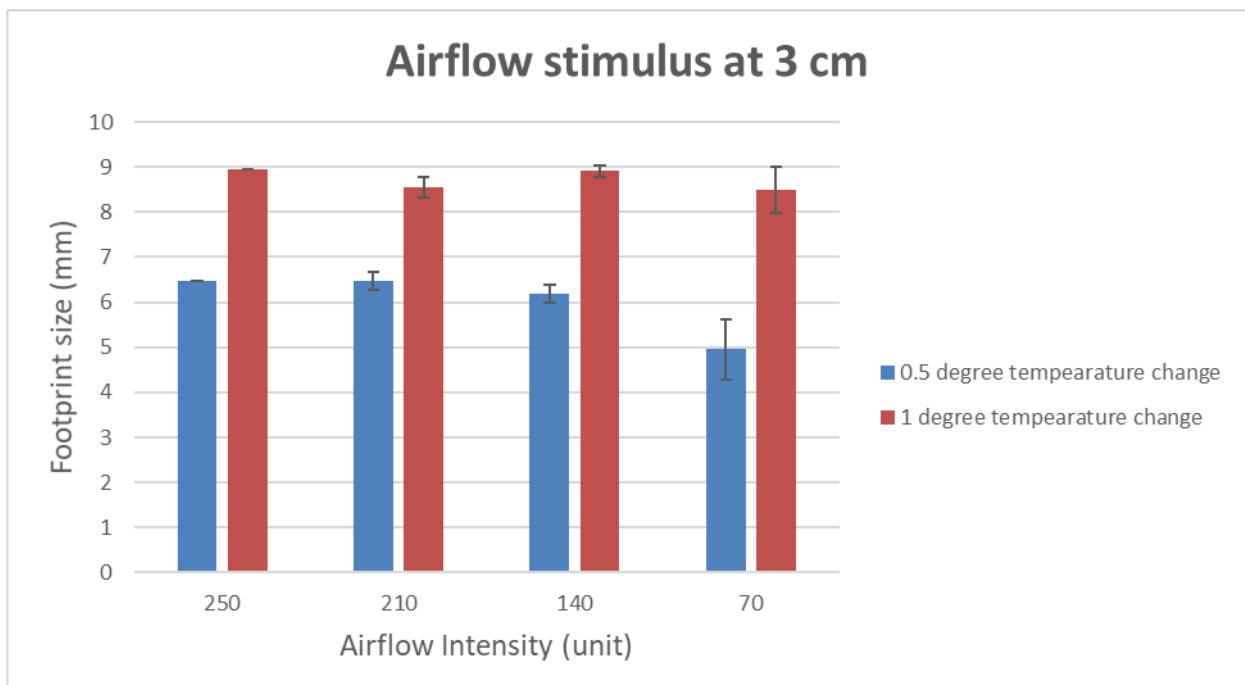
Stimulus Intensity (unit)	Duration (s)	Average tall mark 1 (cm)	SEM	Average tall mark 2 (cm)	SEM	Average tall mark 3 (cm)	SEM
250	1	0.647	0.018	1.541	0.019	2.557	0.018
250	10	1.071	0.027	2.768	0.023	4.073	0.023
210	10	1.512	0.022	3.291	0.025	4.493	0.023
140	1	0.765	0.018	1.769	0.018	2.781	0.018
140	10	1.215	0.019	3.306	0.090	4.846	0.022

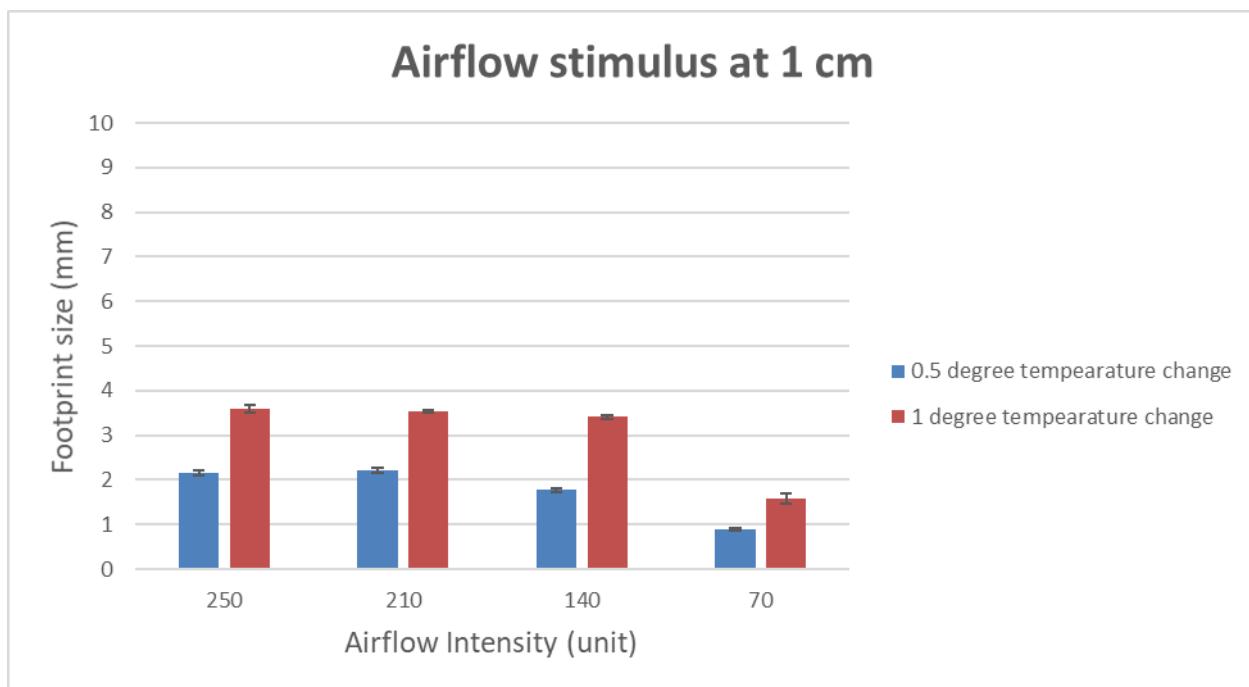
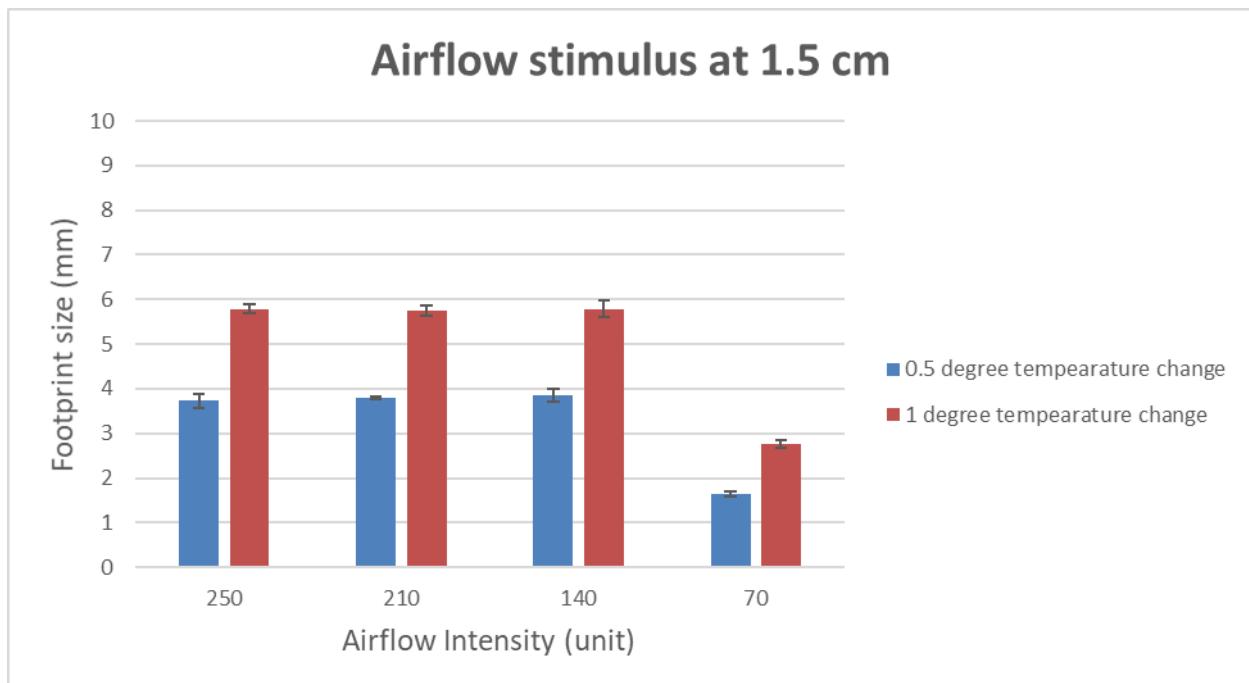
Stimulus Intensity (unit)	Duration (s)	Average Diameter mark 1 (cm)	SEM	Average diameter mark 2 (cm)	SEM	Average diameter mark 3 (cm)	SEM
250	1	0.150	0.008	0.231	0.010	0.356	0.009
250	10	0.230	0.003	0.277	0.009	0.371	0.005
210	10	0.212	0.005	0.294	0.007	0.363	0.011
140	1	0.128	0.003	0.180	0.005	0.222	0.016
140	10	0.204	0.007	0.253	0.006	0.313	0.008

