



Computational Tracking of Dental Splatter: Comparison of High-Speed Drill and 9.3 μ m CO₂ Laser

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Abstract

Objective: The purpose of this study was to track and characterize visible oral splatter generated by a 9.3 μ m CO₂ laser compared to that of a traditional high-speed dental drill when performing a cavity preparation procedure.

Methods: Extracted human second molars were placed in a dental-simulator mannequin head, which was positioned in a local dentist's operatory, along with a high-volume evacuation (HVE) system to simulate a typical clinical environment. A cavity preparation was performed using both a 9.3 μ m CO₂ laser and high-speed drill, while maintaining identical cutting angle and location in the mouth. A high-speed camera was used to capture the splatter ejected from the mouth. The recordings were analyzed with both manual and computational-tracking methods using an image analysis software. A quantitative analysis of the tracked droplets was performed to determine splatter displacement, speed, and rate of droplet formation during the procedure for both the 9.3 μ m CO₂ laser and drill.

Results: Over the visible tracking range during a time of maximal droplet generation, the 9.3 μ m CO₂ laser, relative to the drill, generated 93.5% fewer droplets per second, reduced mean droplet speed by 35%, and reduced average maximum droplet speed by 50%. At a typical distance between the patient and the dentist, 0% of the laser-generated droplets, and 12% drill-generated droplets traveled a net displacement of >30 cm from the tooth. Mean splatter displacement rate, a weighted representation of the combined displacements of droplets over time, was 95% lower for the 9.3 μ m CO₂ laser than for the drill.

Conclusion: Droplet tracking analysis showed that the use of a 9.3 μ m CO₂ laser reduced the amount, speed and displacement of splatter as compared to a conventional high-speed drill. Since splatter may harbor infectious pathogens, the laser reduces the risk of infection to dental practitioners.

Keywords: 9.3 μ m CO₂ laser; High-speed drill; Dental splatter; Dental aerosol; Droplet tracking

Abbreviations: HVE: High-Volume Evacuation; SARS CoV2: Syndrome Coronavirus 2; AGPs: Aerosol Generating Procedures; ATP: Adenosine Triphosphate; DoG: Difference of Gaussian; LAP: Linear Assignment Problem.

Introduction

The recent public health crisis caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has transformed the world. Recent information suggests that the virus has a high risk of transmission through bioaerosols

from infected individuals [1,2]. This is of particular concern in the field of dentistry, in which aerosol generating procedures (AGPs) are performed. Saliva has been demonstrated to be a source of SARS-CoV-2, [3] among many other pathogens, suggesting a significant risk of AGPs to dental practitioners. The coronavirus disease 2019 (COVID-19) pandemic has highlighted the risk of disease transmission through splatter and aerosol in dental procedures.

The proximity between patient and dentist required to perform necessary procedures provides the opportunity

Materials and Methods

Sample Environment

This study was performed in a clinical operatory by a practicing dentist using settings typical of a cavity preparation for both the CO₂ laser and the high-speed drill, as well as a high-volume evacuation (HVE) system. The oral cavity was mimicked using a simulation mannequin head that was positioned on a dental chair in a manner that is consistent with a patient's typical head placement. Extracted second human molars were randomly selected and placed in the mannequin head in the position of the second molar. These samples had no signs of caries or fluorosis and were stored in 0.1% thymol solution for no more than three months after extraction.

System Settings

A high-speed drill, KaVo SMARTtorque™ handpiece with a carbide bur #245, rotating at 400,000 rpm, at a 30 psi air pressure, and a measured 50 mL/min mist water flow rate was used. The laser (Solea, Convergent Dental, Needham, MA) operated with 1.25 mm spot size set at 30% cutting speed, 10 psi air pressure, and a measured 9 mL/min water flow rate with a fine mist spray.

Imaging Setup

A professional videographer (Reel Big Media, Cumberland, RI) recorded the simulated cavity preparation using a digital camera with an EF 24-70mm f/2.8L USM standard zoom lens (24-70L Series, Canon Inc., Tokyo, Japan) at a frame rate of 240 fps and approximate focal length of 35mm. The camera was positioned perpendicular to the direction of a local dentist cutting in order to capture most of the droplets. White light was placed in front of a black backdrop at high and low angles to capture the full length of droplet tracks from the tooth to where the droplets landed.

Video Processing and Software

Three seconds of recorded videos for the laser and drill were loaded and pre-processed in a multimedia software (FreeStudio v. 6.7.1.316, Microsoft Corp). They were then analyzed using the TrackMate [24] plugin in the Fiji [25] package of ImageJ [26]. The images were converted to 8-bit grayscale and were used for automated and manual tracking of single-droplets.

