# Aerosol production during autopsies: Minimising health risks of bone sawing

Master Thesis

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TU Delft Delft University of Technology

Nederlands Forensisch Instituut
*Ministerie van Justitie en Veiligheid*

# Aerosol production during autopsies: Minimising health risks of bone sawing

## Master Thesis

by

J. Pluim

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# Summary

This thesis is part of a collaboration between the Delft University of Technology and the Netherlands Forensic Institute (NFI) concerning the health risks faced by (forensic) pathologists and anthropologists posed by the production of harmful aerosols (solid or liquid airborne particles) when sawing in bone. This thesis provides an overview of a year-longs worth MSc graduation project, including an internship at the department of Forensic Anthropology of the NFI.

Mechanical tools, either powered manually or mechanically are often used to aid in gaining access to the body, or during the task itself, such as scalpels, lasers, scissors, saws, drills or electrocautery tools. Within the field of forensic anthropology and pathology bone saws are mainly used during autopsies and forensic anthropological or archaeological bone examinations, for instance for human identification purposes or tool mark analysis. The inhalation of surgical smoke or aerosols produced during the use of mechanical tools is an often overlooked health hazard, as these aerosols can act as pathways for pathogens such as Hepatitis B and Hepatitis C, Streptococci, and Human Immunodeficiency Virus (HIV). Although the pathogen-carrying ability of aerosols produced during autopsies is considered the highest health risk of aerosolised material, also the non-pathogen-carrying aerosols can pose a hazard when inhaled and deposited in the airways.

Safety protocols have been developed and adapted over the years to minimise these risks, but these protocols are generally dependent on high-tech tools and a well equipped working environment. Problems arise when the setting of the procedure might not be suited for adequate ventilation systems, which may be the case in developing countries without high-tech infrastructure, or after natural or anthropogenic disasters where emergency makeshift mortuaries are often used.

The goal of this thesis is twofold: Firstly, to quantify the number of aerosol bone dust particles that are produced when sawing in bone, with respect to several controllable sawing parameters and environmental conditions. Secondly, with the knowledge obtained in quantifying the production of aerosol bone dust particles, to be able to give an indication of the optimal sawing settings, reducing the risks posed by the aerosol bone particles. This can further be divided into two main parts, reducing the production of aerosol bone dust particles, and limiting the possibility for the aerosols to reach the respiratory tract of the persons involved. These can include different suggestions depending on the environmental setting, such as for places or moments where high-tech solutions are not available.

A custom test setup was designed and manufactured to test the sawing parameters in 8 experiments, where a particle counter was used to determine the production of aerosol particles while varying 5 different parameters: saw blade frequency, saw blade contact load, bone condition, test environment and saw blade type.

Results showed that the number of produced particles was highest with higher saw blade frequencies, lower saw blade contact loads, in dry completely skeletonised bone compared to fresh bone, and using an electrical oscillating saw compared to hand-sawing. Under all conditions, the high amount of aerosol produced posed potential health risks. The tested external ventilation system was adequate in removing the produced number of particles, but these high-tech systems are not always available in developing countries or emergency situations.

In conclusion, the production of aerosols can be reduced by optimising the sawing parameters. However, even the lowest number of aerosol particles produced during the current study was high enough to cause potential health risks to practitioners. Safety precautions should be taken, such as external ventilation, proper breathing gear, and adequate protocols, to truly minimise the risk in all bone sawing scenarios.

# Contents

# 1 Introduction

## 1.1. Short motivation

This thesis is part of a collaboration between the Delft University of Technology and the Netherlands Forensic Institute (NFI) concerning the health risks faced by professionals who work with human tissue. Although similar risks are faced by hospital workers, for instance during (orthopaedic) surgeries, and even extend to veterinarians, the origin of the project lies specifically with health risks faced by (forensic) pathologists and anthropologists posed by the production of harmful aerosols (solid or liquid airborne particles) when sawing in bone.

Mechanical tools, either powered manually or mechanically are often used to aid in gaining access to the body, or during the task itself, such as scalpels, lasers, scissors, saws, drills or electrocautery tools. Within the field of forensic anthropology and pathology bone saws are mainly used during autopsies and forensic anthropological or archaeological bone examinations, for instance for human identification purposes or tool mark analysis. The inhalation of surgical smoke or aerosols produced during the use of mechanical tools is an often overlooked health hazard, as these aerosols can act as pathways for pathogens such as Hepatitis B and Hepatitis C, Streptococci, and Human Immunodeficiency Virus (HIV). Although the pathogen-carrying ability of aerosols produced during autopsies is considered the highest health risk of aerosolised material, also the non-pathogen-carrying aerosols can pose a hazard when inhaled and deposited in the airways.

Safety protocols have been developed and adapted over the years to minimise these risks, but these protocols are generally dependent on high-tech tools and a well equipped working environment. Problems arise when the setting of the procedure might not be suited for adequate ventilation systems, which may be the case in developing countries without high-tech infrastructure, or after natural or anthropogenic disasters where emergency makeshift mortuaries are often used.

## 1.2. Scope & Goal

Although there are plenty similarities between the risks faced by all professionals who come into contact with (deceased) human or animal bodies, this thesis will specifically focus on reducing the risk of aerosol bone dust inhalation by forensic workers. The goal of this thesis is twofold: Firstly, to quantify the number of aerosol bone dust particles that are produced when sawing in bone, with respect to several controllable sawing parameters and environmental conditions. Secondly, with the knowledge obtained in quantifying the production of aerosol bone dust particles, to be able to give an indication of the optimal sawing settings, reducing the risks posed by the aerosol bone particles. This can further be divided into two main parts, reducing the production of aerosol bone dust particles, and limiting the possibility for the aerosols to reach the respiratory tract of the persons involved. These can include different suggestions depending on the environmental setting, such as for places or moments where high-tech solutions are not available.

However important, quantifiable conclusions with respect to the resulting health risk when inhaling aerosol bone dust particles are deemed to be outside the scope of this thesis. It is assumed that lowering the number of produced aerosol particles, including bone dust particles, is desired to minimise the risk faced by those who come into contact with these aerosols. Within this thesis no quantification to the minimum infective dose is given: only the number of produced particles is measured, not the pathogen-carrying effect those particles can potentially have. The same goes for the overall health risk of inhaling small dust particles, pathogen-carrying or not, it is assumed that less is better.

## 1.3. Approach & Outline

This thesis provides an overview of a year-longs worth MSc graduation project, including an internship at the department of Forensic Anthropology of the Netherlands Forensic Institute.

The effects of several sawing parameters on the production of aerosols were systematically studied, resulting in two stand-alone research papers that are included as separate chapters and are the main focus of this thesis. The first paper was published in Forensic Science International in August 2018, the second is submitted to the same journal as of January 2019.

Chapter 2 contains the first paper, and describes a pilot test using an electrical oscillating saw on dry, archaeological bone, in a controlled and boxed environment. This chapter is the result of the first three months of the graduation project, and was performed in collaboration with Lucas Jimenez-Bou, a co-intern and Forensic Science Master's student from the University of Amsterdam.

As a continuation of this graduation project, a step-wise process into a total of eight experiments achieved a closer representation of the production of aerosols during bone sawing in real life working scenarios. Chapter 3 contains the second paper, and describes the influence of five sawing parameters on the production of aerosols that were efficiently studied at the Netherlands Forensic Institute in a total of eight experiments.

Chapter 4 evaluates the findings of this project as a whole, of which the details are presented in the research papers seen in Chapter 2 and 3, along with the limitations within this thesis, recommendations for further research, and concluding remarks. In the appendices more background information is given on the design of the experimental setup (Appendix A), the validation of the experimental protocol (Appendix B), and all the resulting data from the eight experiments (Appendix C). Large parts of appendices A and B are based on my internship report.

# 2

# Aerosol production during autopsies: Pilot tests

This chapter contains the research paper that was submitted to and published in Forensic Science International in August 2018, and reports the findings of the exploratory pilot tests performed during an internship at the Netherlands Forensic Institute in collaboration with Lucas Jimenez-Bou, a co-intern and Forensic Science Master's student from the University of Amsterdam.

During these tests the influence of saw blade frequency and saw blade contact load on the production of aerosols were studied by sawing in dried human femora with an electrical oscillating saw. Detailed background information on the production of the experimental setup and the validation of the experimental protocol are shown in Appendix A and Appendix B respectively.

J.M.E. Pluim, L. Jimenez-Bou, R.R.R. Gerretsen, A.J. Loeve,
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Abstract: When sawing during autopsies on human remains, fine dust is produced, which consists of particles of sizes that may fall within the human respirable range, and can act as vectors for pathogens. The goal of this study was to explore the potential effects of saw blade frequency and saw blade contact load on the number and size of airborne bone particles produced. The methodology involved the use of an oscillating saw with variable saw blade frequencies and different saw blade contact loads on dry human femora. Released airborne particles were counted per diameter by a particle counter inside a closed and controlled environment. Results corroborated with the hypotheses: higher frequencies or lower contact loads resulted in higher numbers of aerosol particles produced. However, it was found that even in the best-case scenario tested on dry bone, the number of aerosol particles produced was still high enough to provide a potential health risk to the forensic practitioners. Protective breathing gear such as respirators and biosafety protocols are recommended to be put into practice to protect forensic practitioners from acquiring pathologies, or from other biological hazards when performing autopsies.
**Keywords: aerosol, bone dust, oscillating saw, autopsy, pathology, biosafety**

## 2.1. Introduction

Autopsies are surgical procedures performed in the field of pathology that are used to find a deceased person's cause or manner of death. Different diagnoses ask for different examinations, some of which requiring incisions in superficial tissues to provide access to deeper internal tissues of the body. Since forensic practitioners are generally aware of potential hazards, protective clothing is used and protocols are followed to minimise contact with pathogens [2, 3]. Some contamination routes may seem more obvious than others due to explicit interaction with contaminants, such as through cuts by scalpels or punctures with needles. However, inhalation of infectious airborne particles during an autopsy could be as harmful as an accidental cut [3–5]. Powered surgical instruments, such as saws and drills, are greatly responsible for aerosolisation (solid or liquid airborne particles) of body tissues, exposure to these aerosols may be considered an often overlooked contamination route [6, 7].

Oscillating saws are routinely used during autopsies, when forensic practitioners are required to make deep incisions through bone or cartilage tissues, oscillating saws have an advantage due to an increased ease of use and accessibility compared to hand or band saws. There is however a concern about the production of suspended particles (aerosols) when operating the saw. Aerosols produced by sawing can be dispersed wide in the surroundings of the site of operation, possibly reaching the respiratory tract of the operator [1, 6, 8, 9]. The aerosol's particle size determines the potential invasion depth of the aerosol in the inhaler's respiratory tract, as reported in Jones and Brosseau [4], and can possibly act as a pathway for hazardous diseases [10]. For example, for particles of 0.1 µm about 2.1% will only reach the head airways, 2.7% will reach the tracheobronchial region and 14% will end up in the alveoli. For particles of 10 µm this would be 81%, 1.5% and 1.9%, respectively. Particles smaller than 10µm are within the respirable range [4] and have the potential to remain suspended in the air for long periods of time, increasing the time during which the air around the working area is contaminated with possibly infective aerosol [9]. Among the hazardous pathogens are Hepatitis B and C, Streptococci, and Human Immunodeficiency Virus (HIV), of which transmissions have already been recorded during autopsy sessions [11–14]. When inhaling such pathogens, a minimal infective dose (MID) is required to actually produce an infection. Most respiratory viruses appear to have infectious potential in humans even with low doses [15]. Concern also emerges towards unknown MIDs, as this serves as motivation for prophylaxis, see Table 2.1.

The goal of this study was to investigate the production of aerosol when sawing dry long bones under different sawing conditions, simulating an autopsy procedure.

Table 2.1: Sizes and Minimal Infective Doses (MID) of zoonotic pathogens. Courtesy of Wenner et al. [1].

| Microorganism | Pathogen | Size | Minimal Infective Dose |
|---|---|---|---|
| Viruses | Influenza A | 120 nm | Unknown |
| | Lyssa (rabies) | 65 x 180 nm | Unknown |
| | Avula (NDV) | 150–250 nm | Unknown |
| | Herpesvirus (eg, herpes B) | 150–200 nm | Unknown (CHV–1) |
| | Coronavirus (eg, SARS-COV) | 80–160 nm | Unknown |
| Bacteria | Mycoplasma sp. | 0.05–0.5 x 0.3–2 µm | <100 CFU (M. pneumoniae) |
| | Francisella tularensis | 0.2–0.7 x 0.2 µm | 5–10 organisms |
| | Brucella sp. | 0.5–0.7 x 0.6–1.5 µm | Unknown |
| | Coxiella burnetti | 0.2–0.4 x 0.4–1 µm | 1–10 |
| | Staphylococcus sp. | 0.5–1.5 µm | >1000000 (S. aureus) |
| | Streptococcus sp. | 0.5–1 µm | Unknown |
| | Mycobacterium tuberculosis | 0.2–0.6 x 1–10 µm | <10 bacilli |
| | Bacillus anthracis | 0.4–1.8 x 0.9–10 µm | 8000—50000 |
| | Leptospira interrogans | 0.1 x 6–12 µm | Unknown |
| | Salmonella sp. | 0.75–1.5 x 2–5 µm | $10^3$–$10^5$ |
| | Escherichia coli | 0.6–1 x 1.2–3 µm | 10 EHEC $10^6$ EPEC $10^8$ ETEC |
| | Yersinia pestis | 0.5–0.8 x 1–3 µm | Unknown |
| Fungi | Histoplasma | 2–4 µm, 8–15 µm | 5 yeast cells |
| | Aspergillus sp. | 3–8 µm | Unknown |
| | Cryptococcus | 3–5 µm | Unknown |
| Parasites | Toxoplasma | 10–12 µm | <10 sporulated oocysts (T. gondii) |
| | Echinococcus sp. (eggs) | 34 x 27 µm | Unknown |

CFU, colony-forming units; CHV-1, Cercopithecine Herpesvirus-1; EHEC, Enterohemorrhagic E. coli; EPEC, Enteropathogenic E. coli; ETEC, Enterotoxic E. coli; NDV, Newcastle Disease Virus; SARS-CoV, SARS Coronavirus.

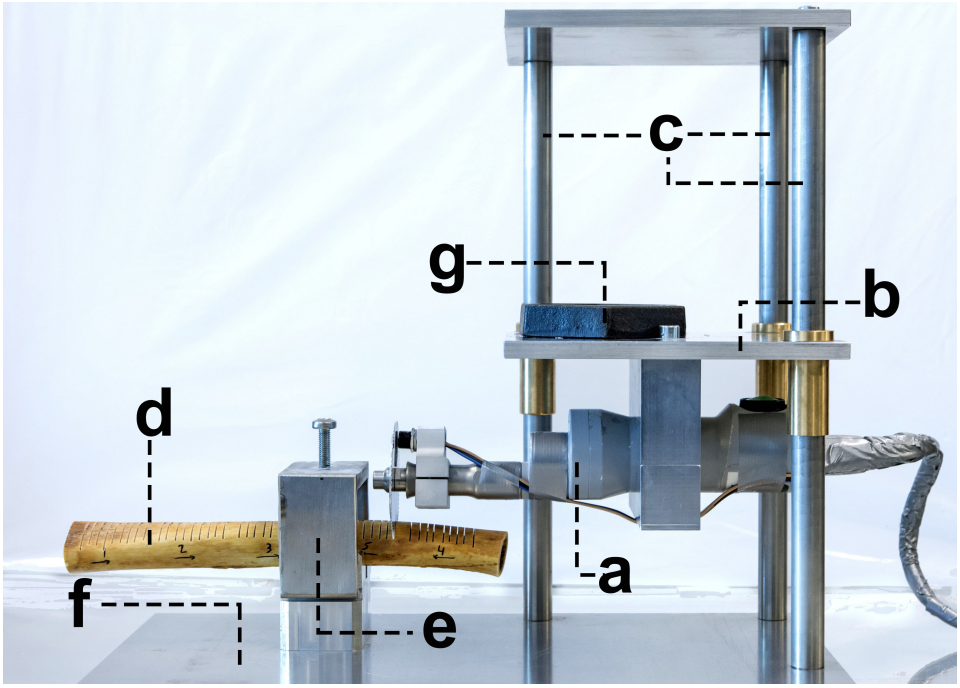



Figure 2.1: Experimental setup used to cut the bone, the setup consisted of: an oscillating saw (a) fastened to a vertical sliding platform (b) guided by 3 stainless steel rods and brass sliding bearings (c). The bone specimen (d) was clamped in a v-groove holder (e), that was connected to an aluminium base plate (f). Interchangeable weights could be attached to the platform (g). The sawing action is further illustrated in Fig. 2.2.

## 2.2. Materials and Methods

### 2.2.1. Experimental setup

A custom setup designed for ease-of-use and cleanability was manufactured. In the explanation of the setup below the letters used to refer to specific parts correspond with those in Figs. 2.1, 2.2, and 2.3.

An oscillating saw (DeSoutter NS3, DeSoutter Medical Limited) (a), with a blade of 76mm in diameter (DeSoutter 16892, DeSoutter Medical Limited), fixed to a sliding platform (b) by a 125x36x40mm aluminium block with a cylindrical hole the diameter of the saw handle. The platform housed three cylindrical brass cylinders that slid along three 20mm diameter surgical stainless steel rods (c) acting as guides, allowing the saw to freely move vertically with a range of 270mm, while being fixated in the other planes. The bone specimen (d) was fastened in a 70x50x60mm aluminium block with a v-shaped groove in its base. An u-shaped clamp with a threaded bolt clamped the femur into the v-groove, securing it to the setup (e). Both the v-groove block and the steel rods were fixed to a 440x260mm aluminium base plate (f). Interchangeable weights (g) were used to vary the contact load of the saw blade against the bone specimen. The sawing depth of the saw blade (h) was controlled to always be 10mm by a depth control stopper (i) placed next to the saw blade. The stopper consisted of a 3mm thick, 56mm diameter (10mm less radius than the saw blade) round aluminium plate. Fig. 2.2 shows the saw blade and stopper reaching the preset limit after a cut. A custom-built tachometer (j) using a hall-effect sensor (Geartooth speed sensor GS100701, ZF Electronics) was clamped to the saw with a 50x40x20 aluminium block (k), and was used to accurately set the initial frequency in each experiment, as well as to observe the frequency change during sawing. Due to the resistance of the bone against the saw it was expected that the sawing frequency would drop during sawing. A close-up view of the saw blade is shown in Fig. 2.2.

The whole platform was placed inside an acrylic glass box (l) of 780x470x500mm, as shown in Fig. 2.3. An entrance hole (110mm diameter) (o) in the side of the box provided access to the setup without having to open the box and cause any disturbance during the measurements. When the entrance hole is not used, a lid was used to seal it. By conducting the experiment inside a closed environment, the invasion of foreign aerosol was minimised, as well as the leakage of produced particles, and the disturbance of any air flow from external interactions, such as from researchers walking by. Also, it was much more feasible to clean the inside of the box than an entire autopsy room.

The number of aerosol particles present in the air in the box was measured using a Fluke 985 particle counter (Fluke corporation, Everett, Washington USA) (m), shown in Fig. 2.3. The particle counter was

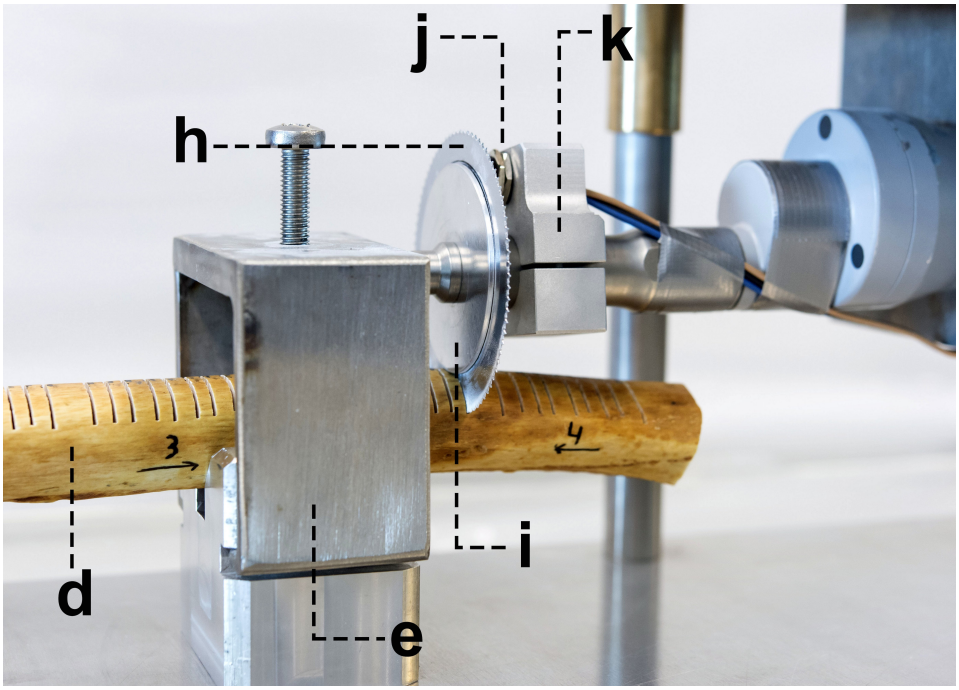



Figure 2.2: Close-up of the saw blade and bone specimen, the setup consisted of: the bone specimen (d) was clamped in place by the v-groove holder (e). The saw blade (h) cut in the bone until the stopper (i) reached the bone for a consistent depth of cut. The Hall-effect sensor (j) acted as a tachometer, and was clamped to the saw with an aluminium block (k).

placed on top of the box with a foam cast to hold it in place. A small hole in the acrylic glass provided access to the particle counter's sensor. The distance between the bone specimen and the particle counter's sensor was about 450mm, to replicate the breathing zone of the saw operator. Particles were counted at a flow of 2.83l/m in six different sizes: 0.3, 0.5, 1.0, 3.0, 5.0, and 10µm. It was decided that sizes over 10µm were not of relevance to the current study, as particles of these sizes are most likely to deposit in the head airway region of the respiratory tract, whereas smaller particles will primarily deposit in the alveoli (see Introduction) [4, 6, 12, 16].

Two human femora from an archaeological bone collection of the Netherlands Forensic Institute (The Hague, the Netherlands) were used. The femur is a long tubular bone with a reasonably consistent cortex thickness, and morphology along the shaft. Both femora were in dry condition and clean of any soft tissues. For this experiment, the bone marrow cavities of the femora were scraped to remove gross trabecular bone tissue, together with other residues that could easily shake loose during cutting and interfere as unwanted suspended particles.

### 2.2.2. Experimental design

For this study, two hypotheses were formulated: H1, the frequency of the saw blade has a positive effect on the number of aerosol particles produced. H2: the contact load of the saw blade has a negative effect on the number of aerosol particles produced. It was hypothesised that by increasing the frequency, or lowering the contact loads, a relative small amount of new bone is encountered by the teeth of the saw blade, removing little bone, producing a smoother cut with more suspended fine particles and less coarse heavy dust. Reversely, it was hypothesised that with lower frequency or higher contact loads, a relatively large amount of new bone is encountered by the teeth of the saw blade, breaking off big chunks of bone, resulting in a rougher cut, more coarse heavy dust and less suspended fine dust.

The experiment was set up such that saw blade frequency and saw blade contact load were the independent variables, and that the number of aerosol particles produced during sawing, was the dependent variable. Three different values were selected for both saw blade frequency and saw blade contact load. A three by three experimental condition (EC) matrix was made, as shown in Table 2.2. The values chosen represent a range of loads and frequencies used in practice, as found during a pilot test with forensic practitioners. A total of 90 cuts were made, with n=10 for each EC.

The load exerted by the saw on the bone was set by adding dumbbell weights of 1 or 2kg (actually 1.003 and 2.004kg respectively) to the saw platform, which together with the saw and its clamping block weighed 3kg. This resulted in three contact loads that were tested: 3kg, 4kg, and 5kg.

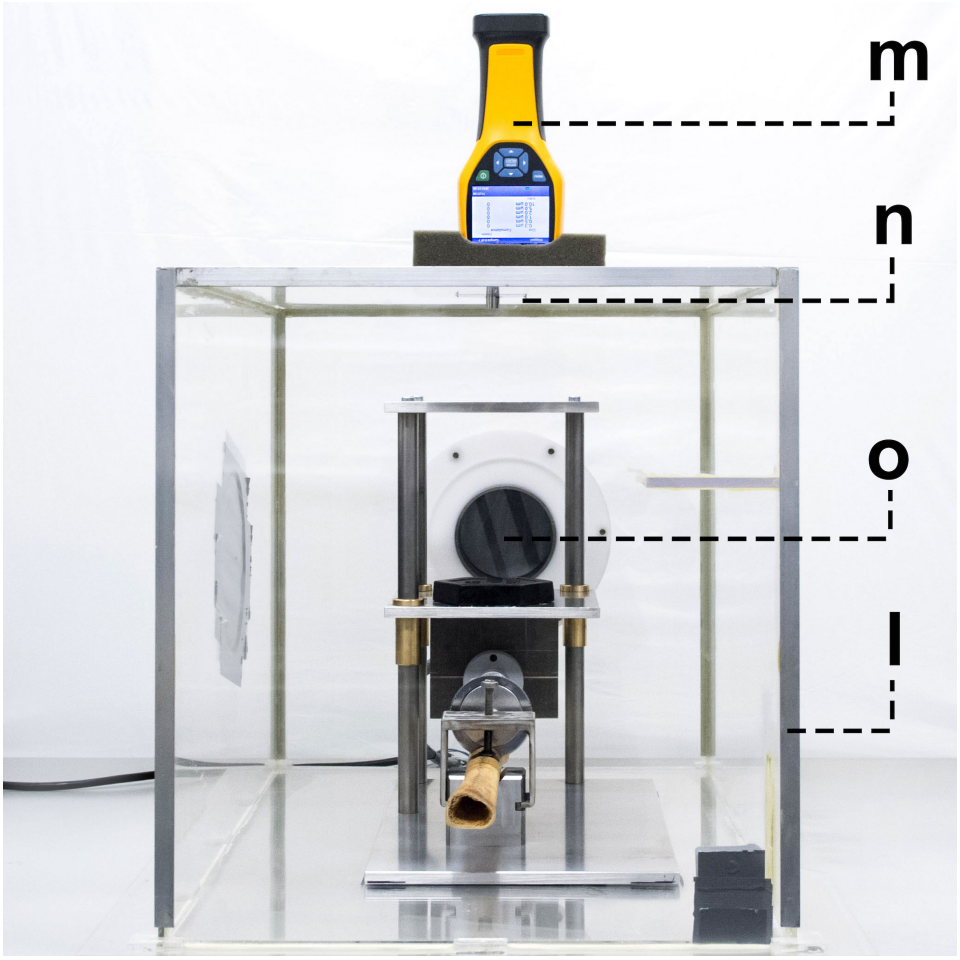



Figure 2.3: Front view of the setup enclosed in the box; an acrylic glass box (l) was used to create an experimental space isolated from the environment. The Fluke 985 particle counter (m) was placed on top of the box with a foam cast, with the nozzle inserted into the box through a hole on top of the box (n). A closable hole with a socketed cap was used for handling the saw during operations inside the box (o).

The saw blade frequency was set using an external control panel. By turning a potentiometer any frequency between 30Hz or 250Hz could be chosen. The saw blade frequencies chosen were 150Hz, 200Hz, and 250Hz.

Other variables that were observed and noted down; temperature, humidity, residual and foreign aerosols, bone weight before and after cutting, and the saw blade frequency during cutting. Any potential effects of bone properties such as mechanical properties, surface topography, marrow cavity, cortex thickness and density, and saw blade wear and any unknown changes over time were considered to be averaged out by creating a randomised block experiment.

Five blocks of 9 cuts, with to each randomly assigned one of the 9 experimental conditions, were made on each of the two selected human femora. Pen markings were made on the bones prior to sawing to provide visual guidance during the tests (see Fig. 2.4). Each cut was spaced 5mm from its neighbouring cuts. This distance was safe enough to avoid flaking, bending or cracking of the bone cortex during sawing. Blocks were spaced 10mm so they could be easily

Table 2.2: The Experimental Condition (EC) matrix. Each number represents an EC, i.e. a combination of saw blade frequency and saw blade contact load. The notation corresponds with the sample notation made on the bone shaft, as seen in Fig. 2.4.

| | **150 Hz** | **200 Hz** | **250 Hz** |
|---|---|---|---|
| **3 kg** | EC 1.1 | EC 1.2 | EC 1.3 |
| **4 kg** | EC 2.1 | EC 2.2 | EC 2.3 |
| **5 kg** | EC 3.1 | EC 3.2 | EC 3.3 |

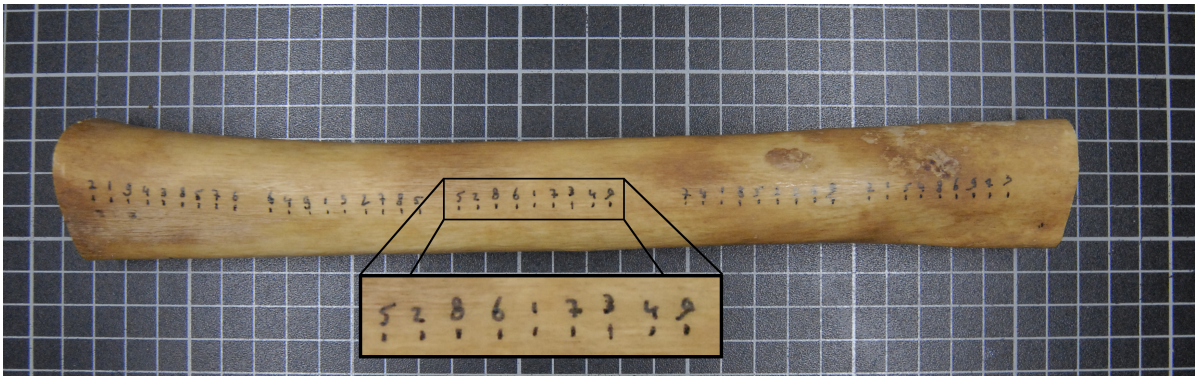


Figure 2.4: Top view of one of the femora that was used in the experiment. The notations of the randomised experimental condition numbers (EC 1 to EC 9) are shown as used during the experiments, divided in 5 blocks. The EC notation was changed to a matrix notation after the experiments for reasons of visibility: EC 1 is changed to 1.1, EC 9 to 3.3, and corresponds to the EC matrix shown in Table 2.2.

distinguished. The total of 10 blocks provided the 10 repetitions of all ECs and within each block the ECs were randomised.

Each cut was coded using the template [Bone type] [Bone ID] [Block] [EC Cut number] to easily identify and record the test runs. The two femora were given the IDs A or B, marked on the back of the bone, each block was numbered 1 to 5 on the side of the bone, and the experimental conditions were numbered 1.1 to 3.3 on the front side of the bone. As an example, a cut in femur A block 2 with experimental condition number 3 was coded as *FEM-A2.3*.

### 2.2.3. Experimental protocol

***Sawing protocol***

The femur was inserted and fastened so the saw blade lined up with the prepared pen marking on the bone. Following the EC number, the desired frequency was selected with the use of the tachometer and the saw control panel, and weights were added to the sliding platform. The box was closed with the particle counter placed on top. Room temperature, room relative humidity, date and time were recorded. The particle counter protocol was started, as described below. At the start of M1 the saw was lifted, switched on and gently brought down to contact the femur and then left to freely move down, until the stopper hit the surface of the femur. Then the saw was switched off. The time between the starting of the saw, and the moment the stopper hit the bone was recorded. After the particle counter protocol was finished, the cleaning protocol started, as described below.

***Particle counter protocol***

The Fluke 985 was programmed for 7 measurements of 60 seconds each. Each measurement was coded as M0, M1, ... , M6. The base measurement (M0) recorded the base levels of particles already suspended inside the box directly after closing the box. This could include both residual aerosol from previous tests, or foreign aerosol from the room or the cleaning process. Including the sawing process described earlier, measurements M1-M6 recorded the suspension and settling down of particles from sawing. After each test run, the particle counter was purged using the manufacturer's filter to guarantee that residual particles in the particle counter were not counted again.

