

Cleaning lateral morphological features of the root canal

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**Cleaning lateral morphological features of the root canal:
the role of streaming and cavitation**

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Cleaning lateral morphological features of the root canal: the role of streaming and cavitation

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Running head: Cavitation and streaming in lateral features

Keywords: lateral canal, cleaning, streaming, cavitation

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Abstract

Aim To investigate the effects of ultrasonic activation file type, lateral canal location and irrigant on the removal of a biofilm-mimicking hydrogel from a fabricated lateral canal. Additionally, the amount of cavitation and streaming was quantified for these parameters.

Methodology An intracanal sonochemical dosimetry method was used to quantify the cavitation generated by an IrriSafe 25 mm length, size 25 file inside a root canal model filled with filtered degassed/saturated water or 3 different concentrations of NaOCl. Removal of a hydrogel, demonstrated previously to be an appropriate biofilm mimic, was recorded to measure the lateral canal cleaning rate from two different instruments (IrriSafe 25 mm length, size 25 and K 21 mm length, size 15) activated with a P5 Suprasson (Satelec) at power P8.5 in degassed/saturated water or NaOCl. Removal rates were compared for significant differences using non-parametric Kruskal–Wallis and/or Mann Whitney U tests. Streaming was measured using high-speed particle imaging velocimetry at 250 kfps, analysing both the oscillatory and steady flow inside the lateral canals.

Results There was no significant difference in amount of cavitation between tap water and oversaturated water ($P=0.538$), although more cavitation was observed than in degassed water. The highest cavitation signal was generated with NaOCl solutions (1.0%, 4.5%, 9.0%) ($P<0.007$) and increased with concentration ($P<0.014$). The IrriSafe file outperformed significantly the K-file in removing hydrogel ($P<0.05$). Up to 64% of the total hydrogel volume was removed after 20 s. The IrriSafe file typically outperformed the K-file in generating streaming. The oscillatory velocities were higher inside the lateral canal 3 mm compared to 6 mm from WL, and were higher for NaOCl than for saturated water, which in turn was higher than for degassed water.

Conclusions Measurements of cavitation and acoustic streaming have provided insight into their contribution to cleaning. Significant differences in cleaning, cavitation and streaming were found depending on the file type and size, lateral canal location and irrigant used. In general, the IrriSafe file outperformed the K-file, and NaOCl performed better than the other irrigants tested. The cavitation and streaming measurements revealed that both contributed to hydrogel removal and both play a significant role in root canal cleaning.

Introduction

One aim of root canal treatment is to remove bacterial biofilm from an infected canal. Any remaining microorganisms have the potential to re-establish a biofilm in the canal (Busscher *et al.* 2010, Ohsumi *et al.* 2015). The biofilm is an agglomeration of bacteria, adhered to a surface and embedded in a self-produced extracellular polymeric substance (EPS). This EPS matrix provides the biofilm with viscoelastic properties, facilitates nutrition and protects it from chemical and mechanical attacks imposed by endodontic cleaning procedures and disinfectants (de Paz 2007, Stewart & Franklin 2008). The viscoelastic properties of the EPS matrix also facilitate the biofilm's ability to deform and adapt under mechanical stress (Körstgens *et al.* 2010)

Inside a root canal, this infecting biofilm is particularly problematic and difficult to remove, especially when it forms in the accessory root canal system such as in lateral canals, which communicate with the surrounding bone. Persistent infection in such confined areas, unreachable by files during root canal preparation (Peters *et al.* 2001) is a common cause of root canal treatment failure (Wu & Wesselink 2005, Ricucci *et al.* 2013).

Biofilm removal has been attempted using syringe irrigation, laser irrigation, and ultrasonic activation (van der Sluis *et al.* 2015). The latter, Ultrasonic Activated Irrigation (UAI), makes use of ultrasonically oscillating files with the aim of improving the chemical and mechanical efficacy of root canal cleaning. In UAI, both cavitation and microstreaming occur even at the lowest clinically significant power settings, in addition their occurrence increases proportional to the ultrasonic power (Macedo *et al.* 2014a,b). The amount of cavitation is dependent on the instrument design, tip size and taper, and also on the type of irrigant and on the confinement of the file within the canal (Macedo *et al.* 2014a,b). Transient cavitation (growth and subsequent implosion of bubbles) has been found both in straight and curved canals, at the entrance of simulated lateral canals and isthmi, and up to 2 mm beyond the tip of the file (Macedo *et al.* 2014b).

Few studies have thus far focused on how the biofilm as a structure responds to mechanical stress. A hydrogel mimicking the viscoelastic properties of a biofilm has been reported (Macedo *et al.* 2014c). Visualization of the removal of a hydrogel from lateral canal anatomies by UAI indicated that microstreaming and transient cavitation may be critical to biofilm cleaning efficacy; however, their

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2
3 central contribution in the cleaning process has not yet been evaluated. Understanding this behaviour
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5 in relation to flow and related fluid dynamical phenomena, e.g. cavitation, is fundamental for
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7 optimizing biofilm removal strategies in root canal cleaning.

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9 The aim of this study therefore was to investigate the effects of file type, lateral canal location and
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11 irrigant on the removal of hydrogel from a lateral canal. In addition, the amount of cavitation and
12
13 streaming was quantified for these parameters. The null hypothesis was that all file types / irrigant
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15 combinations performed equally and that lateral canal location did not affect cleaning efficacy.
16

17 18 19 **Materials & Methods**

20 21 *Cavitation quantification by sonochemical dosimetry*

22
23 A previously described intracanal sonochemical dosimetry method (Macedo *et al.* 2014a) was used to
24
25 measure sonoluminescence (SL) generated by the endodontic files. The SL signal gave a direct
26
27 measure of the amount of transient cavitation occurring around an endodontic file. The endodontic
28
29 file was positioned in a PDMS (PolyDiMethylSiloxane; Sylgard 184, Dow-Corning, Midland, MI,
30
31 USA) root canal model. The canal has an apical diameter of 0.45 mm, a taper of 6% and a length of
32
33 20 mm and was fixed inside a 1.0×1.0×4.0 cm³ cuvette (Plastibrand, Brand, Wertheim, Germany).