**Table 2.** Lateral air pattern visualization measurements of each intensity and time tested. Mean diameter (bottom) and mean altitude of the dust plume (top).

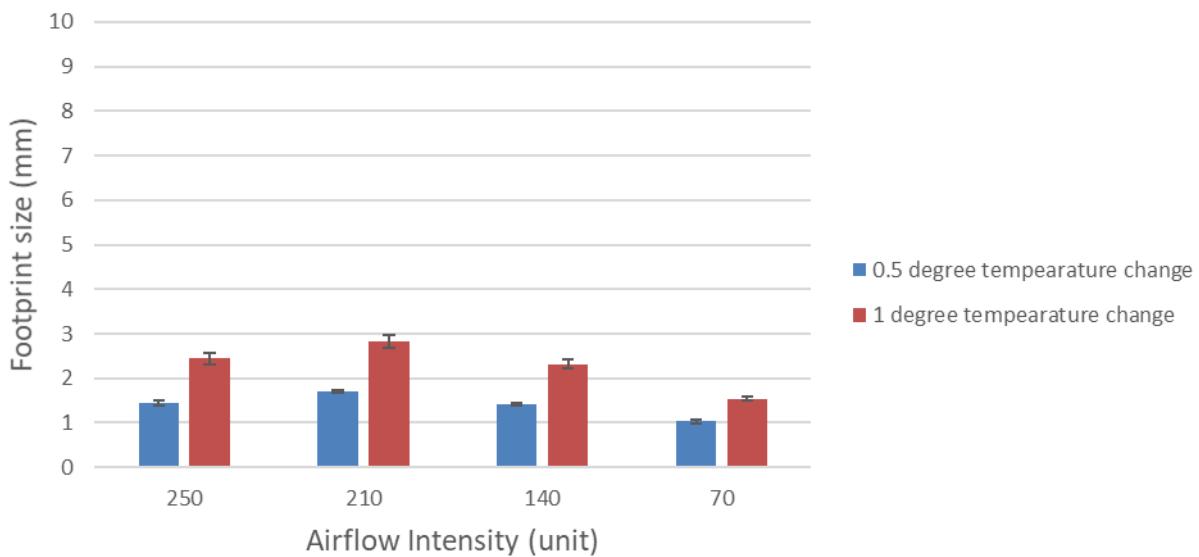
### 3.3. Footprint size of the air-flow stimulus examined with a model eye and a thermal camera

The mean ( $\pm$  error) footprint size (mm) found for each stimulus pressure (unit) and for each distance (cm), tested with 1 s air-flow duration, are shown in Fig. 11. The footprint size was defined as the diameter in which less than  $0.5^{\circ}\text{C}$  or  $1^{\circ}\text{C}$  temperature gradient was measured. In agreement with the air-flow visualization experiment, the larger the distance between the eye and the micro-blower, the larger the diameter of corresponding thermal footprint. Smaller diameters were obtained with the lower intensity value at each trial (70 unit).





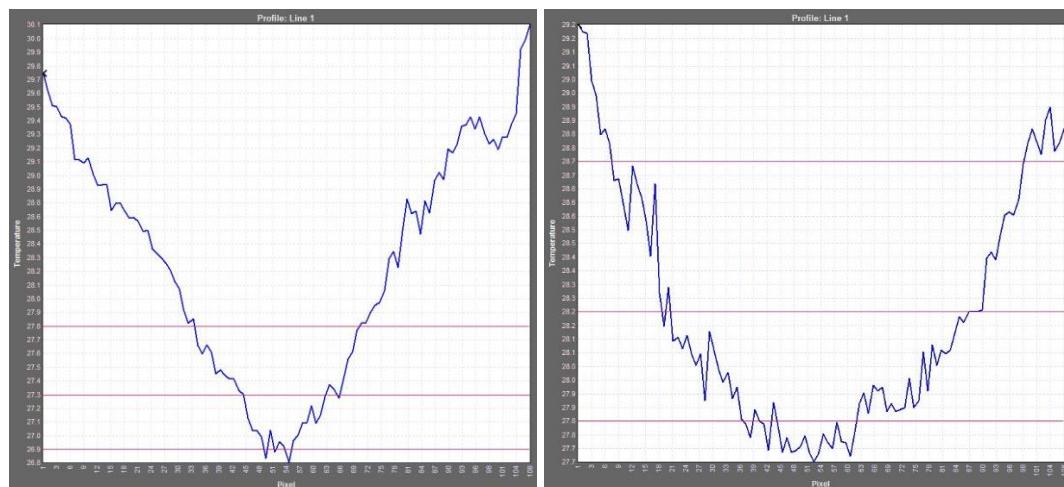
## Airflow stimulus at 0,5 cm



**Fig. 11.** Diameter (mm) of the temperature footprint produced during 1 second for every intensity (unit) evaluated and at each test distance (cm). These values were taken during the maximum thermal difference.

Full results with the combination of all parameters under study are presented in Annex 3. The temperature of the model eye reached 32.17°C. Overall, as the intensity was reduced, temperature measurements stayed higher, only decreasing 1.77°C (trial 70 unit, during 1 s at 3 cm). In contrast, temperature was lower, decreasing 2.83°C, when distance was reduced (trial 70 unit, during 1 s at 0.5 cm). The air-flow stimulus appears more controlled with the brass nozzle adapted on the exit-jet of the aesthesiometer than in the trials without it.

The diameter results were taken from thermic profile plots like the examples shown in Fig. 12, transforming the pixels to millimetres. As may be observed from these figures, the pink lines drawn where the diameter values corresponding to the 0.1°C, 0.5°C and 1°C temperature gradients. Thermography plots showed that temperature changes induced by air at room temperature were always larger in the center of the model cornea and smaller towards the corneal borders



**Fig.12.** Profile plots saved from FLIR ResearchIR Max Version 4.30.1.70 software. Footprint diameter results (in pixels) at 210 unit during 1s test at 1 cm (left) and 3 cm (right). These values were taken during the maximum thermal difference.

#### 4.Discussion

This research is included in a main project leading to a PhD thesis at the University of Waterloo (Canada) called *Studies on corneal sensitivity*. The aim of the work was to gain a better understanding of corneal sensitivity by improving its way of evaluation. The objective of the study was to determine the optimal parameters of the air jet of a new non-contact corneal aesthesiometer and to explore the temperature change caused by this device.