***Cleaning protocol***

Once measurement M6 was finished, the inside of the box was vacuumed for 1 minute via the side opening of the box to avoid scattering of unwanted particles to the outside environment. Next, the box was lifted, the bone specimen was removed from the clamp, vacuum cleaned to remove residual dust and weighted. Both the box and the setup were vacuum cleaned and wiped off using fresh multi-purpose disinfectant wipes, so all residual particles were removed. Both the box and setup were dried with kitchen paper and further left to air-dry for 2 minutes, after which the sawing protocol for the next run could start. Pilot testing showed less suspended fine dust particles in the box after cleaning, than present in the environment outside the box.

### 2.2.4. Data analysis

Statistical analyses were performed in three parts using MATLAB (MATLAB 2014a, The MathWorks Inc.). First for one of each of the produced individual particle sizes (0.3, 0.5, 1.0, 2.0, 5.0, and 10µm), then for the total number of produced aerosol particles, and finally for the total surface area of the produced aerosol particles. In all analyses the production of aerosol was summed over the 6 minutes of measurements (M1 to M6) from the moment the saw was commenced. The base level (M0) was subtracted to separate the background aerosol from the aerosols

Table 2.3: The means and standard deviations over 10 repetitions for each particle size and all experimental conditions.

| | 0.3 um | | 0.5 um | | 1.0 um | | 2.0 um | | 5.0 um | | 10 um | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| **EC 1.1** | 3.47 x$10^6$ | 0.283 x$10^6$ | 3.18 x$10^6$ | 0.395 x$10^6$ | 2.56 x$10^6$ | 0.437 x$10^6$ | 1.99 x$10^6$ | 0.420 x$10^6$ | 0.775 x$10^6$ | 0.235 x$10^6$ | 0.322 x$10^6$ | 0.111 x$10^6$ |
| **EC 1.2** | 3.92 x$10^6$ | 0.182 x$10^6$ | 3.68 x$10^6$ | 0.240 x$10^6$ | 3.03 x$10^6$ | 0.272 x$10^6$ | 2.38 x$10^6$ | 0.268 x$10^6$ | 0.937 x$10^6$ | 0.149 x$10^6$ | 0.379 x$10^6$ | 0.0718 x$10^6$ |
| **EC 1.3** | 4.33 x$10^6$ | 0.153 x$10^6$ | 4.07 x$10^6$ | 0.172 x$10^6$ | 3.34 x$10^6$ | 0.244 x$10^6$ | 2.62 x$10^6$ | 0.292 x$10^6$ | 0.980 x$10^6$ | 0.223 x$10^6$ | 0.376 x$10^6$ | 0.116 x$10^6$ |
| **EC 2.1** | 3.32 x$10^6$ | 0.328 x$10^6$ | 2.98 x$10^6$ | 0.430 x$10^6$ | 2.35 x$10^6$ | 0.448 x$10^6$ | 1.80 x$10^6$ | 0.415 x$10^6$ | 0.691 x$10^6$ | 0.227 x$10^6$ | 0.281 x$10^6$ | 0.110 x$10^6$ |
| **EC 2.2** | 3.59 x$10^6$ | 0.237 x$10^6$ | 3.26 x$10^6$ | 0.292 x$10^6$ | 2.59 x$10^6$ | 0.300 x$10^6$ | 1.97 x$10^6$ | 0.277 x$10^6$ | 0.720 x$10^6$ | 0.147 x$10^6$ | 0.279 x$10^6$ | 0.0705 x$10^6$ |
| **EC 2.3** | 4.01 x$10^6$ | 0.225 x$10^6$ | 3.73 x$10^6$ | 0.200 x$10^6$ | 3.01 x$10^6$ | 0.159 x$10^6$ | 2.32 x$10^6$ | 0.148 x$10^6$ | 0.858 x$10^6$ | 0.117 x$10^6$ | 0.334 x$10^6$ | 0.0686 x$10^6$ |
| **EC 3.1** | 2.92 x$10^6$ | 0.193 x$10^6$ | 2.55 x$10^6$ | 0.295 x$10^6$ | 1.98 x$10^6$ | 0.315 x$10^6$ | 1.49 x$10^6$ | 0.291 x$10^6$ | 0.553 x$10^6$ | 0.146 x$10^6$ | 0.222 x$10^6$ | 0.0622 x$10^6$ |
| **EC 3.2** | 3.19 x$10^6$ | 0.192 x$10^6$ | 2.81 x$10^6$ | 0.269 x$10^6$ | 2.15 x$10^6$ | 0.289 x$10^6$ | 1.60 x$10^6$ | 0.272 x$10^6$ | 0.564 x$10^6$ | 0.150 x$10^6$ | 0.215 x$10^6$ | 0.0762 x$10^6$ |
| **EC 3.3** | 3.51 x$10^6$ | 0.161 x$10^6$ | 3.11 x$10^6$ | 0.255 x$10^6$ | 2.39 x$10^6$ | 0.306 x$10^6$ | 1.77 x$10^6$ | 0.308 x$10^6$ | 0.618 x$10^6$ | 0.184 x$10^6$ | 0.238 x$10^6$ | 0.0906 x$10^6$ |

that were actually generated by sawing. The effects of saw blade frequency and saw blade contact load were analysed using a two-way ANOVA to evaluate their effects on the production of aerosol. Effects were considered significant when p<0.05.

By summation of the number of particles produced for each individual particle size, the total number of produced particles per EC was calculated. By summation of the number of particles produced for each particle size, multiplied by the square of the particle's size times pi, the total surface area of the produced particles per EC were calculated. This calculation assumes that the particles are perfect spheres, for any other shapes the total surface would be bigger, but the ratio between the ECs would stay the same.

# 2.3. Results

## 2.3.1. Individual particle sizes

Stacked bar graphs for each of the 6 particle sizes are shown in Fig. 2.5. For all cuts the number of smaller particles outranked the number of bigger particles. The mean numbers and standard deviations of the individual sizes of aerosol particles produced per each experimental condition are shown in Table 2.3.

A clear trend was visible in the results of all the individual particle sizes, except for the 10µm particles. The highest number of aerosol particles was consistently produced in EC 1.3, with the highest tested frequency (250Hz) and the lowest tested contact load (3kg). The lowest number of aerosol particles was consistently produced in EC 3.1, with the lowest tested frequency (150Hz) and the highest tested contact load (5kg). This only deviated for particle size 10µm, where the highest number of particles was found at EC 1.2 (200Hz, 3kg), and the lowest number of particles at EC 3.2 (250Hz, 5kg).

The two-way ANOVA showed significant effects of frequency and of contact load on the number of aerosols particles for particle sizes 0.3, 0.5, 1.0 and 2.0µm (p<0.001). For particle size 5.0µm the effects were also significant for frequency (p=0.0096) and contact load (p<0.001). For particle size 10.0µm only the effect of contact load was statistically significant (p<0.001), the effect of frequency was not (p=0.21). The interaction effect was in all cases not statistically significant. Table 2.4 shows an overview of all p-values for the effects of frequency and contact load as well as the interaction effect, for all particle sizes.

## 2.3.2. Total produced particles

By summation of the number of particles produced in all particle sizes, the total number of produced particles per EC was calculated, as shown in Fig. 2.6a. By multiplying the square of each particle size with pi and the number of particles counted for that size, and summing these products for all particle sizes, the total surface area of the produced particles per EC was calculated, shown in Fig. 2.6b. The mean numbers, standard deviations and the maximum and minimum value of the total number of aerosol particles produced per each experimental condition are shown in Table 2.5, the mean numbers, standard deviations and the maximum and minimum value of the total surface area of particles produced are shown in Table 2.6.

Stacked bar graphs of the total number of particles produced, and total surface area of the particles

Table 2.4: p-values of the effects of saw blade frequency and saw blade contact load on the number of aerosol particles produced. Significant values are marked in italics.

| | Particle size | | | | | | Total number of particles | Total surface area of particles |
|---|---|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| Effect of saw blade frequency | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p=0.0096* | p=0.21 | *p<0.001* | *p=0.027* |
| Effect of saw blade contact load | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Interaction effect | p=0.37 | p=0.40 | p=0.36 | p=0.37 | p=0.56 | p=0.70 | p=0.38 | p=0.63 |

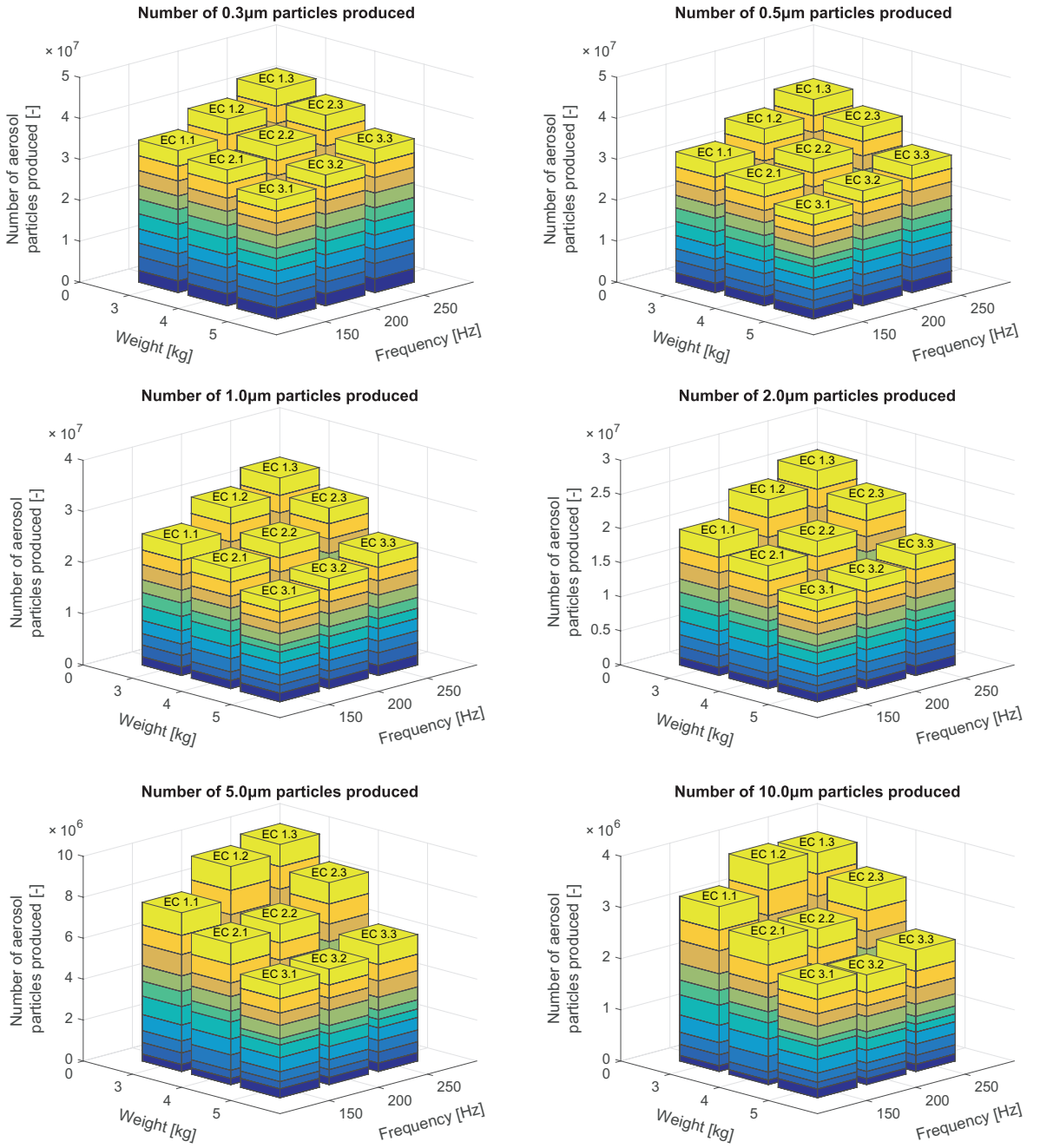
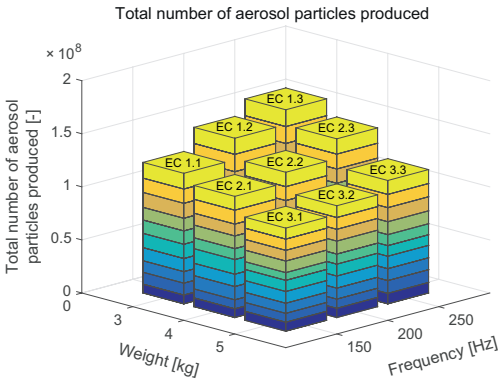



Figure 2.5: Stacked bar graphs of the number of aerosol particles produced per experimental condition (marked columns) during the total of n=10 measurements (coloured layers) for each particle size (0.3µm top left to 10µm bottom right). Each layer corresponds with one block of ECs, the bottom layers were from Block A.1, the top layers Block B.5. Note that for reasons of visibility, the vertical axes are scaled differently for each particle size.

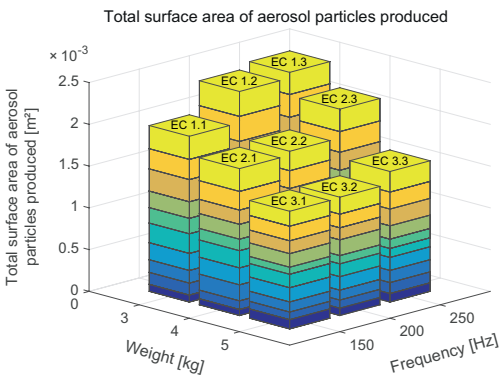
produced show similar trends as the results for individual particle sizes 0.3 to 5.0 µm, the highest number of particles is found at EC 1.3 (250Hz, 3kg), the lowest at EC 3.1 (150Hz, 5kg).

The results of the total number of particles showed significant effects of frequency and contact load ($p<0.001$). Similarly, the results of the total surface area of the particles produced showed significant effects for frequency ($p=0.027$) and contact load ($p<0.001$). The interaction effect was in all cases not statistically significant. Table 2.4 shows all p-values for the effects of saw blade frequency and saw blade contact load as well as the interaction effect, for the total number of particles produced, and the total surface area of the particles produced.

Figure 2.6




(a) Stacked bar graph of the total number of aerosol particles produced per experimental condition (marked columns) during the total of n=10 measurements (coloured layers). Each layer corresponds with one block of ECs, the bottom layers were from Block A.1, the top layers Block B.5.




(b) Stacked bar graph of the total surface area of the aerosol particles produced per experimental condition (marked columns) during the total of n=10 measurements (coloured layers). Each layer corresponds with one block of ECs, the bottom layers were from Block A.1, the top layers Block B.5.

Table 2.5: Means, standard deviations, maximum and minimum numbers of total number of particles for each experimental condition over 10 repetitions.

| | **Total number of particles [n]** | | | |
|---|---|---|---|---|
| | **Mean** | **SD** | **Max Value** | **Min Value** |
| **EC 1.1** | 12.3 x10$^6$ | 1.86 x10$^6$ | 14.8 x10$^6$ | 8.38 x10$^6$ |
| **EC 1.2** | 14.3 x10$^6$ | 1.14 x10$^6$ | 16.4 x10$^6$ | 12.5 x10$^6$ |
| **EC 1.3** | 15.7 x10$^6$ | 1.04 x10$^6$ | 17.3 x10$^6$ | 14.1 x10$^6$ |
| **EC 2.1** | 11.4 x10$^6$ | 1.90 x10$^6$ | 13.8 x10$^6$ | 8.28 x10$^6$ |
| **EC 2.2** | 12.4 x10$^6$ | 1.26 x10$^6$ | 13.9 x10$^6$ | 9.59 x10$^6$ |
| **EC 2.3** | 14.3 x10$^6$ | 0.667 x10$^6$ | 15.2 x10$^6$ | 12.9 x10$^6$ |
| **EC 3.1** | 9.72 x10$^6$ | 1.27 x10$^6$ | 11.0 x10$^6$ | 7.27 x10$^6$ |
| **EC 3.2** | 10.5 x10$^6$ | 1.20 x10$^6$ | 12.2 x10$^6$ | 8.66 x10$^6$ |
| **EC 3.3** | 11.6 x10$^6$ | 1.22 x10$^6$ | 13.3 x10$^6$ | 9.94 x10$^6$ |

Table 2.6: Means, standard deviations, maximum and minimum numbers of total surface area [m$^2$] of the particles for each experimental condition over 10 repetitions.

| | **Total surface area of particles [m$^2$]** | | | |
|---|---|---|---|---|
| | **Mean** | **SD** | **Max Value** | **Min Value** |
| **EC 1.1** | 199 x10$^{-6}$ | 60.1 x10$^{-6}$ | 277 x10$^{-6}$ | 84.2 x10$^{-6}$ |
| **EC 1.2** | 236 x10$^{-6}$ | 38.3 x10$^{-6}$ | 300 x10$^{-6}$ | 187 x10$^{-6}$ |
| **EC 1.3** | 243 x10$^{-6}$ | 58.3 x10$^{-6}$ | 322 x10$^{-6}$ | 150 x10$^{-6}$ |
| **EC 2.1** | 176 x10$^{-6}$ | 58.7 x10$^{-6}$ | 273 x10$^{-6}$ | 72.6 x10$^{-6}$ |
| **EC 2.2** | 181 x10$^{-6}$ | 37.7 x10$^{-6}$ | 233 x10$^{-6}$ | 116 x10$^{-6}$ |
| **EC 2.3** | 215 x10$^{-6}$ | 31.8 x10$^{-6}$ | 268 x10$^{-6}$ | 158 x10$^{-6}$ |
| **EC 3.1** | 141 x10$^{-6}$ | 35.6 x10$^{-6}$ | 179 x10$^{-6}$ | 84.6 x10$^{-6}$ |
| **EC 3.2** | 142 x10$^{-6}$ | 39.7 x10$^{-6}$ | 204 x10$^{-6}$ | 88.0 x10$^{-6}$ |
| **EC 3.3** | 156 x10$^{-6}$ | 47.6 x10$^{-6}$ | 247 x10$^{-6}$ | 103 x10$^{-6}$ |

### 2.3.3. Tachometer readings

The measured saw blade frequency throughout the cutting of the bone showed a drop, as seen in Fig. 2.7. The resistance on the saw blade when in contact with the bone surface seemed to hamper with the torque of the oscillating saw's engine. At higher frequencies the drops seemed bigger than with lower frequencies, whereas higher contact loads used in a given frequency significantly increased the drop in frequency (two-way ANOVA, $p<0.001$).

### 2.3.4. Sawing time

A significant effect for both saw blade contact load and saw blade frequency was found ($p<0.001$): as the saw blade frequency or saw blade contact load decreased, the sawing time increased (see Fig. 2.8. For reasons of visibility, in this plot the ECs are arranged in a different order than in the rest of the figures).

## 2.4. Discussion

Oscillating saws are routinely used during autopsy procedures, leading to production of considerable amounts of bone dust, and putting forensic practitioners and others involved at risk of being contaminated by pathogen-carrying aerosols. As previous studies have already demonstrated, there can be an alarmingly high production of bone aerosol when using oscillating saws [1, 6, 8, 17]. In the current study, the effects of saw blade frequency and saw blade contact load on aerosol production were demonstrated. The results suggest that, for all particle sizes, contact loads and sawing frequencies tested, increasing the contact load exerted by the saw blade on the bone has an inverse effect on the number of aerosol particles produced. The same effect was seen when considering the total number of aerosol particles, as well as the total surface area of a particles produced. Analysing the effect of frequency on particle production, it was demonstrated that higher frequencies re-

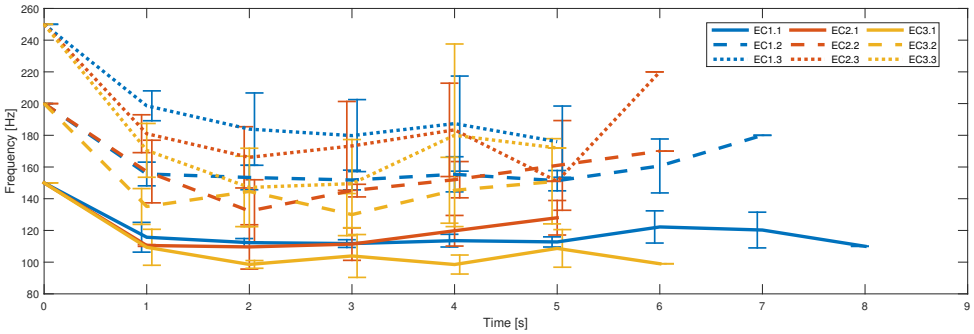



Figure 2.7: Saw blade frequency during sawing measured by the tachometer displayed over time, averaged over 10 EC repetitions. The error bars show the standard deviations.

sult in a higher number of aerosol particles produced for the smaller particle sizes, except for particles of size 10.0µm. The deviating results for 10.0µm aerosol particles could be explained as these bigger particles are more susceptible to disturbances, such as air resistance. Therefore these bigger particles might have taken more time than smaller particles to evenly diffuse throughout the box and reach the particle counter.

Among the selected frequencies and contact loads in the experimental condition matrix, EC 3.1 showed to be the most promising for minimising the number of aerosol particles produced. The selection of lower frequencies in conjunction with high contact loads showed to be the optimal setting. There was however a limit to those parameters, as it was necessary to observe the saw's torque threshold: frequencies lower than 150Hz or contact loads higher than 5kg could lead to engine halts or failure to cut through the bone. Similarly, the cutting time was affected by the used saw blade contact loads and saw blade frequency, which together with a possible change in quality of cut could be a reason why forensic practitioners use these sawing parameters.

Despite having produced the lowest number of aerosol particles, EC 3.1 still generated a significant number of suspended particles, averaging a total number of $9.715 \times 10^6$ particles over 16.98 litres of sampled air. Considering that in resting conditions humans breathe about 6 litres of air per minute [18], this would result in the inhalation of $3.43 \times 10^6$ particles. Together with Jones and Brosseau [4] observations on deposition of aerosol in the human respiratory track, different amounts of particles will be deposited in different parts of the respiratory tract, all potentially providing health risks. Depending on a pathogen's survivability and the time period during which a person is in contact with the bone dust, it is plausible to assume that a pathogen could cause an infection on its host, as seen by Yezli and Otter [15] and Wenner et al. [1] in studies on Minimal Infection Dose.

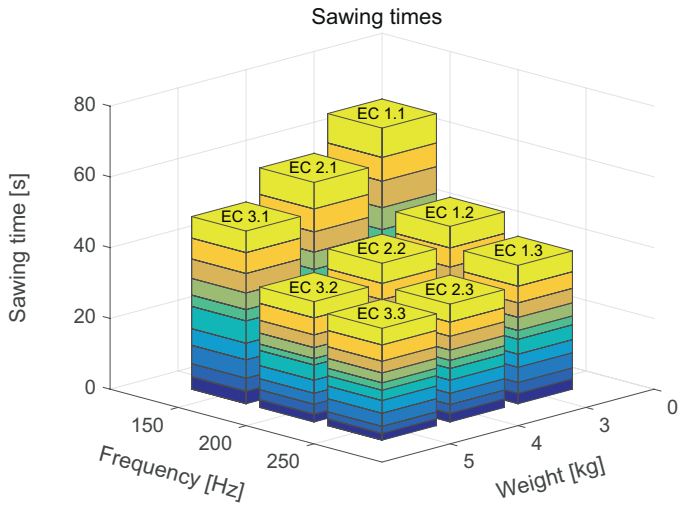



Figure 2.8: Stacked bar graph of the cumulative sawing time over 10 repetitions (coloured layers) per experimental condition (marked columns). Each layer corresponds with one block of ECs, the bottom layers were from Block A.1, the top layers Block B.5. Note that for reasons of visibility, in this plot the ECs are arranged in a different order than in the rest of the figures.

The total number of aerosol particles produced is dominated by the production of the 0.3µm particles, as they were roughly 10 times more prevalent than the 10.0µm particles. The stacked bar graphs of the 0.3µm particles and of the total number of particles show similar trends between ECs. Reversely, the total surface area of the produced particles was dominated by the production of 10.0µm particles, as their surface area is roughly 1000 times bigger than that of the 0.3µm particles. The stacked bar graphs of the 10.0µm particles and of the total surface area of particles show similar trends between ECs.

The total number of particles produced is of interest as it indicates a number of potential pathways for pathogens to the human body. Similarly, the total surface area gives an indication of the possible amount of pathogens that could be attached to one particle. As different pathogens have different sizes and MIDs, as shown in Table 2.1, it could well be that some of the counted particles are the pathogen themselves, or one particle can carry enough pathogens to acquire the MID. Lastly, even non-pathogen-carrying particles might pose health risks through mechanisms similar to the risk of inhaling asbestos particles.

The effects of sawing in dry bone could differ from fresh bone due to increased presence of organic matter in fresh bone. This organic matter is mainly composed of collagen, granting greater bone elasticity, whereas diminishing organic content in dry bone changes the elastic properties from viscoelastic to brittle [19, 20].

Even though the current experiment was performed in a closed and significantly smaller environment than as in common practice, – a room with a ventilation system and constant turbulence disturbances by human movement–, the resulting production values still clearly show the potential risk of aerosols produced during autopsy. Adding that the particles can remain airborne for periods longer than 15 min [8, 21], and as the particle gets smaller the more likely it will remain suspended [1, 17], there is strong reason to further test the potential negative health effects of aerosol bone dust particles.

The results obtained from the current study stress the importance of biosafety guidelines. Despite finding significant effects of saw blade frequency and contact load on the aerosolisation of dry bone, which suggests that aerosol production could be reduced, the optimal experimental condition (EC 3.1) still resulted in a number of particles that is considered a risk to anyone potentially inhaling the bone dust. Combining these findings with recommendations from other studies could further reduce the intake of bone dust when using oscillating saws: i.e adaptations with protective casing around the saw blade [6] or moistening the saw blade to reduce spread of particles [1, 22]. Similarly, the use of protective gear, such as specialised, well-fitted respirators and filtering face pieces [16, 23], as well as guideline inspections to ensure proper infrastructure of autopsy facilities could greatly reduce the number of aerosol particles reaching the respiratory tracts of forensic practitioners.

Unfortunately, many pathology institutes suffer from precarious conditions and governmental negligence [3, 5, 24]. However the results provided by the current study could help minimise the occupational risk in autopsy practice, as from the results some clear suggestions can be distilled: decrease aerosol production by reducing the saw blade frequency and by increasing the contact load on the bone subject, or more radically, but probably not ideal; switch to hand sawing. Similarly, workers in environments without means to acquire ventilation systems, or where the reduction the spreading of dust is hard to achieve, e.g. in field work, emergency response work, or practices in less developed or poorer countries, could improve their bone sawing protocols with the results of the current study. Future studies should investigate realistic scenarios faced by forensic practitioners, such as aerosolisation of fresh bone instead of dry archaeological bone. Furthermore, testing different bone types (e.g. long, short, flat, irregular or sesamoid bones), and testing additional sawing parameters (such as the morphology of the saw blade), and their effects on aerosol production could be investigated. Lastly, following the steps of Pereira et al. [17], future studies should study influences of ventilation systems and air flows in autopsy rooms.

## 2.5. Conclusion

Overall, increasing the saw blade frequency or decreasing the saw blade contact load resulted in a higher production of aerosol bone dust. Future studies are needed to determine the influence of other sawing parameters, other sawing materials, and other practice environments. For now, the results suggest that in order to limit bone aerosol production when using oscillating saws, one should try to keep the saw blade frequency as low and saw blade contact force as high as possible within the limits of safety and practicality.

## 2.6. Acknowledgements

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# 3

# Aerosol production during autopsies: Daily practice

After the publication of the results of the pilot tests as seen in Chapter 2, it was decided to expand the experimental design of the pilot tests as a continuation of the MSc graduation project. The goal was to study the effects of saw blade frequency and saw blade contact load under a closer representation of the sawing procedure in daily practice, by looking into the effects of the bone condition, sawing environment and saw blade type. Detailed background information on the production of the experimental setup and the validation of the experimental protocol are shown in Appendix A and Appendix B respectively. The current Chapter shows the resulting paper that is submitted to Forensic Science International, the same journal as Chapter 2, as of January 2019.

J.M.E. Pluim, A.J. Loeve, R.R.R. Gerretsen
"Minimising aerosol bone dust during autopsies",
Submitted to Forensic Science International as of January 2019

Abstract: When sawing in bone for medical or medico-legal procedures, fine, airborne dust is produced (aerosols) that can pose a health hazard when inhaled by practitioners. The goal of the current study was to find the influence of saw blade frequency and saw blade contact load, the degree of skeletonisation of the bone, the test environment with external air flows such as ventilation systems, and the type of saw blade used on the production of aerosol particles. A custom test setup was designed and manufactured to test the sawing parameters in 8 experiments, with 2 to 9 experimental conditions tested in each, where a particle counter was used to determine the production of aerosol particles while varying the 5 chosen parameters. Results showed that the number of counted particles was highest with higher saw blade frequencies, lower saw blade contact loads, in dry completely skeletonised bone compared to fresh bone, and using an electrical oscillating saw compared to hand-sawing. Under all conditions, the high amount of aerosol counted posed potential health risks. The tested external ventilation system was adequate in removing the produced number of particles, but these high-tech systems are not always available in developing countries or emergency situations. In conclusion, the production of aerosols can be reduced by optimising the sawing parameters. However, even the lowest number of aerosol particles counted during the current study was high enough to cause potential health risks to practitioners. Safety precautions should be taken, such as external ventilation, proper breathing gear, and adequate protocols, to truly minimise the risk in all bone sawing scenarios.
**Keywords: aerosol, bone dust, sawing parameters, autopsy, pathology, biosafety**

Table 3.1: Overview of the variables tested in eight performed experiments. The saw blade frequencies and saw blade contact loads used within the eight experiments are shown in Table 3.2

| | | Tested variables, experiment numbers and their code names, nr of ECs and nr of reps | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tested variable: | Bone condition | | | | | Test environment | | | Saw blade type | | | | |
| | Experiment number: | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 6 | Exp. 7 | Exp. 1 | Exp. 4 | Exp. 5 | Exp. 1 | Exp. 6 | Exp. 2 | Exp. 7 | Exp. 8 |
| | Experiment code: | 'DCE' | 'GCE' | 'FCE' | 'DCB' | 'GCB' | 'DCE' | 'DOE' | 'DAE' | 'DCE' | 'DCB' | 'GCE' | 'GCB' | 'GCM' |
| | Nr of ECs: | 9EC | 4EC | 4EC | 2EC | 2EC | 9EC | 4EC | 4EC | 9EC | 2EC | 2EC | 2EC | 2EC |
| | Nr of reps: | 10reps | 5reps | 10reps | 5reps | 5reps | 10reps | 5reps | 5reps | 10reps | 5reps | 5reps | 5reps | 5reps |
| Bone condition | Dry bone cat D.4[1] | **D** | | | **D** | | D | D | D | D | D | | | |
| | Greasy bone cat D.3[1] | | **G** | | | **G** | | | | | | G | G | G |
| | Fresh bone cat A.1[1] | | | **F** | | | | | | | | | | |
| Test environment | Closed environment | C | C | C | C | C | **C** | | | C | C | C | C | C |
| | Open environment | | | | | | | **O** | | | | | | |
| | Active ventilation | | | | | | | | **A** | | | | | |
| Saw blade type | Electric oscillating saw | E | E | E | | | E | E | E | **E** | | **E** | | |
| | Satterlee bone-saw | | | | B | B | | | | | **B** | | **B** | |
| | Metal-saw | | | | | | | | | | | | | **M** |

The experiment codes (Exp. 'CODE') provide a concise reference to the variables tested in each experiment when referred to later in text. For example, in Exp. 1 'DCE' a *Dry bone* was used, in a *Closed environment* using an *Electric oscillating saw*.
The grouped columns show which experiments are compared to find the influence of bone condition, test environment, and saw blade type, with the independent variable shown in **bold**.