34
35 The Ultrasonic Activated Irrigation was performed with 25 mm long, size 25 IrriSafe files (Satelec
36
37 Acteon, Merignac, France), driven with a commercial endodontic ultrasound device (P-Max, Satelec
38
39 Acteon) at its maximum power “Red 10”. The instruments were centered and fixed 1 mm from
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41 working length (WL). The ultrasound device was driven by a pulse generator (TGP110, TTI,
42
43 Huntingdon, UK) in 18 series for a period of 10 s with a duty cycle of 30%, consisting of 3 s ON and
44
45 7 s OFF. After each 3 series of measurements, the file and irrigant were replaced and none of the files
46
47 fractures during the experiments.

48
49 Six irrigants were included in the measurements: i.) Filtered water (Millipore Corporation, Billerica,
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51 MA, USA); ii.) Degassed filtered water which had been degassed using a vacuum pump for a
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53 minimum of 30 minutes; iii.) Oversaturated filtered water obtained by pumping air into filtered water
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55 for at least 30 minutes; iv.) 1% NaOCl solution (Sigma-Aldrich, St. Louis, MO, USA); v.) 4.5%
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57 NaOCl solution; vi.) 9% NaOCl Solution. NaOCl solutions were obtained by dilution of a 10-15%
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3 NaOCl (Sigma-Aldrich) with filtered water. Each measurement was repeated 3 times. The
4
5 concentration of the various NaOCl solutions was measured immediately prior to starting the
6
7 experiments using a standard titration method (Vogel 1962).
8

9 In order to measure the SL intensity, a photomultiplier tube (PMT; R508, Hamamatsu Photonics,
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11 Hamamatsu, Japan) was mounted within a light-tight box and adjacent to the cuvette containing the
12
13 root canal model. The PMT received an electrical voltage of 1.6 kV from a DC power supply (6516A,
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15 Hewlett-Packard, Palo Alto, CA, USA). Its output was recorded at a rate of 300 kHz with a high-
16
17 speed data acquisition device (DAQ; USB-6356, National Instruments, Austin, TX, USA). The
18
19 average and standard deviation of each of the 18 measurements was calculated as previously
20
21 described (Macedo *et al.* 2014a). A calibration measurement demonstrated that there was a linear
22
23 response of the PMT up to an output voltage of 1 V and an interclass correlation coefficient score of
24
25 0.994 for single measurements with $p < 0.001$ was obtained.
26

27 *Hydrogel removal from a lateral canal*

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29 A second set of transparent root canal models included lateral canals, as described before for the
30
31 investigation of root canal cleaning (Macedo *et al.* 2014c). The models exhibited the very same
32
33 dimensions as the one described above, but with a lateral canal with a diameter of 200 μm and
34
35 positioned 3 mm or 6 mm from the apex.
36

37 A hydrogel, demonstrated previously to be an appropriate biofilm mimic, was prepared as described
38
39 (Macedo *et al.* 2014a). Prior to being used, it was stored in an oven at 30°C (Hybridisation oven S1
40
41 20H, Stuart Scientific, Stone, UK). The viscoelastic properties were confirmed to be similar to those
42
43 described by Macedo *et al.* (2014a).
44

45 The hydrogel was placed in the lateral canal using a 30G needle (Becton, Dickson and Company,
46
47 Oxford, UK) and remained there for at least 1 minute to cool and solidify at room temperature. After
48
49 that, the root canal was filled with irrigant. The ultrasonic file was positioned in the centre of the
50
51 model with the file tip aligned to the entrance of the lateral canal and the oscillation direction was in
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53 plane with the lateral canal. The file was driven by an ultrasonic device (P5 Suprasson, Satelec
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55 Acteon) at a power setting of 8.5/20 (42.5%), corresponding with a power setting of “Yellow 5” as
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57 used in a previous study (Macedo *et al.* 2014a).
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3 The root canal models were imaged using a bright-field microscope (Leitz Dialux 22, Leica, Wetzlar,
4 Germany) and a digital SLR camera equipped with a 12.3 megapixel CMOS sensor (D5000, Nikon,
5 Tokyo, Japan) at a recording rate of 24 fps. The sample was magnified by a 10× objective, resulting in
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7 900 μm of the lateral canal visible in the recordings, which corresponds to a hydrogel volume of 28
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9 nL.
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12 The experiment was performed using two instruments (IrriSafe length 25 mm, size 25 and length 21
13 mm, size 15 Satelec Acteon) and three irrigants (n=10) resulting in 6 experimental groups. Activation
14 was performed for 20 s, following the recommended UAI protocol (van der Sluis *et al.* 2010, Macedo
15 *et al.* 2014d). Studies were undertaken on a lateral canal positioned 6 mm from the apex. The irrigants
16 studied included saturated water, degassed water and sodium hypochlorite (NaOCl, Septodont,
17 Maidstone, UK). Saturated water was prepared by pumping air into filtered water for at least 30
18 minutes; degassed water was prepared by placing filtered water in a vacuum chamber for at least 30
19 minutes. Filtered water was prepared by running tap water through carbon, 5 μm sediment and DI
20 filters (Osmotics, Aylsham, UK). NaOCl was determined to have a concentration of 4.5% by a
21 standard titration protocol (Vogel 1962).
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33 An additional study (n=5) was performed using the two instruments on a lateral canal located 3 mm
34 from the apex. Both saturated and degassed water were compared resulting in 4 experimental groups.
35
36 In a control group, NaOCl was used as irrigant in the absence of UAI, in order to study the chemical
37 reaction between NaOCl and the hydrogel.
38
39

40 The area occupied by the hydrogel was calculated into volume for each frame of the videos using a
41 Matlab script (The Mathworks, Natick, MA, USA). Mean and average volume were calculated for
42 each group and plotted as a function of time.
43
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45
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47 Non-parametric Kruskal–Wallis with Mann Whitney U as *post hoc* tests were performed to compare
48 the volume of hydrogel removed, for each root canal model, and for the 3 different irrigation solutions
49 (degassed and saturated filtered water and 4.5% NaOCl solution). Mann Whitney U tests were used to
50 compare the volume of hydrogel removal in lateral canals at two positions (3 vs. 6 mm), by two file-
51 types (IS length 25 mm, size 25 and K length 21 mm, size 15,) and between activated and non-
52 activated irrigation with NaOCl at 4.5%. For all tests, P-values < 0.05 were considered statistically
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3 significant. The statistical values were calculated for 1 to 10 and 11 to 20 seconds of hydrogel
4
5 removal.