The stability of the force exerted by the air jet was determined with the **microbalance experiment**. With the trials where the stimulus duration was 10 seconds, a well defined plateau was observed, although this test duration was only for visualisation purposes, not for trials *in vivo*. Besides, confirming previous reports [11,16], the maximum measured force (g) was similar for all time trials (10 s and 1 s) and test distances. In contrast, the largest change in maximum force was found between different intensities. However, as the normal cornea is able to detect stimuli with lower intensities than 250 unit [13], future *in vivo* measurements are required to determine the optimal intensity values for the new aesthesiometer. In addition, a stimulus duration of 1 s was sufficient to reach the maximum force, in agreement with published literature [18], which suggests this duration as optimal for *in vivo* measurements. In effect, corneal nerves respond to the initial impact of the air-pulse on the corneal surface, rather than to the actual volume of air in the stimulus pulse [18].

The results showed that with the 0.5 mm air-jet brass nozzle the force was smaller than without the nozzle (0.75 mm air-jet). This small difference between the diameters of the exit-jet could produce a difference with the dispersion of the air-flow directed to the surface of the microbalance, resulting in larger values (g). Nevertheless, for the best performance of the micro-blower we suggest using the brass nozzle in the new aesthesiometer, as with this configuration the smallest diameter of stimulation was found. From the results displayed in Figure 8 and 9, it may be observed that the best outcome was obtained with a distance of 1.5 cm.

The technique of recording the results needs to be improved because some interesting measures were lost, like the exact time the air-flow reached the maximum value (g). It is known that this time is less than 1 s [15], but the exact value is as yet undetermined, even considering its relevance for *in vivo* trials. Finally, some problems were also found with the 0.5 cm trial without the nozzle because of the set up of the microbalance and aesthesiometer having a negative impact on the microbalance sensitivity.

With the pattern of the air-flow using lycopodium powder in the **lycopodium powder displacement test**, the expected results were that the diameter of the craters would increase with the intensity of the stimulus. The measurements confirmed this initial hypothesis. However, it was also expected to find a more significant powder displacement when the distances between the air jet and the powder surface increased, which the results did not show. With the no nozzle test, the diameter was clearly larger when the distance of the test increased, whereas for the brass nozzle trials the working distances and the stimulus duration did not appear to create relevant changes in the displacement results. One improvement of the design of this experiment could be the control over the thickness of the lycopodium powder layer over all the surface, a factor that might have altered the results.

In addition, the **air flow pattern** showed a gradual dispersion of the lycopodium powder, as was expected. However, the pattern of the air observed in this experiment may not necessarily replicate the *in vivo* conditions on the corneal surface, which is covered by a thin tear film layer. The best distance for the study was not well defined in this case. However, from the results summarized in Table 2, it may be



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evidenced an optimal air pressure of 140 unit, with which the diameter of the dust plume was always the smallest, irrespective of the duration of the stimulus, that is, less dispersion occurred.

Lastly, with the **thermal camera** the measurements have shown that the air-flow produced a well defined pattern of reduction of the temperature on the model cornea. Two aspects may be discussed in this regard: which was the temperature change of the corneal surface and which diameter of the cornea was affected, so the cornea may be stimulated mechanically and thermally in accordance with previous reports [16].

In our case, the continuous flow of air at room temperature ( $22^{\circ}\text{C}$ ) touched the model eye corneal surface ( $29.84 \pm 2.34^{\circ}\text{C}$ ) and its temperature decreased without evaporation due to the lack of tear film. In a real case, the literature indicates that normal ocular surface temperature ranges from  $32.9$  to  $36^{\circ}\text{C}$  [19]. Because of the tear film, which is a good heat conductor, the footprint size found in a real-life experiment should be larger than the value found in this experiment, although the actual results may depend on the tear quality of the subject so that a future trial *in vivo* should include both healthy subjects and subjects with dry eye disease.

Other interesting findings of this experiment were, as it is shown on the results and coinciding with the other parts of the research, that the stimulus duration did not affect the thermal pattern and that the temperature gradient across the surface remained constant for the duration of the stimulus. Once the air-flow was interrupted, the temperature of the surface gradually recovered its previous baseline values.

Secondly, the working distances were related to the footprint size as it was found in the air-flow pattern experiment: at long distances between the model eye and the exit jet the diameter was larger and this value decreased as distances shortened. Thirdly, the intensity did not have any relation with the diameter values but only with small changes in temperature. The highest intensities resulted in a more significant decrease in corneal temperature than low intensities.

Finally, the results can confirm that the area of cooling produced by the air-flow was localised during the trials. Unlike reported by previous authors [13,15], thermography images done by Belmonte and their colleagues on their research in 1999 showed that temperature changes induced by air at room temperature were always restricted to the center of the cornea and did not extend to the corneal borders too [16]. However, the aesthesiometer footprint is larger than that of the Cochet-Bonnet device and stimulates a less specific number of sensory receptors [5], so in this research we aim at a smaller diameter. From the results shown in Figure 11, the best parameters may be a yet undetermined intensity applied at a 1 cm distance and during 1 second.

## 5. Conclusion

Although further work is necessary to determine the optimal parameters of the air-jet of the aesthesiometer, an association was found between the diameter of the powder displacement and the footprint size found with the thermal camera. The tree experiments have results in common: the distance and the duration of the stimulus is not the cause of the variation of the diameter, leaving intensity as the most important parameter. However, more information and repeated trials are needed to have clear results for the improvement of the new device. The characterisation of the new device units is also necessary for the comparison between studies, although there is still no consistency in stimulus parameters used in the published literature [8,13,15,20,21]. Overall, as previously documented [22,23], infrared thermography may be considered a good imaging modality to study the ocular surface temperature.

## 6. Limitations and future work

The general limitations found in the present research were that this was the beginning of a larger project still unfinished and we could not do the research *in vivo*. For the microbalance experiment, we should find a better way of recording the results, which may allow finding a relation between force and time. For the lycopodium powder, to have a control over the dust thickness and to determine the error with the craters. Finally, with the thermal camera experiment, to repeat the procedures with the instillation of saline or tear substitutes on the model eye before the trials.