## 3.1. Introduction

When operating on the human body, e.g. during (orthopaedic) surgeries or (forensic) autopsies, (electro-) mechanical tools are often used to aid in gaining access to the body, or during the task itself, such as scalpels, lasers, scissors, saws, drills or electrocautery tools. Although the most common health hazards during these procedures are well known, e.g. cutting by sharps or needle puncture incidents, the inhalation of surgical smoke or aerosols (solid or liquid airborne particles) produced during the use of mechanical tools is often overlooked and can lead to e.g. respiratory irritations, transmission of infections, and genotoxicity [2–7]. Safety awareness concerning the aerosolisation of particles exists for known high risk airborne transmissible pathogens such as Tuberculosis (TB) [8–10] or Severe Acute Respiratory Syndrome (SARS) [11, 12]. However, the health risks associated with the aerosolisation of pathogens in the skin, blood or other bodily material remain uncertain. These aerosolised pathogens could include Hepatitis B and Hepatitis C [13, 14], Streptococci [15, 16], and Human Immunodeficiency Virus (HIV) [14, 17], of which airborne transmissions are rare but have been reported, or proven plausible during surgery or autopsy sessions [18–22]. Although the pathogen-carrying ability of aerosols produced during autopsies is considered the highest health risk of aerosolised material, also the non-pathogen-carrying aerosols can pose a hazard when inhaled and deposited in the airways, for instance due to an increased risk of cardiovascular mortality [23, 24]. Similar to many more commonly found aerosols, be it industry smog, car exhaust gas, cigarette smoke or urban pollution, the inhalation of surgical smoke is unwanted and should be minimised [25].

The current study focuses on aerosol bone dust particles produced when sawing in bone during forensic autopsies, although parallels can be drawn to clinical or veterinary (orthopaedic) surgeries and autopsies. Aerosols produced by sawing have been shown to be dispersed wide in the surroundings of the operation site, possibly reaching the respiratory tract of the operator [8, 26–32]. Particles smaller than 10µm are within the respirable range and have the potential to remain suspended in the air for hours after the sawing action, increasing the time during which the air around the working area is contaminated with possibly infective aerosol [33]. How deep the particles can reach in the respiratory tract when inhaled is determined by the particle's size [3, 34]. Studies have shown that fine particles (0.1 µm) deposit in the head airways (2.1%), the tracheobronchial region (2.7%) or alveoli (14%), whereas for coarse particles (10 µm) this would be 81%, 1.5% and 1.9%, respectively [34].

The goal of this study was to investigate the effects of several sawing parameters that are relevant in daily practice during forensic autopsies on the production of aerosols, in order to inherently minimise the health risk faced by forensic practitioners.

## 3.2. Materials and Methods

### 3.2.1. Hypotheses

A pilot study was performed by Pluim et al. (2018) [35] on the influence of saw blade frequency and saw blade contact load on the production of aerosol in dry bone. The current study improves upon the premises

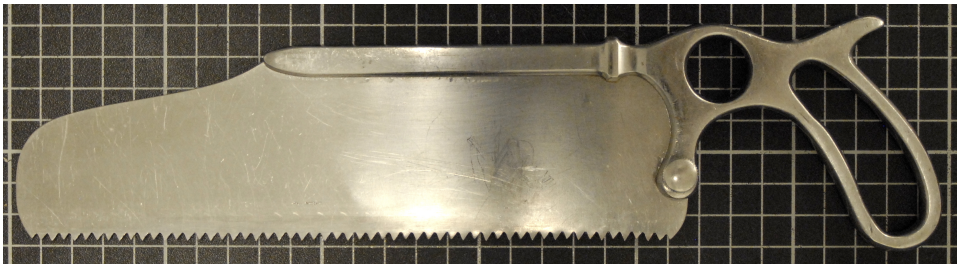

Figure 3.1: Satterlee type bone saw used in Exp. 6 'DCB' and Exp. 7 'GCB', with a 200x60x0.8mm, 9 teeth per inch saw blade (FH325R, Aesculap AG, Germany).

and methodology of Pluim et al. (2018) [35] by looking deeper into potentially relevant sawing parameters. Three parameters were chosen that closely represent the variety in sawing parameters faced in daily practice, and were varied in eight different experiments. In each of these experiments the influences of saw blade frequency and saw blade contact load were studied. Three parameters were chosen:

***Influence of the bone condition***

Sawing is routinely performed on bones of various conditions, from fresh bone *in vivo* or *ex vivo*, to completely skeletonised dry archaeological bone [1], differing greatly in composition and mechanical properties. This is done e.g. to access the skull, or for sample harvesting for anthropological examinations such as identification purposes. Fresh, *in vivo* bone is composed of minerals such as hydroxyapatite (60-70%), organic matter such as type I collagen (10-30%), and water (10-20%) [36], holds bone marrow, blood and other bodily fluids, and is surrounded by soft tissues. Whereas in decomposed, dry bone only the mineral structure remains. Fracture tests have shown that fresh bone shows visco-eleastic bending, whereas dry bone fails in a brittle manner: dry bone has a much higher Young's Modulus (stiffness), but its impact energy is reduced to a far greater extent than the increase in stiffness [37, 38]. It was expected that bone particles will break off more easily in dry bone, whereas the elasticity of fresh bone will limit the breaking off of particles. It was hypothesised therefore that the dryer the bone is, the higher the number of counted dust particles would be.

***Influence of the test environment***

Since forensic practitioners are generally aware of potential hazards, protective clothing and masks are generally prescribed, ventilation systems are used, and protocols are followed to minimise contact with pathogens [19–21, 39–41]. However, in practice not all safety precautions are or can be taken consistently. There is discussion on the efficacy of surgical masks against the inhalation of aerosols as these masks are usually designed to prevent the transmission of course particles [42–46]. Ideally, an autopsy room equipped with validated safety precautions such as ventilation systems is used for these procedures. However, adequate ventilation systems may not always be available, which may be the case in developing countries without high-tech infrastructure, or after natural or anthropogenic disasters where emergency makeshift mortuaries are often used. It was hypothesised that the number of aerosol dust particles measured in a closed and controlled environ-

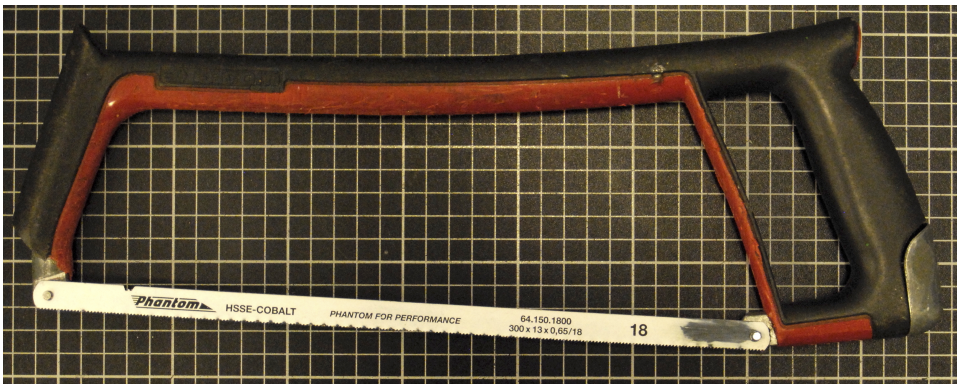


Figure 3.2: Hack saw used in Exp. 9 'GCM', with a 300x13x0.65mm, 18 teeth per inch, metal-saw blade (Phantom, Van Ommen B.V., The Netherlands).

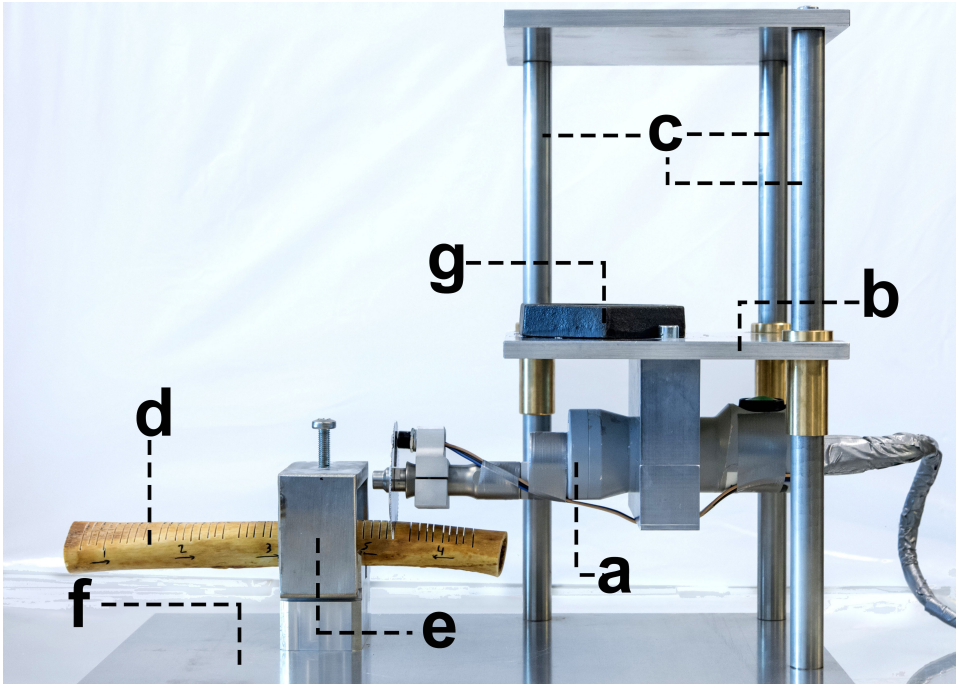


Figure 3.3: Experimental setup used to cut the bone, the setup consisted of: an oscillating saw (a) fastened to a vertical sliding platform (b) guided by 3 stainless steel rods and brass sliding bearings (c). The bone specimen (d) was clamped in a v-groove holder (e), that was connected to an aluminium base plate (f). Interchangeable weights could be attached to the platform (g). The sawing action is further illustrated in Fig. 3.4.

ment is higher than the number of aerosol dust particles measured in an open environment, and that the tested active ventilation system completely removes the produced aerosol dust particles during sawing, as it was specifically designed for this purpose.

***Influence of the saw blade type***

Electric oscillating saws are routinely used during autopsies by forensic practitioners to make deep incisions through bone or cartilage tissues. Oscillating saws provide better usability and accessibility compared to hand or band saws. However, electric saws may be impractical, for example in developing countries or after natural or anthropogenic disasters. Additionally, in high risk autopsies on patients with known diseases, such as TB or Creutzfeldt-Jakob disease (CJD) [47], or delicate tasks, such as opening the skull cap the hand-saw is preferred. Often-used hand-saws include a Satterlee type rough toothed bone-saw such as shown in Fig. 3.1, and a hack saw with a fine toothed metal-saw blade such as shown in Fig. 3.2. Saw characteristics are found in literature mostly in the context of orthopaedic surgeries, where sawing and drilling are focused on minimising the damage done to the bone during these procedures [36]. Additionally, saw blade kerf marks in bone are studied forensically, for instance to identify which (class of) saw was used in a dismemberment case. This can include characteristics such as hand vs mechanically powered, but also saw tooth characteristics such as size, shape and set of the saw teeth [48–56]. The amount of aerosol that is produced by sawing is believed to be influenced by similar parameters, although only one study was found that compared the production of aerosol particles by different saw characteristics [28]. It was hypothesised that more respirable dust was counted after sawing with smaller saw blade teeth.

### 3.2.2. Experimental design

Table 3.1 gives an overview of the eight performed experiments and the sawing parameters that were tested. The data from the pilot tests from Pluim et al. (2018) [35] are included as 'Exp. 1 DCE'. Within each experiment the influence of saw blade frequency and saw blade contact load were tested. By comparing the number of counted aerosol particles between the experiments the influence of the following additional variables was tested:

- Bone condition: between Exp. 1, 2 and 3, and between Exp. 6 and 7
- Test environment: between Exp. 1, 4 and 5
- Saw blade type: between Exp. 1 and 6, and between Exp. 2, 7 and 8.

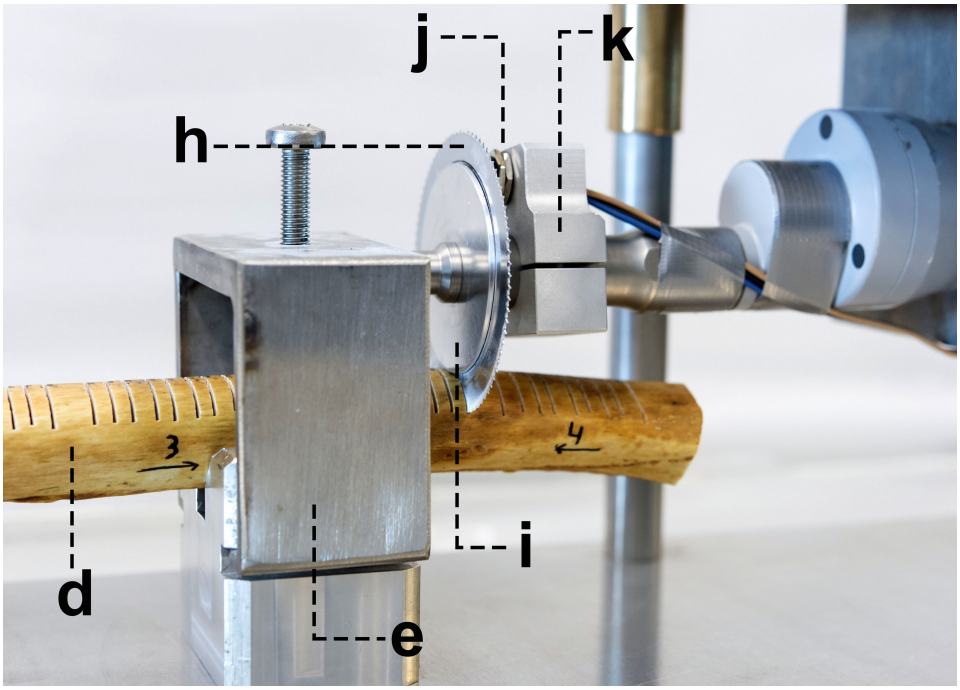


Figure 3.4: Close-up of the saw blade and bone specimen in the setup: the bone specimen (d) was clamped in place by the v-groove holder (e). The saw blade (h) cut into the bone until the stopper (i) reached the bone and provided a consistent depth of cut. The Hall-effect sensor (j) acted as a tachometer, and was clamped to the saw with an aluminium block (k).

Four human femora from an anthropological bone collection of the Netherlands Forensic Institute (The Hague, the Netherlands) were used as sawing specimens. Three femora were in dry condition (Cat. D.4 [1]), one still had a greasy, adipocere residue (Cat. D.3 [1]), all were clean of any soft tissues. The bone marrow cavities of the femora were scraped to remove gross trabecular bone tissue that could shake loose during cutting and affect the particle count. For the fresh bone specimens, a total of ten metacarpal and metatarsal bones were obtained from fresh (Cat. A.1 [1]) porcine specimens, stored in a freezer (-20 °C) between 4-9 days and thawed overnight before usage.

A setup was designed and manufactured for ease-of-use and cleanability, the letters used below to indicate specific parts correspond with those in Figs. 3.3, 3.4, 3.5 and 3.6. An electric oscillating saw (DeSoutter NS3, DeSoutter Medical Limited, UK) (a), fixed to a sliding platform (b), allowed a free vertical sawing motion (Fig. 3.3). The bone specimen (d) was fastened in a v-shaped groove block (e), secured to the setup base plate (f). A 1kg and 2kg dumbbell weight (g) were use to set the saw blade contact load to 3, 4 or 5kg (with the platform being 3kg). The sawing depth was set at 10mm by a stopper (i). Fig. 3.4 shows the maximum cutting depth. A custom-built tachometer (j) provided an accurate reading of the saw blade frequency. The chosen saw blade frequencies of the electric oscillating saw were 150Hz and 250Hz. A third saw blade frequency of 200Hz was tested in Exp. 1 'DCE' [35]. A standard 290mm Satterlee type bone saw (FH325R, Aesculap AG, Germany) with a 200x60x0.8mm, 9 teeth per inch saw blade was used in Exp. 6 and Exp. 7. A hack saw with a 300x13x0.65mm, 18 teeth per inch, metal-blade (Phantom, Van Ommen B.V., The Netherlands) was used in Exp. 8. These are shown in Fig. 3.1 and Fig. 3.2 respectively. When sawing by hand a much lower saw blade frequency is used, and as the blades had different dimensions, the saw blade frequency was converted to saw blade speed for proper comparison. A saw blade frequency equivalent to 15Hz and 25Hz of the electric oscillating saw was achieved by limiting the hand sawing range to 110mm and using a metronome at 87 BPM and 145 BPM, where every beat would indicate a change in sawing direction.

For the experiments in the closed environment (Exp. 1 through 3 and Exp. 6 through 8), the entire setup was placed inside an acrylic glass box (l), as shown in Fig. 3.5. Access to the setup was provided by a hole (o) in the side of the box. After each repetition, the box and setup were vacuum cleaned and wiped off using fresh multi-purpose disinfectant wipes, so all residual particles were removed. The experiment in the open environment (Exp. 4 'DOE') was conducted in a 4x8x3.5m furnished room that was normally used for anthropological examinations and storage. The setup without the box was placed in the middle of the room on a large tabletop. As

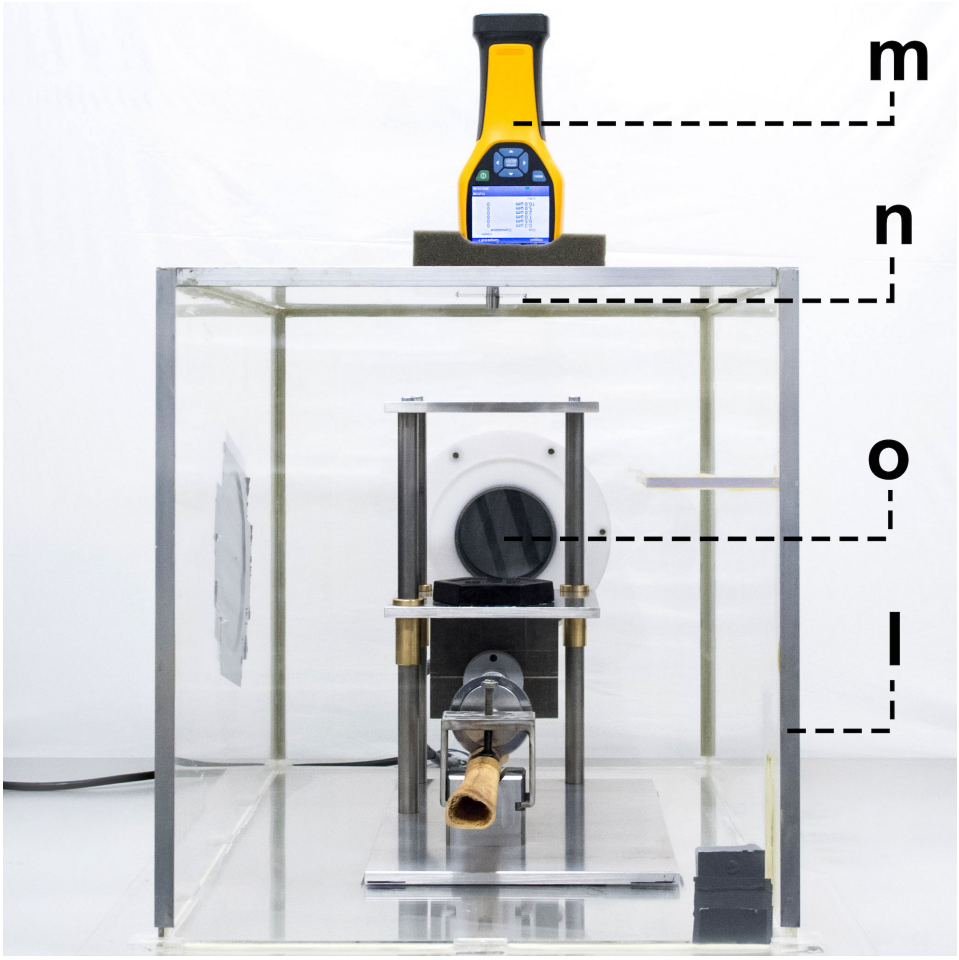


Figure 3.5: Front view of the setup enclosed in the box; an acrylic glass box (l) was used to create an experimental space isolated from the environment. The Fluke 985 particle counter (m) was placed on top of the box with a foam cast, with the nozzle inserted into the box through a hole on top of the box (n). A closable hole with a socketed cap (o) was used for handling the saw during operations inside the box.

the room was ventilated by the integral system of the building, the refreshing rate was unfortunately uncontrollable and unknown. The experiment set under active ventilation (Exp. 5 'DAE') was conducted in an 6x7x3.5m autopsy room. The sliding platform was placed directly on a 100x310cm custom built autopsy table (Elcee Holland BV, Dordrecht, The Netherlands) that was equipped with a built-in ventilation system with a ventilation capacity of 3000$m^3$/h through 252, 2cm diameter holes spaced 7cm apart, as shown in Fig. 3.6.

A Fluke 985 particle counter, shown in Fig. 3.5 (m), (Fluke corporation, Everett, Washington, USA) counted the number of aerosol particles. The particle counter's sensor was placed at a height comparable to where the saw operator's head would be. Particle sizes 0.3, 0.5, 1.0, 3.0, 5.0, and 10µm were counted at a 0.1cfm (0.1 cubic foot per minute, equivalent to 2.83l/m or 4.72 x$10^{-5}m^3$/s) flow rate. The particle counter was programmed for 7 measurements of 60 seconds each, coded as M0, M1, ... , M6. The first minute measurement (M0) was used to record the base level of particles already suspended in the environment. At the start of M1 the saw was started, and was shut off after the preset depth of cut was reached. In M1-M6 the suspension and settling down of particles from sawing was recorded. During the experiments using the electric oscillating saw (Exp. 1 through 5) the saw blade frequency and saw blade contact load were set to be the independent variables. In the experiments with the hand-saw (Exp. 6 through 8) only the saw blade frequency was set to be an independent variable, while the saw blade contact load was kept as constant as possible during manual sawing. The number of aerosol particles counted during sawing was the dependent variable. By comparing between the eight experiments as shown in Table 3.1, the influence of bone condition, the test environment, and the saw blade type were independent variables.


Figure 3.6: Front view of the setup; the electric oscillating saw (a) mounted under the weighted (g) sliding platform, with the saw blade (h) ready to make a test cut in the bone (d). The whole setup was placed directly on a 100x310cm custom built autopsy table that was equipped with a built-in ventilation system with a ventilation capacity of 3000 $m^3$/h through 252, 2cm diameter holes spaced 7cm apart.

Table 3.2 shows the Experimental Condition (EC) matrices that were used. For the experiments with the electrical oscillating saw in dry and greasy bone (Exp. 2, 4 and 5), five blocks of 4 cuts (one for each EC in bold: 1.1, 1.3, 3,1 and 3.3) were selected randomly on each of the two human femora. For the experiments using a hand operated saw (Exp. 6 through 8), five blocks of 2 cuts (one for each EC: 4.4 and 4.5.) were selected randomly on each of the two human femora). For the experiments with fresh bone (Exp. 3 'FCE'), ten blocks of 4 cuts (one for each EC in bold: 1.1, 1.3, 3,1 and 3.3) were selected on the porcine metacarpals and metatarsals, where each block corresponded with a new bone. Exp. 1 used 10 blocks of 9 cuts (one for each EC: 1.1 through 3.3). Within each block the order of the ECs was randomised.

Other variables that were monitored were temperature, humidity, residual aerosols (i.e. from pre-

Table 3.2: The Experimental Condition (EC) matrices of the sawing conditions used within the eight experiments mentioned in Table 3.1. Each EC represents a combination of saw blade frequency, and saw blade contact load.

| | 150Hz | 200Hz | 250Hz |
|---|---|---|---|
| 3kg | **EC 1.1** | EC 1.2 | **EC 1.3** |
| 4kg | EC 2.1 | EC 2.2 | EC 2.3 |
| 5kg | **EC 3.1** | EC 3.2 | **EC 3.3** |

| | 15Hz | 25Hz |
|---|---|---|
| unfixed load | EC 4.4 | EC 4.5 |

Experiments with the electric oscillating saw used EC 1.1 through EC 3.3 (Exp. 1), or only the 4 corner values shown in bold **EC 1.1**, **EC 1.3**, **EC 3.1**, and **EC 3.3** (Exp. 2 through 5). Experiments with hand-saws used EC 4.4 and EC 4.5 (Exp. 6 through 8).
The saw blade frequency used for manual sawing, EC 4.4 and 4.5, are equivalent to 15Hz and 25Hz of electric oscillating saw blade respectively

vious experiments) and foreign aerosols (e.g. dust from outside the testing environment), bone weight before and after cutting, and the saw blade frequency during cutting. Any potential effects of bone properties such as mechanical properties, surface topography, marrow cavity, cortex thickness and density, and saw blade wear, and any unknown changes over time were considered to be averaged out by creating a randomised blocked experiment. A more detailed description of the Exp 1. 'DCE' can be found in Pluim et al. (2018) [35].

### 3.2.3. Data protocol

In all analyses, the number of counted aerosol particles was determined over six minutes of measurements (M1 to M6) at a sampling flow rate of 0.1cfm (cubic foot per minute, equivalent to 2.83l/m or 4.72 x$10^{-5}$$m^3$/s). The number of counted aerosol particles was normalised to the weight of bone that was removed per cut. The base level (M0) was subtracted from each minute measurement to separate the background aerosol from the aerosols that were actually generated by sawing. The data reported in Table 3.10, and Fig. 3.7 and 3.8 is displayed in number of particles per 0.1 cubic foot per minute [n/0.1cfm]. Before the statistical analyses, the six minutes of measurements (M1 to M6) were summed and stacked (a layer for each repetition) per EC as seen in Fig. 3.9, and are displayed in number of particles per 0.6 cubic foot per minute [n/0.6cfm]. Effects were considered significant when $p \leq 0.05$.

The total number of counted particles per EC was calculated by summation of the number of particles counted for each individual particle size. This gave an indication of the total production of particles, and the number of potential pathways they formed for pathogen to reach the forensic practitioner. The total surface area of the counted particles per EC was calculated, under the assumption that the particles were spheres, by summation of the number of particles counted for each particle diameter multiplied by the square of the particle's size times 1/4 pi. The total surface area of the counted particles gave an indication of the possible amount of pathogen that could be attached to produced particles.

Statistical analyses were performed in two parts using MATLAB (MATLAB 2015a, The MathWorks Inc.). First a two-way ANOVA was done for the effect of saw blade frequency and saw blade contact load on the number of one of each of the counted individual particle sizes (0.3, 0.5, 1.0, 2.0, 5.0, and 10µm), on the total number of counted aerosol particles, and on the total surface area of the counted aerosol particles. Secondly, a three-way ANOVAN compared the effects of saw blade frequency and saw blade contact load between the eight experiments, thus the effects of the bone condition, the test environment, or the saw blade type as third variable.

## 3.3. Results

The mean numbers and standard deviations of the counted particles per sample flow rate are shown in Table 3.10, for all experimental conditions, all individual particle sizes, as well as the total number and total surface area of counted particles. The particles sizes for which the effects of saw blade frequency or saw blade contact load were statistically significant are marked.

Typical responses of a single measurement are shown in Fig. 3.7 for EC3.1 in Exp. 2 'GCE' and Fig. 3.8 for EC3.1 in Exp. 5 'DAE'. Fig. 3.9 shows a typical overview of the number of 0.3µm particles per EC in Exp. 1 'DCE'.

The results from the pilot tests (Exp. 1 'DCE') showed that there is a significant effect of saw blade frequency and saw blade contact load on the number of aerosol particles that are counted after sawing in dry bone, in a closed environment, with an electric oscillating saw: a lower saw blade frequency or higher saw blade contact load results in the lowest number of counted particles [35].

### 3.3.1. Influence of the bone condition

The number of particles counted after sawing in greasy bone (Exp. 2 'GCE') showed a clear trend between the experimental conditions for all particle

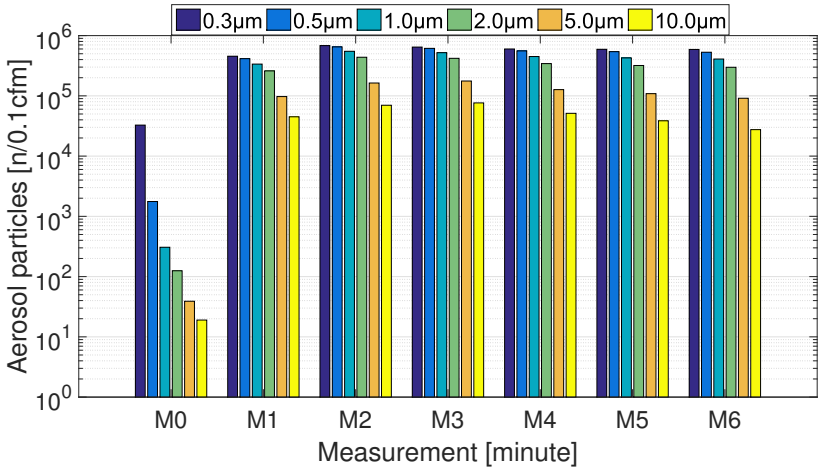


Figure 3.7: Typical response over 6 minutes of particle counting (M1...M6) at a sampling flow rate of 0.1cfm (2.83l/m), including the base level (M0), of a sawing action in greasy bone, in a closed environment, using an electric oscillating saw (Exp. 2 'GCE', EC3.1). Note that the vertical axis is logarithmic, that the base level is not yet subtracted from M1...M6, and that the 6 measurements (M1...M6) are not yet summed per the data analysis protocol.

sizes. The effects of saw blade frequency and saw blade contact load in greasy bone were statistically significant for all particle sizes, for the total number of counted particles and for the total surface area of counted particles ($p<0.05$). The highest mean number of particles was consistently counted for EC1.3 (3kg, 250Hz), whereas at EC3.1 (5kg, 150Hz) the lowest mean number of aerosol particles was counted (roughly half of EC1.3). The interaction effect was in all cases not statistically significant ($p>0.05$).

For sawing in fresh bone (Exp. 3 'FCE') statistically significant effects of saw blade frequency were found for the total number of particles counted and for individual particle sizes 0.3µm - 1µm ($p<0.05$). The effect of saw blade contact load was only statistically significant for particle size 0.3µm ($p<0.05$). Mean numbers of counted particles for all particle sizes were highest at EC1.3 (3kg, 250Hz), between 3.2 and 8.7 times higher than other ECs with lower saw blade frequencies and equal or higher saw blade contact loads. The interaction effect was in all cases not statistically significant ($p>0.05$).

Comparing the numbers of aerosol particles counted after using the electric oscillating saw in dry bone (Exp. 1 'DCE'), greasy bone (Exp. 2 'GCE'), and fresh bone (Exp. 3 'FCE') showed a statistically significant effect of bone condition: a higher number of particles was counted after sawing in dry bone than in greasy bone ( $p<0.001$) or fresh bone ($p<0.001$). With the counted number of particles in dry bone

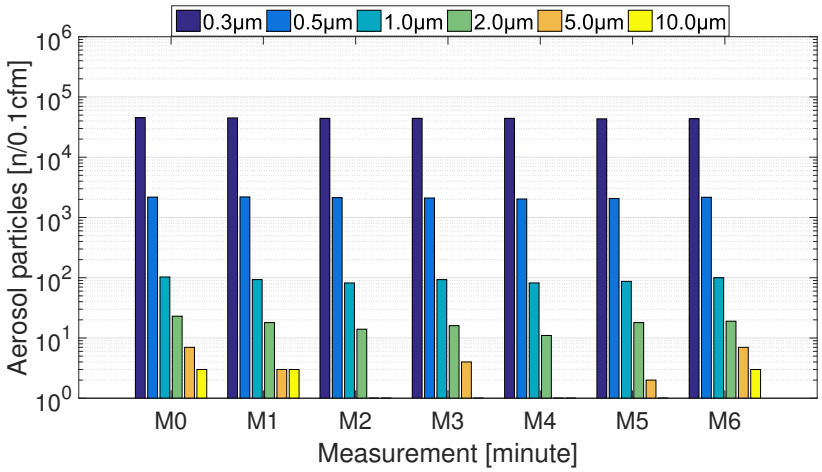


Figure 3.8: Typical response over 6 minutes of particle counting (M1...M6) at a sampling flow rate of 0.1cfm (2.83l/m), including the base level (M0), of a sawing action in dry bone, under active ventilation in the autopsy room, using an electric oscillating saw (Exp. 5 'DAE', EC3.1). Note that the vertical axis is logarithmic, that the base level is not yet subtracted from M1...M6, and that the 6 measurements (M1...M6) are not yet summed per the data analysis protocol.