6 7 *Velocity measurements with Particle Imaging Velocimetry (PIV)*

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9 The streaming around an unconfined endodontic file was recorded using a high-speed camera (HPV-
10
11 1, Shimadzu Corp., Kyoto, Japan), capable of recording 100 frames at speeds up to 10^6 frames/sec.
12
13 The camera was attached to a microscope (Leitz Dialux 22, Leica) with a $20\times$ magnification
14
15 objective, with a measurement depth of field of $100\ \mu\text{m}$. Illumination for bright field imaging was
16
17 provided by a continuous wave light source (KL2500, Schott, Germany).

18
19 An IrriSafe length 25 mm, size 25 or K length 21 mm, size 15 file was positioned inside the PDMS
20
21 root canal models described above, filled with degassed or saturated demi water, or with a NaOCl
22
23 solution (4.5%). The file tip was aligned with the lateral canal entrance. Activation was performed
24
25 using an ultrasound device (P5 Suprasson, Satelec Acteon) operated at a power setting of 8.5/20.
26
27 Monodisperse hollow glass spheres of diameter $10\ \mu\text{m}$ (Sphericel, Potters Industries, Barnsley, UK;
28
29 mean density of $1.1 \cdot 10^3\ \text{kg/m}^3$, Stokes number $O(1)$ for the highest velocities occurring indicating
30
31 that they follow the flow well) were added to the liquids.

32
33 The flow was analyzed from the high-speed recordings using a particle imaging velocimetry
34
35 algorithm developed in-house (Verhaagen *et al.* 2013a). The oscillatory component was analyzed by
36
37 calculating the ensemble average over 2-3 frames for each area of 16×16 pixels down the lateral
38
39 canal. The resulting velocity vs. distance plots were verified to show translatory oscillations of
40
41 approx. 30 kHz; the rms value was used as final value for the oscillatory velocity, while the steady
42
43 component of the velocity was calculated from the mean of the velocimetry result.

44
45 Measurements were performed ten times for each file and each liquid. Due to the large range of the
46
47 velocities (which could not be captured in a single video), videos were made at two recording speeds:
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49 at 250 kfps, for the oscillatory component, and at 63 kfps, for the steady component.

50 51 52 53 **Results**

54 55 56 *Hydrogel removal*

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3 The hydrogel located in the lateral canal detached in fragments. Figure 1 shows the hydrogel removal
4 as a function of time, averaged over the 10 repeated experiments.

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7 For a lateral canal at 6 mm, the greatest removal rate occurred with NaOCl, followed by saturated and
8 then degassed water (Figure 1). The differences between the three irrigants after 20 s were significant
9 for the IrriSafe file ($P < 0.05$, Figure 1B), at which moment 28/43/64% was removed for degassed and
10 saturated water and NaOCl, respectively. For the K-file, only degassed water and NaOCl were
11 significantly different ($P < 0.05$, Figure 1A). In the absence of activation, the removal of hydrogel by
12 the chemical reaction between NaOCl and the hydrogel occurred at a much lower rate ($P < 0.001$).

13
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15 The IrriSafe file outperformed the K-file in the lateral canal positioned at 6 mm, removing more
16 hydrogel with NaOCl ($P < 0.05$) and saturated water ($P < 0.01$) in 20 s (Figure 1) (43% and 28%,
17 respectively, for saturated water). When averaging all irrigants for this lateral canal, the difference
18 between the two file types was highly significant ($P = 0.001$).

19
20
21 There were no significant differences between degassed and saturated water in a lateral canal
22 positioned at 3 mm (Figure 2A, B). With the lateral canal at 3 mm vs. 6 mm, more hydrogel was
23 removed (49% and 27%, respectively, after 20 s with degassed water), although this difference was
24 not always significant.

25
26
27 In many of the recordings involving saturated water and NaOCl, small transient bubbles (cavitation)
28 could be observed at the removal interface (supplementary video). Larger, stable bubbles were also
29 observed in some recordings, which were found to negatively influence the hydrogel removal rate
30 (Figure 3).

31 32 33 34 35 36 37 38 39 40 41 42 43 44 *Cavitation quantification*

45
46 The SL intensity generated by IrriSafe 25 mm length, size 25 files in the six irrigant solutions is
47 plotted in Figure 4. It is evident that cavitation did not occur in degassed water with no difference
48 with the background noise ($P = 0.597$). There was no significant difference in the SL value between the
49 tap water regular and oversaturated water ($P = 0.538$), although their SL values were both higher than
50 those for degassed water ($P < 0.043$). The highest SL signal was generated with NaOCl solutions
51 ($P < 0.007$), which increased with concentration ($P < 0.014$).

52 53 54 55 56 57 58 59 60 *Streaming*

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3 The flow pattern in the main root canal was similar to the flow patterns reported previously
4 (Verhaagen *et al.* 2013a), showing oscillatory streaming in phase with the file oscillation; the steady
5 streaming was shaped as jets occurring sideways from the file tip (Figure 5). In the lateral canal, up to
6 two vortices could be identified, driven by the jets (Verhaagen *et al.* 2013b). No qualitative
7 differences were observed between the IrriSafe file and K-file and between the three liquids.
8

9
10
11 The steady (time-averaged) velocities decrease from approximately 0.3 m/s to below 0.05 m/s
12 following penetration of 300 μm into the lateral canal (Figure 6). The IrriSafe outperformed the K-file
13 in fluid velocities in the lateral canal both at 3 and 6 mm (Figure 6A vs. B). The velocities were
14 higher in the lateral canal at 3 mm than at 6 mm (Figure 6A vs. C). The differences between the three
15 irrigants, however, were not significant.
16

17
18 The oscillatory component of the velocity shows the 30 kHz oscillation of the liquid (Figure 5 insert),
19 with rms velocities decreasing from approximately 0.5 m/s to below 0.1 m/s 450 μm into the lateral
20 canal (Figure 7). The IrriSafe file outperformed the K-file when using saturated water or NaOCl; with
21 degassed water, no significant differences were found. The oscillatory velocities were higher inside
22 the lateral canal at 3 mm compared to that at 6 mm. Velocities were found to be higher for NaOCl
23 than that for saturated water, which was in turn higher than that for degassed water; this effect was
24 present with both files and most prominent with the lateral canal at 6 mm. This measurement was
25 repeated twice and in separate laboratories where the above findings were confirmed. The
26 contribution of alignment or intermittent transient cavitation on the file tip to the streaming was
27 therefore ruled out.
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45 **Discussion**