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## 8. Annex

## *8.1. Annex 1*

Microbalance results (g)												
No Nozzle		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Average	SEM
250, 10s, 3cm		0,104	0,099	0,096	0,101	0,096	0,094	0,093	0,090	0,099	0,094	0,097
210, 10s, 3cm		0,087	0,086	0,084	0,083	0,088	0,089	0,087	0,085	0,084	0,083	0,086
140, 10s, 3cm		0,045	0,043	0,044	0,043	0,043	0,043	0,045	0,045	0,044	0,043	3,505E-04
70, 10s, 3cm		0,008	0,007	0,007	0,007	0,008	0,008	0,007	0,008	0,008	0,007	0,007
250, 10s, 1.5cm		0,083	0,075	0,078	0,082	0,079	0,078	0,081	0,081	0,077	0,082	0,079
210, 10s, 1.5cm		0,072	0,068	0,068	0,071	0,067	0,071	0,067	0,072	0,069	0,068	0,069
140, 10s, 1.5cm		0,037	0,035	0,037	0,036	0,037	0,038	0,036	0,036	0,035	0,036	2,432E-04
70, 10s, 1.5cm		0,007	0,006	0,006	0,006	0,006	0,006	0,007	0,007	0,007	0,006	3,416E-05
250, 10s, 1cm		0,066	0,064	0,062	0,061	0,062	0,063	0,061	0,064	0,062	0,062	0,063
210, 10s, 1cm		0,059	0,057	0,056	0,055	0,054	0,053	0,055	0,054	0,055	0,055	5,182E-04
140, 10s, 1cm		0,034	0,034	0,033	0,033	0,035	0,033	0,034	0,033	0,033	0,034	0,034
70, 10s, 1cm		0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	3,590E-05
250, 5s, 3cm		0,104	0,096	0,096	0,094	0,093	0,100	0,100	0,098	0,096	0,096	0,097
210, 5s, 3cm		0,088	0,086	0,086	0,087	0,088	0,087	0,086	0,088	0,086	0,085	0,087
140, 5s, 3cm		0,046	0,045	0,043	0,044	0,047	0,046	0,046	0,046	0,044	0,045	4,058E-04
70, 5s, 3cm		0,008	0,007	0,008	0,007	0,008	0,008	0,008	0,008	0,007	0,007	4,761E-05
250, 5s, 1.5cm		0,084	0,078	0,081	0,079	0,081	0,081	0,079	0,080	0,076	0,077	0,080
210, 5s, 1.5cm		0,074	0,072	0,071	0,068	0,070	0,068	0,068	0,072	0,073	0,072	0,071
140, 5s, 1.5cm		0,037	0,037	0,036	0,035	0,035	0,037	0,037	0,036	0,036	0,036	2,097E-04
70, 5s, 1.5cm		0,006	0,007	0,007	0,007	0,007	0,007	0,007	0,007	0,007	0,007	3,350E-05
250, 5s, 1cm		0,007	0,065	0,065	0,064	0,063	0,063	0,065	0,065	0,063	0,063	0,058
210, 5s, 1cm		0,060	0,058	0,057	0,058	0,058	0,057	0,056	0,055	0,056	0,059	0,057
140, 5s, 1cm		0,033	0,033	0,033	0,032	0,035	0,035	0,034	0,034	0,034	0,033	0,034
70, 5s, 1cm		0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	5,783E-05
250, 1.5s, 3cm		0,101	0,100	0,099	0,099	0,099	0,099	0,098	0,101	0,100	0,101	0,100
210, 1.5s, 3cm		0,090	0,087	0,089	0,090	0,090	0,090	0,089	0,090	0,089	0,089	3,391E-04
140, 1.5s, 3cm		0,047	0,047	0,046	0,046	0,046	0,046	0,047	0,046	0,047	0,046	1,439E-04
70, 1.5s, 3cm		0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	2,906E-05
250, 1.5s, 1.5cm		0,080	0,078	0,081	0,082	0,081	0,079	0,079	0,079	0,080	0,081	0,080
210, 1.5s, 1.5cm		0,070	0,071	0,071	0,071	0,072	0,072	0,071	0,072	0,072	0,071	0,071
140, 1.5s, 1.5cm		0,037	0,037	0,038	0,038	0,037	0,037	0,037	0,037	0,037	0,037	1,009E-04
70, 1.5s, 1.5cm		0,007	0,007	0,007	0,007	0,007	0,007	0,007	0,007	0,007	0,007	4,069E-05
250, 1.5s, 1cm		0,068	0,066	0,066	0,066	0,065	0,065	0,066	0,065	0,067	0,066	0,066
210, 1.5s, 1cm		0,061	0,060	0,059	0,060	0,058	0,058	0,060	0,059	0,060	0,059	0,059
140, 1.5s, 1cm		0,034	0,035	0,034	0,033	0,035	0,034	0,033	0,034	0,034	0,034	1,955E-04
70, 1.5s, 1cm		0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	4,269E-05
250, 1s, 3cm		0,107	0,104	0,100	0,100	0,101	0,100	0,102	0,100	0,100	0,101	0,101
210, 1s, 3cm		0,088	0,090	0,089	0,089	0,089	0,089	0,088	0,089	0,087	0,089	0,089
140, 1s, 3cm		0,046	0,046	0,045	0,046	0,047	0,047	0,046	0,046	0,047	0,047	2,195E-04
70, 1s, 3cm		0,008	0,008	0,008	0,007	0,007	0,008	0,007	0,007	0,007	0,008	8,622E-05
250, 1s, 1.5cm		0,082	0,080	0,082	0,081	0,080	0,079	0,080	0,079	0,080	0,081	0,080
210, 1s, 1.5cm		0,071	0,072	0,071	0,072	0,071	0,072	0,072	0,072	0,071	0,071	1,318E-04
140, 1s, 1.5cm		0,037	0,036	0,037	0,037	0,037	0,037	0,037	0,037	0,037	0,036	0,037
70, 1s, 1.5cm		0,007	0,006	0,006	0,007	0,007	0,007	0,006	0,007	0,006	0,007	5,588E-05
250, 1s, 1cm		0,069	0,067	0,066	0,066	0,065	0,067	0,067	0,067	0,066	0,067	3,093E-04
210, 1s, 1cm		0,061	0,060	0,059	0,060	0,059	0,058	0,059	0,059	0,059	0,059	2,711E-04
140, 1s, 1cm		0,035	0,034	0,034	0,035	0,035	0,035	0,035	0,035	0,035	0,035	1,035E-04
70, 1s, 1cm		0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	7,180E-05