(Exp. 1 'DCE') being up to 1.9 times higher than in greasy bone (Exp. 2 'GCE'), and between 8.4 and 1647 times higher than those found in fresh bone (Exp. 3 'FCE'). Especially the larger particles were counted in higher numbers after sawing in dry bone (Exp. 1 'DCE') compared to fresh bone (Exp. 3 'FCE'). Similarly, comparing the counted number of particles after hand-sawing in dry bone (Exp. 6 'DCB') and greasy bone (Exp. 7 'GCB') also showed a statistically significant effect of bone condition: between 1.5 and 2.6 times more particles counted after sawing in dry bone (Exp. 6 'DCB') than in greasy bone (Exp 7. 'GCB') ($p<0.001$).

### 3.3.2. Influence of the test environment

When sawing in an open environment (Exp. 4 'DOE') or under active ventilation (Exp. 5 'DAE'), the effects of saw blade frequency and saw blade contact load proved not statistically significant ($p>0.05$), nor was the the interaction effect ($p>0.05$).

Comparing the closed environment (Exp. 1 'DCE'), the open environment (Exp. 4 'DOE') and active ventilation (Exp. 5 'DAE') proved a statistical significant effect of the test environment: a higher number of particles was counted in the closed environment than in the open environment ($p<0.001$), or with active ventilation ($p<0.001$). The mean numbers counted in the closed environment (Exp. 1 'DCE') were between 23.8 and 109 times higher than in the open environment (Exp. 4 'DOE'), and between $1.1 \times 10^4$ and $3.8 \times 10^6$ higher than with the active ventilation (Exp. 5 'DAE'). Especially the larger particles were present in higher numbers in the closed environment (Exp. 1 'DCE') compared to active ventilation (Exp. 5 'DAE').

### 3.3.3. Influence of the saw blade type

When using either the bone-saw or metal-saw in dry or greasy bone, no statistically significant effect of saw blade frequency on the counted number of aerosol particles was found ($p>0.05$).

By comparing the counted number of aerosol particles with the Satterlee bone-saw (Exp. 7 'GCB') and metal-saw (Exp. 8 'GCM') the effect of the saw blade type proved statistically significant for all particle sizes, the total number and total surface area of particles counted ($p<0.05$). The mean number of particles was between 1.8 and 2.7 times higher with the Satterlee bone-saw (Exp. 7 'GCB') than with the metal-saw (Exp. 8 'GCM').

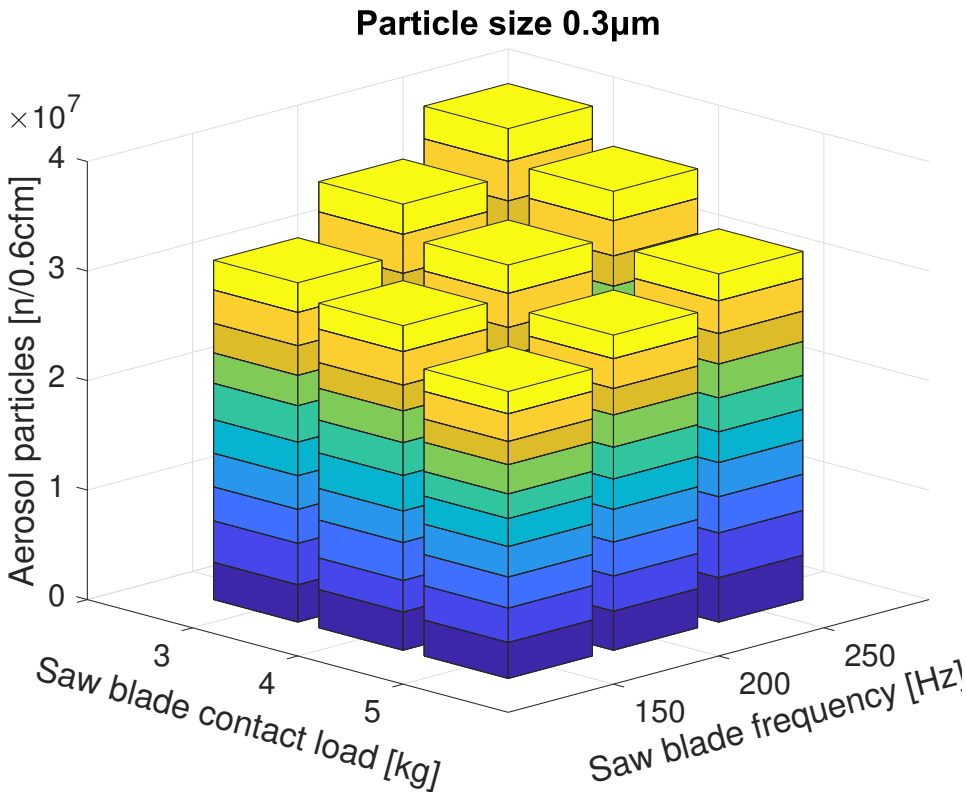


Figure 3.9: Typical stacked bar graph overview of the number of 0.3µm aerosol particles counted per EC (columns) during the n=10 measurements (layers) in Exp. 1 'DCE'. Particles were counted at a sampling flow rate of 0.1cfm (2.83l/m), had the respective base level subtracted and were summed over six minutes of measurements as per the data protocol. Note that in Exp. 1 'DCE', 9 EC were tested in 10 measurements, where as in Exp. 2 through 8 less EC (2 or 4) were tested in fewer measurements (5 to 10).

Figure 3.10: The means and standard deviations for each particle size and all experimental conditions. Calculated over n=5 for Exp. 2 and 4 through 8, and n=10 for Exp. 1 and 3.

| | | Number of particles per size [n/0.1cfm] | | | | | | Total number of particles [n/0.1cfm] | Total surface area of particles [$m^2$/0.1cfm] |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| | | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| Exp. 1 'DCE' | Base level | ***(3.84 ± 2.40 ) x10$^{4}$*** | ***(3.52 ± 1.67 ) x10$^{3}$*** | ***(4.63 ± 1.76 ) x10$^{2}$*** | ***(1.82 ± 0.857) x10$^{2}$*** | ***(2.92 ± 2.05 ) x10$^{1}$*** | ***(7.69 ± 7.04 ) x10$^{0}$*** | ***(4.26 ± 2.56 ) x10$^{4}$*** | ***(2.21 ± 0.986) x10$^{-8}$*** |
| EC 1.1 | 3kg, 150Hz | ***(3.10 ± 0.339) x10$^{6}$*** | ***(2.99 ± 0.254) x10$^{6}$*** | ***(2.40 ± 0.194) x10$^{6}$*** | ***(1.85 ± 0.183) x10$^{6}$*** | ***(7.13 ± 1.28 ) x10$^{5}$*** | ***(2.94 ± 0.683) x10$^{5}$*** | ***(1.13 ± 0.080) x10$^{7}$*** | ***(1.82 ± 0.336) x10$^{-4}$*** |
| EC 1.3 | 3kg, 250Hz | ***(3.99 ± 0.732) x10$^{6}$*** | ***(3.89 ± 0.626) x10$^{6}$*** | ***(3.19 ± 0.429) x10$^{6}$*** | ***(2.49 ± 0.302) x10$^{6}$*** | ***(9.20 ± 1.60 ) x10$^{5}$*** | ***(3.51 ± 0.882) x10$^{5}$*** | ***(1.48 ± 0.207) x10$^{7}$*** | ***(2.28 ± 0.428) x10$^{-4}$*** |
| EC 3.1 | 5kg, 150Hz | ***(2.62 ± 0.412) x10$^{6}$*** | ***(2.41 ± 0.319) x10$^{6}$*** | ***(1.87 ± 0.248) x10$^{6}$*** | ***(1.40 ± 0.207) x10$^{6}$*** | ***(5.16 ± 0.983) x10$^{5}$*** | ***(2.06 ± 0.389) x10$^{5}$*** | ***(9.02 ± 1.17 ) x10$^{6}$*** | ***(1.31 ± 0.228) x10$^{-4}$*** |
| EC 3.3 | 5kg, 250Hz | ***(3.18 ± 0.523) x10$^{6}$*** | ***(2.95 ± 0.432) x10$^{6}$*** | ***(2.27 ± 0.293) x10$^{6}$*** | ***(1.67 ± 0.212) x10$^{6}$*** | ***(5.74 ± 1.03 ) x10$^{5}$*** | ***(2.18 ± 0.529) x10$^{5}$*** | ***(1.09 ± 0.139) x10$^{7}$*** | ***(1.45 ± 0.262) x10$^{-4}$*** |
| Exp. 2 'GCE' | Base level | ***(3.24 ± 1.14 ) x10$^{4}$*** | ***(1.75 ± 0.557) x10$^{3}$*** | ***(3.19 ± 1.30 ) x10$^{2}$*** | ***(1.40 ± 0.730) x10$^{2}$*** | ***(3.36 ± 1.68 ) x10$^{1}$*** | ***(1.34 ± 0.477) x10$^{1}$*** | ***(3.47 ± 1.20 ) x10$^{4}$*** | ***(2.02 ± 0.549) x10$^{-8}$*** |
| EC 1.1 | 3kg, 150Hz | ***(2.57 ± 0.240) x10$^{6}$*** | ***(2.40 ± 0.301) x10$^{6}$*** | ***(1.86 ± 0.311) x10$^{6}$*** | ***(1.41 ± 0.285) x10$^{6}$*** | ***(5.65 ± 1.50 ) x10$^{5}$*** | ***(2.54 ± 0.790) x10$^{5}$*** | ***(9.06 ± 1.35 ) x10$^{6}$*** | ***(1.50 ± 0.412) x10$^{-4}$*** |
| EC 1.3 | 3kg, 250Hz | ***(3.56 ± 0.422) x10$^{6}$*** | ***(3.37 ± 0.463) x10$^{6}$*** | ***(2.65 ± 0.427) x10$^{6}$*** | ***(2.00 ± 0.358) x10$^{6}$*** | ***(7.37 ± 1.43 ) x10$^{5}$*** | ***(3.05 ± 0.675) x10$^{5}$*** | ***(1.26 ± 0.183) x10$^{7}$*** | ***(1.91 ± 0.371) x10$^{-4}$*** |
| EC 3.1 | 5kg, 150Hz | ***(1.94 ± 0.262) x10$^{6}$*** | ***(1.58 ± 0.249) x10$^{6}$*** | ***(1.10 ± 0.210) x10$^{6}$*** | ***(7.69 ± 1.65 ) x10$^{5}$*** | ***(2.78 ± 0.776) x10$^{5}$*** | ***(1.19 ± 0.408) x10$^{5}$*** | ***(5.79 ± 0.966) x10$^{6}$*** | ***(7.42 ± 2.16 ) x10$^{-5}$*** |
| EC 3.3 | 5kg, 250Hz | ***(2.72 ± 0.286) x10$^{6}$*** | ***(2.47 ± 0.337) x10$^{6}$*** | ***(1.85 ± 0.296) x10$^{6}$*** | ***(1.35 ± 0.240) x10$^{6}$*** | ***(4.95 ± 1.04 ) x10$^{5}$*** | ***(2.12 ± 0.512) x10$^{5}$*** | ***(9.10 ± 1.29 ) x10$^{6}$*** | ***(1.31 ± 0.280) x10$^{-4}$*** |
| Exp. 3 'FCE' | Base level | ***(4.16 ± 1.48 ) x10$^{4}$*** | **(2.72 ± 1.13 ) x10$^{3}$** | **(2.37 ± 0.695) x10$^{2}$** | (9.75 ± 3.27 ) x10$^{1}$ | (2.01 ± 1.04 ) x10$^{1}$ | (7.23 ± 4.91 ) x10$^{0}$ | **(4.46 ± 1.60 ) x10$^{4}$** | (1.97 ± 0.637) x10$^{-8}$ |
| EC 1.1 | 3kg, 150Hz | ***(9.93 ± 10.1 ) x10$^{4}$*** | **(4.97 ± 5.24 ) x10$^{4}$** | **(1.82 ± 2.20 ) x10$^{4}$** | (7.25 ± 9.58 ) x10$^{3}$ | (8.10 ± 12.0 ) x10$^{2}$ | (1.78 ± 2.28 ) x10$^{2}$ | **(1.75 ± 1.85 ) x10$^{5}$** | (3.35 ± 4.21 ) x10$^{-7}$ |
| EC 1.3 | 3kg, 250Hz | ***(4.75 ± 4.74 ) x10$^{5}$*** | **(2.46 ± 2.72 ) x10$^{5}$** | **(1.02 ± 1.25 ) x10$^{5}$** | (4.68 ± 6.11 ) x10$^{4}$ | (6.94 ± 9.69 ) x10$^{3}$ | (1.55 ± 2.20 ) x10$^{3}$ | **(8.79 ± 9.39 ) x10$^{5}$** | (2.27 ± 2.91 ) x10$^{-6}$ |
| EC 3.1 | 5kg, 150Hz | ***(9.28 ± 6.45 ) x10$^{4}$*** | **(4.39 ± 3.70 ) x10$^{4}$** | **(1.80 ± 1.84 ) x10$^{4}$** | (8.90 ± 10.2 ) x10$^{3}$ | (1.75 ± 2.39 ) x10$^{3}$ | (4.56 ± 6.60 ) x10$^{2}$ | **(1.66 ± 1.31 ) x10$^{5}$** | (5.09 ± 6.21 ) x10$^{-7}$ |
| EC 3.3 | 5kg, 250Hz | ***(1.47 ± 1.58 ) x10$^{5}$*** | **(6.91 ± 8.10 ) x10$^{4}$** | **(2.46 ± 3.27 ) x10$^{4}$** | (1.01 ± 1.47 ) x10$^{4}$ | (1.37 ± 2.49 ) x10$^{3}$ | (3.35 ± 7.11 ) x10$^{2}$ | **(2.53 ± 2.88 ) x10$^{5}$** | (5.13 ± 8.00 ) x10$^{-7}$ |
| Exp. 4 'DOE' | Base level | (6.04 ± 1.25 ) x10$^{4}$ | (3.82 ± 0.693) x10$^{3}$ | (9.89 ± 2.86 ) x10$^{2}$ | (5.00 ± 1.72 ) x10$^{2}$ | (1.31 ± 0.484) x10$^{2}$ | (4.76 ± 1.68 ) x10$^{1}$ | (6.59 ± 1.29 ) x10$^{4}$ | (5.47 ± 1.15 ) x10$^{-8}$ |
| EC 1.1 | 3kg, 150Hz | (7.88 ± 2.73 ) x10$^{4}$ | (6.77 ± 2.98 ) x10$^{4}$ | (5.05 ± 2.71 ) x10$^{4}$ | (3.94 ± 2.42 ) x10$^{4}$ | (1.98 ± 1.53 ) x10$^{4}$ | (1.03 ± 0.872) x10$^{4}$ | (2.67 ± 1.26 ) x10$^{5}$ | (5.53 ± 4.34 ) x10$^{-6}$ |
| EC 1.3 | 3kg, 250Hz | (7.31 ± 3.01 ) x10$^{4}$ | (5.06 ± 2.11 ) x10$^{4}$ | (3.27 ± 1.38 ) x10$^{4}$ | (2.27 ± 0.969) x10$^{4}$ | (9.51 ± 4.50 ) x10$^{3}$ | (4.70 ± 2.33 ) x10$^{3}$ | (1.93 ± 0.732) x10$^{5}$ | (2.67 ± 1.22 ) x10$^{-6}$ |
| EC 3.1 | 5kg, 150Hz | (7.69 ± 3.01 ) x10$^{4}$ | (5.74 ± 1.43 ) x10$^{4}$ | (4.11 ± 1.22 ) x10$^{4}$ | (3.11 ± 1.04 ) x10$^{4}$ | (1.44 ± 0.591) x10$^{4}$ | (7.60 ± 3.46 ) x10$^{3}$ | (2.28 ± 0.699) x10$^{5}$ | (4.11 ± 1.73 ) x10$^{-6}$ |
| EC 3.3 | 5kg, 250Hz | (1.34 ± 0.718) x10$^{5}$ | (9.11 ± 6.31 ) x10$^{4}$ | (5.76 ± 4.57 ) x10$^{4}$ | (3.87 ± 3.42 ) x10$^{4}$ | (1.43 ± 1.55 ) x10$^{4}$ | (7.02 ± 8.37 ) x10$^{3}$ | (3.42 ± 2.36 ) x10$^{5}$ | (4.11 ± 4.49 ) x10$^{-6}$ |
| Exp. 5 'DAE' | Base level | (5.35 ± 0.453) x10$^{4}$ | (3.18 ± 0.558) x10$^{3}$ | (1.39 ± 0.279) x10$^{2}$ | (2.81 ± 0.891) x10$^{1}$ | (6.75 ± 3.45 ) x10$^{0}$ | (3.35 ± 2.18 ) x10$^{0}$ | (5.69 ± 0.510) x10$^{4}$ | (2.00 ± 0.200) x10$^{-8}$ |
| EC 1.1 | 3kg, 150Hz | (8.07 ± 11.0 ) x10$^{2}$ | (1.80 ± 1.72 ) x10$^{2}$ | (6.51 ± 13.5 ) x10$^{0}$ | (4.84 ± 10.8 ) x10$^{-1}$ | (2.45 ± 5.48 ) x10$^{0}$ | (1.08 ± 1.66 ) x10$^{0}$ | (9.98 ± 12.1 ) x10$^{2}$ | (9.26 ± 13.0 ) x10$^{-10}$ |
| EC 1.3 | 3kg, 250Hz | (5.66 ± 8.37 ) x10$^{2}$ | (8.02 ± 9.67 ) x10$^{1}$ | (5.17 ± 6.43 ) x10$^{0}$ | (9.69 ± 16.4 ) x10$^{0}$ | (4.52 ± 10.1 ) x10$^{0}$ | (2.30 ± 3.85 ) x10$^{0}$ | (6.68 ± 8.65 ) x10$^{2}$ | (1.44 ± 2.39 ) x10$^{-9}$ |
| EC 3.1 | 5kg, 150Hz | (5.72 ± 8.59 ) x10$^{2}$ | (2.23 ± 2.92 ) x10$^{2}$ | (1.02 ± 1.31 ) x10$^{1}$ | (3.72 ± 4.62 ) x10$^{0}$ | (3.29 ± 5.00 ) x10$^{0}$ | (9.42 ± 21.1 ) x10$^{-1}$ | (8.13 ± 10.5 ) x10$^{2}$ | (9.70 ± 11.2 ) x10$^{-10}$ |
| EC 3.3 | 5kg, 250Hz | (3.67 ± 6.45 ) x10$^{2}$ | (1.40 ± 1.86 ) x10$^{2}$ | (2.91 ± 4.12 ) x10$^{0}$ | (6.84 ± 15.3 ) x10$^{-1}$ | (1.37 ± 3.06 ) x10$^{0}$ | (1.03 ± 2.29 ) x10$^{0}$ | (5.12 ± 6.55 ) x10$^{2}$ | (6.61 ± 9.39 ) x10$^{-10}$ |
| Exp. 6 'DCB' | Base level | (2.90 ± 0.351) x10$^{4}$ | (2.09 ± 0.641) x10$^{3}$ | (4.97 ± 2.53 ) x10$^{2}$ | (1.85 ± 1.08 ) x10$^{2}$ | (2.14 ± 1.48 ) x10$^{1}$ | (5.00 ± 3.68 ) x10$^{0}$ | (3.18 ± 0.437) x10$^{4}$ | (1.70 ± 0.516) x10$^{-8}$ |
| EC 4.4 | 15Hz | (9.97 ± 3.86 ) x10$^{5}$ | (6.41 ± 2.46 ) x10$^{5}$ | (3.51 ± 1.25 ) x10$^{5}$ | (2.11 ± 0.672) x10$^{5}$ | (6.24 ± 1.68 ) x10$^{4}$ | (2.18 ± 0.716) x10$^{4}$ | (2.28 ± 0.836) x10$^{6}$ | (1.63 ± 0.449) x10$^{-5}$ |
| EC 4.5 | 25Hz | (7.24 ± 2.60 ) x10$^{5}$ | (4.55 ± 1.71 ) x10$^{5}$ | (2.46 ± 0.977) x10$^{5}$ | (1.48 ± 0.623) x10$^{5}$ | (4.43 ± 2.19 ) x10$^{4}$ | (1.53 ± 0.830) x10$^{4}$ | (1.63 ± 0.614) x10$^{6}$ | (1.15 ± 0.556) x10$^{-5}$ |
| Exp. 7 'GCB' | Base level | (2.35 ± 0.581) x10$^{4}$ | (2.18 ± 0.861) x10$^{3}$ | (5.47 ± 2.17 ) x10$^{2}$ | (2.42 ± 0.804) x10$^{2}$ | (4.21 ± 1.50 ) x10$^{1}$ | (1.39 ± 0.718) x10$^{1}$ | (2.65 ± 0.685) x10$^{4}$ | (2.08 ± 0.549) x10$^{-8}$ |
| EC 4.4 | 15Hz | (4.37 ± 1.15 ) x10$^{5}$ | (2.66 ± 0.640) x10$^{5}$ | (1.47 ± 0.325) x10$^{5}$ | (9.18 ± 1.91 ) x10$^{4}$ | (2.65 ± 0.693) x10$^{4}$ | (8.41 ± 3.37 ) x10$^{3}$ | (9.77 ± 2.35 ) x10$^{5}$ | (6.68 ± 1.89 ) x10$^{-6}$ |
| EC 4.5 | 25Hz | (2.98 ± 1.09 ) x10$^{5}$ | (1.91 ± 0.664) x10$^{5}$ | (1.12 ± 0.400) x10$^{5}$ | (7.39 ± 2.77 ) x10$^{4}$ | (2.57 ± 1.19 ) x10$^{4}$ | (9.98 ± 5.36 ) x10$^{3}$ | (7.11 ± 2.60 ) x10$^{5}$ | (6.67 ± 3.17 ) x10$^{-6}$ |
| Exp. 8 'GCM' | Base level | (2.35 ± 0.581) x10$^{4}$ | (2.18 ± 0.861) x10$^{3}$ | (5.47 ± 2.17 ) x10$^{2}$ | (2.42 ± 0.804) x10$^{2}$ | (4.21 ± 1.50 ) x10$^{1}$ | (1.39 ± 0.718) x10$^{1}$ | (2.65 ± 0.685) x10$^{4}$ | (2.08 ± 0.549) x10$^{-8}$ |
| EC 4.4 | 15Hz | (1.63 ± 0.478) x10$^{5}$ | (1.07 ± 0.271) x10$^{5}$ | (6.50 ± 1.90 ) x10$^{4}$ | (4.41 ± 1.48 ) x10$^{4}$ | (1.44 ± 0.662) x10$^{4}$ | (4.67 ± 2.60 ) x10$^{3}$ | (3.98 ± 1.16 ) x10$^{5}$ | (3.49 ± 1.58 ) x10$^{-6}$ |
| EC 4.5 | 25Hz | (1.39 ± 0.512) x10$^{5}$ | (9.07 ± 3.02 ) x10$^{4}$ | (5.56 ± 1.40 ) x10$^{4}$ | (3.79 ± 0.685) x10$^{4}$ | (1.24 ± 0.132) x10$^{4}$ | (4.13 ± 1.05 ) x10$^{3}$ | (3.40 ± 1.01 ) x10$^{5}$ | (3.03 ± 0.329) x10$^{-6}$ |

Particles were counted at a sampling flow rate of 0.1cfm (0.1 cubic foot per minute, equivalent to 2.83l/m or 4.72 x10$^{-5}$m$^3$/s), and had the respective base level subtracted as per the data protocol.
The means and stds were calculated over n=5 for Exp. 2 and 4 through 8, and n=10 for Exp. 1 and 3.
The experiment code (Exp. # 'CODE') refers to the variables tested in each experiment as shown in Table 3.1. The variables are **D**ry, **G**reasy or **F**resh bone, in a **C**losed, **O**pen or **A**ctively ventilated environment, using an **E**lectric oscillating, **S**atterlee or **M**etal-saw.
'EC...' refers to the chosen Experimental Conditions of the independent variables saw blade frequency and saw blade contact load shown in Table 3.2 (EC 1.1 though 3.3), or the saw blade frequency in Table 3.2 (EC 4.4 and 4.5).
'Base level' shows the average of all base levels, i.e. the first minute of measurement of each repetition of each different test.
Text in **bold** shows that the effect of saw blade frequency was statistically significant (p<0.05). Text in *italics* shows that the effect of saw blade contact load was statistically significant (p<0.05).

## 3.4. Discussion

The current study investigated the influence of 5 sawing parameters on the production of aerosol dust particles, to inherently minimise the health risk faced by forensic practitioners during sawing operations. Within the experiments, the influence of saw blade frequency and saw blade contact load were studied under conditions better matching those in actual autopsy practice than in the pilot tests described in Pluim et al. (2018) [35]. Furthermore, comparisons between the experiments showed the effect of the bone condition, the test environment, and the saw blade type.

### 3.4.1. Influence of the bone condition

The results show that the condition of the bone has a clear effect on the counted number of aerosol particles of particles. Within the tests with different bone conditions, the effect of saw blade frequency and saw blade contact load remained similar to earlier findings: a lower saw blade frequency or higher saw blade contact load result in a reduction of counted particles [35]. In fresh bone (Exp. 3 'FCE') only the combination of high saw blade frequency and low saw blade contact load (EC1.3 3kg, 250Hz) resulted in an extreme high number of aerosol dust particles. The counted number of aerosol particles with either a low saw blade frequency or a high saw blade contact load (or both) was much less.

Between the tests with different bone conditions, sawing in dry bone (Exp. 1 'DCE' and Exp. 6 'DCB') more particles were counted compared to sawing in greasy bone (Exp. 2 'GCE' and Exp. 7 'GCB'). It seems that the greasy substance itself was not aerosolised, as the decrease in the counted number of particles compared to dry bone is the same for all particle sizes. The decrease in the counted number of particles might be explained by an ability of the greasy substance to limit the spread of the particles by binding them.

After sawing in fresh bone (Exp. 3 'FCE') much less particles were counted than in dry bone (Exp. 1 'DCE'). It is suggested that the increased elasticity of fresh bone prevents particles from easily breaking off. The particles that are broken off, consist of mostly coarse dust that is not aerosolised. The smaller particles were counted in only slightly lower numbers (8.4 times fewer), whereas the larger particles were counted much less (1647 times fewer) in fresh bone than in dry bone. This could be explained by the organic materials (10-30%) and water (10-20%) present in fresh bone [36], that might themselves have been aerosolised by the sawing action, resulting in a relatively higher number of particles of the smallest sizes. As the organic materials, such as bone marrow, blood and other bodily fluids contain the potentially hazardous pathogens, especially the smallest particles are of risk to the forensic practitioner.

These results corroborate with the proposed hypothesis: the dryer the bone, the higher the counted number of of respirable dust particles. Nonetheless, it should be advised that sawing, especially in fresh bone, should be carried out only under high safety precautions: using adequate ventilation systems, protective breathing gear and following validated protocols to minimise the production of aerosols that reach the respiratory tract of the forensic practitioner.

### 3.4.2. Influence of the test environment

Although a clear effect of the test environment on the counted number of particles was shown, no effect of saw blade frequency and saw blade contact load on the counted number of aerosols was found in the open environment (Exp. 4 'DOE') or at the autopsy table with active ventilation (Exp. 5 'DAE'): A high variance between the repetitions, and no clear differences between the ECs suggest that external influences are more dominantly responsible for the aerosol's ability to reach the respiratory tract of the forensic practitioner, than the saw blade frequency and saw blade contact load. These external influences could include air flow from personnel movement, door openings or active ventilation systems. Following these findings, the importance of external protection against the inhalation of aerosols, e.g. wearing protective breathing gear or working in a properly ventilated workplace, seems to outweigh the choice in saw blade frequency and saw blade contact load.

The number of particles that were counted in the open environment (Exp. 4 'DOE') was slightly lower than those counted in the closed environment (Exp. 1 'DCE'), as particles in a small confined space will spread out less, reach the particle counter quicker and in higher numbers than in an open environment. The number of particles counted for the active airflow of the ventilated autopsy table (Exp. 5 'DAE') was similar or lower than the base level measurements, suggesting that the counted particles are within the variance of the number of particles generally in the air rather than necessarily aerosol produced by sawing.

These results show that a properly designed air removal system can be capable of removing the harmful aerosol dust particles from the surrounding air, corroborating with the hypothesis. There is however a wide variety of ventilation systems compared to the built-in down-draft ventilation system at the tested autopsy table. For instance, varied placements and airflow-orientations of ventilation systems are in use, such as ceiling-mounted top-down laminar airflow, wall-mounted horizontal laminar airflow, or down-

draft ventilation in tables [57]. Similarly, the furnishings in the room [58], the practitioner's movement [59] or generated heat by the practitioner [57] and equipment are of influence on the airflow in the room and the spreading of aerosols. Local Exhaust Ventilation (LEV) directly attached to the operating saw can significantly reduce the aerosols produced by sawing, but is often left unused because of decreased accessibility and ergonomic issues [60].

Additionally, the quality of these air removal systems needs to be tested under more stressing circumstances. The cuts that were made during these tests required much shorter sawing times, and much less material removal than regular scenarios faced in daily practice. Similarly, the air intake point of the particle counter was set to be similar to the breathing zone of practitioner in upright position, whereas in daily practice this point could be closer to the saw, for instance when the cutting location is in the middle of the body (e.g. an extraction of the pubic joint for age estimation) and the practitioner has to bend over, possibly rendering the air removal system less effective. Additionally, large parts of the autopsy table and its air extraction holes could be blocked by the specimen, making active ventilation locally less effective.

The number of particles counted over the autopsy table (Exp. 5 'DAE'), even though they might be background noise and not necessarily have been produced by sawing, is still well over the amount that is advised during surgeries [59]. Although the risk of surgical-site wound infection in the patient is not of importance during autopsies, the practitioner in surgeries and autopsies both face similar health risks posed by the produced aerosols, making the comparison to the ventilation and air filtration systems used in hospitals useful in the design of autopsy rooms. Similarly, improved room design and standardisation of requirements and protocols can be adapted from clean room ventilation technology [59].

The use of irrigation fluids on the saw blade is often used to reduce the amount of aerosols that are produced by mechanical tools, to cool the cutting surface, and remove blood and larger debris. However, this method is not suitable for use in forensic cases as trace evidence is often crucial and irrigation fluids might wash away traces or contaminate other areas. More importantly, it has been shown that the irrigation fluids themselves can become infected with pathogens [17] and can get aerosolised into respirable particles. Additionally, studies on irrigation fluids in the field of material science and machining have shown that the fluids might increase the roughness of the cutting surface and decrease tool wear, but that the total amount of aerosol increases significantly [61, 62].

By comparing the base level measurements to the relative humidity (RH) in the room in a 2-way ANOVA, it was found that the RH might have had a significant effect on the number of particles counted in the base level measurement for particle sizes 0.3µm - 2µm ($p<0.05$), but not for particle sizes 5µm - 10µm ($p>0.05$). These results suggest that the water vapour particles in the air that make up the RH are similar in size to the smallest particles counted in these experiments. These water vapour particles might prevent the produced aerosol particles to disperse easily by binding to them. By removing the base level from each subsequent measurement, and depending on a randomised blocked experiment, it is believed that the changes of RH did not have play a significant role on the tested influences in the current study.

When the setting of the sawing procedure is not suited for the use of adequate ventilation systems, which may be the case in developing countries without high-tech infrastructure, or after natural or anthropogenic disasters where emergency makeshift mortuaries are often used, other precautions should be taken: most importantly following validated protocols and wearing protective and adequate breathing gear, aside from the reduction of aerosols by optimising sawing parameters.

### 3.4.3. Influence of the saw blade type

No statistically significant effect of saw blade frequency was found for the Satterlee bone-saw (Exp. 6 'DCB' and Exp. 7 'GCB') or the metal-saw (Exp. 8 'GCM'), suggesting that the effect of lowering the saw blade frequency to decrease the production of aerosol as seen in the experiments using the electric oscillating saw (Exp. 1 through 5), is limited at the low saw blade frequencies used when hand-sawing. Unfortunately, the influence of the saw blade contact load cannot be compared between the oscillating saw and hand-saw, as when hand-sawing no constant saw blade contact load could be kept within and between the strokes due to the inherent inconsistencies of manual sawing.