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47 A number of studies have shown that ultrasonic activation of the irrigant has yielded an improved
48 outcome over syringe irrigation in cleaning isthmuses and irregularities in the canal wall (Lee *et al.*
49 2004, Gutarts *et al.* 2005, Rödiger *et al.* 2010, Paqué *et al.* 2011). Both streaming and cavitation have
50 been proposed to play a significant role in the cleaning process (van der Sluis *et al.* 2007). This study
51 measured both the amount of cavitation and the streaming in relation to the removal of a biofilm-
52 mimicking hydrogel. Up to 64% of the hydrogel could be removed from a lateral canal within a time-
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3 span of 20 s. The rate of hydrogel removal decreased as hydrogel removal progressed into the lateral
4 canal. Simultaneously, the flow velocities of the vortices in the lateral canal decreased with distance
5 into the lateral canal.
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9 The IrriSafe file outperformed the K-file in removing the hydrogel from the lateral canal, for all
10 irrigants and lateral canal positions. It has previously been shown that there is a greater amount of
11 cavitation around an IrriSafe file than a K-file (Macedo *et al.* 2014a). Additionally, the IrriSafe file
12 has a larger cross-section, leading to a closer proximity of the file to the root canal walls. It has also
13 been demonstrated before that this can lead to higher velocities, as well as to an increased pressure
14 and higher shear stresses; all aspects are beneficial for cleaning (Verhaagen *et al.* 2013a). These
15 findings reject the null hypothesis.
16
17

18
19 The experimental data shows that cleaning a lateral canal takes place even when using degassed water
20 alone, in which, according to the sonochemiluminescence data, little (if any) cavitation occurs since
21 degassing causes a reduction of the nuclei available for cavitation generation (Brennen 1995). This
22 indicates that streaming around the files plays a significant role in hydrogel removal.
23
24

25
26 The cleaning rate was increased when NaOCl or oversaturated water was used instead of degassed
27 water. This may have resulted from their increased gas content and/or the microbubble-stabilizing
28 surfactant action of salts in NaOCl (Wall *et al.* 1999). The increased amount of cavitation in NaOCl
29 and saturated water may initially appear as the cause leading to improved cleaning, especially since
30 the streaming is assumed to be equal as the fluidic properties differ minimally between the liquids.
31 However, the measurements revealed significant differences in fluid velocities in the three different
32 liquids. The oscillatory component of the velocities was found to increase when using NaOCl
33 compared with saturated or degassed water (in that order); no significant differences were found for
34 the steady flow component. This finding for oscillatory velocity was unexpected as the acoustic
35 streaming theory predicts that the oscillatory part of the flow is dominated by potential flow, which is
36 independent of the fluidic properties. Possibly the small differences in fluidic properties (van der Sluis
37 *et al.* 2010) have an effect on file oscillation and/or acoustic streaming effects. The effect of
38 misalignment with respect to the lateral canal was ruled out by repeating experiments in different
39 laboratories and by repositioning the file prior to experimental analysis. This approach should have
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3 eliminated variations in the alignment. 2D numerical simulations (Verhaagen *et al.* 2013a) using a
4 model including a lateral canal also suggested that the file-to-canal alignment could not have led to
5 this difference (unpublished data). Additionally, the effect of cavitation on the streaming was
6 eliminated through additional repeats of the PIV experiments at different settings. Further research on
7 these intriguing aspects are therefore needed.
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12 In all the measurements involving the lateral canal at 6 mm from WL, significantly more hydrogel
13 was removed with NaOCl as the irrigant; both the chemical dissolution potential and the effect on
14 bubble formation are potential contributors and depend on the concentration of NaOCl. This finding is
15 in line with previous studies (van der Sluis *et al.* 2010), but contrary to that of a previous report
16 (Macedo *et al.* 2014c), which observed greater cleaning with water than with NaOCl. However, in that
17 study, the lateral canal was located at the 3 mm position, and a greater concentration of NaOCl was
18 used, both variables may therefore have resulted in greater amounts of inhibiting bubbles. To ensure
19 reproducibility of the present set of experiments, the root canal models were ensured to be absent of
20 large, stable air bubbles prior to cleaning.
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25 With the lateral canal 3 mm from WL, more stable bubbles were formed that hindered hydrogel
26 removal, see Fig. 3. This outcome could be due to the reduced confinement and closer file proximity
27 to the root canal walls, leading to the higher velocities measured, as well as increased pressures and
28 shear stresses (Verhaagen *et al.* 2012). Higher pressures may have also increased the probability of
29 stable bubble formation. Previous studies observing this phenomenon suggest that the bubbles could
30 be generated by rectified diffusion that is enhanced at higher acoustic pressures (Crum 1980, Macedo
31 *et al.* 2014c). On the other hand, higher acoustic pressures that are generated in smaller confinements
32 may result in greater forces being exerted on the hydrogel (as long as compressible bubbles are
33 absent).
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38 The setup used in this study is an idealised situation that is useful for studying the effect of individual
39 parameters such as file type, irrigant, and amount of cavitation and streaming on hydrogel removal. In
40 practice, there may be several factors that affect the cleaning of a root canal. For example, whereas
41 contact with the wall was avoided in the present setup, it is very likely in narrow root canal
42 preparations for the file to contact the root canal wall during activation, which has been shown to
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3 affect file oscillation and therefore streaming and cavitation (Boutsioukis *et al.* 2013a). Local
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5 complex root canal and lateral canal geometry may further complicate the streaming and therefore the
6
7 cleaning by ultrasonic activation. Additionally, during clinical practice, the irrigant will not be as
8
9 clean as used in this study but will contain debris and other contaminants that may affect the
10
11 streaming, cavitation process and cleaning rate.

12
13 Furthermore, in the clinical situation, stable bubbles may enter into lateral canals prohibiting efficient
14
15 cleaning, similar to a bubble being entrapped near the apex (vapour lock) (Boutsioukis *et al.* 2013b).