Two types of analysis were performed in TrackMate on each video: manual and automated. Manual tracking provided an overview of droplet characteristics and a visualization of droplet paths. Manual tracking also provided

for disease transmission from viruses or bacteria [4,5]. Saliva, air and mist mix with live pathogens during AGPs, creating infection transmission risk from patient to dentist [6]. This mixture generates a wide array of small and large droplets, which may form an aerosol or splatter [7,8]. The differences between aerosols, droplets, and splatter have not been uniformly defined, with opposing literature outlining differing definitions, sizes and characteristics of these droplet categories [2]. Regardless, droplets of all sizes pose a risk to dental practitioners [4,9,10]. Although larger and heavier droplets fall at a faster rate, they are still of great concern; these droplets they may contain a higher yield of pathogens that could infect exposed mucous membranes, and may continually release aerosolized live pathogens while evaporating from surfaces [11-13]. Previous work has been done to investigate and characterize the splatter generated during various AGPs, including using fluorescein dye [14,15] and adenosine triphosphate (ATP) bioluminescence [16] to visualize the splatter. Additional studies have been done to characterize live pathogens contained in bioaerosols and splatter, often using air sampling techniques [17,18].

Cavity preparation, a commonly performed AGP, is a procedure in which a significant amount of dental material mixed with saliva is forcibly ejected into the air due to a combination of tissue cutting and mist/air cooling. Since high-speed dental drills cut tooth enamel and dentin via mechanical rotation of up to 440,000 rpm, cooling is generally required to prevent the tooth from overheating or damaging the pulp [19]. Cooling is achieved through a combination of mist water flow (typical values of 30-60 mL/min) and air (typical values of 25-38 psi) [20]. Conversely, the 9.3 μm CO₂ laser is directly absorbed by hydroxyapatite in the tooth and cuts by rapidly vaporizing and ejecting superheated dental material from the surface [21]. It also uses much lower mist water flow (8-15 mL/min) and air (10-25 psi) for cooling compared to high-speed drills. An additional benefit of the high surface temperatures achieved through laser irradiation is a likely decontamination of irradiated pathogens, which are expected to be inactivated or killed at such high temperatures [22,23].

This study aimed to compare the generation of splatter from a 9.3 μm CO₂ laser to that from a high-speed drill on dental enamel in vitro through computational analysis of imaged droplets during a simulated cavity preparation. To the best of our knowledge, this is the first time a quantitative analysis was performed on splatter tracked during a cavity preparation in a comparison of these devices. Characterization of the speed, displacement, and rate of droplets generated during a cavity preparation is important to provide clarity on how dental practitioners may mitigate the inherent risks of disease transmission during AGPs.

a validation of the model outputs provided by the automated tracking method on droplet generation rate, displacement, and speed, to ensure that only droplets from splatter were analyzed by the algorithm. The automated method provided a more accurate quantitative analysis to capture most of the particles in a consistent way.

The video for the laser revealed short bursts of detectable splatter, and long periods of minimal to no splatter. To provide a fair and robust comparison of splatter produced by the drill and laser, 50 frames (out of 719) for each video were analyzed, matching the parts of the videos with a maximum generation of splatter. Additionally, each video was cropped and zoomed in to focus only on the region of interest that included the mouth and splatter droplets, excluding unnecessary background noise.

Manual Splatter Droplet Tracking

Each droplet was manually selected over each frame for the duration of its path, and its coordinates were recorded. For visualization, the droplet tracks were overlaid on top of each still frame image and the video frame rate was slowed by 97% to better demonstrate the droplets moving in slow motion. Displacement and speed maxima were calculated for the fastest apparent droplets from these coordinates and from the video frame rate to provide a validation for the computational analysis from the automated method. This manual method of tracking was used to provide a histogram of droplet displacements for both the laser and drill.

Automated Splatter Droplet Tracking

Spot detection: A spot detection algorithm, difference of gaussian (DoG), was used for splatter droplet detection. In this algorithm the image is filtered using two gaussian filters with standard deviations: $\sigma_{-1} = 1/(1 + \sqrt{2}) \times d$ and $\sigma_{-2} = \sqrt{2} \times \sigma_{-1}$

where d is the estimated spot diameter. The result of the second filter (largest sigma) is subtracted from the result of the first filter (smallest sigma). This yields a smoothed image with sharp local maxima at droplet locations. Expected droplet sizes were provided to allow the algorithm to detect droplets in the frames. Empirically, these values provided good results matched by the manual tracking in this study and at least one previous study [15].