Contrary to hypothesised, the larger saw teeth of the Satterlee bone-saw (Exp. 7 'GCB') seemed to produce higher numbers of aerosol particles than the smaller teeth of the metal-saw (Exp. 8 'GCM'). It was expected that the rougher toothed saw would break off larger pieces of bone, and thus less fine dust particle were counted than after sawing with the smaller toothed metal-saw. Although both hand-saws were used under the same saw blade frequency and a constant as possible saw blade contact load, it was noted that the rough toothed Satterlee bone-saw regularly got stuck and had much greater difficulty cutting through the bone than the metal-saw. This is also reflected in the increased sawing time, on average the Satterlee bone hand-saw took roughly 1.2-1.3

times as long to achieve the same depth of cut, possibly collaborating to the unexpected higher counted number of aerosol particles: the removal of the same amount of bone over a longer time suggest that more sawing strokes were needed and the particles that were broken off were smaller. Unknown differences in saw blade sharpness or other unknown differences between the blades could have influenced the increase of counted number of particles using the Satterlee bone hand-saw over the fine toothed metal-saw blade, along the general increased inconsistencies due to manual sawing.

Saw blade kerf mark analysis in forensic science has shown that saw blade characteristics are of influence on the traces that are left on the bone and can be used as means to identify classes of, or individual saw blades. This can include characteristics such as hand versus mechanically powered, but also saw tooth characteristics such as tooth size, tooth shape and set, or the direction of the cut [48–56]. The current study shows there is potential for further research into similar sawing characteristics to intrinsically minimise the amount of produced aerosol, for instance by the use of a slower hand-saw compared to machine operations, as fewer aerosol is counted when sawing by hand than when using an fast electric oscillating saw. Similar studies have shown differences in aerosol production between more large-scale characteristics, such as power, mechanical working principle (band-, table-, oscillating-, or reciprocating- saws) [28], but hardly into more small-scale characteristics such as saw tooth size, shape and set. Further research is needed to determine the influence of these saw blade characteristics on the production of aerosols, and to design a blade that intrinsically produces the lowest amount of dust. It should be expected however that the protection against aerosols, for instance by research into breathing gear, seems more promising than a slight reduction in aerosol production by using a low aerosol producing saw blade.

Within the field of orthopaedic surgery research has been performed to find alternative methods of cutting bone, such as laser cutting [63] or water jetting [64]. Advantages of these methods over conventional sawing could include reduced tissue necrosis and improved cutting accuracy. Unfortunately, from a standpoint of aerosol production these methods are likely similarly or even more hazardous than conventional sawing, as both produce high amounts of surgical smoke or water vapour particles that are possibly infused with bodily fluids or pathogen. Furthermore, these methods might not be suited for forensic practice, because of the possible contamination of forensic traces.

## 3.5. Conclusion

The production of aerosol dust particles by sawing in bone can pose health risks for those near the site of operation, even for long periods of time after the procedure has finished. The fine particles are within the respirable range and can cause harm in the respiratory tract, or potentially transfer harmful pathogens. It was found that active ventilation systems within the tested autopsy table can remove nearly all of these aerosol dust particles from the air. The choice of sawing parameters can minimise the production of aerosols: sawing by hand using a sharp, fine toothed hack saw was found to be the best option. When an electric oscillating saw is used, decreasing the saw blade frequency or increasing the saw blade contact load can be used to minimise the production. However, even for the parameters with the lowest production this intrinsic decrease in particles is slight, and the number of aerosol bone particles that are produced still pose a serious health hazard to anyone near the sawing site. Adequate protective breathing gear, ventilation systems and safety protocols should be used to minimise the risks faced by practitioners.

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# 4

# Discussion, recommendations, and conclusions

This thesis was set up as a larger collaboration between the Delft University of Technology and the Netherlands Forensic Institute, studying the health risks faced by (forensic) pathologists and anthropologists posed by the production of harmful aerosols when sawing in bone. The goal of this thesis was twofold: Firstly, to quantify the number of aerosol bone dust particles that are produced when sawing in bone, and secondly to reduce the risks of those aerosol bone dust particles by choosing the optimal sawing parameters, by reducing the production of aerosol bone dust particles or limiting the amount of ways the aerosols reach the respiratory tract of the persons involved.

The tested sawing parameters provided a clear insight in the production of aerosol bone dust particle under different tested sawing conditions. It was found that the production of aerosol dust particles can be kept to a minimum by using a sharp, low speed hand-saw. When an electric oscillating saw was used, decreasing the saw blade frequency or increasing the saw blade contact load resulted in the lowest number of aerosol particles, although this remained higher than the hand-saw alternative. However, further research is needed to determine the exact influence of different saw blade characteristics such as the sawing working principle (manual or machine powered) or saw tooth characteristics such as size, kerf, and set. Different types of saw blades operated under different sawing conditions could produce even fewer numbers of aerosol dust particles than found in these experiments, but might be limited in practical use requirements. The optimal sawing parameters when using the electric oscillating saw are limited by the amount of torque of the saw, something that would be even more challenging when larger saw teeth are used, suggesting that the electric oscillating saw is not likely to ever be the lowest aerosol producing alternative. The testing of a wider variety of hand saw

designs is needed to determine the least aerosol producing saw blade, although it is expected that even the least aerosol producing saw blade produces hazardous amounts of aerosol particles.

The differences in bone condition did not account for larger variations in the production of aerosols, suggesting that all sawing procedures on any state of (human) bone should be conducted under strict safety protocols. Although the pathogen carrying-effect of the aerosol is expected to be minimal in dry archaeological bone compared to fresh *in vivo* bone, the inhalation of any aerosol particle should be kept to a minimum. When there are strong indications of an infected specimen, extra caution should be taken.

The results suggested that the removal of aerosol particles from the environment had a much higher efficacy in preventing the inhalation of aerosol particles that the reduction in production of aerosol by the choice in saw blade parameters. Further experiments are needed to test the efficacy of specific ventilation systems; any professional setting should be thoroughly tested and be responsible for the safety of their workers. The tested ventilation system at the Netherlands Forensic Institute (NFI) seemed to be adequate in the removal of all aerosol particles, be it under not so stressing circumstances, but not all sawing procedures within the NFI were conducted at this table. It is strongly advised that every sawing operation should be only performed under validated air removal systems. More importantly, the awareness of the importance of proper surgical masks should be radically improved. There are plenty of scenarios where adequate ventilation systems are not feasible, which may be the case in developing countries without high-tech infrastructure, or after natural or anthropogenic disasters where emergency makeshift mortuaries are often used. The surgical masks that are used now are primarily designed to reduce the risk of the professional worker infecting the patient, not necessarily the other way around. Furthermore, the standard surgical masks are not designed to protect against the finest produced particles, and often only worn in a loose fitting matter. It is recommended that the efficacy of different types of masks is tested, along with the importance of proper face-fitting, for specific settings and individual workers.

Although this was deemed to be outside the scope of this thesis, the knowledge on the actual hazard of aerosol bone dust particles is crucial in spreading the awareness of the problem. Under the assumption that the number of inhaled aerosol dust particles should be kept as low as possible, the influences of the tested sawing parameters could be determined independently of the potential inhalation of aerosol particles. The actual risks however, depend much more on the effect these aerosol particles have on the human body. As these are largely unknown, it remains hard to determine the actual risk of the inhalation of aerosol dust particles, and therefor limits the reach of all recommendations in this thesis. Protocols and safety awareness will not change much if the true risk of the inhalation of aerosol dust particles is unknown, especially as these will probably lead to increased cost and decreased easy of use and comfort. This will also strengthen the importance of adequate ventilation systems and proper breathing gear that match the risk of the task: no worker should wear a cumbersome high performance gas-mask when this is not needed, but even more importantly, workers should have access to better breathing gear than the standard surgical mask when the risk is indeed proven.

In conclusion, it was found that production of aerosol dust particles by sawing in bone can pose health risks for those near the site of operation, even for long periods of time after the procedure finished. The fine particles are within the breathable range and can cause harm in the respiratory tract, or potentially transfer harmful pathogens. The choice of sawing parameters can reduce the production of aerosols, but is still large enough to cause potential health hazards. It was found that active ventilation systems within the tested autopsy table can remove the vast majority of aerosol particles from the air. Further research is needed to determine the true risk of the inhalation of aerosol bone dust particles, in the mean time all precautions should be taken to minimise the risks faced by practitioners by implementing adequate ventilation systems, protective breathing gear, clothing and safety protocols.

# A

# Experimental design

*This appendix is based on my internship report.*
As mentioned in Chapter 1, this thesis continues on a greater project collaboration of the Delft University of Technology and Netherlands Forensic Institute with a focus on creating a controlled testing environment to study the production of potentially harmful aerosol particles that are produced during forensic autopsies and anthropological examinations. Two groups of bachelor students ('Bachelor Eind-Project' 2013 and 2014 respectively [1, 2]) started with a first version of the setup and testing protocol, but ran into several problems resulting in too few correct measurements for sufficient statistical power.

In order to study the influences of the various proposed parameters in a controlled and methodological way, a new setup was designed and built using the base of the existing setup. Furthermore, a systematic testing and cleaning protocol was drafted and carried out. Both the setup and the protocol were adapted to fit the testing on dry human femora in a closed environment using an electrical oscillating saw (Chapter 2), as well as using dry or greasy human femora, fresh porcine metatarsals and metacarpals, in a closed or open environment, or under active ventilation, using an electrical oscillating saw or two types of hand saws (Chapter 3).

This chapter provides a more in depth overview of the design process and production of the setup, as well as the experimental protocol used in this thesis. Some figures and explanations may have already been introduced in one of the former Chapters, for the sake of completion they also appear in this Appendix.

Table A.1: Overview of the variables tested in eight performed experiments. The saw blade frequencies and saw blade contact loads used within the eight experiments are shown in Table A.2

| | | Tested variables, experiment numbers and their code names, nr of ECs and nr of reps | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tested variable: | Bone condition | | | | | Test environment | | | Saw blade type | | | | |
| | Experiment number: | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 6 | Exp. 7 | Exp. 1 | Exp. 4 | Exp. 5 | Exp. 1 | Exp. 6 | Exp. 2 | Exp. 7 | Exp. 8 |
| | Experiment code: | 'DCE' | 'GCE' | 'FCE' | 'DCB' | 'GCB' | 'DCE' | 'DOE' | 'DAE' | 'DCE' | 'DCB' | 'GCE' | 'GCB' | 'GCM' |
| | Nr of ECs: | 9EC | 4EC | 4EC | 2EC | 2EC | 9EC | 4EC | 4EC | 9EC | 2EC | 2EC | 2EC | 2EC |
| | Nr of reps: | 10reps | 5reps | 10reps | 5reps | 5reps | 10reps | 5reps | 5reps | 10reps | 5reps | 5reps | 5reps | 5reps |
| Bone condition | Dry bone cat D.4[3] | **D** | | | **D** | | D | D | D | D | D | | | |
| | Greasy bone cat D.3[3] | | **G** | | | **G** | | | | | | G | G | G |
| | Fresh bone cat A.1[3] | | | **F** | | | | | | | | | | |
| Test environment | Closed environment | C | C | C | C | C | **C** | | | C | C | C | C | C |
| | Open environment | | | | | | | **O** | | | | | | |
| | Active ventilation | | | | | | | | **A** | | | | | |
| Saw blade type | Electric oscillating saw | E | E | E | | | E | E | E | **E** | | **E** | | |
| | Satterlee bone-saw | | | | B | B | | | | | **B** | | **B** | |
| | Metal-saw | | | | | | | | | | | | | **M** |

The experiment codes (Exp. 'CODE') provide a concise reference to the variables tested in each experiment when referred to later in text. For example, in Exp. 1 'DCE' a *Dry bone* was used, in a *Closed environment* using an *Electric oscillating saw*.
The grouped columns show which experiments are compared to find the influence of bone condition, test environment, and saw blade type, with the independent variable shown in **bold**.

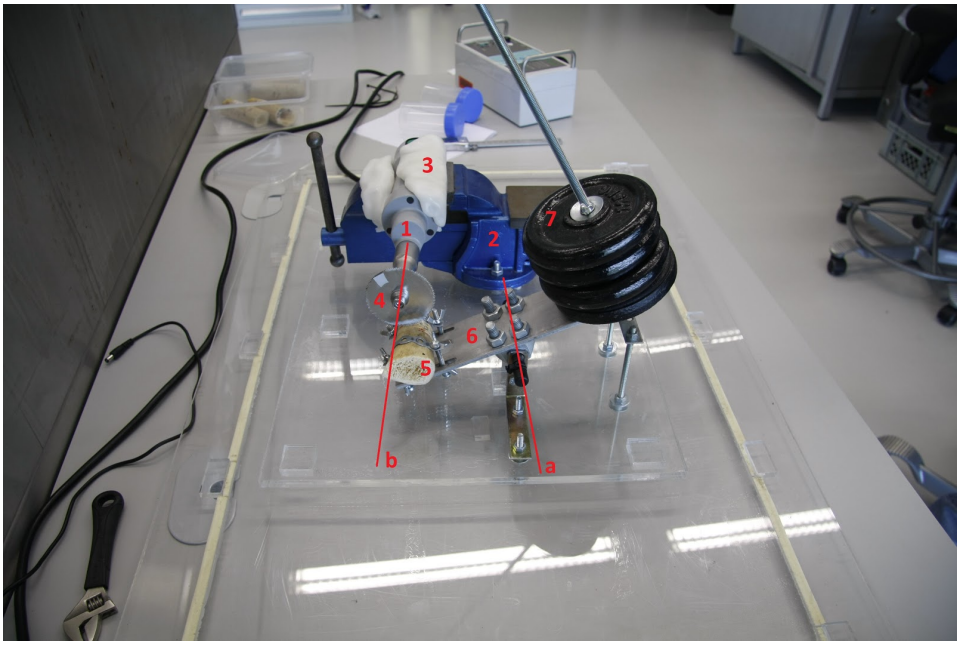


Figure A.1: The internal mechanism of the setup made and used by the TU Delft bachelor groups Hockers et al. (2013) [1] and van Doeveren et al. (2014) [2]. Picture by van Doeveren et al. (2014) [2]

# A.1. Experimental Setup

## A.1.1. Design by bachelor group 2013

The setup shown in Fig. A.1 was designed and made by the first group of TU Delft bachelor students (Hockers et al. (2013) [1]). Shown are the base of the setup with the mechanics of the saw action. During testing a transparent acrylic box was placed around to contain the dust, which is shown in Fig. A.2. The principles of this first iteration are shortly discussed, the parts that were used in the improved version are explained in more detail in section A.1.2. The electrical oscillating bone saw (1) was held by a vise (2) using smart plastics (3) that allowed for the round form of the saw to be clamped. The saw blade (4) was forced against the bone segment (5) by a lever mechanism (6) loaded with interchangeable weights (7). Although this setup did work and was able to saw into the bone, several problems limited its performance, the ease of use and the cleanability; In order to study the any sawing variables or characteristics in a methodological way, a large amount of cuts needed to be made. Between each two successive cuts the whole setup needed to be cleaned, to make sure no residual bone dust would bias the subsequent measurement. The exposed threads, the sharp edges of the vise, and irregular shapes of the weights provided hard to clean surfaces. Furthermore, the lever mechanism provided some mechanical difficulties. The axis of the lever (line a) and the axis of the saw (line b) were not exactly parallel, which resulted in unwanted internal forces and friction of the saw blade in the bone. This could possibly have influenced the true contact load of the saw on the bone, and subsequently the amount of sawdust. Secondly, by stacking the dumbbells, the centre of mass of the weights lied high above the lever which when tilted moved closer to the axis. This changed the contact load characteristics during

Table A.2: The Experimental Condition (EC) matrices of the sawing conditions used within the eight experiments mentioned in Table A.1. Each EC represents a combination of saw blade frequency, and saw blade contact load.

| | 150Hz | 200Hz | 250Hz |
|---|---|---|---|
| 3kg | **EC 1.1** | EC 1.2 | **EC 1.3** |
| 4kg | EC 2.1 | EC 2.2 | EC 2.3 |
| 5kg | **EC 3.1** | EC 3.2 | **EC 3.3** |

| | 15Hz | 25Hz |
|---|---|---|
| unfixed load | EC 4.4 | EC 4.5 |

Experiments with the electric oscillating saw used EC 1.1 through EC 3.3 (Exp. 1), or only the 4 corner values shown in bold **EC 1.1**, **EC 1.3**, **EC 3.1**, and **EC 3.3** (Exp. 2 through 5). Experiments with hand-saws used EC 4.4 and EC 4.5 (Exp. 6 through 8).
The saw blade frequency used for manual sawing, EC 4.4 and 4.5, are equivalent to 15Hz and 25Hz of electric oscillating saw blade respectively

sawing as the depth of cut increased from a uniform load to a increasing load. For small deflections these influences would be negligible, but as the lever started out at an almost 45 degree angle, these influences do need to be taken into account.

Fig. A.2 shows the outside acrylic box that was placed over the internal mechanism. This box made sure that the dust is contained within an easier to clean environment than an entire room, and provided a much more stable level of background noise particles. There were two gloves (1) attached with hose clamps to holes in the box (2) that allowed for operations during testing. The gloves however turned out to be very difficult to clean, and the acrylic screen around the gloves was easily cracked. The particle counter (3) was attached on top of the box.

### A.1.2. Improvements to the setup

The internal mechanism of the setup was redesigned, and produced with the help of Hannes Habraken. The design was focused on ease of use, and ability to be thoroughly cleaned. Specific parts of the setup are discussed below, the parts are lettered to refer to Fig. A.3, A.4, A.5, and A.6, which correspond to the letters and figures used in Chapters 2 and 3.

The setup can be categorised into roughly three parts, that could be adapted to suit the testing of the three main independent variables: the influence of the bone condition, the testing environment, and the saw blade type. The specific components of the setup are elaborated further below. The first part of the experimental setup was the sliding platform, shown in Fig. A.3. The oscillating saw (a) was fixed to the sliding platform (b). The platform housed three brass cylinders that slid along stainless steel rods (c), allowing the saw to freely move vertically. Interchangeable weights (g) were used to vary the contact force of the saw blade against bone specimen. The depth of cut of the saw blade (h) was controlled by a height control stopper (i). A tachometer (j) was clamped to the saw (k), and used to measure the varied sawing frequency. A detailed view of the saw blade is shown in Fig. A.4. During the experiments with hand saws (Exp. 6 through 8) the sliding platform was removed entirely, to make room for the use of a hand saw.

The second part was the base plate. A bone specimen (d) could be fastened using a v-groove block and a clamping mechanism (e). Both the v-groove block and the steel rods of the sliding platform were fixed to an aluminium base plate (f). This part was used in all the 8 experiments.

The third part of the setup consisted of the acrylic box made by a TU Delft bachelors group (Hockers


Figure A.2: The acrylic box made and used by the TU Delft bachelor group Hockers et al. (2013) [1] and van Doeveren et al. (2014) [2], shown here without the internal mechanism. Picture by van Doeveren et al. (2014) [2]

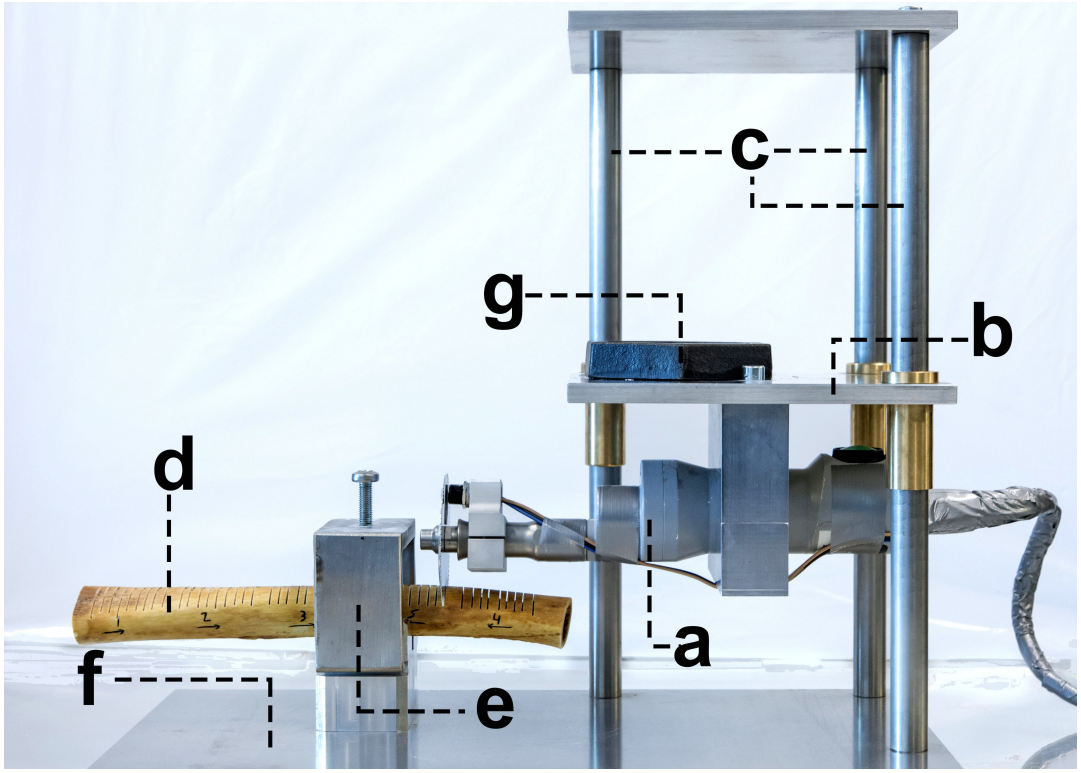


Figure A.3: Experimental setup used to cut the bone, the setup consisted of: an oscillating saw (a) fastened to a vertical sliding platform (b) guided by 3 stainless steel rods and brass sliding bearings (c). The bone specimen (d) was clamped in a v-groove holder (e), that was connected to an aluminium base plate (f). Interchangeable weights could be attached to the platform (g). The sawing action is further illustrated in Fig. A.4.

et al. (2013) [1]). The whole platform could be placed inside the acrylic glass box (l), in which the amount of aerosol could be measured by a particle counter (m), shown in Fig. A.5. During the experiments in the open environment (Exp. 4 and 5) the sliding platform and the base plate were used without the acrylic box, either in a closed room or on an autopsy table with active airflow by built-in ventilation.

a, h Oscillating saw and saw blade: in Exp. 1 through 5 cuts were made using an oscillating saw (DeSoutter NS3, DeSoutter Medical Limited) with a blade of 76mm in diameter (DeSoutter 16892, DeSoutter Medical Limited). The frequency of the saw could be set using an external control panel. By turning a potentiometer any frequency between 30Hz or 250Hz could be chosen, although no scale was displayed.

b, c, f Vertical sliding platform: The sliding platform was built from 10mm thick aluminium plates, shown in Fig. A.3. A 200mm square plate moved vertically by three 60mm long brass sliding bearings on 20mm diameter surgical stainless steel rods, so the total vertical range was 270mm. The three stainless steel rods were connected to a 300x200mm aluminium base plate on the bottom, and a stationary 200m aluminium square plate on top. The oscillating saw was fastened by clamping two 125x36x40mm thick aluminium blocks with a cylindrical hole the diameter of the saw handle.

d Bone sample: four human femora from an anthropological bone collection of the Netherlands Forensic Institute (The Hague, the Netherlands) were used, as shown in Fig. A.13 and A.14. The femur is a long tubular bone with a reasonably consistent morphology along the shaft, and with few irregularities in cortex thickness. The total length of two femur shafts allowed for a high amount of cuts and therefor repetitions, requiring fewer bone pieces for this study compared to the size of other human bone parts. Three femora were in dry condition (Cat. D.4 [3]), one still showed a greasy residue (Cat. D.3 [3]), all were clean of any soft tissues. The bone marrow cavities of the femora were scraped to remove gross trabecular bone tissue, together with other residues that could easily shake loose during cutting and interfere as unwanted suspended particles. For the fresh bone specimens, a total of ten metacarpals and metatarsal bones, such as shown in Fig. A.12, were removed from five fresh (Cat. A.1 [3]) porcine

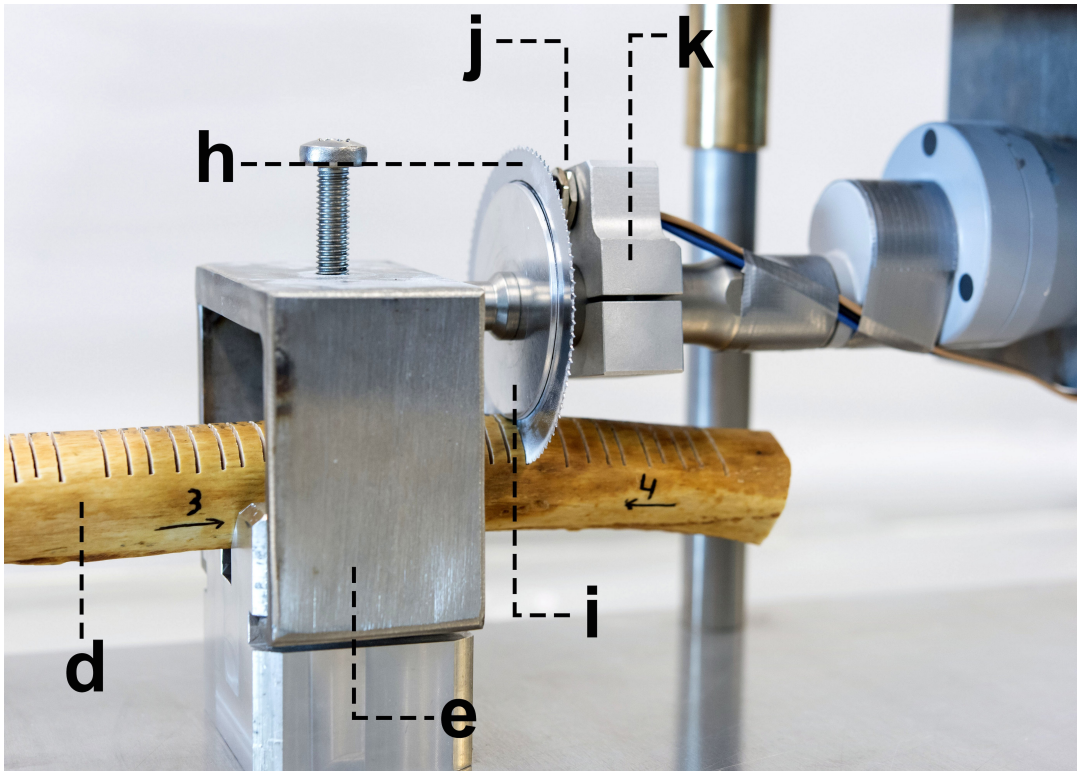


Figure A.4: Close-up of the saw blade and bone specimen, the setup consisted of: the bone specimen (d) was clamped in place by the v-groove holder (e). The saw blade (h) cut in the bone until the stopper (i) reached the bone for a consistent depth of cut. The Hall-effect sensor (j) acted as a tachometer, and was clamped to the saw with an aluminium block (k).

feet (front and back respectively) such as shown in Fig. A.11, stored in a freezer (-20 °C) between 4-9 days and thawed overnight before usage.

e Bone holder: The bone holder consisted of a 70x50x60mm square aluminium block with a v-shaped groove cut out, similar to the cylindrical cross sectional shape of the femora used. An u-shaped clamp with a threaded bolt clamped the femur in the v-groove, securing it to the setup. The holder is shown in detail in Fig. A.4.

g Weights: The weights used in Exp. 1 through 5 were standard cast iron dumbbell weights of 1kg and 2kg. The true weights measured were 1.003kg and 2.004kg respectively. They were placed on top of the vertical sliding platform when needed, and secured with a piece of duct tape to prevent vibrations. The setup itself weighed 3kg, resulting in the possible experimental weights of 3kg, 4kg and 5kg. With neglecting the friction in the sliding platform, the weight of the setup can directly be translated to the contact force of the saw blade on the bone. During the experiments using handsaws (Exp. 6 through 8) it proved impossible to use the weights as constant contact load: the added weight made it very hard to start manual sawing.

i Stopper: To guarantee a consistent depth of cut, a stopper was installed adjacent to the saw blade. The stopper consisted of an approximately 3mm thick, 56mm diameter (10mm less radius than saw blade) aluminium round plate. The thickness of the stopper was enough to halt the saw from descending beyond 10mm depth of cut, so the cuts through the bone were as consistently as possible regarding any differences in bone morphology. During preliminary testing the contact between stopper and bone surface showed no influence on the total production of aerosol. Additionally, the saw was shut down and sawing time was recorded as soon as the stopper hit the bone. Fig. A.4 shows the saw blade and stopper reaching its limit after a cut.

j, k Tachometer: A custom-built tachometer, as shown in Fig. A.4 was used to accurately set the initial frequency of each experiment, as well as to observe the frequency during cutting. Due to the influences of drag forces, the true frequency of the saw blade was expected to be lower during sawing than the

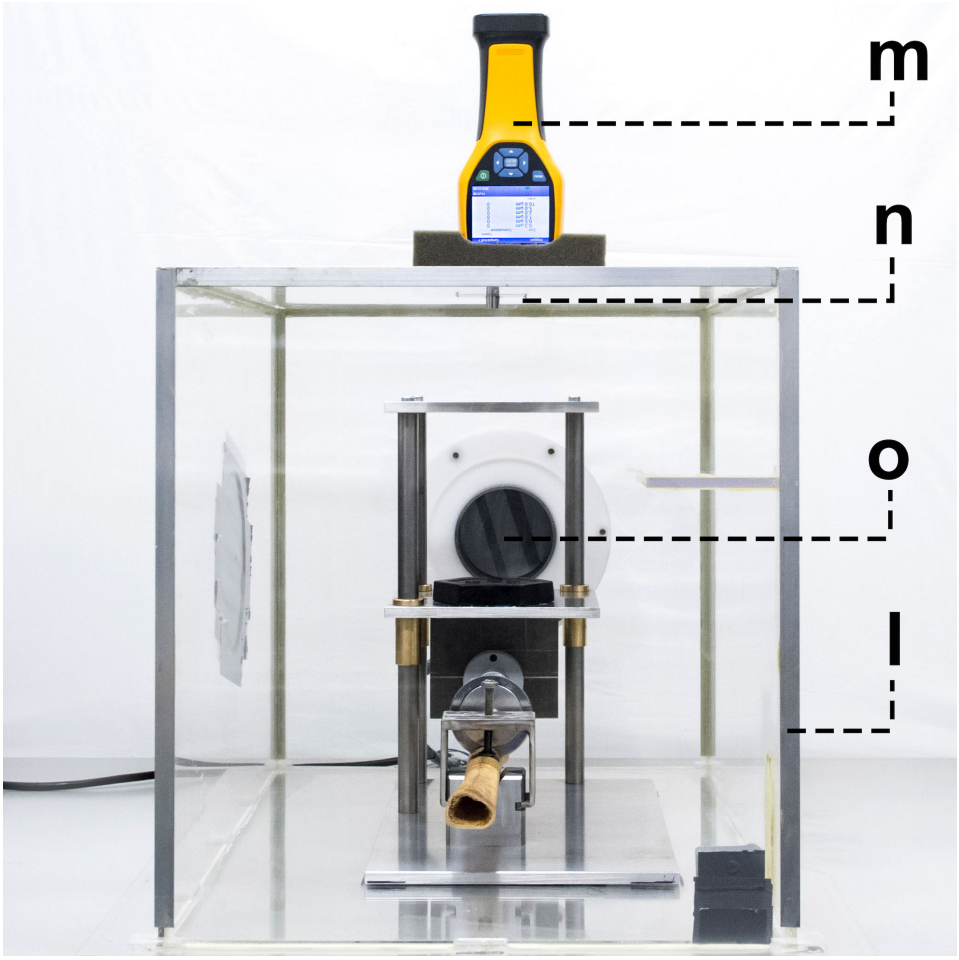


Figure A.5: Front view of the setup enclosed in the box; an acrylic glass box (l) was used to create an experimental space isolated from the environment. The Fluke 985 particle counter (m) was placed on top of the box with a foam cast, with the nozzle inserted into the box through a hole on top of the box (n). A closable hole with a socketed cap was used for handling the saw during operations inside the box (o).