16
17 While a vapour lock can be removed with minimal effort (Boutsioukis *et al.* 2013b) a bubble within a
18
19 lateral canal will be more difficult to remove due to its location. The clinical significance of the
20
21 inhibiting stable bubbles should be investigated, and the conditions for formation of these bubbles and
22
23 ways to remove them should be considered in future work.
24

25 26 27 **Conclusions**

28
29 The removal of a biofilm-mimicking hydrogel from a lateral canal by Ultrasonically Activated
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31 Irrigation was shown with up to 64% of the hydrogel removed within 20 s. Measurements of
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33 cavitation and acoustic streaming have provided insight into their contribution to cleaning. Significant
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35 differences in cleaning, cavitation and streaming were found depending on the file size, lateral canal
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37 location and irrigant used. In general, the IrriSafe file outperformed the K-file, and NaOCl was more
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39 effective than the other irrigants tested. The cavitation and streaming measurements showed that both
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41 contributed to the hydrogel removal and indicated that both play a significant role in root canal
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43 cleaning.
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45 46 47 **Acknowledgements**

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3 microstreaming and the effect of file-to-canal alignment. We also acknowledge the loan of equipment
4
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11 **Conflict of Interest statement**
12

13 Dr. van der Sluis reports grants from Government financed project (Technology Foundation STW),
14 during the conduct of the study. The other authors have stated explicitly that there are no conflicts of
15 interest in connection with this article
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For Peer Review

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Figure legends

Figure 1 Hydrogel removal (percentage of hydrogel that was visible) versus time, for the K-file (A) and IrriSafe file (B), for three different liquids. Lateral canal at 6 mm from WL. The plots show the means (solid lines) and standard deviations (typically 10% of the mean; indicated with shaded areas).

Figure 2 Hydrogel removal versus time for degassed and saturated water, comparing the lateral canal at 3 or 6 mm from WL.

Figure 3 Hydrogel removal versus time in the presence or absence of bubbles.

Figure 4 Sonoluminescence signal (background subtracted), representing the amount of cavitation, for various liquids. Error bars denote standard deviation.

Figure 5 Example of an averaged PIV result near the tip of a K-file next to a lateral canal. The figure shows (top) the velocity vectors and (bottom) the velocity magnitude (m/s). The inset shows the oscillatory velocity at the indicated location inside the lateral canal, demonstrating the 30 kHz oscillation of the liquid. The average (steady) velocity is indicated with a dashed line.

Figure 6 Steady velocity magnitudes as a function of distance within the lateral canal and for three different liquids. (A) K-file, lateral canal at 6 mm. (B) IrriSafe file, lateral canal at 6 mm. (C) K-file, lateral canal at 3 mm.

Figure 1 Rms oscillatory velocities within the lateral canal showing both the mean (solid lines) and standard deviations (shaded areas). (A) Comparison of the two file types, using NaOCl and the lateral canal at 6 mm. (B) Comparison of the lateral canal at two different positions, using degassed water and the IrriSafe file. (C) Comparison of the three liquids, using the IrriSafe file and the lateral canal at 6 mm.

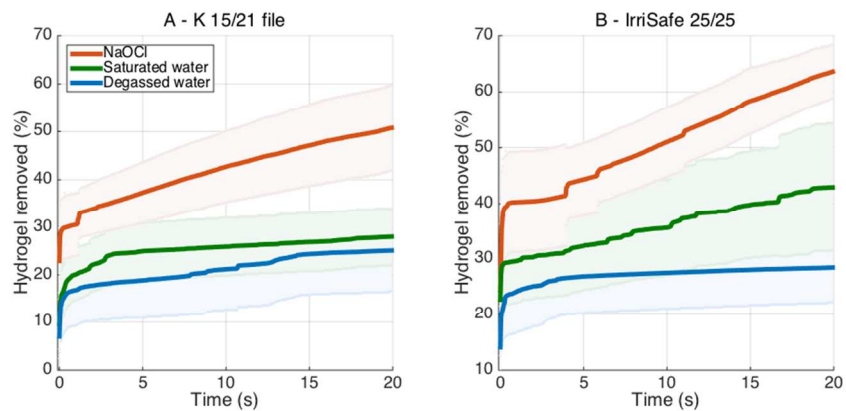


Figure 1

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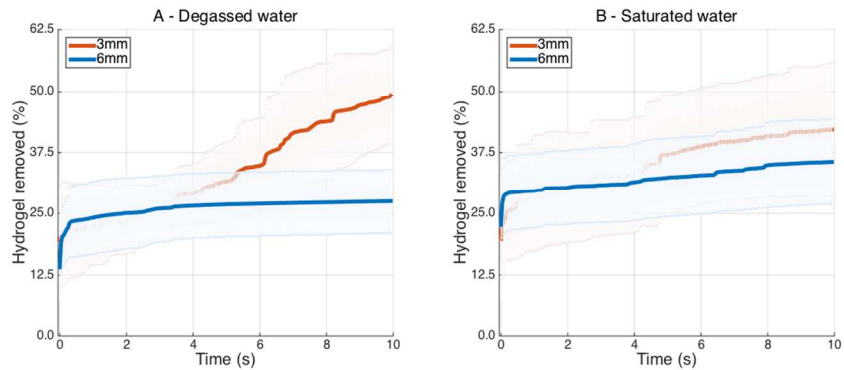


Figure 2

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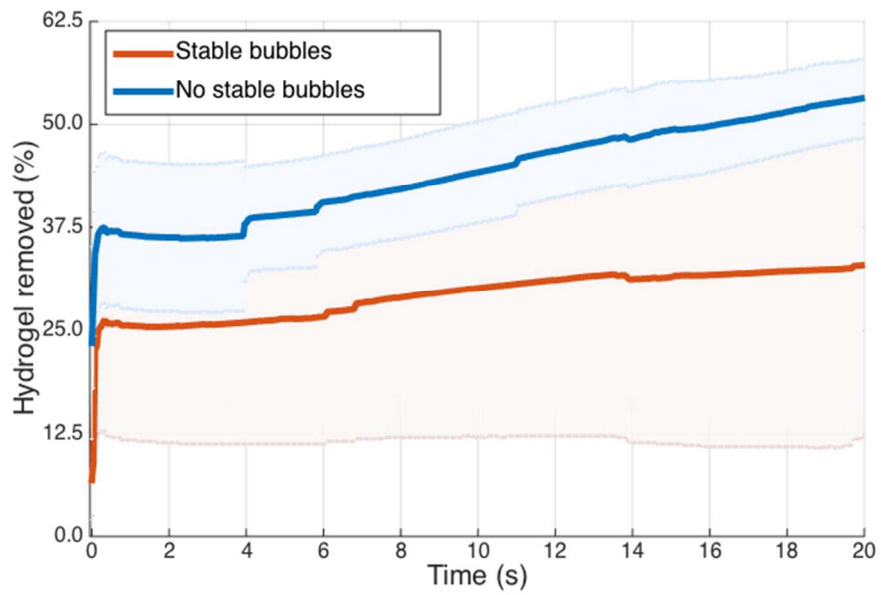