Track analysis: The linear assignment problem (LAP), which relies on the Munkres & Kuhn algorithm, [27] was employed for droplet tracking across frames. Droplet track features were generated by the track analyzers, which yielded features including number of frames for each track, number of gaps allowed for each track, and longest gap

distance allowed, based on selected input parameters. The input parameters used for tracking were selected through a combination of careful observation from manual tracking, visualization and trial-and-error. The largest inter-frame distance a droplet was observed to travel was 1.5 cm, which was used as a threshold for droplet to droplet linking for calculation of distance and speed, helping to reduce spurious noise. For this reason, the frame-to-frame linking distance and track segment gap closing distance were limited to 1.5 cm. Additionally, based on empirical observation from manual tracking and visualization, splatter droplets occasionally divided into two or more droplets, so track segment splitting was enabled in the automated analysis. Contrarily, no merging event of two or more droplets was observed through manual tracking or visualization. Furthermore, due to the 3-dimensional stochastic nature of splatter generated in this study, it is unlikely that such small droplets would be coincidental in time and space so as to merge mid-air. For these reasons, track segment merging was disabled. The algorithm provided outputs for the path of each droplet track. These outputs included mean droplet speeds, maximum inter-frame speeds, rate of droplets generated, and mean and maximum droplet displacements.

Post-Analysis filtering: Due to noise in the imaging and analysis methods, some post-analysis filtering was necessary to enhance the accuracy of droplet track data. Droplets were excluded if they did not appear in at least five consecutive frames (path too short for analysis) or if they appeared in more than 45 frames. These were deemed to be outside of acceptable ranges for the reported parameters, based on previous work [15] and from calculated durations of number of frames tracked splatter was visible from the manual method in this study.

Results

Part 1: Manual Splatter Droplet Tracking:

Figure 1 shows a visualization of the manually tracked, randomly selected set of droplets over a duration of 0.21 seconds during a simulated cavity preparation on an extracted human molar using either the drill (Figure 1A) or the 9.3 μm CO_2 laser (Figure 1B) at a maximum splatter output. The droplet tracks are overlaid on an image of the background from a still frame. This visualization from manual tracking is also shown in Video 1 for the drill and Video 2 for the laser. The purpose of this visualization was to highlight a sample set of droplets for both the laser and drill to demonstrate the general trend of displacement and speed. Therefore, not every droplet was tracked manually. Generally, droplets were selected with a focus on those moving either slowly, quickly, or farther from the tooth (origin) to allow for a characteristic calculation of relevant parameters.

100 droplets were selected for the randomized manual tracking of the drill splatter. 20 droplets were selected for the randomized manual tracking of laser splatter. Each track was assigned a random color and represents an individual

droplet following its traversal through the video frames. For the laser, the number of droplets was low, which allowed for most visible droplets to be selected (Videos 1,2).

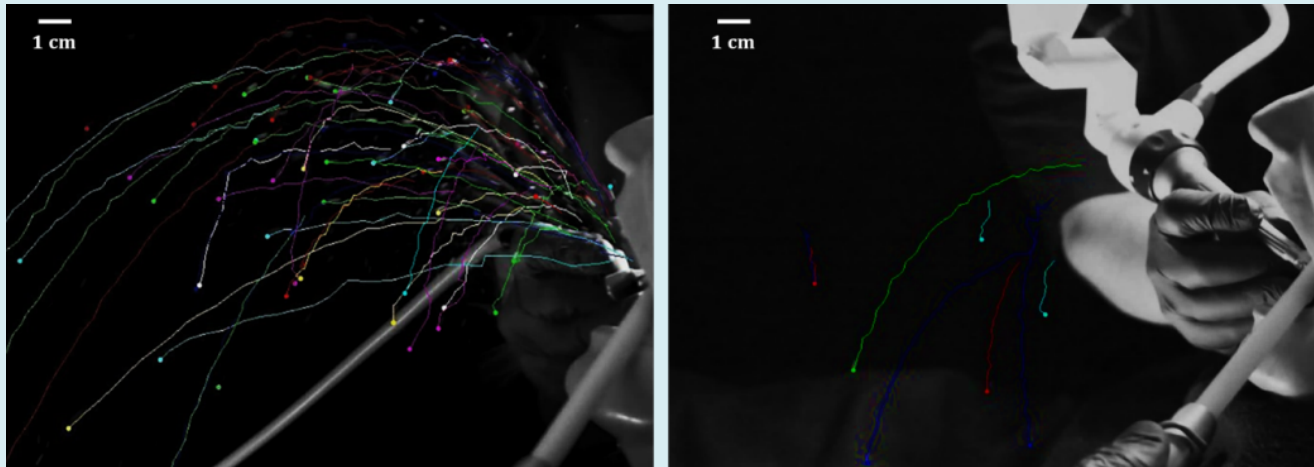
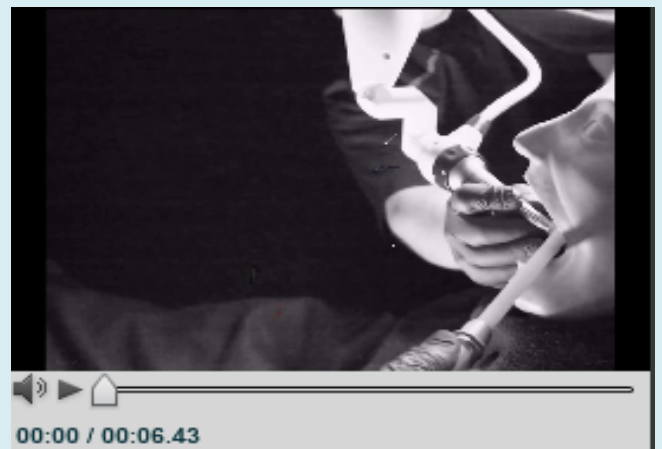