initial frequency. The first iteration was designed and made by the second group of TU Delft bachelor students (van Doeveren et al. (2014) [2]), and adapted to fit on the new setup. The tachometer used a Hall-effect sensor (Geartooth speed sensor GS100501, Cherry switches) that detected changes in the magnetic field. A strip of magnetic metal was fixated to the saw blade so that it oscillated approximately 1mm away from the Hall-effect sensor. The sensor was connected to an Arduino circuit board (Arduino UNO R2, arduino.cc) that registered the input from the sensor over time, so a frequency could be calculated. The tachometer was held in place by an aluminium block clamped to the shaft of the saw as seen in Fig. A.4. During Exp. 1 the frequency readings from the Arduino were displayed on a LCD panel, variations during sawing were manually recorded. During Exp. 2 through 5 the frequency readings from the Arduino were logged into a data text file on a PC with the help of Arjan van Dijke, which made the sampling frequency much higher.

l, o Acrylic glass Box: During Exp. 1 through 3 and 6 through 8 the setup was placed inside an acrylic glass box with dimensions of 780x470x500mm, as seen in Fig. A.5. A particle counter was placed on top of the box with a foam cast to hold it in place. A small hole in the acrylic glass provided access to the particle counter's sensor. The distance between the bone specimen and the particle counter's sensor was about 450mm, to replicate the breathing zone of the saw operator. A hole in the side of the box provided access to the setup without having to open the box and cause any disturbance during the measurements. When the hole was not being used, a lid was used to seal it. By conducting the experiment inside a closed environment, it was possible to minimise the invasion of foreign aerosol inside the box, minimise the leakage of produced particles, and reduce the disturbance of the air flow

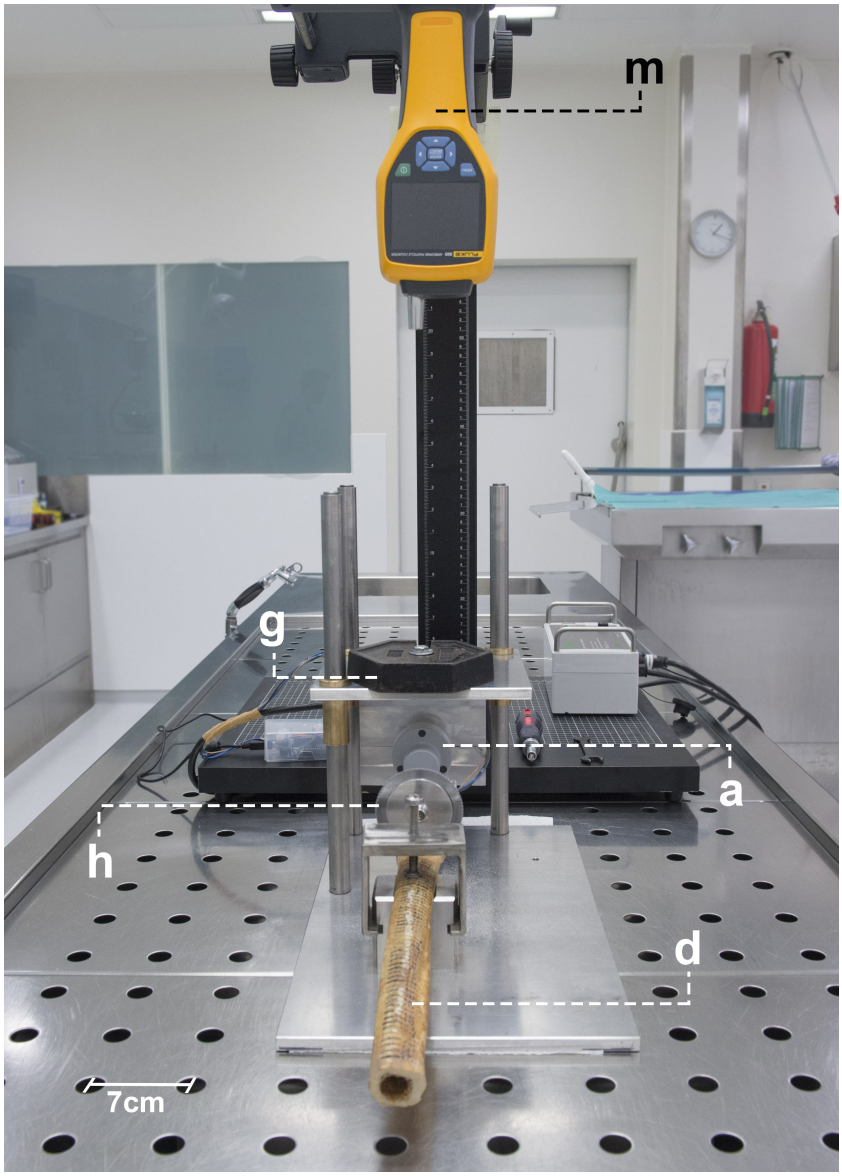


Figure A.6: Front view of the setup; the sliding platform was placed directly on a 100x310cm custom built autopsy table that was equipped with a built-in ventilation system with a ventilation capacity of 3000 $m^3$/h through 252, 2cm diameter holes spaced 7cm apart.

from external interactions, such as walking by, or any other air flows. Also, it was more convenient and time saving to clean the inside of the box than the entire autopsy room. The attachment of the gloves has been modified. A new thicker piece of acrylic replaces the cracked one, and an extra piece of pvc piping of a slightly bigger diameter so that they can easily be slid over the existing pvc entrances to the box. It was decided not to use the gloves, but instead just close the cap quickly after the start of the measurement.

m, n Particle Counter: The aerosol production measurements were carried out using a Fluke 985 particle counter (Fluke corporation, Everett, Washington USA) calibrated by the manufacturer. The particle counter used a light source of 775 nm to 795 nm, 90 mW class 3B laser to detect a size range of particles coming from a flow controlled nozzle. Counted particles were divided in six different size ranges: 0.3, 0.5, 1.0, 3.0, 5.0, and 10 µm at a flow of 0.1cfm (0.1 cubic foot per minute, equivalent to 2.83l/m or 4.72 x$10^{-5}$$m^3$/s). It was decided that measurements over 10 µm were not of relevance to this study, as particles in this size range are most likely to deposit in the head airway region of the respiratory tract, whereas smaller particles will primarily deposit in the alveoli. During the experiments without the acrylic box (Exp. 4 and 5) the particle counter was suspended on the same height from the cutting sam-

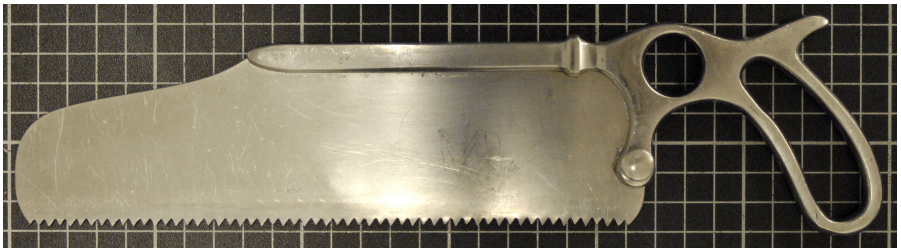

Figure A.7: Satterlee type bone saw used in Exp. 6 'DCB' and Exp. 7 'GCB', with a 200x60x0.8mm, 9 teeth per inch saw blade (FH325R, Aesculap AG, Germany). Each square of the gridline background is 10x10mm.

ple as with the other experiments by a photography stand (Kaiser RS1, Kaiser fototechnik, Germany).

- Satterlee type bone handsaw: A standard 290mm Satterlee type bone saw (FH325R, Aesculap AG, Germany) with a 200x60x0.8mm, 9 teeth per inch saw blade was used in Exp. 6 and Exp. 7.

- Metal handsaw: A hack saw with a 300x13x0.65mm, 18 teeth per inch, metal saw blade (Phantom, Van Ommen B.V., The Netherlands) was used in Exp. 8.

- Metronome: An digital metronome (`https://www.google.com/search?q=metronome`) was used at 87 BPM and 145 BPM to aid achieving constant sawing speeds during manual sawing, where every beat would indicate a change in sawing direction. The blades were limited to a sawing range of 110mm. This corresponded with 1/10th of the electric oscillating saw blade speeds, and fell within the range of commonly used fast and slow sawing motions. As the blades had different dimensions, the saw blade frequency was converted to saw blade speed for proper comparison.

- Autopsy table: The experiment set under active ventilation (Exp. 5 'DAE') was conducted in an 6x7x3.5m autopsy room. The sliding platform was placed directly on a 100x310cm custom built autopsy table (Elcee Holland BV, Dordrecht, The Netherlands) that was equipped with a built-in ventilation system with a ventilation capacity of 3000 $m^3$/h through 252, 2cm diameter holes spaced 7cm apart, such as shown in Fig. A.6.

- Scale: The scale (Kern EMB 600-2, Kern & Sohn GmbH) was placed next to the acrylic glass box, so that the bone could be weighed directly after each cut. The weight of the bone after the antecedent cut was assumed to be the weight of the bone before the next cut.

- Temperature and RH: A simple temperature and relative humidity sensor (Medisana 60079 HG 100, Medisana) that also included a digital clock, was mounted at a central spot in the room where the testing took place, making sure that no external heat sources, lamps, or previous measurements could influence its sensors.

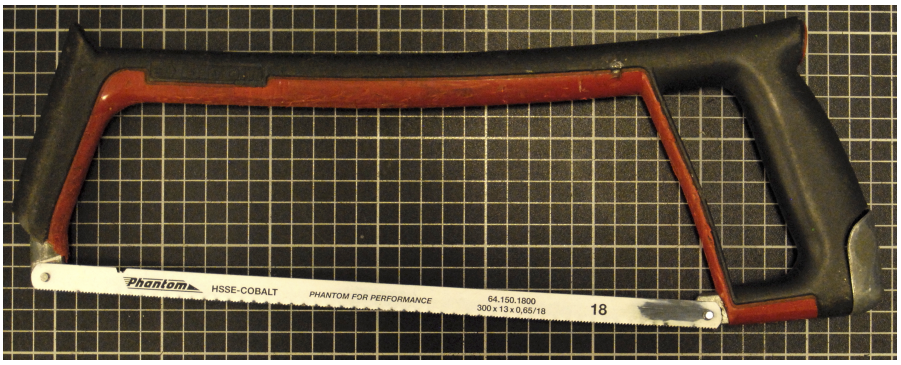


Figure A.8: Hack saw used in Exp. 9 'GCM', with a 300x13x0.65mm, 18 teeth per inch, metal saw blade (Phantom, Van Ommen B.V., The Netherlands). Each square of the gridline background is 10x10mm.

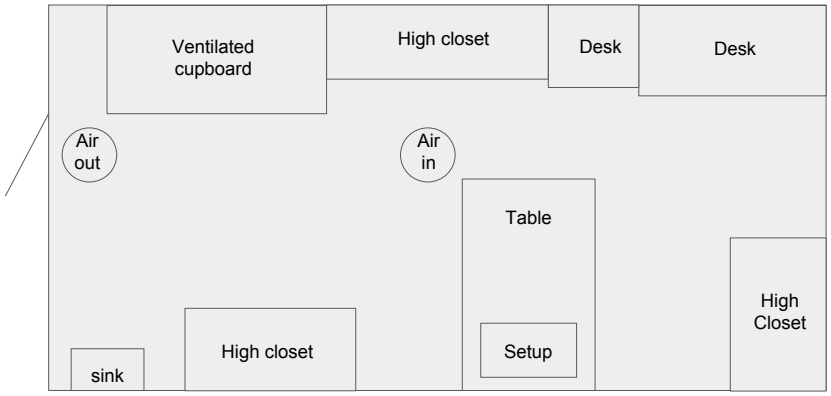


Figure A.9: Schematic overview of the room at the Netherlands Forensic Institute in which most of the experiments took place. Only during Exp. 4 'DOE', the acrylic box was not used around the setup so that the influence of the sawing environment was studied. The room was approximately 4x8x3.5m and was usually used for storage (closets) and basic anthropological examinations (table).

## A.2. Experimental protocol

To allow for consistent and time efficient testing, a strict experimental protocol was compiled and carried out. This included several practice and validation runs (described below in Chapter B, and a tactical placement of relevant tools, such as the computer, notepads, scale, temperature and humidity sensor etc.

Worksheets were prepared to note all relevant information during testing. This included the date and time of the start of each test run, the temperature and relative humidity at that moment, the weight of the bone before and after each cut, and the imported data from the particle counter. A simple temperature and relative humidity sensor (Medisana 60079 HG 100, Medisana) that also included a digital clock, was mounted at a central spot in the room where the testing took place, making sure that no external heat sources, lamps, or previous measurements could influence its sensors. The scale (Kern EMB 600-2, Kern & Sohn GmbH) was placed next to the acrylic glass box, so that the bone could be weighed directly after each cut. The weight of

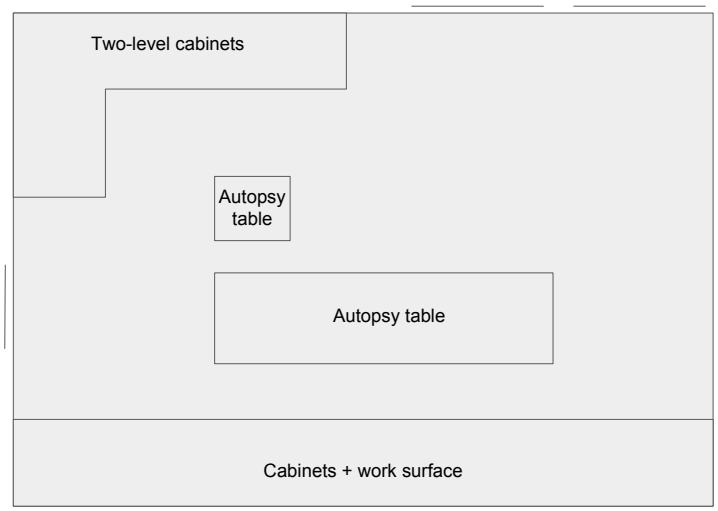


Figure A.10: Schematic overview one of the autopsy rooms at the Netherlands Forensic Institute, in which Exp. 5 'DAE' took place to study the influence of forced air ventilation. The room was approx. 6x7x3.5m and contained a 1x3.1m custom built autopsy table (Elcee Holland BV, Dordrecht, The Netherlands), along with a smaller approx. 1x1m extension table. Several standard sized kitchen-style cabinets allowed for storage and extra work surface. The door on the left gave access to the hallways and dressing rooms, the doors on the top gave access to the second autopsy room, and a service elevator to the cooled storage respectively.

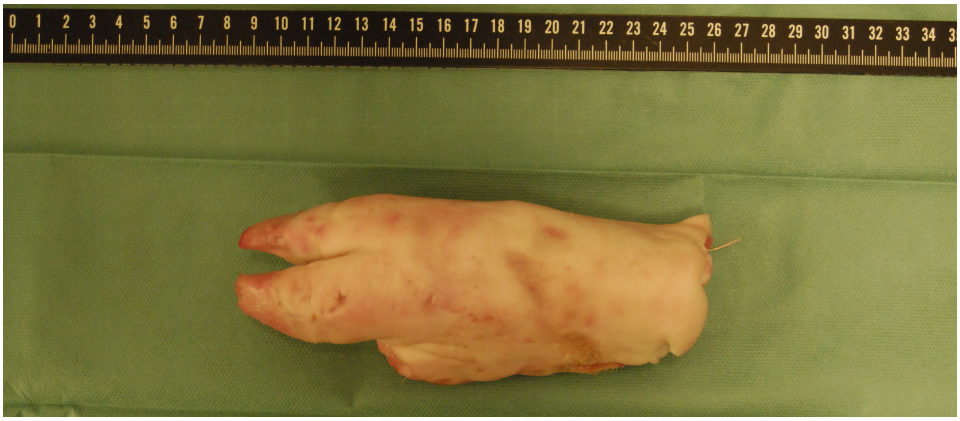


Figure A.11: Top view of one of the porcine specimen that was used. The metacarpals and metatarsal bones were removed from the feet before testing.

the bone after the antecedent cut was assumed to be the weight of the bone before the next cut.

The locations for the cuts were marked on the bone specimen, along with the respective experimental condition notation. Additionally, an overview of the order of experiments and their experimental conditions was hung in sight for quick reference during testing. This helped ensuring that before each cut, the experimental conditions were correctly set. The order of cuts was a randomised blocked design, so that any unwanted influences would be averaged out. The blocks were defined such that each set of experimental conditions would be tested within the closest proximity of each other (both location on the bone and with respect to time). Within the blocks the order of experimental condition was randomised.

The following general experimental protocol was used for most of the tested parameters, with some changes to accommodate for the specific differences required for the various experiments.

### A.2.1. Sawing protocol

The bone was inserted and fastened so the saw blade lined up with the prepared pen marking on the bone. During the experiments with the electrical oscillating saw, the desired frequency was selected with the use of the tachometer and the saw control panel, and weights were added to the sliding platform following the specific tested EC. The box was closed with the particle counter inserted on top (during the experiments in the open environment this step was skipped). Room temperature, room relative humidity, date and time were recorded. The particle counter protocol was started, as described below. At the start of M1 the saw was lifted, switched on and gently brought down to contact the bone and then left to freely move down, until the stopper hit the surface of the bone. Then the saw was switched off. The time between the starting of the saw, and the moment the stopper hit the bone was recorded. During the experiments using handsaws, the sawing stopped after the depth of cut was reached, or 1 minute had passed.

After the particle counter protocol was finished, the cleaning protocol started, as described below.

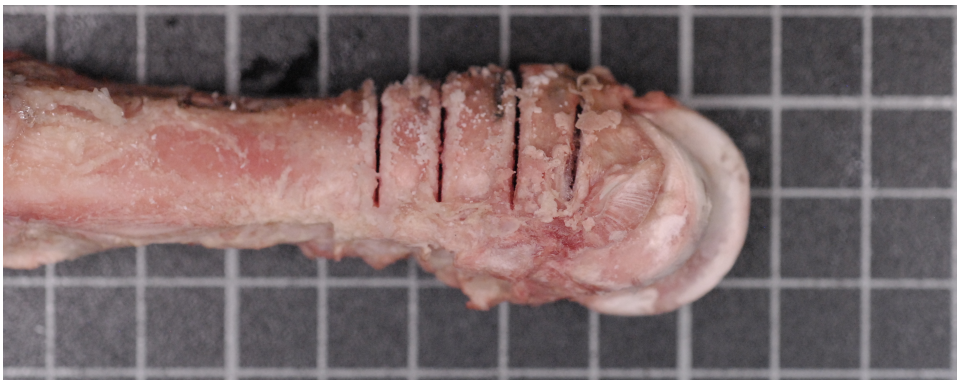

Figure A.12: Side view of one of the porcine specimen used in Exp. 3 'FCE', with 4 cuts. Each square of the gridline background is 10x10mm.

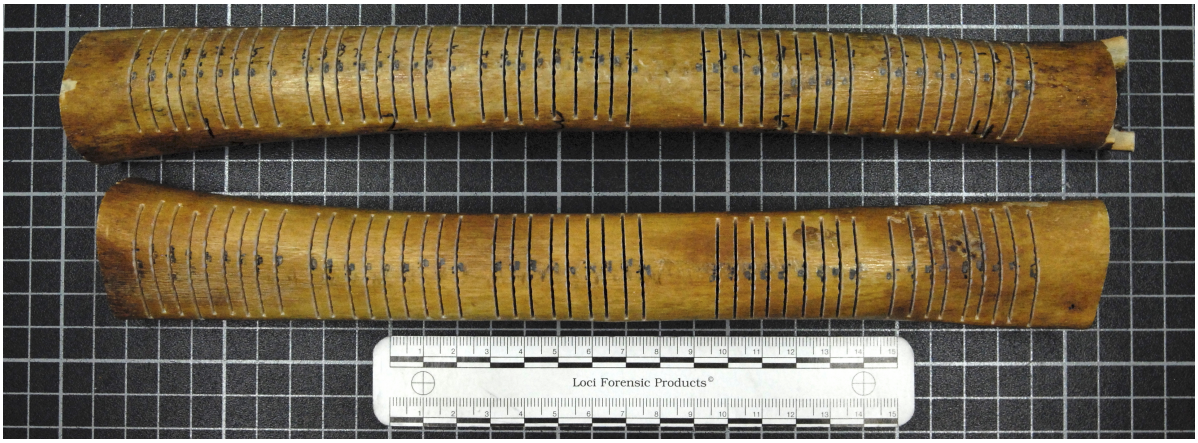


Figure A.13: Top view of femora A (bottom) and B (top) that were used in Exp. 1 'DCE', both were in dry condition (Cat. D.4 [3]). Each square of the gridline background is 10x10mm.

### A.2.2. Particle counter protocol

The Fluke 985 particle counter (Fluke corporation, Everett, Washington USA) was programmed for 7 measurements of 60 seconds each. Each measurement was coded as M0, M1, ... , M6. The base measurement (M0) recorded the base levels of particles already suspended inside the box directly after closing the box. This could include both residual aerosol from previous tests, or foreign aerosol from the room or the cleaning process. Measurements M1-M6 recorded the suspension and settling down of particles from sawing. After each test run, the particle counter was purged using the manufacturer's filter to guarantee that residual particles in the particle counter were not counted again.

### A.2.3. Cleaning protocol

Once measurement M6 was finished, the inside of the box was vacuumed for 1 minute via the side opening of the box to avoid scattering of unwanted particles to the outside environment. Next, the box was lifted, the bone specimen was removed from the clamp, vacuum cleaned to remove residual dust, and weighed. Both the box and the setup were vacuum cleaned and wiped off using fresh multi-purpose disinfectant wipes, so all residual particles were removed. Both the box and setup were dry with kitchen paper and further left to air-dry for 2 minutes, after which the sawing protocol for the next run could start. Validation testing showed less suspended fine dust particles in the box after cleaning than present in the environment outside the box.

Figure A.14: Top view of femora C (bottom) and D (top) that were used in Exp. 2 and 4 through 8. Femur C was in dry condition (Cat. D.4 [3]), femur D still had a greasy adipocere residue (Cat. D.3 [3]). Note that some pieces of bone between cuts in femur D (top) broke off during hand sawing, these were taken into account when weighing the removed bone. Each square of the gridline background is 10x10mm.

### A.2.4. Data protocol

In all analyses, the number of counted aerosol particles was determined over six minutes of measurements (M1 to M6) at a sampling flow rate of 0.1cfm (cubic foot per minute, equivalent to 2.83l/m or 4.72 x$10^{-5}$$m^3$/s). Validation testing such as described in Chapter B showed that 6 minutes of measuring proved sufficient. After the pilot test of Chapter 3 it was decided to normalise the number of produced aerosol particles to the weight of bone that was removed per cut, because of the high variance in removed weight between the human and porcine bone specimens. In Chapter 2 this normalisation was not used, resulting in slight differences in the data of the same experiment (Exp. 1 'DCE') in Chapters 2 and 3, although this had no consequences for the outcome of the statistical tests. The base level (M0) was subtracted from each minute measurement to separate the background aerosol from the aerosols that were actually generated by sawing. The data reported in Table 3.10, and Fig. 3.7 and 3.8 is displayed in number of particles per 0.1 cubic foot per minute [n/0.1cfm]. Before the statistical analyses, the six minutes of measurements (M1 to M6) were summed and stacked (a layer for each repetition) per EC as seen in Fig. 3.9, and are displayed in number of particles per 0.6 cubic foot per minute [n/0.6cfm]. Effects were considered significant when $p \leq 0.05$.

The total number of produced particles per EC was calculated by summation of the number of particles produced for each individual particle size. This gives an indication of the total production of particles, and the amount of potential pathways they form for pathogen to reach the forensic practitioners. The total surface area of the produced particles per EC was calculated under the assumption that the particles were spheres, by summation of the number of particles produced for each particle size multiplied by the square of the particle's size times pi. The total surface area of the produced particles gives an indication of the possible amount of pathogen that could be attached to produced particle.

Statistical analyses were performed in two parts using MATLAB (MATLAB 2015a, The MathWorks Inc.). First for one of each of the individual produced particle sizes (0.3, 0.5, 1.0, 2.0, 5.0, and 10µm), the total number of produced aerosol particles, and the total surface area of the produced aerosol particles. In all analyses the production of aerosol was summed over the 6 minutes of measurements (M1 to M6) from the moment sawing was commenced. The base level (M0) was subtracted to separate the background aerosol from the aerosols that were actually generated by sawing. Secondly, a three-way ANOVAN compared the influences of saw blade frequency and saw blade contact load between the eight experiments, thus the influences of the bone condition, the test environment, and the saw blade type as third variable. Effects were considered significant when $p<0.05$.

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# B

# Validation of experimental design

*This appendix is based on my internship report.*
In order to circumvent start up problems during the experiments, as well as to validate the choice of the experimental conditions and protocols, several exploratory tests were performed. As the setup had gone through significant modifications (as described in Chapter A), recommendations and experiences from the previous groups would not be sufficient to forecast all possible complications. A secondary goal of the exploratory tests was to familiarise with pre-established protocols to ensure smooth testing, such as the workings and placement of devices such as the particle counter.

The choice of sawing parameters was validated in the first exploratory tests, followed by an analysis of the interference of cleaning methods with the detection of suspended particles, of which disruption due to humidity levels or residual particles from previous experiments might mislead the counting of particles and hamper base levels (background levels). Furthermore, the spreading and deposition of flour particles over time was analysed to determine how long the produced bone particles should be counted. Finally the functionality of the setup was validated, to see if its construction provided any other challenges and performs accordingly to what was designed.

In the subsequent bar-graphs used to visualise the counted number of aerosol particles under the various conditions (Fig. B.1, B.2, B.3, B.4, B.5), the following things are shown: The z-axis shows the total number of counted aerosol particles, measured with the Fluke 985 particle counter (Fluke corporation, Everett, Washington, USA). This device counts 6 sizes of aerosol particles, ranging from 0.3 µm (dark blue), 0.5µm (blue), 1µm (light blue), 2µm (green), 5µm (orange), to 10µm (yellow). The graph stacks the number of particles of different sizes (colours) together from smallest (0.3 µm) on the bottom to largest (10µm) on top, so that the total number of particles is also shown. The x-axis shows the sequence of testing, in this case the minutes spent during testing. The usual protocol consists of 12, one minute measurements. For the flour tests this was elongated to a 30 minute and 60 minute test. The Y-axis shows the given run, this will be different for each test and will be mentioned in the captions. The runs will be numbered along with a figure for referencing. Some of the graphs have been made transparent, so that later runs can be seen behind previous runs, and to keep the chronological order of testing intact.

## B.1. Sawing Parameters

The choice of sawing parameters was validated by a small number of exploratory tests. These tests are meant to provide a proof of principle, and to limit the chances of failed measurements.

### B.1.1. Saw blade frequency

***Oscillating saw***

The saw blade frequency of the electric oscillating saw could be set by an external control panel by turning a potentiometer, so that any frequency between 30Hz and 250Hz could be chosen. In practice, only the highest setting of 250Hz is chosen, as this provides the fastest and easiest cut. Preliminary testing showed that the saw in the sliding platform was unable to cut autonomously through the bone on frequencies of or lower than 100Hz. The ultimately chosen saw blade frequencies were in line with the ones tested by the bachelor groups in 2013 and 2014, on 150Hz, 200Hz, and 250Hz. It was found that the saw blade was unable to get up to the

correct saw blade frequency when the saw was turned on with the saw blade resting on the bone specimen. Although this was to be expected, it did meant that it was necessary to reach into the closed environment to start the sawing process, which could possibly influence the air flows inside the closed environment. By keeping this motion as constant and as short as possible, the effects of the disturbance to the air in the closed environment are considered to be minimal.

***Hand-saws***
The saw blade frequency of the hand saws was initially set to be as similar as possible to the saw blade frequency of the oscillating saw. However, when sawing by hand a much lower saw blade frequency is used, and as the blades had different dimensions, the saw blade frequency was converted to saw blade speed for proper comparison. It proved that a saw tooth speed of 1/10th of the electric oscillating saw fell into the range of hand saw tooth speeds ordinarily used in practice. So a saw blade frequency equivalent to 15Hz and 25Hz of the electric oscillating saw was achieved by limiting the hand sawing range to 110mm and using a metronome at 87 BPM and 145 BPM, where every beat would indicate a change in sawing direction.

### B.1.2. Saw blade contact load

***Oscillating saw***
The saw blade contact load used in daily practice was obtained by asking the forensic practitioners to test cut a bone sample on a scale. The loads used varied, but were hardly ever larger than 5kg. Within their experience, it was best to not apply too much force, and 'let the saw do the work'. During preliminary testing it was found that the weight of the sliding platform itself (3kg), on the lowest chosen frequency, was enough to let the saw cut autonomously through the bone. The bachelor groups in 2013 and 2014 had used a load of 5kg, although their setup had some limitations in transferring the actual load to the bone (see Chapter A. The resulting loads of 3kg, 4kg and 5kg were chosen, and applied by the sliding platform itself (3k) and two dumbbell weights of 1kg and 2kg.

***Hand-saws***
The saw blade contact load during hand sawing was initially set to be the same as during experiments using the electric oscillating saw. By a small adaptation to the sliding platform, the oscillating saw was removed and a tract was supplied for consistent use of the hand saw. It was found however that any external load on the hand saw caused it to be very hard to start sawing. The saw blade teeth will sink into the bone under the external load, and are very hard to move afterwards. It is possible to start the sawing action prior to reaching the bone, this however resulted in a very awkward and non fluent sawing motion. It was decided to 'let the saw do the work', and use no external saw blade contact load for the manual sawing tests. This did however limit the ability to compare results of manual sawing and the use of the electrical oscillating saw.

## B.2. Cleaning Methods

By the end of every experimental run it is critical to ensure that aerosol leftovers are removed from the inside of the box, as otherwise those particles will be recounted and provide erroneous results. Secondly, the removal of the aerosol residue itself should not influence the particle counter, for instance with the water droplets released by the use of wet towels. To detect the influence of water droplets, two sets of experiments were performed. For the first set of runs, base level of aerosol was measured prior to any cleaning in Run B.1.1. Run B.1.2 followed with sealing of the box immediately after cleaning with wet wipes. Then Run B.1.3 sealing the box after cleaning and air drying (longer than 5 min) and base levels again after letting the box air for longer than 20 min. As seen in Fig. B.1, there is a clear difference between the base level aerosol measurements (Run B.1.1 and B.1.4) and the aerosol measurement when sealing the box immediately after cleaning with wet wipes without drying (Run B.1.2). This suggests that water particles are indeed detectable by the particle counter. During Run B.1.2 a high presence of moisture was visible on the surface of the box of which lasted throughout the entire run. Given that the box has no exit routes from where the humid air could escape, the air inside of the box probably remained saturated hence limiting water evaporation and explaining why particles remained constant in Run B.1.2. In Run B.1.3, aerosol levels drop close to base levels of (Run B.1.1 and B.1.4) indicating that allowing the box to air for at least 5 minutes might be enough to expel unwanted water droplets by themselves. Run B.1.4, similar to Run B.1.1 was a base level of the box after allowing it to dry for longer than 20 min, since it was done last there is a possibility that some water droplets from previous cleaning were still present in the room where the experiment was conducted, which explains why the base levels was slightly higher than Run B.1.1, and remained constant over the 12 minutes of measurements.