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Brass nozzle											Average	SEM
<b>250, 10s, 3cm</b>											0,000	0,000E+00
Mass (g)	0,043	0,040	0,042	0,037	0,041	0,038	0,040	0,039	0,038	0,038 Mass (g)	0,039	5,993E-04
First Mass at the start of the Stability plateau (g) A	0,041	0,039	0,041	0,037	0,039	0,036	0,039	0,038	0,037	0,038 A	0,038	5,052E-04
Middle Mass at the start of the Stability plateau (g) I	0,041	0,038	0,039	0,035	0,038	0,035	0,038	0,036	0,036	0,036 B	0,037	5,650E-04
Final Mass at the start of the Stability plateau (g) C	0,038	0,035	0,036	0,032	0,035	0,032	0,036	0,035	0,033	0,033 C	0,034	5,749E-04
<b>140, 10s, 3cm</b>											0,000	0,000E+00
Mass (g)	0,016	0,016	0,015	0,016	0,015	0,015	0,016	0,016	0,014	0,015 Mass (g)	0,015	1,397E-04
First Mass at the start of the Stability plateau (g) A	0,015	0,015	0,015	0,015	0,015	0,015	0,015	0,015	0,014	0,015 A	0,015	1,306E-04
Middle Mass at the start of the Stability plateau (g) I	0,014	0,014	0,014	0,015	0,015	0,013	0,014	0,014	0,013	0,014 B	0,014	2,422E-04
Final Mass at the start of the Stability plateau (g) C	0,013	0,014	0,014	0,014	0,014	0,013	0,014	0,014	0,013	0,013 C	0,013	1,600E-04
<b>250, 10s, 1.5cm</b>											0,000	0,000E+00
Mass (g)	0,037	0,037	0,039	0,036	0,035	0,036	0,034	0,036	0,034	0,034 Mass (g)	0,036	5,271E-04
First Mass at the start of the Stability plateau (g) A	0,037	0,036	0,031	0,036	0,035	0,036	0,033	0,035	0,034	0,033 A	0,035	5,651E-04
Middle Mass at the start of the Stability plateau (g) I	0,035	0,033	0,028	0,034	0,033	0,033	0,032	0,034	0,032	0,032 B	0,033	5,423E-04
Final Mass at the start of the Stability plateau (g) C	0,033	0,032	0,028	0,031	0,032	0,032	0,032	0,032	0,031	0,031 C	0,031	4,457E-04
<b>140, 10s, 1.5cm</b>											0,000	0,000E+00
Mass (g)	0,015	0,014	0,014	0,014	0,014	0,014	0,014	0,014	0,015	0,014 Mass (g)	0,014	5,121E-05
First Mass at the start of the Stability plateau (g) A	0,014	0,014	0,014	0,014	0,014	0,014	0,014	0,013	0,014	0,014 A	0,014	5,821E-05
Middle Mass at the start of the Stability plateau (g) I	0,013	0,013	0,012	0,013	0,013	0,013	0,013	0,012	0,013	0,013 B	0,013	9,452E-05
Final Mass at the start of the Stability plateau (g) C	0,013	0,012	0,012	0,013	0,013	0,012	0,013	0,012	0,013	0,013 C	0,013	7,303E-05
<b>250, 10s, 1cm</b>											0,000	0,000E+00
Mass (g)	0,038	0,034	0,034	0,033	0,033	0,033	0,033	0,034	0,034	0,033 Mass (g)	0,034	4,387E-04
First Mass at the start of the Stability plateau (g) A	0,037	0,034	0,033	0,033	0,033	0,033	0,032	0,034	0,033	0,032 A	0,033	4,510E-04
Middle Mass at the start of the Stability plateau (g) I	0,036	0,032	0,031	0,031	0,031	0,031	0,030	0,032	0,031	0,031 B	0,032	4,698E-04
Final Mass at the start of the Stability plateau (g) C	0,034	0,031	0,030	0,029	0,030	0,030	0,029	0,029	0,030	0,029 C	0,030	4,403E-04
<b>140, 10s, 1cm</b>											0,000	0,000E+00
Mass (g)	0,014	0,014	0,014	0,014	0,015	0,014	0,014	0,014	0,014	0,014 Mass (g)	0,014	6,360E-05
First Mass at the start of the Stability plateau (g) A	0,014	0,014	0,013	0,013	0,014	0,014	0,014	0,014	0,014	0,014 A	0,014	9,165E-05
Middle Mass at the start of the Stability plateau (g) I	0,013	0,013	0,013	0,012	0,013	0,013	0,013	0,013	0,013	0,013 B	0,013	8,825E-05
Final Mass at the start of the Stability plateau (g) C	0,013	0,012	0,012	0,012	0,013	0,012	0,012	0,012	0,012	0,013 C	0,012	1,041E-04
<b>250, 10s, 0.5cm</b>											0,000	0,000E+00
Mass (g)	0,035	0,035	0,035	0,032	0,031	0,031	0,030	0,032	0,032	0,031 Mass (g)	0,032	5,556E-04
First Mass at the start of the Stability plateau (g) A	0,035	0,034	0,035	0,032	0,031	0,030	0,030	0,032	0,031	0,030 A	0,032	5,988E-04
Middle Mass at the start of the Stability plateau (g) I	0,032	0,032	0,032	0,029	0,029	0,028	0,028	0,029	0,029	0,028 B	0,030	5,555E-04
Final Mass at the start of the Stability plateau (g) C	0,031	0,030	0,030	0,029	0,028	0,027	0,027	0,027	0,027	0,027 C	0,028	4,610E-04
<b>140, 10s, 0.5cm</b>											0,000	0,000E+00
Mass (g)	0,014	0,014	0,014	0,013	0,013	0,013	0,014	0,013	0,014	0,013 Mass (g)	0,014	8,667E-05
First Mass at the start of the Stability plateau (g) A	0,014	0,013	0,013	0,013	0,013	0,013	0,013	0,013	0,013	0,013 A	0,013	1,043E-04
Middle Mass at the start of the Stability plateau (g) I	0,013	0,012	0,013	0,012	0,012	0,012	0,012	0,012	0,012	0,012 B	0,012	8,570E-05
Final Mass at the start of the Stability plateau (g) C	0,012	0,012	0,012	0,012	0,012	0,012	0,012	0,012	0,012	0,012 C	0,012	5,812E-05
<b>250, 5s, 3cm</b>											0,000	0,000E+00
Mass (g)	0,040	0,040	0,038	0,040	0,042	0,041	0,040	0,040	0,040	0,041 Mass (g)	0,040	3,538E-04
First Mass at the start of the Stability plateau (g) A	0,039	0,040	0,038	0,039	0,041	0,040	0,040	0,039	0,039	0,041 A	0,040	2,968E-04
Middle Mass at the start of the Stability plateau (g) I	0,038	0,038	0,036	0,037	0,038	0,038	0,038	0,036	0,036	0,039 B	0,037	2,766E-04
Final Mass at the start of the Stability plateau (g) C	0,037	0,038	0,035	0,037	0,037	0,038	0,038	0,037	0,036	0,037 C	0,037	3,236E-04
<b>140, 5s, 3cm</b>											0,000	0,000E+00
Mass (g)	0,016	0,016	0,017	0,016	0,016	0,016	0,016	0,016	0,016	0,017 Mass (g)	0,016	9,568E-05
First Mass at the start of the Stability plateau (g) A	0,016	0,016	0,016	0,016	0,016	0,016	0,016	0,015	0,015	0,016 A	0,016	1,055E-04
Middle Mass at the start of the Stability plateau (g) I	0,015	0,016	0,016	0,016	0,016	0,015	0,016	0,015	0,015	0,016 B	0,016	1,713E-04
Final Mass at the start of the Stability plateau (g) C	0,014	0,016	0,016	0,015	0,016	0,014	0,016	0,015	0,015	0,015 C	0,015	2,368E-04
<b>250, 5s, 1.5cm</b>											0,000	0,000E+00
Mass (g)	0,039	0,036	0,037	0,036	0,035	0,036	0,036	0,035	0,036	0,036 Mass (g)	0,036	3,459E-04
First Mass at the start of the Stability plateau (g) A	0,039	0,036	0,037	0,036	0,035	0,036	0,036	0,035	0,036	0,036 A	0,036	3,483E-04
Middle Mass at the start of the Stability plateau (g) I	0,038	0,036	0,035	0,036	0,034	0,036	0,035	0,034	0,036	0,035 B	0,067	3,174E-02
Final Mass at the start of the Stability plateau (g) C	0,037	0,034	0,035	0,036	0,036	0,038	0,034	0,033	0,034	0,034 C	0,095	4,039E-02
<b>140, 5s, 1.5cm</b>											0,000	0,000E+00
Mass (g)	0,015	0,015	0,015	0,015	0,015	0,015	0,015	0,015	0,015	0,015 Mass (g)	0,015	7,860E-05
First Mass at the start of the Stability plateau (g) A	0,015	0,015	0,015	0,015	0,015	0,015	0,015	0,014	0,014	0,014 A	0,015	1,009E-04
Middle Mass at the start of the Stability plateau (g) I	0,015	0,015	0,014	0,014	0,015	0,014	0,015	0,014	0,014	0,014 B	0,014	1,267E-04
Final Mass at the start of the Stability plateau (g) C	0,014	0,014	0,013	0,014	0,014	0,012	0,014	0,014	0,013	0,014 C	0,014	1,668E-04
<b>250, 5s, 1cm</b>											0,000	0,000E+00
Mass (g)	0,038	0,036	0,037	0,035	0,035	0,035	0,036	0,035	0,035	0,034 Mass (g)	0,035	3,489E-04
First Mass at the start of the Stability plateau (g) A	0,038	0,035	0,037	0,034	0,035	0,034	0,035	0,035	0,035	0,034 A	0,035	3,728E-04
Middle Mass at the start of the Stability plateau (g) I	0,036	0,034	0,035	0,033	0,033	0,034	0,034	0,034	0,033	0,033 B	0,034	3,358E-04
Final Mass at the start of the Stability plateau (g) C	0,036	0,034	0,035	0,032	0,032	0,032	0,033	0,033	0,032	0,032 C	0,033	4,144E-04
<b>140, 5s, 1cm</b>											0,000	0,000E+00
Mass (g)	0,015	0,015	0,015	0,016	0,015	0,015	0,015	0,015	0,015	0,015 Mass (g)	0,015	1,106E-04
First Mass at the start of the Stability plateau (g) A	0,015	0,014	0,015	0,015	0,015	0,014	0,014	0,014	0,014	0,015 A	0,015	1,164E-04
Middle Mass at the start of the Stability plateau (g) I	0,014	0,014	0,014	0,014	0,014	0,014	0,014	0,014	0,014	0,015 B	0,014	1,062E-04
Final Mass at the start of the Stability plateau (g) C	0,014	0,013	0,013	0,014	0,014	0,013	0,014	0,014	0,013	0,013 C	0,013	1,232E-04
<b>250, 5s, 0.5cm</b>											0,000	0,000E+00
Mass (g)	0,035	0,033	0,033	0,033	0,033	0,033	0,034	0,033	0,033	0,033 Mass (g)	0,033	2,308E-04
First Mass at the start of the Stability plateau (g) A	0,035	0,032	0,033	0,033	0,032	0,033	0,033	0,033	0,032	0,033 A	0,033	2,902E-04
Middle Mass at the start of the Stability plateau (g) I	0,033	0,032	0,032	0,032	0,030	0,031	0,031	0,031	0,030	0,031 B	0,031	2,564E-04
Final Mass at the start of the Stability plateau (g) C	0,032	0,030	0,031	0,030	0,030	0,030	0,031	0,030	0,029	0,031 C	0,030	2,802E-04
<b>140, 5s, 0.5cm</b>											0,000	0,000E+00
Mass (g)	0,014	0,014	0,014	0,014	0,014	0,014	0,014	0,014	0,014	0,014 Mass (g)	0,014	6,633E-05
First Mass at the start of the Stability plateau (g) A	0,014	0,013	0,013	0,013	0,013	0,013	0,013	0,013	0,014	0,013 A	0,013	4,422E-05
Middle Mass at the start of the Stability plateau (g) I	0,013	0,013	0,013	0,013	0,013	0,013	0,013	0,013	0,013	0,013 B	0,013	4,069E-05
Final Mass at the start of the Stability plateau (g) C	0,013	0,012	0,013	0,013	0,012	0,012						