Figure 1: The manually tracked droplets are shown over the duration of 0.21 seconds to provide a visualization of the splatter from drill (A) and laser (B) on a human molar.



Splatter Tracks Visualization of Procedure with Drill.avi

Video 1: This video is an illustration of manual tracking for a representative selection of 100 droplets over 50 frames for a simulated cavity preparation using a standard high-speed dental drill with HVE. Each droplet track was assigned a random color.



Splatter Visualization for Laser.avi

Video 2: This video is an illustration of manual tracking for a representative selection of 20 droplets over 50 frames for a simulated cavity preparation using a 9.3 μm CO_2 laser with HVE. Each droplet track was assigned a random color. The nearest droplets were hidden by background noise and not shown in the video.

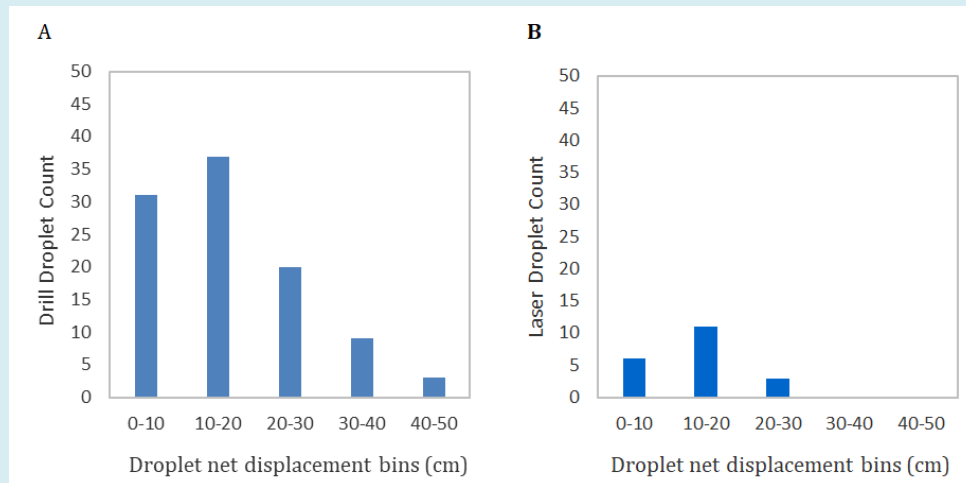


Figure 2: Net displacement tracked for 20 and 100 droplets generated by the drill (A) and laser (B), respectively. Tracking was done over 0.21 sec interval where maximum number of droplets were generated by both techniques.

Figure 2 shows the net displacement of a sample of droplets tracked during a 0.21 sec interval during which maximum number of droplets were generated by the laser and the drill. Out of the 20 droplets tracked for the laser, none reached the >30 cm, which is usually the least distance between the patient and the dentist. On the other hand, 12% of the droplets ejected by the drill reached a distance of >30 cm, with the majority of them, about 57%, falling within 10-30cm range.

Part 2: Automated Splatter Droplet Tracking

Figure 3 shows the number of droplets that were detected over each of the 719 frames at intervals of every ~0.0042 seconds for the drill and laser. Generally, there were large spans of time in which no splatter from the laser was visible. Additionally, due to the nature of the experiment method, there was high variability over time in the number of released droplets, and in their detection, for both the drill and laser. In this section of video, after ~0.5 seconds, no droplets from the laser were detected; however, the drill continued to output a significant amount of splatter.