Figure 3

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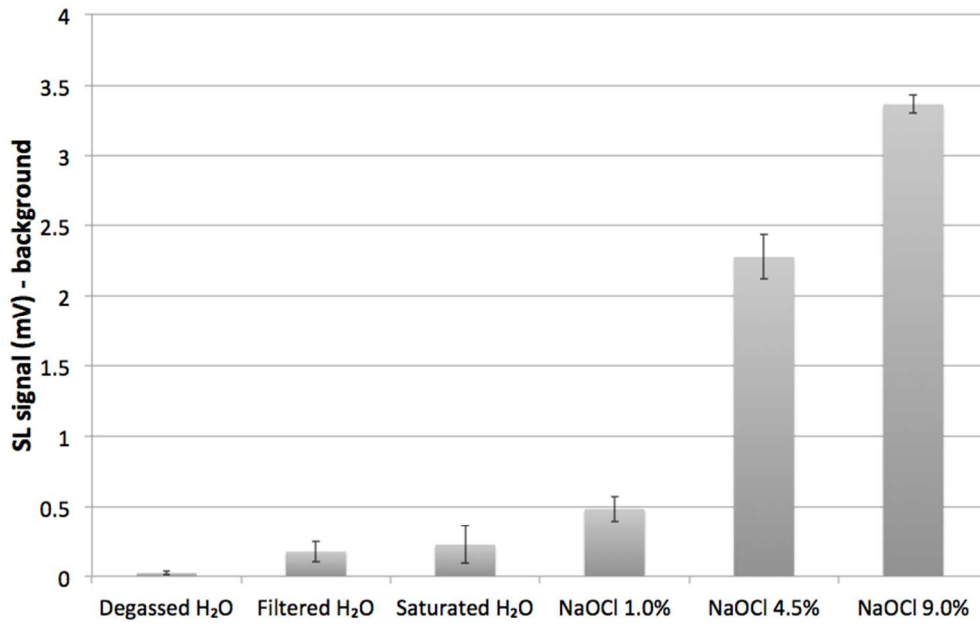


Figure 4

310x194mm (72 x 72 DPI)

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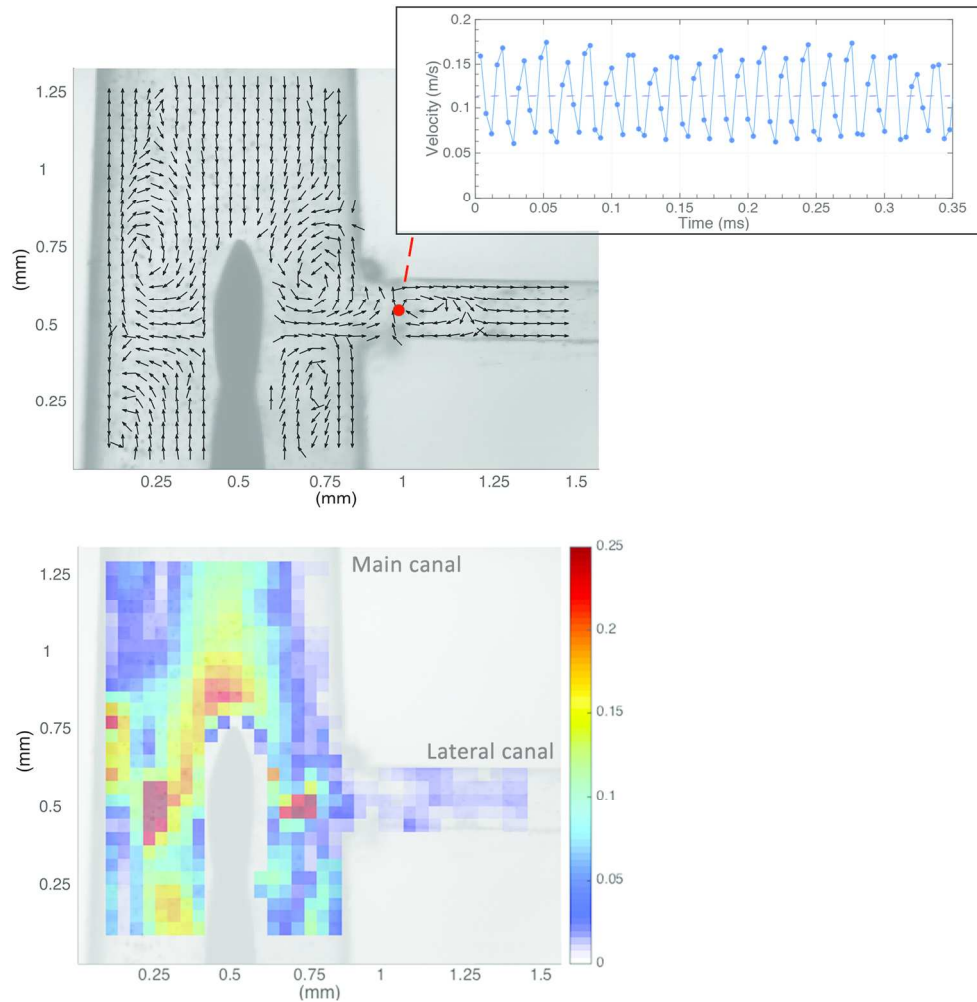


Figure 5

150x150mm (300 x 300 DPI)