												Average	SEM
												start	
												0	0
<b>Without nozzle</b>													
<b>250, 10s, 3cm</b>													
Mass (g)	0,1036	0,0993	0,0964	0,1006	0,0959	0,0944	0,0932	0,0897	0,0988	0,0935	Mass (g)	0,09654	0,00129634
First Mass at the start of the Stability plateau (g) A	0,103	0,099	0,093	0,1	0,096	0,093	0,092	0,089	0,094	0,0926	A	0,09524	0,00135402
Middle Mass at the start of the Stability plateau (g) B	0,097	0,092	0,089	0,093	0,091	0,089	0,089	0,087	0,089	0,087	B	0,09014	0,00098964
Final Mass at the start of the Stability plateau (g) C	0,093	0,088	0,088	0,088	0,087	0,085	0,086	0,085	0,086	0,0853	C	0,08724	0,00078205
<b>140, 10s, 3cm</b>													
Mass (g)	0,0454	0,0433	0,0437	0,0426	0,0433	0,0428	0,0454	0,0454	0,0438	0,0431	Mass (g)	0,04388	0,00035049
First Mass at the start of the Stability plateau (g) A	0,045	0,043	0,043	0,042	0,042	0,042	0,045	0,045	0,044	0,0423	A	0,04325	0,00040832
Middle Mass at the start of the Stability plateau (g) B	0,043	0,04	0,041	0,041	0,04	0,04	0,043	0,044	0,043	0,0408	B	0,04153	0,00044072
Final Mass at the start of the Stability plateau (g) C	0,041	0,04	0,04	0,039	0,038	0,038	0,042	0,042	0,042	0,0395	C	0,04008	0,0005035
<b>250, 10s, 1.5cm</b>													
Mass (g)	0,0826	0,0749	0,0781	0,0815	0,0794	0,078	0,0806	0,0813	0,0767	0,0816	Mass (g)	0,07947	0,00078656
First Mass at the start of the Stability plateau (g) A	0,081	0,074	0,077	0,081	0,079	0,076	0,078	0,081	0,076	0,0808	A	0,07831	0,00083459
Middle Mass at the start of the Stability plateau (g) B	0,079	0,072	0,071	0,075	0,075	0,071	0,075	0,076	0,073	0,076	B	0,07418	0,00083464
Final Mass at the start of the Stability plateau (g) C	0,075	0,067	0,066	0,073	0,071	0,066	0,068	0,068	0,066	0,054	C	0,06754	0,00181415
<b>140, 10s, 1.5cm</b>													
Mass (g)	0,037	0,0354	0,0369	0,0358	0,0366	0,0376	0,0364	0,0356	0,0353	0,0359	Mass (g)	0,03625	0,00024324
First Mass at the start of the Stability plateau (g) A	0,036	0,034	0,035	0,035	0,036	0,037	0,036	0,035	0,034	0,0353	A	0,03532	0,00027072
Middle Mass at the start of the Stability plateau (g) B	0,034	0,032	0,034	0,032	0,034	0,034	0,034	0,033	0,033	0,0331	B	0,03323	0,00022214
Final Mass at the start of the Stability plateau (g) C	0,032	0,031	0,033	0,031	0,033	0,033	0,032	0,032	0,032	0,0312	C	0,03193	0,00021084
<b>250, 10s, 1cm</b>													
Mass (g)	0,0661	0,0643	0,0624	0,0606	0,0615	0,0628	0,0607	0,0644	0,0623	0,0621	Mass (g)	0,06272	0,00055253
First Mass at the start of the Stability plateau (g) A	0,065	0,063	0,061	0,064	0,058	0,059	0,057	0,063	0,059	0,0605	A	0,06097	0,00087281
Middle Mass at the start of the Stability plateau (g) B	0,063	0,059	0,058	0,055	0,056	0,056	0,056	0,06	0,058	0,0562	B	0,0577	0,0007919
Final Mass at the start of the Stability plateau (g) C	0,06	0,057	0,056	0,056	0,054	0,055	0,044	0,058	0,056	0,0549	C	0,055	0,00131732
<b>140, 10s, 1cm</b>													
Mass (g)	0,034	0,0339	0,033	0,0334	0,0347	0,0334	0,0335	0,0332	0,0329	0,0338	Mass (g)	0,03358	0,00016984
First Mass at the start of the Stability plateau (g) A	0,033	0,032	0,032	0,033	0,034	0,032	0,033	0,032	0,032	0,0312	A	0,03243	0,00025036
Middle Mass at the start of the Stability plateau (g) B	0,032	0,031	0,03	0,03	0,032	0,031	0,031	0,03	0,03	0,0295	B	0,0307	0,00026499
Final Mass at the start of the Stability plateau (g) C	0,03	0,03	0,03	0,029	0,031	0,03	0,03	0,03	0,029	0,0291	C	0,02965	0,00016482
<b>250, 5s, 3cm</b>													
Mass (g)	0,1036	0,0963	0,0961	0,0936	0,0925	0,0997	0,1002	0,0984	0,0956	0,0962	Mass (g)	0,09722	0,00104497
First Mass at the start of the Stability plateau (g) A	0,104	0,096	0,096	0,093	0,092	0,1	0,1	0,097	0,094	0,0941	A	0,0965	0,00114358
Middle Mass at the start of the Stability plateau (g) B	0,101	0,093	0,092	0,091	0,091	0,095	0,094	0,093	0,092	0,0917	B	0,09332	0,0009259
Final Mass at the start of the Stability plateau (g) C	0,1	0,092	0,09	0,09	0,089	0,093	0,092	0,091	0,089	0,0901	C	0,09163	0,00097275
<b>140, 5s, 3cm</b>													
Mass (g)	0,0455	0,0446	0,0425	0,0444	0,0472	0,0463	0,0458	0,0455	0,0443	0,0453	Mass (g)	0,04514	0,00040585
First Mass at the start of the Stability plateau (g) A	0,045	0,044	0,042	0,044	0,047	0,045	0,045	0,044	0,044	0,0451	A	0,04445	0,00040586
Middle Mass at the start of the Stability plateau (g) B	0,043	0,041	0,041	0,041	0,045	0,043	0,044	0,043	0,044	0,0444	B	0,04289	0,00046677
Final Mass at the start of the Stability plateau (g) C	0,041	0,041	0,041	0,04	0,044	0,042	0,043	0,043	0,044	0,0439	C	0,08224	0,04019799
<b>250, 5s, 1.5cm</b>													
Mass (g)	0,084	0,0781	0,0812	0,079	0,0812	0,0811	0,0793	0,0795	0,076	0,0768	Mass (g)	0,07962	0,00074711
First Mass at the start of the Stability plateau (g) A	0,083	0,077	0,078	0,078	0,081	0,08	0,079	0,079	0,075	0,0753	A	0,07851	0,00076295
Middle Mass at the start of the Stability plateau (g) B	0,08	0,075	0,008	0,077	0,077	0,077	0,076	0,076	0,072	0,0735	B	0,06904	0,00685135
Final Mass at the start of the Stability plateau (g) C	0,08	0,075	0,008	0,075	0,074	0,075	0,074	0,073	0,071	0,0733	C	0,06794	0,00673267
<b>140, 5s, 1.5cm</b>													
Mass (g)	0,0366	0,0368	0,0363	0,0354	0,0354	0,0374	0,0368	0,0364	0,0361	0,0356	Mass (g)	0,03628	0,00020966
First Mass at the start of the Stability plateau (g) A	0,036	0,036	0,035	0,035	0,035	0,037	0,036	0,036	0,036	0,0348	A	0,03546	0,00022862
Middle Mass at the start of the Stability plateau (g) B	0,034	0,034	0,033	0,033	0,033	0,035	0,034	0,034	0,034	0,0332	B	0,03366	0,00019505
Final Mass at the start of the Stability plateau (g) C	0,033	0,033	0,033	0,033	0,033	0,035	0,034	0,033	0,033	0,0331	C	0,03319	0,00017349
<b>250, 5s, 1cm</b>													
Mass (g)	0,0069	0,0652	0,0648	0,0635	0,0631	0,0628	0,0653	0,0645	0,0631	0,0632	Mass (g)	0,058244	0,00570818
First Mass at the start of the Stability plateau (g) A	0,066	0,064	0,063	0,062	0,062	0,06	0,061	0,063	0,062	0,0616	A	0,06247	0,00047189
Middle Mass at the start of the Stability plateau (g) B	0,065	0,061	0,06	0,06	0,06	0,059	0,058	0,06	0,058	0,0584	B	0,05981	0,0006806
Final Mass at the start of the Stability plateau (g) C	0,063	0,06	0,058	0,058	0,058	0,057	0,058	0,059	0,057	0,0574	C	0,05842	0,00056071
<b>140, 5s, 1cm</b>													
Mass (g)	0,0334	0,0334	0,0329	0,0323	0,0349	0,0347	0,0344	0,0343	0,0343	0,0332	Mass (g)	0,03378	0,00027113
First Mass at the start of the Stability plateau (g) A	0,033	0,032	0,032	0,032	0,034	0,034	0,034	0,033	0,034	0,0329	A	0,0329	0,00025122
Middle Mass at the start of the Stability plateau (g) B	0,032	0,031	0,031	0,031	0,032	0,032	0,032	0,031	0,032	0,0313	B	0,03142	0,00013646
Final Mass at the start of the Stability plateau (g) C	0,032	0,031	0,03	0,03	0,032	0,031	0,032	0,023	0,03	0,031	C	0,03016	0,00082707