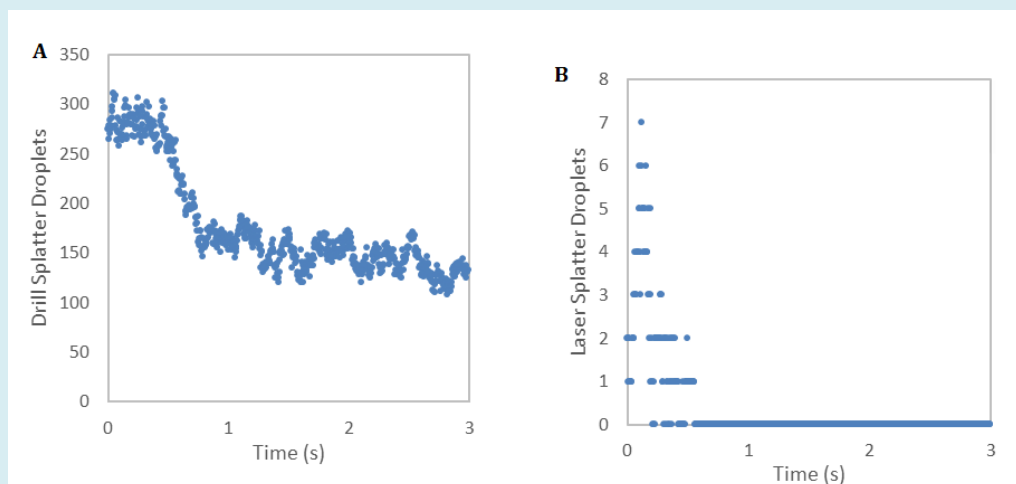


Figure 3: Number of splatter droplets detected in each frame over time through automated tracking for drill (A) and laser (B). The number of detected splatter droplets was substantially higher for the drill. Variability of detected droplets was high over time, and splatter droplets were not detected after 0.5 seconds for the laser over this recorded duration.

A summary of the automated droplet tracking data from the first 50 frames of peak splatter generation obtained for both the drill and 9.3 μm CO_2 laser is shown in Table 1. The number of splatter droplets was substantially higher for the drill, which produced a mean rate of 1032 droplet per second, whereas the laser produced at least 93% fewer droplets over time. Compared to the laser, the drill produced droplets with a mean speed 35% faster and an average maximum

droplet speed 50% faster. The mean net displacement of the droplets was 25% greater for the drill than the laser, and the maximum net displacement was 50% larger. A meaningful measure of relative risk is provided by the multiplication of mean displacement and droplet generation rate, defined as the mean splatter displacement rate. At peak splatter generation, the laser produced a mean splatter displacement rate that was 95% less than the drill.

	Drill	Laser
Mean displacement (cm)	16.8	12.7
Maximum displacement (cm)	44.4	22
Mean speed (cm/s)	120.7	78.1
Maximum speed detected (cm/s)	369.3	351.5
Mean of maximum speeds (cm/s)	242.8	120.3
Rate (droplets/s)	1032	67
Mean splatter displacement rate (cm*droplets/s)	17337.6	850.9

Table 1: Summary of Data from Automated Droplet Tracking for Drill and Laser.

The mean speed for splatter droplets from the drill and laser over the selected range of 50 frames are shown in Figure 4. In this span of time, the drill produced 215 detected droplets with variable speed, between 40.7-258.6 cm/s, with some individual droplets exhibiting mean speeds far higher

than the average across all droplets (120.7 cm/s). Conversely, the laser produced only 14 detected droplets, with relatively slow and consistent individual speeds, between 45.5-114.5 cm/s, and an average speed across these droplets of 78.1 cm/s.