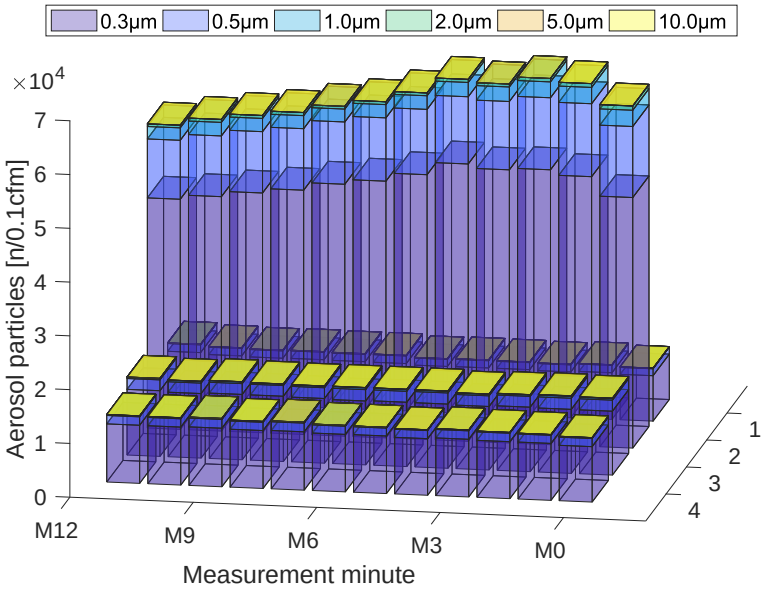



Figure B.1: The total number of aerosol particles is plotted in a stacked bar graph (stacked from smallest particles on bottom to largest on top: 0.3 µm (dark blue), 0.5µm (blue), 1µm (light blue), 2µm (green), 5µm (orange), to 10µm (yellow)). This test consisted of 4 runs over 12 minutes. Run B.1.1 describes aerosol measurements of the base level, B.1.2 shows aerosol measurements of the cleaning with wet wipes without drying, B.1.3 shows aerosol measurements of the cleaning with wet wipes with drying, and B.1.4 again describes aerosol measurements of the base level.

The second and final set analyses the impact of using kitchen rolls to remove water particles from the box. In Fig. B.2, Run B.2.2 shows the effect of cleaning with wet wipes, using the paper towels then sealing the box immediately (no air drying time). Results seem satisfactory as total aerosol level drops drastically compared to Run B.2.1. The efficacy of using paper towel suggests that air drying time can be reduced, allowing room for more experiments in the same amount of time.

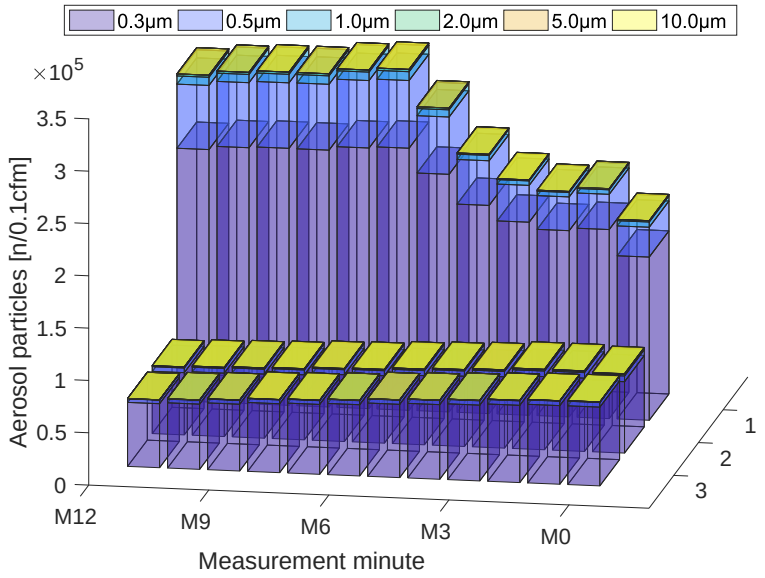



Figure B.2: The total number of aerosol particles is plotted in a stacked bar graph (stacked from smallest particles on bottom to largest on top: 0.3 µm (dark blue), 0.5µm (blue), 1µm (light blue), 2µm (green), 5µm (orange), to 10µm (yellow))). This test consisted of 3 runs over 12 minutes. Run B.2.1 describe aerosol measurements of cleaning with wet wipes without drying. Run B.2.2 and B.2.3 show cleaning with wet wipes and drying with paper towel, and base level measurements respectively.

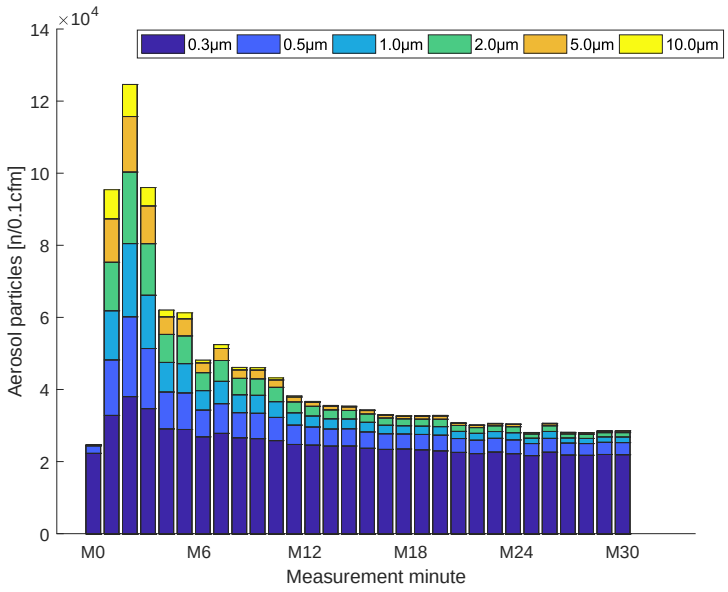



Figure B.3: The total number of aerosol particles is plotted in a stacked bar graph (stacked from smallest particles on bottom to largest on top: 0.3 µm (dark blue), 0.5µm (blue), 1µm (light blue), 2µm (green), 5µm (orange), to 10µm (yellow)). This test consisted of 1 run over 30 minutes.

## B.3. Dust Settling Time

To find out how long the measurements of the number of particles in the air should continue, a simple test using common baking flour had been set up. Just as during the proposed bone sawing tests, the first minute of the test was a base level test, to see the number of aerosol particles currently in the box. These can be used to correct the subsequent measurements for any deviations in the room. After a minute, a small number of flour particles was introduced in the box, using a glove filled with a set amount of flour and by shooting it into the box. Although the amount of (aerosol) particles will be different between bone saw dust and flour, the assumption was made that the extracted information is transferable. The same test was performed

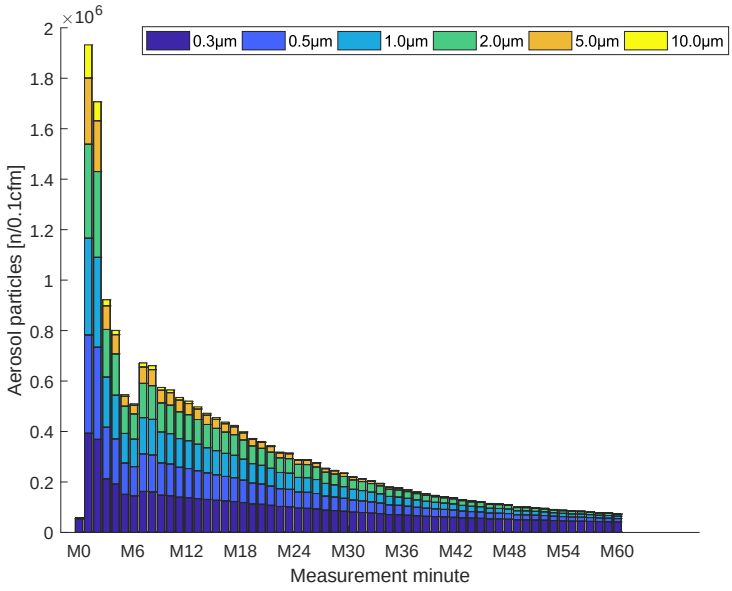



Figure B.4: The total number of aerosol particles is plotted in a stacked bar graph (stacked from smallest particles on bottom to largest on top: 0.3 µm (dark blue), 0.5µm (blue), 1µm (light blue), 2µm (green), 5µm (orange), to 10µm (yellow)). This test consisted of 1 run over 60 minutes.

twice, once measuring for 30 minutes (Fig. B.3) and once measuring for 60 minutes (Fig. B.4). Both used the same protocol, although the amount of flour used was not carefully compared. As shown in both figures, the number of particles increases quickly after insertion of the flour, and then slowly settles down. After 5 minutes, the number of particles has halved, but it takes many more minutes for all of the dust to settle down. Although the base levels (the first minute of each test) are very different, both show the amount of dust at the end of the measurement has not reached the same level as at the start of the test. As this study only looks into the relative number of aerosol particles produced when comparing different variable settings, there does not seem to be a need to record the number of particles for longer than 5 minutes after sawing. Continuing measuring over time will only provide some information about the behaviour of particles in this specific box, but would hardly be transferable to a different situation, e.g. the room itself. It was concluded from these tests that a measurement protocol of 7 minutes (including one minute of base level measurements) should suffice.

## B.4. Vacuuming Influence

A last test was conducted where a vacuum cleaner was used for one minute, after 5 minutes of testing, while the measurement continued. This shows the added use of vacuuming the box after the tests have been conducted, to limit the amount of aerosol that will spread when opening the box, and limiting the amount of dust that needs to be removed by cleaning with wet wipes considerably.

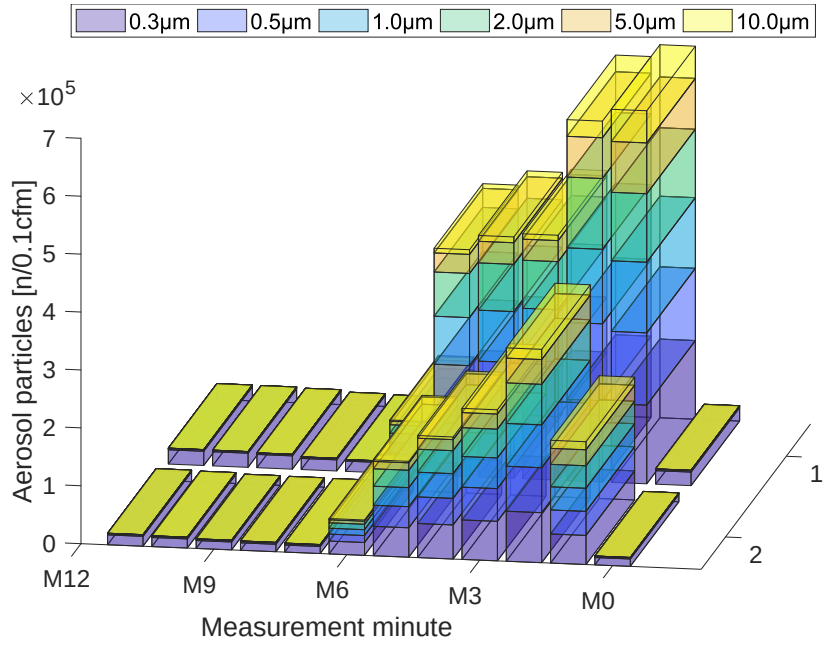



Figure B.5: The total number of aerosol particles is plotted in a stacked bar graph (stacked from smallest particles on bottom to largest on top: 0.3µm (dark blue), 0.5µm (blue), 1µm (light blue), 2µm (green), 5µm (orange), to 10µm (yellow)). This test consisted of 2 runs over 12 minutes, with both had the vacuum activated after 5 minutes.

# C

# Data of all experiments

## C.1. Tables: 2-way ANOVA Exp. 1 though 8

Table C.1: Exp. 1 'DCE': p-values of the effects of saw blade frequency and saw blade contact load on the number of aerosol particles produced per particle size, the total number of particles, and the total surface area of particles. Significant values are printed in italics.

| Exp. 1 'DCE' | Particle size | | | | | | Total number of particles | Total surface area of particles |
|---|---|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| Effect of saw blade frequency | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.05* | *p<0.001* | *p<0.001* |
| Effect of saw blade contact load | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Interaction effect | p=0.89 | p=0.73 | p=0.30 | p=0.10 | p=0.08 | p=0.20 | p=0.42 | p=0.11 |

Table C.2: Exp. 2 'GCE': p-values of the effects of saw blade frequency and saw blade contact load on the number of aerosol particles produced per particle size, the total number of particles, and the total surface area of particles. Significant values are printed in italics.

| Exp. 2 'GCE' | Particle size | | | | | | Total number of particles | Total surface area of particles |
|---|---|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| Effect of saw blade frequency | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.05* | *p<0.05* | *p<0.001* | *p<0.05* |
| Effect of saw blade contact load | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Interaction effect | p=0.46 | p=0.79 | p=0.91 | p=0.98 | p=0.69 | p=0.47 | p=0.84 | p=0.59 |

Table C.3: Exp. 3 'FCE': p-values of the effects of saw blade frequency and saw blade contact load on the number of aerosol particles produced per particle size, the total number of particles, and the total surface area of particles. Significant values are printed in italics.

| Exp. 3 'FCE' | Particle size | | | | | | Total number of particles | Total surface area of particles |
|---|---|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| Effect of saw blade frequency | *p<0.05* | *p<0.05* | *p<0.05* | p=0.05 | p=0.09 | p=0.11 | *p<0.05* | p=0.06 |
| Effect of saw blade contact load | *p<0.05* | p=0.05 | p=0.07 | p=0.09 | p=0.17 | p=0.23 | p=0.05 | p=0.12 |
| Interaction effect | p=0.06 | p=0.07 | p=0.07 | p=0.07 | p=0.05 | p=0.06 | p=0.06 | p=0.06 |

Table C.4: Exp. 4 'DOE': p-values of the effects of saw blade frequency and saw blade contact load on the number of aerosol particles produced per particle size, the total number of particles, and the total surface area of particles. Significant values are printed in italics.

| Exp. 4 'DOE' | Particle size | | | | | | Total number of particles | Total surface area of particles |
|---|---|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| Effect of saw blade frequency | p=0.21 | p=0.62 | p=0.96 | p=0.66 | p=0.33 | p=0.29 | p=0.75 | p=0.35 |
| Effect of saw blade contact load | p=0.16 | p=0.38 | p=0.55 | p=0.70 | p=0.96 | p=0.94 | p=0.40 | p=1.00 |
| Interaction effect | p=0.13 | p=0.15 | p=0.19 | p=0.24 | p=0.34 | p=0.39 | p=0.16 | p=0.35 |

Table C.5: Exp. 5 'DAE': p-values of the effects of saw blade frequency and saw blade contact load on the number of aerosol particles produced per particle size, the total number of particles, and the total surface area of particles. Significant values are printed in italics.

| Exp. 5 'DAE' | Particle size | | | | | | Total number of particles | Total surface area of particles |
|---|---|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| Effect of saw blade frequency | p=0.58 | p=0.32 | p=0.36 | p=0.43 | p=0.98 | p=0.58 | p=0.48 | p=0.88 |
| Effect of saw blade contact load | p=0.59 | p=0.57 | p=0.88 | p=0.46 | p=0.69 | p=0.55 | p=0.70 | p=0.60 |
| Interaction effect | p=0.96 | p=0.93 | p=0.52 | p=0.13 | p=0.50 | p=0.63 | p=0.97 | p=0.56 |

Table C.6: Exp. 6 'DCB': p-values of the effects of saw blade frequency and saw blade contact load on the number of aerosol particles produced per particle size, the total number of particles, and the total surface area of particles. Significant values are printed in italics.

| Exp. 6 'DCB' | Particle size | | | | | | Total number of particles | Total surface area of particles |
|---|---|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| Effect of saw blade frequency | p=0.23 | p=0.20 | p=0.18 | p=0.17 | p=0.18 | p=0.22 | p=0.20 | p=0.17 |

Table C.7: Exp. 7 and 8: p-values of the effects of saw blade frequency and saw blade contact load on the number of aerosol particles produced per particle size, the total number of particles, and the total surface area of particles. Significant values are printed in italics.

| Exp. 7 and 8 | Particle size | | | | | | Total number of particles | Total surface area of particles |
|---|---|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| Effect of saw blade frequency | p=0.05 | p=0.06 | p=0.09 | p=0.17 | p=0.68 | p=0.74 | p=0.08 | p=0.80 |
| Effect of saw blade type | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.05* | *p<0.05* | *p<0.001* | *p<0.05* |
| Interaction effect | p=0.16 | p=0.21 | p=0.32 | p=0.49 | p=0.86 | p=0.51 | p=0.24 | p=0.80 |

## C.2. Tables: 3-way ANOVAN between Experiments

Table C.8: ANOVA: p-values of the comparisons between the different experiments. Significant values are printed in italics.

| | Particle size | | | | | |
|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm |
| Bone condition | | | | | | |
| Exp. 1 vs Exp. 2 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Exp. 1 vs Exp. 3 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Exp. 2 vs Exp. 3 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Exp. 6 vs Exp. 7 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Test environment | | | | | | |
| Exp. 1 vs Exp. 4 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Exp. 1 vs Exp. 5 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Exp. 4 vs Exp. 5 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Saw blade type | | | | | | |
| Exp. 1 vs Exp. 6 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Exp. 1 vs Exp. 7/8 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |
| Exp. 2 vs Exp. 7/8 | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* | *p<0.001* |

## C.3. Tables: Relative Humidity vs Base level

Table C.9: Relative Humidity vs Base level: p-values of the effects of ambient Relative Humidity (RH) on the number of aerosol particles in the base level measurement (M0) per particle size, the total number of particles, and the total surface area of particles. Significant values are printed in italics.

| | Particle size | | | | | | Total number of particles | Total surface area of particles |
|---|---|---|---|---|---|---|---|---|
| | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| Effect of Relative Humidity | *p<0.001* | *p<0.001* | *p=0.014* | *p=0.034* | p=0.18 | p=0.096 | *p<0.001* | p=0.89 |

# C.4. Table: Results Exp. 1 through 8

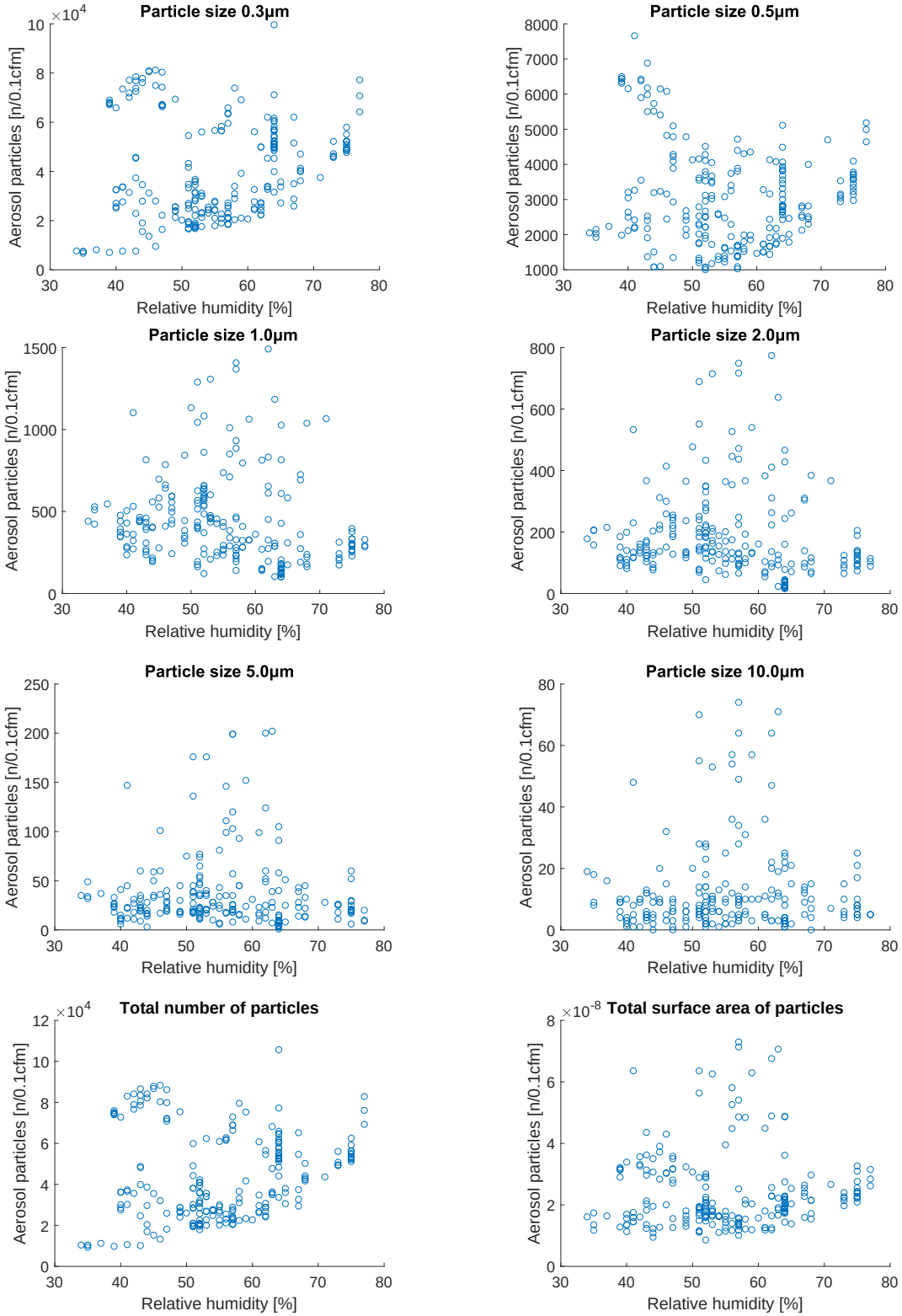
Table C.10: The means and standard deviations of the counted number of particles for each particle size and all experimental conditions.

| | | Number of particles per size [n/0.1cfm] | | | | | | Total number of particles [n/0.1cfm] | Total surface area of particles [$m^2$/0.1cfm] |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.3µm | 0.5µm | 1.0µm | 2.0µm | 5.0µm | 10.0µm | | |
| | | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| Exp. 1 'DCE' | Base level | ***(3.84 ± 2.40 ) x10$^4$*** | ***(3.52 ± 1.67 ) x10$^3$*** | ***(4.63 ± 1.76 ) x10$^2$*** | ***(1.82 ± 0.857) x10$^2$*** | ***(2.92 ± 2.05 ) x10$^1$*** | ***(7.69 ± 7.04 ) x10$^0$*** | ***(4.26 ± 2.56 ) x10$^4$*** | ***(2.21 ± 0.986) x10$^{-8}$*** |
| EC 1.1 | 3kg, 150Hz | ***(3.10 ± 0.339) x10$^6$*** | ***(2.99 ± 0.254) x10$^6$*** | ***(2.40 ± 0.194) x10$^6$*** | ***(1.85 ± 0.183) x10$^6$*** | ***(7.13 ± 1.28 ) x10$^5$*** | ***(2.94 ± 0.683) x10$^5$*** | ***(1.13 ± 0.080) x10$^7$*** | ***(1.82 ± 0.336) x10$^{-4}$*** |
| EC 1.3 | 3kg, 250Hz | ***(3.99 ± 0.732) x10$^6$*** | ***(3.89 ± 0.626) x10$^6$*** | ***(3.19 ± 0.429) x10$^6$*** | ***(2.49 ± 0.302) x10$^6$*** | ***(9.20 ± 1.60 ) x10$^5$*** | ***(3.51 ± 0.882) x10$^5$*** | ***(1.48 ± 0.207) x10$^7$*** | ***(2.28 ± 0.428) x10$^{-4}$*** |
| EC 3.1 | 5kg, 150Hz | ***(2.62 ± 0.412) x10$^6$*** | ***(2.41 ± 0.319) x10$^6$*** | ***(1.87 ± 0.248) x10$^6$*** | ***(1.40 ± 0.207) x10$^6$*** | ***(5.16 ± 0.983) x10$^5$*** | ***(2.06 ± 0.389) x10$^5$*** | ***(9.02 ± 1.17 ) x10$^6$*** | ***(1.31 ± 0.228) x10$^{-4}$*** |
| EC 3.3 | 5kg, 250Hz | ***(3.18 ± 0.523) x10$^6$*** | ***(2.95 ± 0.432) x10$^6$*** | ***(2.27 ± 0.293) x10$^6$*** | ***(1.67 ± 0.212) x10$^6$*** | ***(5.74 ± 1.03 ) x10$^5$*** | ***(2.18 ± 0.529) x10$^5$*** | ***(1.09 ± 0.139) x10$^7$*** | ***(1.45 ± 0.262) x10$^{-4}$*** |
| Exp. 2 'GCE' | Base level | ***(3.24 ± 1.14 ) x10$^4$*** | ***(1.75 ± 0.557) x10$^3$*** | ***(3.19 ± 1.30 ) x10$^2$*** | ***(1.40 ± 0.730) x10$^2$*** | ***(3.36 ± 1.68 ) x10$^1$*** | ***(1.34 ± 0.477) x10$^1$*** | ***(3.47 ± 1.20 ) x10$^4$*** | ***(2.02 ± 0.549) x10$^{-8}$*** |
| EC 1.1 | 3kg, 150Hz | ***(2.57 ± 0.240) x10$^6$*** | ***(2.40 ± 0.301) x10$^6$*** | ***(1.86 ± 0.311) x10$^6$*** | ***(1.41 ± 0.285) x10$^6$*** | ***(5.65 ± 1.50 ) x10$^5$*** | ***(2.54 ± 0.790) x10$^5$*** | ***(9.06 ± 1.35 ) x10$^6$*** | ***(1.50 ± 0.412) x10$^{-4}$*** |
| EC 1.3 | 3kg, 250Hz | ***(3.56 ± 0.422) x10$^6$*** | ***(3.37 ± 0.463) x10$^6$*** | ***(2.65 ± 0.427) x10$^6$*** | ***(2.00 ± 0.358) x10$^6$*** | ***(7.37 ± 1.43 ) x10$^5$*** | ***(3.05 ± 0.675) x10$^5$*** | ***(1.26 ± 0.183) x10$^7$*** | ***(1.91 ± 0.371) x10$^{-4}$*** |
| EC 3.1 | 5kg, 150Hz | ***(1.94 ± 0.262) x10$^6$*** | ***(1.58 ± 0.249) x10$^6$*** | ***(1.10 ± 0.210) x10$^6$*** | ***(7.69 ± 1.65 ) x10$^5$*** | ***(2.78 ± 0.776) x10$^5$*** | ***(1.19 ± 0.408) x10$^5$*** | ***(5.79 ± 0.966) x10$^6$*** | ***(7.42 ± 2.16 ) x10$^{-5}$*** |
| EC 3.3 | 5kg, 250Hz | ***(2.72 ± 0.286) x10$^6$*** | ***(2.47 ± 0.337) x10$^6$*** | ***(1.85 ± 0.296) x10$^6$*** | ***(1.35 ± 0.240) x10$^6$*** | ***(4.95 ± 1.04 ) x10$^5$*** | ***(2.12 ± 0.512) x10$^5$*** | ***(9.10 ± 1.29 ) x10$^6$*** | ***(1.31 ± 0.280) x10$^{-4}$*** |
| Exp. 3 'FCE' | Base level | ***(4.16 ± 1.48 ) x10$^4$*** | **(2.72 ± 1.13 ) x10$^3$** | **(2.37 ± 0.695) x10$^2$** | (9.75 ± 3.27 ) x10$^1$ | (2.01 ± 1.04 ) x10$^1$ | (7.23 ± 4.91 ) x10$^0$ | **(4.46 ± 1.60 ) x10$^4$** | (1.97 ± 0.637) x10$^{-8}$ |
| EC 1.1 | 3kg, 150Hz | ***(9.93 ± 10.1 ) x10$^4$*** | **(4.97 ± 5.24 ) x10$^4$** | **(1.82 ± 2.20 ) x10$^4$** | (7.25 ± 9.58 ) x10$^3$ | (8.10 ± 12.0 ) x10$^2$ | (1.78 ± 2.28 ) x10$^2$ | **(1.75 ± 1.85 ) x10$^5$** | (3.35 ± 4.21 ) x10$^{-7}$ |
| EC 1.3 | 3kg, 250Hz | ***(4.75 ± 4.74 ) x10$^5$*** | **(2.46 ± 2.72 ) x10$^5$** | **(1.02 ± 1.25 ) x10$^5$** | (4.68 ± 6.11 ) x10$^4$ | (6.94 ± 9.69 ) x10$^3$ | (1.55 ± 2.20 ) x10$^3$ | **(8.79 ± 9.39 ) x10$^5$** | (2.27 ± 2.91 ) x10$^{-6}$ |
| EC 3.1 | 5kg, 150Hz | ***(9.28 ± 6.45 ) x10$^4$*** | **(4.39 ± 3.70 ) x10$^4$** | **(1.80 ± 1.84 ) x10$^4$** | (8.90 ± 10.2 ) x10$^3$ | (1.75 ± 2.39 ) x10$^3$ | (4.56 ± 6.60 ) x10$^2$ | **(1.66 ± 1.31 ) x10$^5$** | (5.09 ± 6.21 ) x10$^{-7}$ |
| EC 3.3 | 5kg, 250Hz | ***(1.47 ± 1.58 ) x10$^5$*** | **(6.91 ± 8.10 ) x10$^4$** | **(2.46 ± 3.27 ) x10$^4$** | (1.01 ± 1.47 ) x10$^4$ | (1.37 ± 2.49 ) x10$^3$ | (3.35 ± 7.11 ) x10$^2$ | **(2.53 ± 2.88 ) x10$^5$** | (5.13 ± 8.00 ) x10$^{-7}$ |
| Exp. 4 'DOE' | Base level | (6.04 ± 1.25 ) x10$^4$ | (3.82 ± 0.693) x10$^3$ | (9.89 ± 2.86 ) x10$^2$ | (5.00 ± 1.72 ) x10$^2$ | (1.31 ± 0.484) x10$^2$ | (4.76 ± 1.68 ) x10$^1$ | (6.59 ± 1.29 ) x10$^4$ | (5.47 ± 1.15 ) x10$^{-8}$ |
| EC 1.1 | 3kg, 150Hz | (7.88 ± 2.73 ) x10$^4$ | (6.77 ± 2.98 ) x10$^4$ | (5.05 ± 2.71 ) x10$^4$ | (3.94 ± 2.42 ) x10$^4$ | (1.98 ± 1.53 ) x10$^4$ | (1.03 ± 0.872) x10$^4$ | (2.67 ± 1.26 ) x10$^5$ | (5.53 ± 4.34 ) x10$^{-6}$ |
| EC 1.3 | 3kg, 250Hz | (7.31 ± 3.01 ) x10$^4$ | (5.06 ± 2.11 ) x10$^4$ | (3.27 ± 1.38 ) x10$^4$ | (2.27 ± 0.969) x10$^4$ | (9.51 ± 4.50 ) x10$^3$ | (4.70 ± 2.33 ) x10$^3$ | (1.93 ± 0.732) x10$^5$ | (2.67 ± 1.22 ) x10$^{-6}$ |
| EC 3.1 | 5kg, 150Hz | (7.69 ± 3.01 ) x10$^4$ | (5.74 ± 1.43 ) x10$^4$ | (4.11 ± 1.22 ) x10$^4$ | (3.11 ± 1.04 ) x10$^4$ | (1.44 ± 0.591) x10$^4$ | (7.60 ± 3.46 ) x10$^3$ | (2.28 ± 0.699) x10$^5$ | (4.11 ± 1.73 ) x10$^{-6}$ |
| EC 3.3 | 5kg, 250Hz | (1.34 ± 0.718) x10$^5$ | (9.11 ± 6.31 ) x10$^4$ | (5.76 ± 4.57 ) x10$^4$ | (3.87 ± 3.42 ) x10$^4$ | (1.43 ± 1.55 ) x10$^4$ | (7.02 ± 8.37 ) x10$^3$ | (3.42 ± 2.36 ) x10$^5$ | (4.11 ± 4.49 ) x10$^{-6}$ |
| Exp. 5 'DAE' | Base level | (5.35 ± 0.453) x10$^4$ | (3.18 ± 0.558) x10$^3$ | (1.39 ± 0.279) x10$^2$ | (2.81 ± 0.891) x10$^1$ | (6.75 ± 3.45 ) x10$^0$ | (3.35 ± 2.18 ) x10$^0$ | (5.69 ± 0.510) x10$^4$ | (2.00 ± 0.200) x10$^{-8}$ |
| EC 1.1 | 3kg, 150Hz | (8.07 ± 11.0 ) x10$^2$ | (1.80 ± 1.72 ) x10$^2$ | (6.51 ± 13.5 ) x10$^0$ | (4.84 ± 10.8 ) x10$^{-1}$ | (2.45 ± 5.48 ) x10$^0$ | (1.08 ± 1.66 ) x10$^0$ | (9.98 ± 12.1 ) x10$^2$ | (9.26 ± 13.0 ) x10$^{-10}$ |
| EC 1.3 | 3kg, 250Hz | (5.66 ± 8.37 ) x10$^2$ | (8.02 ± 9.67 ) x10$^1$ | (5.17 ± 6.43 ) x10$^0$ | (9.69 ± 16.4 ) x10$^0$ | (4.52 ± 10.1 ) x10$^0$ | (2.30 ± 3.85 ) x10$^0$ | (6.68 ± 8.65 ) x10$^2$ | (1.44 ± 2.39 ) x10$^{-9}$ |
| EC 3.1 | 5kg, 150Hz | (5.72 ± 8.59 ) x10$^2$ | (2.23 ± 2.92 ) x10$^2$ | (1.02 ± 1.31 ) x10$^1$ | (3.72 ± 4.62 ) x10$^0$ | (3.29 ± 5.00 ) x10$^0$ | (9.42 ± 21.1 ) x10$^{-1}$ | (8.13 ± 10.5 ) x10$^2$ | (9.70 ± 11.2 ) x10$^{-10}$ |
| EC 3.3 | 5kg, 250Hz | (3.67 ± 6.45 ) x10$^2$ | (1.40 ± 1.86 ) x10$^2$ | (2.91 ± 4.12 ) x10$^0$ | (6.84 ± 15.3 ) x10$^{-1}$ | (1.37 ± 3.06 ) x10$^0$ | (1.03 ± 2.29 ) x10$^0$ | (5.12 ± 6.55 ) x10$^2$ | (6.61 ± 9.39 ) x10$^{-10}$ |
| Exp. 6 'DCB' | Base level | (2.90 ± 0.351) x10$^4$ | (2.09 ± 0.641) x10$^3$ | (4.97 ± 2.53 ) x10$^2$ | (1.85 ± 1.08 ) x10$^2$ | (2.14 ± 1.48 ) x10$^1$ | (5.00 ± 3.68 ) x10$^0$ | (3.18 ± 0.437) x10$^4$ | (1.70 ± 0.516) x10$^{-8}$ |
| EC 4.4 | 15Hz | (9.97 ± 3.86 ) x10$^5$ | (6.41 ± 2.46 ) x10$^5$ | (3.51 ± 1.25 ) x10$^5$ | (2.11 ± 0.672) x10$^5$ | (6.24 ± 1.68 ) x10$^4$ | (2.18 ± 0.716) x10$^4$ | (2.28 ± 0.836) x10$^6$ | (1.63 ± 0.449) x10$^{-5}$ |
| EC 4.5 | 25Hz | (7.24 ± 2.60 ) x10$^5$ | (4.55 ± 1.71 ) x10$^5$ | (2.46 ± 0.977) x10$^5$ | (1.48 ± 0.623) x10$^5$ | (4.43 ± 2.19 ) x10$^4$ | (1.53 ± 0.830) x10$^4$ | (1.63 ± 0.614) x10$^6$ | (1.15 ± 0.556) x10$^{-5}$ |
| Exp. 7 'GCB' | Base level | (2.35 ± 0.581) x10$^4$ | (2.18 ± 0.861) x10$^3$ | (5.47 ± 2.17 ) x10$^2$ | (2.42 ± 0.804) x10$^2$ | (4.21 ± 1.50 ) x10$^1$ | (1.39 ± 0.718) x10$^1$ | (2.65 ± 0.685) x10$^4$ | (2.08 ± 0.549) x10$^{-8}$ |
| EC 4.4 | 15Hz | (4.37 ± 1.15 ) x10$^5$ | (2.66 ± 0.640) x10$^5$ | (1.47 ± 0.325) x10$^5$ | (9.18 ± 1.91 ) x10$^4$ | (2.65 ± 0.693) x10$^4$ | (8.41 ± 3.37 ) x10$^3$ | (9.77 ± 2.35 ) x10$^5$ | (6.68 ± 1.89 ) x10$^{-6}$ |
| EC 4.5 | 25Hz | (2.98 ± 1.09 ) x10$^5$ | (1.91 ± 0.664) x10$^5$ | (1.12 ± 0.400) x10$^5$ | (7.39 ± 2.77 ) x10$^4$ | (2.57 ± 1.19 ) x10$^4$ | (9.98 ± 5.36 ) x10$^3$ | (7.11 ± 2.60 ) x10$^5$ | (6.67 ± 3.17 ) x10$^{-6}$ |
| Exp. 8 'GCM' | Base level | (2.35 ± 0.581) x10$^4$ | (2.18 ± 0.861) x10$^3$ | (5.47 ± 2.17 ) x10$^2$ | (2.42 ± 0.804) x10$^2$ | (4.21 ± 1.50 ) x10$^1$ | (1.39 ± 0.718) x10$^1$ | (2.65 ± 0.685) x10$^4$ | (2.08 ± 0.549) x10$^{-8}$ |
| EC 4.4 | 15Hz | (1.63 ± 0.478) x10$^5$ | (1.07 ± 0.271) x10$^5$ | (6.50 ± 1.90 ) x10$^4$ | (4.41 ± 1.48 ) x10$^4$ | (1.44 ± 0.662) x10$^4$ | (4.67 ± 2.60 ) x10$^3$ | (3.98 ± 1.16 ) x10$^5$ | (3.49 ± 1.58 ) x10$^{-6}$ |
| EC 4.5 | 25Hz | (1.39 ± 0.512) x10$^5$ | (9.07 ± 3.02 ) x10$^4$ | (5.56 ± 1.40 ) x10$^4$ | (3.79 ± 0.685) x10$^4$ | (1.24 ± 0.132) x10$^4$ | (4.13 ± 1.05 ) x10$^3$ | (3.40 ± 1.01 ) x10$^5$ | (3.03 ± 0.329) x10$^{-6}$ |