## 8.2. Annex 2

No Nozzle 1,5s								
Vertical diameter	250	SEM	210	SEM	140	SEM	70	SEM
3 cm	1,381	0,034	1,241	0,019				
1,5 cm	1,488	0,012	1,523	0,000	0,875	0,009	0,875	
1 cm	1,680	0,057	1,549	0,125	1,228	0,264	0,955	
0,5 cm	1,820	0,059	1,720	0,006	1,195	0,003	1,198	
Horizontal diameter	250	SEM	210	SEM	140	SEM	70	SEM
3 cm	1,384	0,006	1,253	0,012				
1,5 cm	1,529	0,003	1,586	0,012	0,908	0,033		
1 cm	1,767	0,022	1,592	0,127	0,978	0,022		
0,5 cm	1,914	0,032	1,732	0,044	1,209	0,012	0,234	
Nozzle 1,5s	250	SEM	210	SEM	140	SEM	70	SEM
Vertical diameter	250	SEM	210	SEM	140	SEM	70	SEM
3 cm	1,168	0,055	1,060	0,013	0,764	0,088		
1,5 cm	0,825	0,249	1,045	0,003	0,913	0,006	0,388	0,026
1 cm	1,202	0,046	1,130	0,020	0,874	0,033	0,365	0,148
0,5 cm	1,248	0,023	1,112	0,088	0,950	0,036	0,415	0,006
Horizontal diameter	250	SEM	210	SEM	140	SEM	70	SEM
3 cm	1,197	0,065	1,092	0,007	0,739	0,033		
1,5 cm	1,128	0,042	1,087	0,006	0,914	0,064	0,371	0,038
1 cm	1,236	0,052	1,174	0,023	0,902	0,007	0,405	0,124
0,5 cm	1,316	0,055	1,274	0,019	0,981	0,032	0,423	0,000
No Nozzle 0,9s	250	SEM	210	SEM	140	SEM	70	SEM
Vertical diameter	250	SEM	210	SEM	140	SEM	70	SEM
3 cm	1,047	0,161	1,219	0,084				
1,5 cm	1,473	0,060	1,416	0,048	0,821	0,038	0,886	
1 cm	1,765	0,088	1,622	0,021	0,960	0,058	0,824	
0,5 cm	1,709	0,079	1,737	0,063	0,878	0,022	0,920	
Horizontal diameter	250	SEM	210	SEM	140	SEM	70	SEM
3 cm	1,119	0,040	1,215	0,036				
1,5 cm	1,484	0,028	1,537	0,032	0,841	0,045		
1 cm	1,773	0,124	1,593	0,029	0,929	0,105		
0,5 cm	1,778	0,038	1,659	0,031	0,970	0,050		
Nozzle 0,9s	250	SEM	210	SEM	140	SEM	70	SEM
Vertical diameter	250	SEM	210	SEM	140	SEM	70	SEM
3 cm	1,054	0,052	0,926	0,036				
1,5 cm	1,098	0,009	1,082	0,019	0,826	0,065	0,436	0,034
1 cm	1,253	0,046	3,145	2,023	0,894	0,007	0,388	0,069
0,5 cm	1,417	0,045	1,320	0,057	0,951	0,018	0,409	0,126
Horizontal diameter	250	SEM	210	SEM	140	SEM	70	SEM
3 cm	1,125	0,078	0,936	0,019	0,000	0,000		
1,5 cm	1,146	0,012	1,133	0,012	0,856	0,053	0,426	0,047
1 cm	1,275	0,072	1,226	0,042	0,894	0,003	0,397	0,085
0,5 cm	1,474	0,040	1,392	0,097	0,970	0,033	0,429	0,131