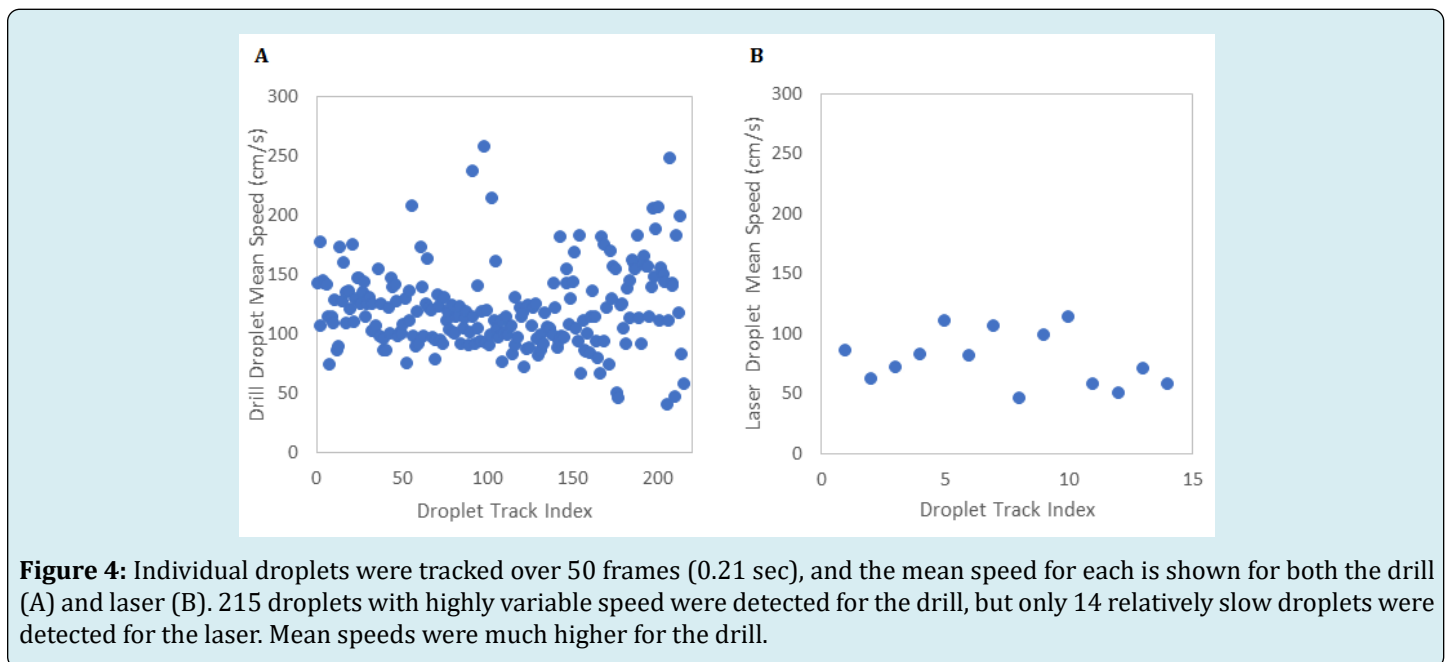


Figure 4: Individual droplets were tracked over 50 frames (0.21 sec), and the mean speed for each is shown for both the drill (A) and laser (B). 215 droplets with highly variable speed were detected for the drill, but only 14 relatively slow droplets were detected for the laser. Mean speeds were much higher for the drill.

The maximum speeds for each splatter droplet detected from the drill and laser over the selected range of 50 frames are shown in Figure 5. Droplets ejected by the drill exhibited maximum speed ranging between 46.6-369.6 cm/s, whereas

the laser produced droplets with maximum speeds that ranged between 45.5-147.5 cm/s with the exception of two measurements thought to be noise, as they were not matched by validation during visualization. For validation

of the automated method, the manual calculation of track speeds for the drill resulted in a maximum droplet speed of

375 cm/s.

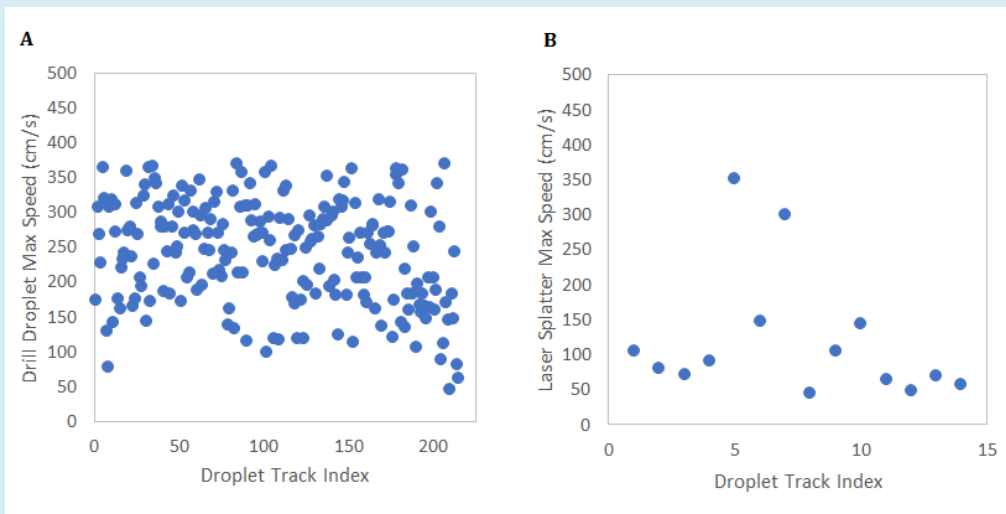


Figure 5: Individual droplets were tracked over 50 frames (0.208 sec), and the maximum speed between frames was recorded for each track for both drill (A) and laser (B). Maximum droplet speeds varied more and averaged higher for the drill.