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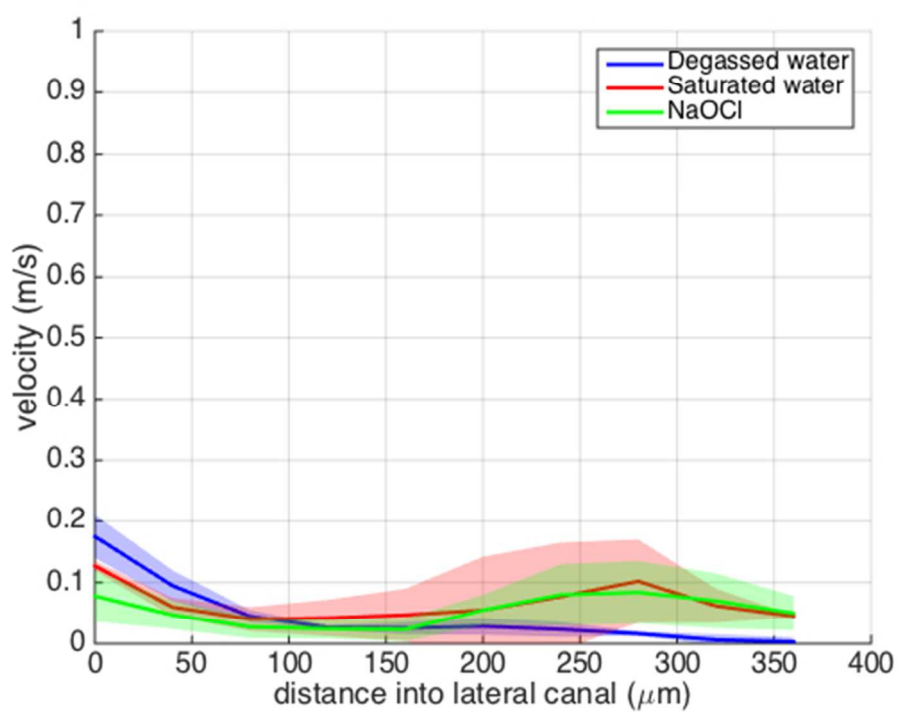


Figure 6A

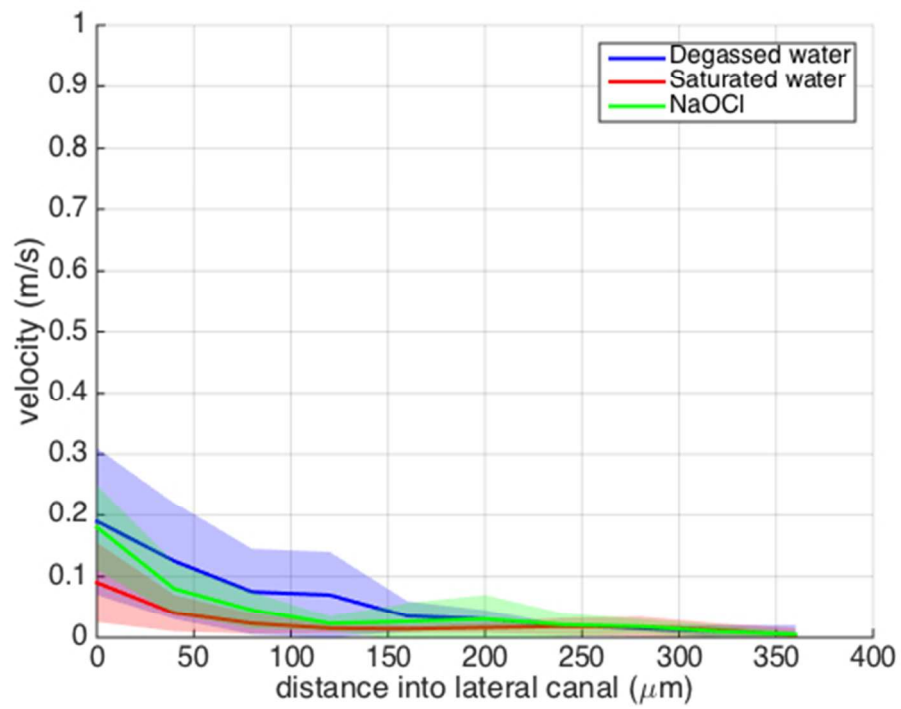


Figure 6B

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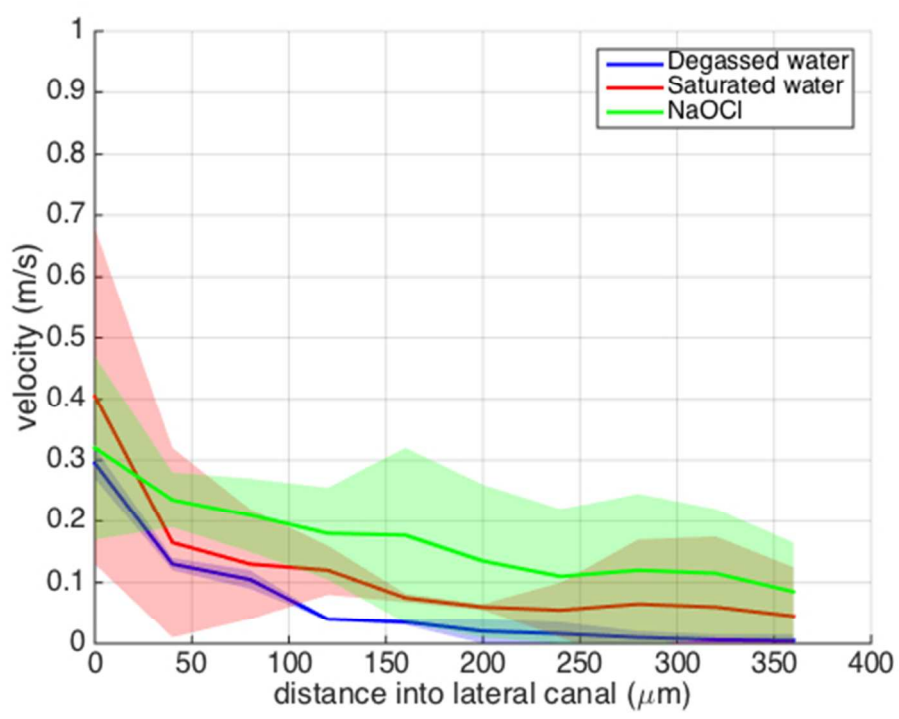