Particles were counted at a sampling flow rate of 0.1cfm (0.1 cubic foot per minute, equivalent to 2.83l/m or 4.72 x10$^{-5}$ $m^3$/s), and had the respective base level subtracted as per the data protocol.
The means and stds were calculated over n=5 for Exp. 2 and 4 through 8, and n=10 for Exp. 1 and 3.
The experiment code (Exp. # 'CODE') refers to the variables tested in each experiment as shown in Table 3.1. The variables are **D**ry, **G**reasy or **F**resh bone, in a **C**losed, **O**pen or **A**ctively ventilated environment, using an **E**lectric oscillating, **S**atterlee or **M**etal-saw.
'EC...' refers to the chosen Experimental Conditions of the independent variables saw blade frequency and saw blade contact load shown in Table 3.2 (EC 1.1 though 3.3), or the saw blade frequency in Table 3.2 (EC 4.4 and 4.5).
'Base level' shows the average of all base levels, i.e. the first minute of measurement of each repetition of each different test.
Text in **bold** shows that the effect of saw blade frequency was statistically significant (p<0.05). Text in *italics* shows that the effect of saw blade contact load was statistically significant (p<0.05).

## C.5. Figures: Relative Humidity vs Base level

Figure C.1: Relative Humidity vs Base level: scatter plots of the ambient Relative Humidity (RH) vs the number of aerosol particles in the base level measurement (M0) per particle size, the total number of particles, and the total surface area of particles. Note that for reasons of visibility the vertical axes of each sub-figure are scaled depending on the maximum number of particles counted for each particle size.



# C.6. Figures: Results Exp. 1 through 8

Figure C.2: Exp. 1 'DCE' : Stacked bar graphs of the number of aerosol particles counted during Exp. 1 'DCE', marked per EC (columns) during n=5 measurements (each coloured layer indicates one test run), displayed per particle size, the total number, and total surface area. Note that for reasons of visibility the vertical axes of each sub-figure are scaled depending on the maximum number of particles counted for each particle size.

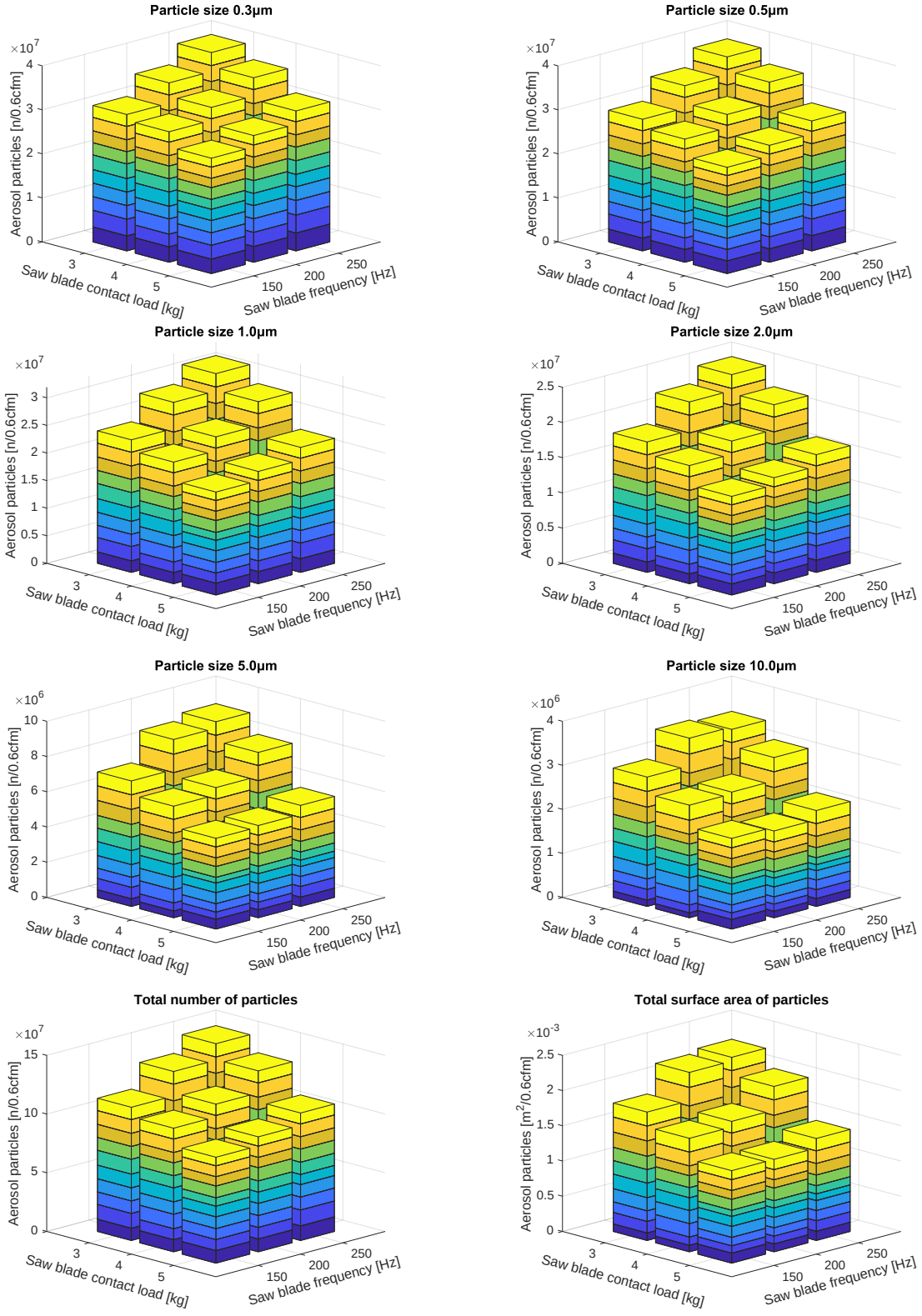


Figure C.3: Exp. 2 'GCE' : Stacked bar graphs of the number of aerosol particles counted during Exp. 2 'GCE', marked per EC (columns) during n=5 measurements (each coloured layer indicates one test run), displayed per particle size, the total number, and total surface area. Note that for reasons of visibility the vertical axes of each sub-figure are scaled depending on the maximum number of particles counted for each particle size.

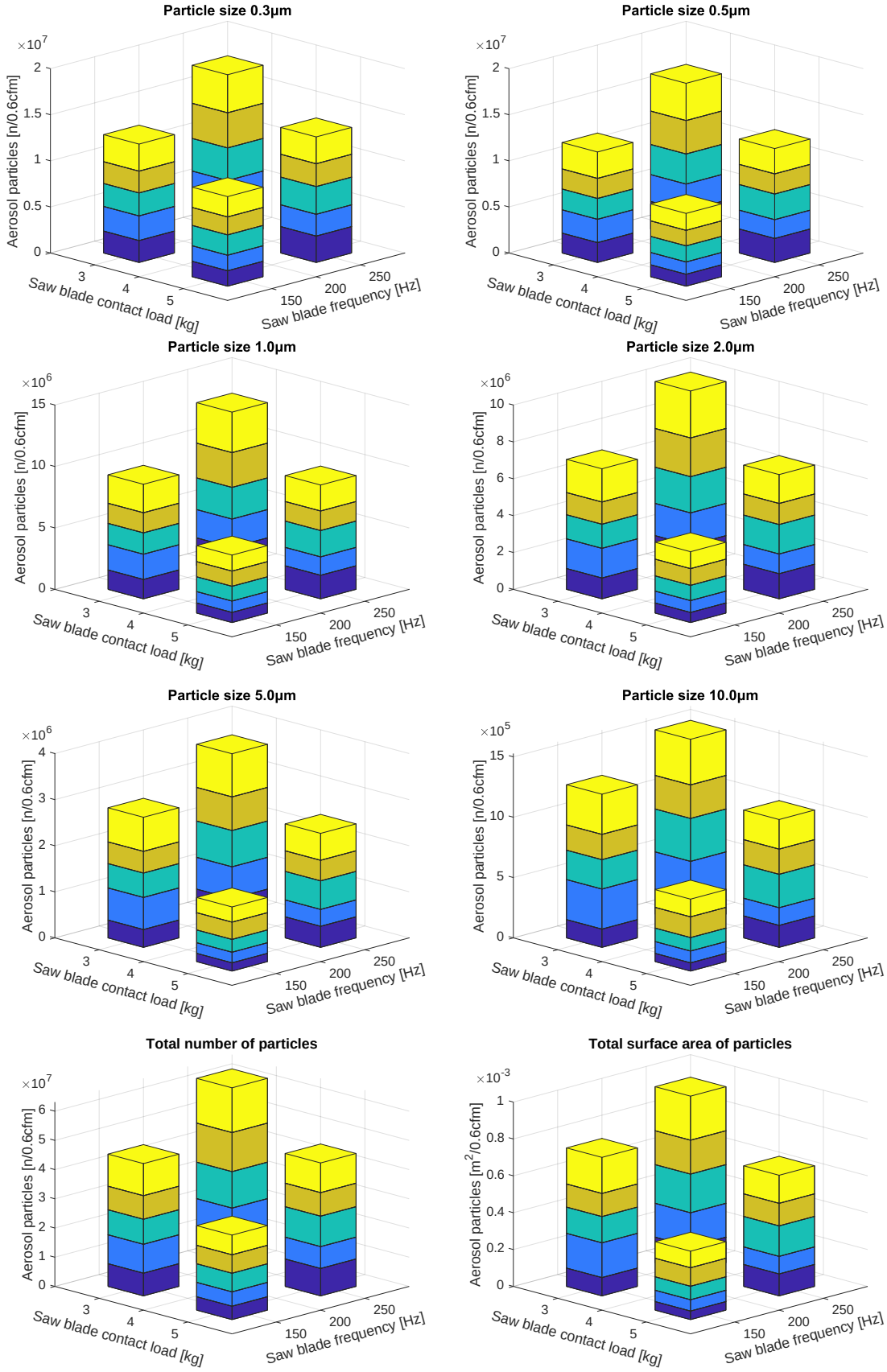


Figure C.4: Exp. 3 'FCE' : Stacked bar graphs of the number of aerosol particles counted during Exp. 3 'FCE', marked per EC (columns) during n=5 measurements (each coloured layer indicates one test run), displayed per particle size, the total number, and total surface area. Note that for reasons of visibility the vertical axes of each sub-figure are scaled depending on the maximum number of particles counted for each particle size.

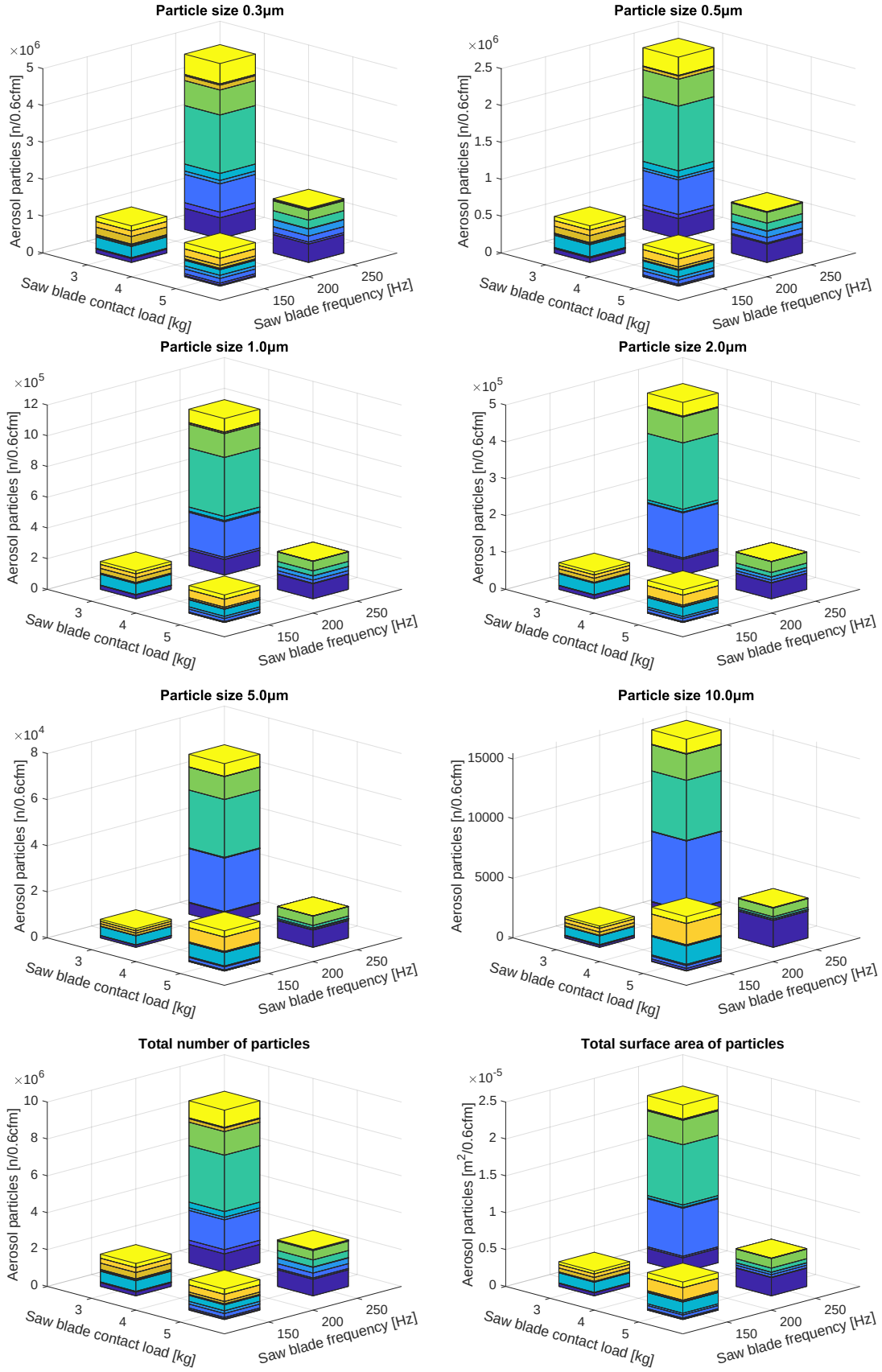


Figure C.5: Exp. 4 'DOE' : Stacked bar graphs of the number of aerosol particles counted during Exp. 4 'DOE', marked per EC (columns) during n=5 measurements (each coloured layer indicates one test run), displayed per particle size, the total number, and total surface area. Note that for reasons of visibility the vertical axes of each sub-figure are scaled depending on the maximum number of particles counted for each particle size.

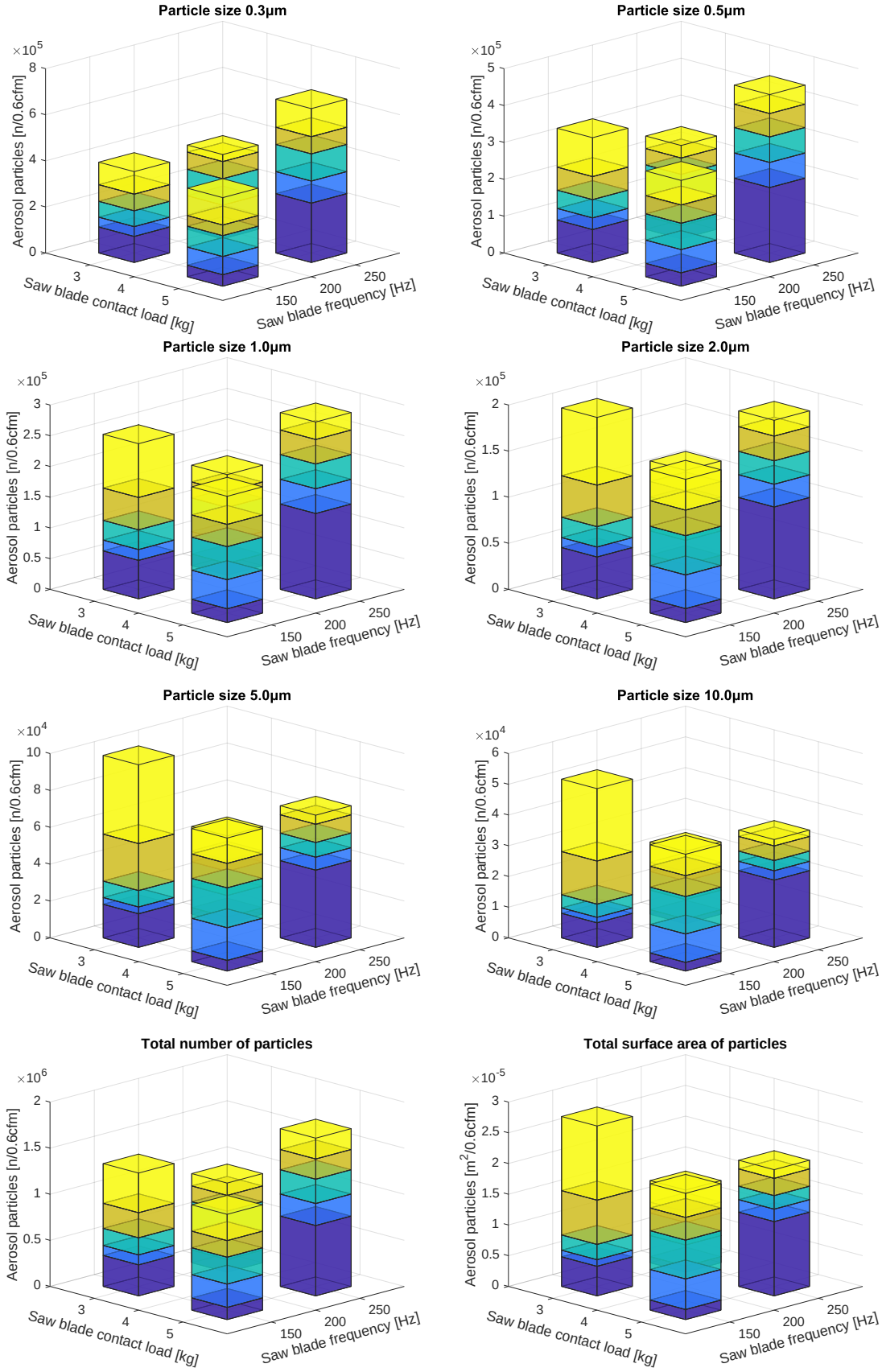


Figure C.6: Exp. 5 'DAE' : Stacked bar graphs of the number of aerosol particles counted during Exp. 5 'DAE', marked per EC (columns) during n=5 measurements (each coloured layer indicates one test run), displayed per particle size, the total number, and total surface area. Note that for reasons of visibility the vertical axes of each sub-figure are scaled depending on the maximum number of particles counted for each particle size.

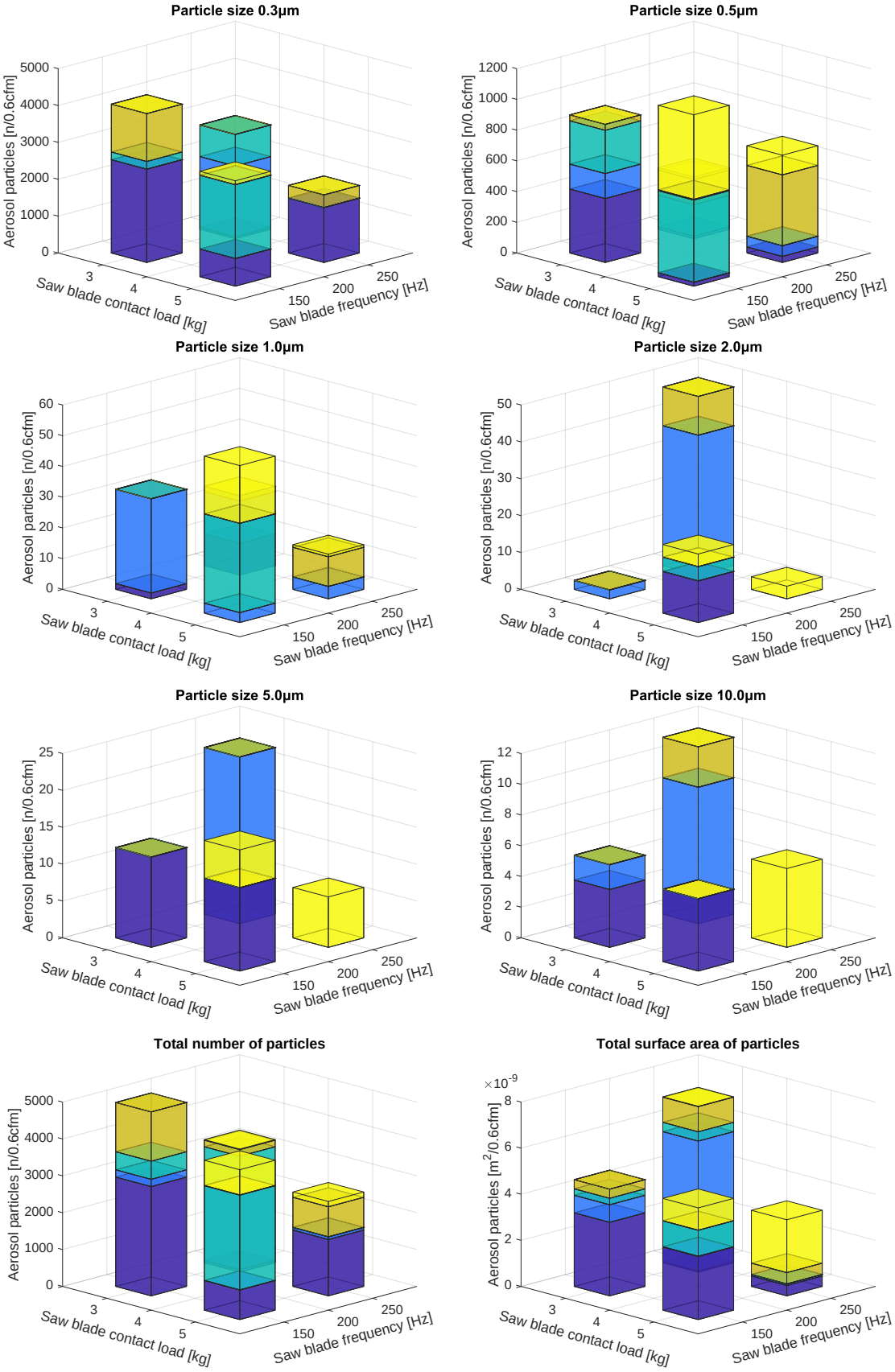


Figure C.7: Exp. 6 'DCB' : Stacked bar graphs of the number of aerosol particles counted during Exp. 6 'DCB', marked per EC (columns) during n=5 measurements (each coloured layer indicates one test run), displayed per particle size, the total number, and total surface area. Note that for reasons of visibility the vertical axes of each sub-figure are scaled depending on the maximum number of particles counted for each particle size.

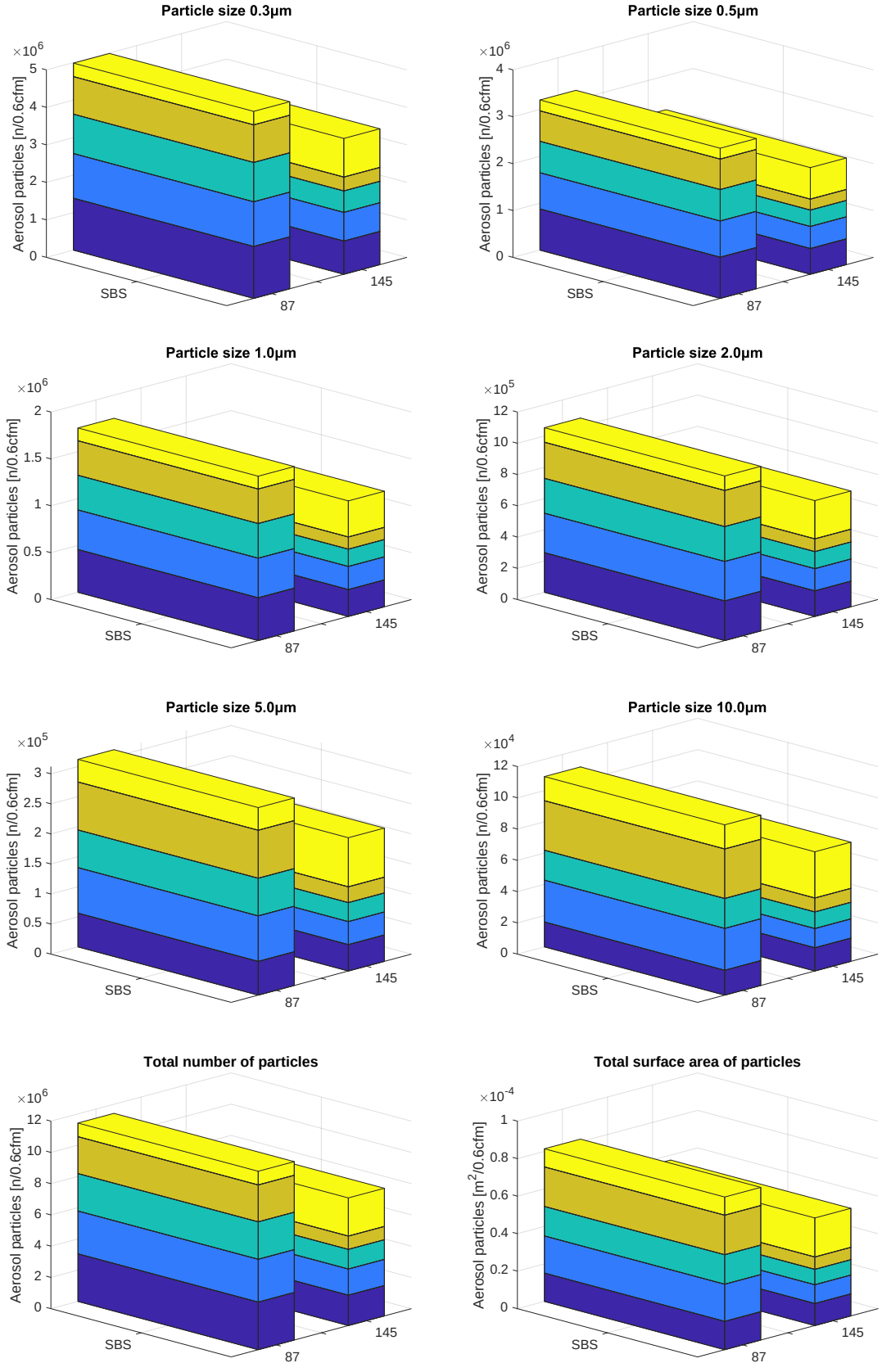


Figure C.8: Exp. 7 and 8 : Stacked bar graphs of the number of aerosol particles counted during Exp. 7 and 8, marked per EC (columns) during n=5 measurements (each coloured layer indicates one test run), displayed per particle size, the total number, and total surface area. Note that for reasons of visibility the vertical axes of each sub-figure are scaled depending on the maximum number of particles counted for each particle size.