Discussion

Bioaerosols and splatter released into the air during dental procedures may serve as vectors for disease transmission, putting healthcare workers at great risk [10,18]. Practitioners may fear performing dental work on patients who could be infected with transmissible diseases. It is therefore imperative to identify devices that can allow dentists to perform their work with relative safety and comfort. In this study, we aimed to investigate and understand the differences in splatter generated during a simulated cavity preparation by 9.3 μm CO_2 laser irradiation on human enamel compared to a conventional high-speed dental drill.

High-speed drills use substantial mechanical force to cut teeth, which results in the ejection of potentially pathogenic tissue. This is exacerbated by a relatively high air pressure and water flow that are required to cool the tooth concurrently. Many of these droplets are ejected from the mouth in a ballistic manner, as illustrated in Figure 1A. Contrarily, the laser vaporizes dental tissue efficiently while using less air and mist cooling, resulting in less splatter, and presumably, aerosols. Furthermore, heat generated from the laser on the tooth surface may be expected to reduce the survival of pathogens in the irradiated areas. In this study, the laser generated significantly fewer detected droplets by a factor of 93% compared to the high-speed drill, and these droplets travelled significantly slower and with less distance than those generated by the drill. Additionally, the

laser produced a mean splatter displacement rate 95% less than the drill, which provided an overall assessment of the relative risk of splatter.

In general, droplet tracks in computational tracking software are commonly misrepresented by noise inherent to the process [28]. For this reason, manual tracking was used as an empirical validation of the model outputs from the automated droplets tracking methods. One limitation of this work is that some of the droplets generated by the drill and laser may have been smaller and travel nearer the tooth, preventing detection by the camera. Fortunately, these smaller droplets may be expected to harbor lower pathogen loads to the dentist, so presumably they are of less risk. However, because the laser produced relatively few detectable droplets, there may be some meaningful loss in these data of potentially transmissible pathogen-contaminated droplets. This was considered not to be a concern due to the massive difference between detected droplets from the drill and those of the laser. Nonetheless, some of the droplets were captured in Figure 2, and provided a more accurate measure mean net displacements.

Due to limitations in the imaging methods, it was not possible to accurately measure droplet sizes. The camera exposure time needed to obtain these images had the effect of blurring droplet edges, which caused an overestimation of droplet sizes. Optical scattering noise also affected this measurement. These sources of error also may have artificially reduced the maximum droplet speeds for the drill,

as some faster droplets may have been untracked or hidden by other droplets. Contrarily, for the laser, noise may have caused an overestimation of the maximum droplet speeds for at least two droplets. This overestimation may have occurred through the software misrepresenting a droplet path and jumping off the actual trajectory of the droplet being tracked, and then returning to the original path. Additionally, the analysis was inherently limited to just those droplets detected by the camera and does not account for droplets that were aerosolized but beyond the resolution limit of the camera. Despite these limitations, values calculated by the software were in relative agreement with a recent study, [15] and clearly show a significant difference between splatter generated by the drill compared to the laser.

Further studies are needed to demonstrate the presence of live pathogens in the splatter and aerosol generated by a high-speed drill and 9.3 μm CO_2 laser to characterize the relative importance of these droplets in the transmission of bacteria and viruses in the dental environment.

Conclusion

Relative to the conventional high-speed dental drill, the 9.3 μm CO_2 laser reduces the speed, displacement, and number of droplets produced during a cavity preparation. The 9.3 μm CO_2 laser may therefore significantly reduce the risk of infection to dental practitioners from droplets that harbor bacteria or viruses that are ejected during aerosol-generating procedures.