Figure 6C

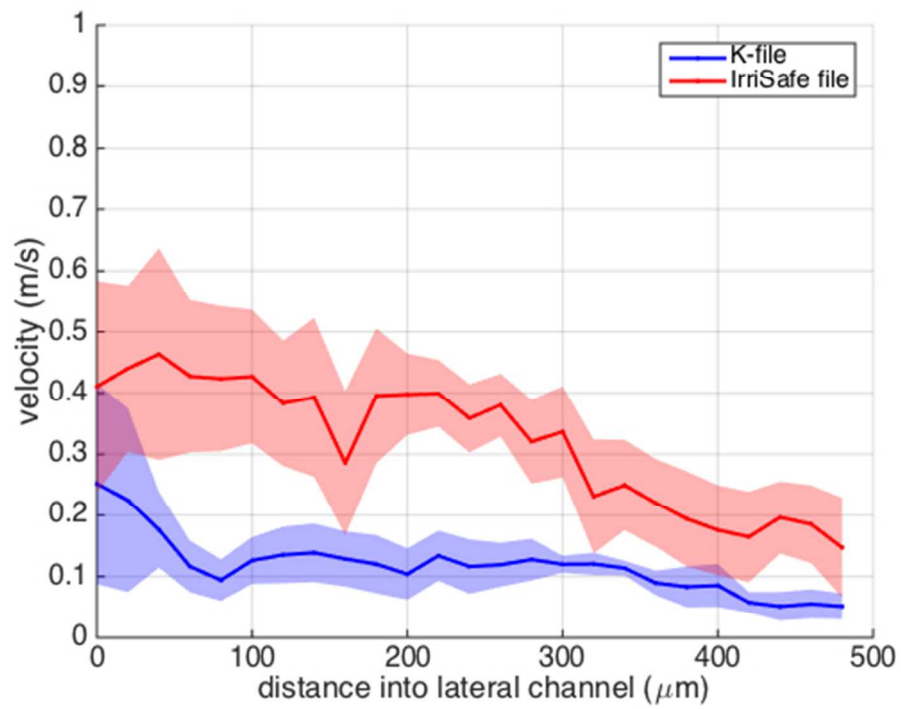


Figure 7A

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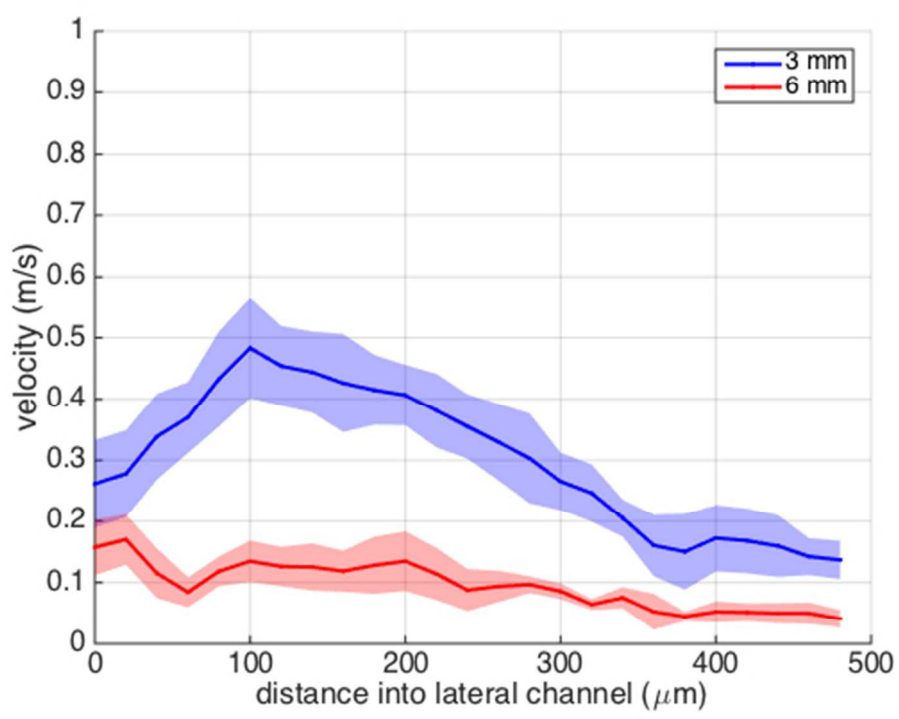


Figure 7B

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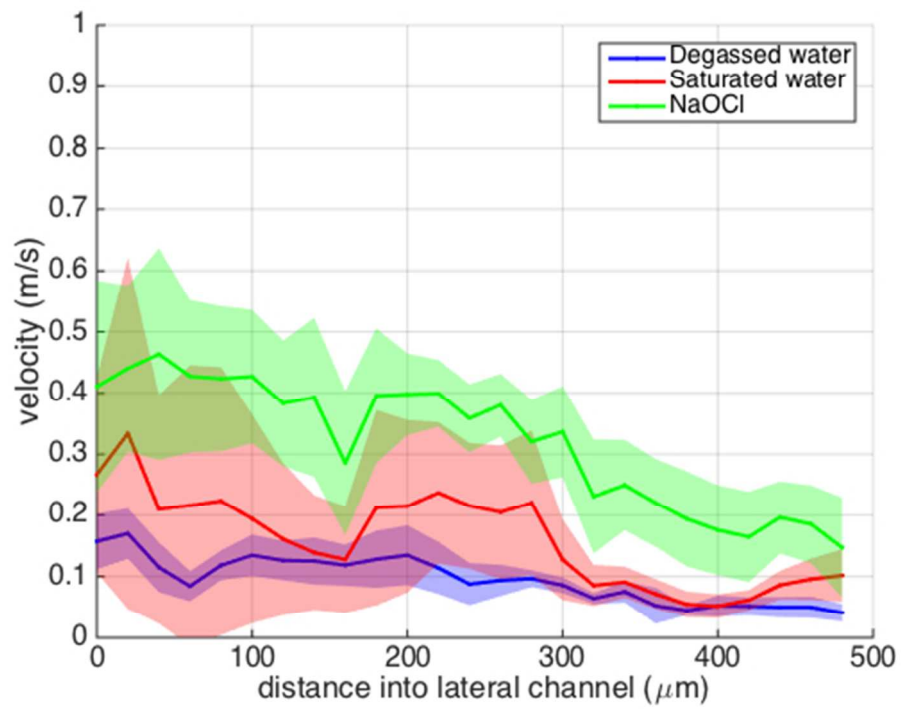


Figure 7C