### 8.3. Annex 3

Brass Nozzle	Baseline temp.	SEM	SD	Temp. range	SEM	SD	Diam. of 0.1°C gradient	SEM	SD	Diam. of 0.5°C gradient	SEM	SD	Diam. of 1.0°C gradient	SEM	SD
250, 1sec, 3cm	29,433	0,267	0,462	1,733	0,067	0,115	3,345	0,225	0,390	6,464	0,193	0,334	8,940	0,232	0,402
210, 1sec, 3cm	29,800	0,153	0,265	1,700	0,058	0,100	1,512	0,499	0,865	6,464	0,201	0,348	8,554	0,129	0,223
140, 1sec, 3cm	29,900	0,058	0,100	1,667	0,033	0,058	2,219	0,754	1,305	6,175	0,675	1,170	8,908	0,505	0,875
70, 1sec, 3cm	29,900	0,058	0,100	1,767	0,033	0,058	2,219	0,365	0,633	4,953	0,716	1,241	8,490	0,675	1,170
250, 1sec, 1.5cm	29,767	0,088	0,153	2,400	0,000	0,000	1,093	0,196	0,339	3,731	0,161	0,279	5,789	0,096	0,167
210, 1sec, 1.5cm	29,733	0,033	0,058	2,267	0,033	0,058	1,801	0,225	0,390	3,795	0,032	0,056	5,757	0,116	0,201
140, 1sec, 1.5cm	29,700	0,058	0,100	2,300	0,058	0,100	1,126	0,257	0,446	3,859	0,147	0,255	5,789	0,193	0,334
70, 1sec, 1.5cm	29,867	0,033	0,058	2,033	0,067	0,115	0,547	0,179	0,310	1,640	0,056	0,096	2,766	0,085	0,147
250, 1sec, 1cm	30,500	0,058	0,100	2,800	0,000	0,000	0,868	0,147	0,255	2,155	0,064	0,111	3,602	0,085	0,147
210, 1sec, 1cm	30,467	0,033	0,058	2,667	0,033	0,058	0,804	0,116	0,201	2,219	0,056	0,096	3,538	0,032	0,056
140, 1sec, 1cm	30,633	0,033	0,058	2,833	0,033	0,058	0,675	0,096	0,167	1,769	0,032	0,056	3,409	0,032	0,056
70, 1sec, 1cm	30,333	0,133	0,231	2,633	0,088	0,153	0,193	0,056	0,096	0,900	0,032	0,056	1,576	0,116	0,201
250, 1sec, 0.5cm	30,933	0,033	0,058	3,100	0,058	0,100	0,354	0,161	0,279	1,447	0,056	0,096	2,444	0,129	0,223
210, 1sec, 0.5cm	30,600	0,115	0,200	3,000	0,058	0,100	0,772	0,147	0,255	1,704	0,032	0,056	2,830	0,140	0,243
140, 1sec, 0.5cm	30,467	0,033	0,058	2,867	0,067	0,115	0,611	0,085	0,147	1,415	0,032	0,056	2,315	0,096	0,167
70, 1sec, 0.5cm	30,700	0,058	0,100	2,833	0,338	0,586	0,482	0,000	0,000	1,029	0,032	0,056	1,544	0,056	0,096
250, 1.5sec, 1cm	30,033	0,120	0,208	2,900	0,058	0,100	0,515	0,032	0,056	2,219	0,201	0,348	3,698	0,085	0,147
210, 1.5sec, 1cm	29,833	0,120	0,208	2,667	0,067	0,115	0,579	0,096	0,167	2,123	0,147	0,255	3,731	0,161	0,279
140, 1.5sec, 1cm	29,700	0,058	0,100	2,767	0,033	0,058	0,386	0,096	0,167	1,833	0,193	0,334	3,505	0,161	0,279
70, 1.5sec, 1cm	29,733	0,033	0,058	2,533	0,219	0,379	0,289	0,096	0,167	0,933	0,064	0,111	1,608	0,032	0,056
250, 0.5sec, 1cm	30,200	0,000	0,000	2,467	0,088	0,153	0,740	0,275	0,476	2,605	0,193	0,334	3,923	0,129	0,223
210, 0.5sec, 1cm	30,133	0,033	0,058	2,500	0,100	0,173	0,482	0,096	0,167	2,219	0,111	0,193	3,763	0,201	0,348
140, 0.5sec, 1cm	30,133	0,033	0,058	2,600	0,058	0,100	0,547	0,032	0,056	1,930	0,147	0,255	3,763	0,201	0,348
70, 0.5sec, 1cm	30,200	0,058	0,100	1,933	0,120	0,208	0,354	0,140	0,243	1,061	0,056	0,096	2,090	0,225	0,390

No Nozzle	Baseline temp.	SEM	SD	Temp. range	SEM	SD	Diam. of 0.1°C gradient	SEM	SD	Diam. of 0.5°C gradient	SEM	SD	Diam. of 1.0°C gradient	SEM	SD
250, 1sec, 3cm	30,233	0,088	0,153	2,167	0,033	0,058	1,962	0,211	0,365	6,689	0,032	0,056	10,484	0,032	0,056
210, 1sec, 3cm	30,000	0,058	0,100	2,133	0,033	0,058	1,801	0,225	0,390	6,561	0,243	0,421	10,452	0,116	0,201
140, 1sec, 3cm	30,000	0,208	0,361	2,033	0,033	0,058	1,930	0,435	0,754	6,946	0,193	0,334	10,677	0,032	0,056
70, 1sec, 3cm	30,400	0,000	0,000	1,933	0,033	0,058	1,319	0,614	1,063	8,362	0,371	0,642	10,452	0,032	0,056
250, 1sec, 1.5cm	30,567	0,067	0,115	2,400	0,000	0,000	1,897	0,307	0,531	4,920	0,255	0,442	7,815	0,295	0,511
210, 1sec, 1.5cm	30,300	0,058	0,100	2,333	0,033	0,058	1,962	0,161	0,279	5,338	0,032	0,056	8,201	0,111	0,193
140, 1sec, 1.5cm	30,200	0,058	0,100	2,333	0,067	0,115	1,962	0,085	0,147	5,113	0,147	0,255	8,522	0,116	0,201
70, 1sec, 1.5cm	30,300	0,000	0,000	2,000	0,000	0,000	2,476	0,161	0,279	6,689	0,129	0,223	9,937	0,000	0,000
250, 1sec, 1cm	30,267	0,033	0,058	1,867	0,133	0,231	1,222	0,354	0,613	4,406	0,196	0,339	6,754	0,096	0,167
210, 1sec, 1cm	30,167	0,067	0,115	1,533	0,033	0,058	1,351	0,386	0,668	4,277	0,032	0,056	6,850	0,096	0,167
140, 1sec, 1cm	30,233	0,033	0,058	1,467	0,033	0,058	1,351	0,279	0,482	4,760	0,032	0,056	7,976	0,464	0,803
70, 1sec, 1cm	30,233	0,033	0,058	1,333	0,033	0,058	1,286	0,225	0,390	5,178	0,280	0,486	8,683	0,147	0,255
250, 1sec, 0.5cm	31,500	0,058	0,100	1,633	0,067	0,115	2,123	0,966	1,674	6,593	0,085	0,147	8,683	0,255	0,442
210, 1sec, 0.5cm	31,167	0,033	0,058	1,800	0,000	0,000	2,669	1,326	2,296	6,689	0,474	0,821	8,876	0,255	0,442
140, 1sec, 0.5cm	31,067	0,088	0,153	1,633	0,033	0,058	3,152	0,358	0,620	6,561	0,056	0,096	9,069	0,056	0,096
70, 1sec, 0.5cm	31,400	0,058	0,100	1,533	0,033	0,058	2,251	1,227	2,125	7,718	0,386	0,668	10,066	0,064	0,111
250, 1.5sec, 1cm	30,300	0,115	0,200	1,633	0,088	0,153	1,512	0,129	0,223	4,695	0,232	0,402	6,786	0,263	0,456
210, 1.5sec, 1cm	30,233	0,033	0,058	1,467	0,033	0,058	1,286	0,371	0,642	4,663	0,196	0,339	6,432	0,140	0,243
140, 1.5sec, 1cm	30,200	0,000	0,000	1,567	0,033	0,058	0,997	0,064	0,111	4,727	0,147	0,255	6,850	0,096	0,167
70, 1.5sec, 1cm	30,233	0,033	0,058	1,367	0,067	0,115	1,158	0,201	0,348	5,371	0,129	0,223	8,362	0,064	0,111
250, 0.5sec, 1cm	30,467	0,033	0,058	1,300	0,000	0,000	2,315	0,310	0,537	5,306	0,096	0,167	8,136	0,225	0,390
210, 0.5sec, 1cm	30,433	0,033	0,058	1,367	0,033	0,058	1,415	0,161	0,279	5,049	0,140	0,243	8,201	0,255	0,442
140, 0.5sec, 1cm	30,467	0,033	0,058	1,367	0,033	0,058	1,994	0,371	0,642	5,435	0,280	0,486	8,522	0,170	0,295
70, 0.5sec, 1cm	30,533	0,033	0,058	1,167	0,033	0,058	1,576	0,864	1,496	6,496	0,140	0,243	9,133	0,032	0,056



#### 8.4. Annex 4

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Elisabet Simó Bertran

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Reference to a book:

[3] Strunk Jr W, White EB. *The elements of style*. 4th ed. New York: Longman; 2000.

Reference to a chapter in an edited book:

[4] Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.

Reference to a website:

[5] Cancer Research UK. Cancer statistics reports for the UK, <http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/>; 2003 [accessed 13 March 2003].

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