References

1. Van Doremalen N, Bushmaker T, Morris DH, Dylan H Morris, Myndi G Holbrook, et al. (2020) Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 382(16): 1564-1567.
2. Jayaweera M, Perera H, Gunawardana B, Manatunge J (2020) Transmission of COVID-19 virus by droplets and aerosols: A critical review on the unresolved dichotomy. *Environ Res* 188: 109819.
3. Xu J, Li Y, Gan F, Du Y, Yao Y (2020) Salivary glands: potential reservoirs for COVID-19 asymptomatic infection. *J Dent Res* 99(8): 989.
4. Leggat PA, Kedjarune U (2001) Bacterial aerosols in the dental clinic: A review. *Int Dent J* 51(1): 39-44.
5. Bennett AM, Fulford MR, Walker JT, Bradshaw DJ, Martin MV, et al. (2000) Microbial aerosols in general dental practice. *Br Dent J* 189(12): 664-667.
6. Slots J, Slots H (2011) Bacterial and viral pathogens in saliva: Disease relationship and infectious risk. *Periodontol* 2000 55(1): 48-69.
7. Liu L, Wei J, Li Y, Ooi A (2017) Evaporation and dispersion of respiratory droplets from coughing. *Indoor Air* 27(1): 179-190.
8. Grayson SA, Griffiths PS, Perez MK, Piedimonte G (2017) Detection of airborne respiratory syncytial virus in a pediatric acute care clinic. *Pediatr Pulmonol* 52(5): 684-688.
9. Bentley CD, Burkhart NW, Crawford JJ (1994) Evaluating spatter and aerosol contamination during dental procedures. *J Am Dent Assoc* 125(5): 579-584.
10. Harrel SK, Molinari J (2004) Aerosols and splatter in dentistry: A brief review of the literature and infection control implications. *J Am Dent Assoc* 135(4): 429-437.
11. Rautemaa R, Nordberg A, Wuolijoki Saaristo K, Meurman JH (2006) Bacterial aerosols in dental practice-a potential hospital infection problem? *J Hosp Infect* 64(1): 76-81.
12. Miller RL, Micik RE, Abel C, Ryge G (1971) Studies on dental aerobiology: II. microbial splatter discharged from the oral cavity of dental patients. *J Dent Res* 50(3): 621-625.
13. Chanpong B, Tang M, Rosenczweig A, Lok P, Tang R (2020) Aerosol-generating procedures and simulated cough in dental anesthesia. *Anesth Prog*.
14. Dahlke WO, Cottam MR, Herring MC, Leavitt JM, Ditmyer MM, et al. (2012) Evaluation of the spatter-reduction effectiveness of two dry-field isolation techniques. *J Am Dent Assoc*. 143(11): 1199-1204.
15. Allison Jr, Currie CC, Edwards Dc, Bowes C, Coulter J, et al. (2020) Evaluating aerosol and splatter following dental procedures.
16. Watanabe A, Tamaki N, Yokota K, Matsuyama M, Kokeguchi S (2018) Use of ATP bioluminescence to survey the spread of aerosol and splatter during dental treatments. *J Hosp Infect* 99(3): 303-305.
17. Zemouri C, Volgenant CMC, Buijs MJ, Crielaard W, Rosemaa NAM, et al. (2020) Dental aerosols: microbial composition and spatial distribution. *J Oral Microbiol* 12(1): 1762040.
18. Zemouri C, De Soet H, Crielaard W, Laheij A (2017) A scoping review on bio-Aerosols in healthcare & the dental environment. *PLoS One* 12(5): e0178007.
19. Zach L, Cohen G (1965) Pulp response to externally

- applied heat. *Oral Surgery, Oral Med Oral Pathol* 19(4): 515-530.
20. Cavalcanti BN, Serairdarian PI, Rode SM (2005) Water flow in high-speed handpieces. *Quintessence Int* 36(5): 361-364.
 21. Fried D, Seka WD, Glana RE, Featherstone JD (1996) Thermal response of hard dental tissues to 9- through 11- μ m CO₂-laser irradiation. *Opt Eng* 35(7): 1976-1984.
 22. Cazares LH, Van Tongeren SA, Costantino J, Kenny T, Garza NL, et al. (2015) Heat fixation inactivates viral and bacterial pathogens and is compatible with downstream MALDI mass spectrometry tissue imaging. *BMC Microbiol* 15(1).
 23. Jabbar Ali B, Hamzah B, Naji E (2015) Effect of CO₂ laser on peri-implant infections. *Hamzah al World J Pharm Res World J Pharm Res SJIF Impact Factor* 5 4(7): 110-122.
 24. Tinevez JY, Perry N, Schindelin J, Hoopes GM, Reynolds GD, et al. (2017) TrackMate: An open and extensible platform for single-particle tracking. *Methods* 115: 80-90.
 25. Schindelin J, Arganda Carreras I, Frise E, Kaynig V, Longair M, et al. (2012) Fiji: An open-source platform for biological-image analysis. *Nat Methods* 9(7): 676-682.
 26. Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9(7): 671-675.
 27. Munkres J (1957) Algorithms for the Assignment and Transportation Problems. *J Soc Ind Appl Math* 5(1): 32-38.
 28. Chenouard N, Smal I, De Chaumont F, Maska M, Sbalzarini IF, et al. (2014) Objective comparison of particle tracking methods. *Nat Methods* 11(3): 281-289.

