EVIDENCE FOR TRAPPED SURFACE BUBBLES AS THE CAUSE FOR THE TWINKLING ARTIFACT IN ULTRASOUND IMAGING

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Abstract—The mechanism of the twinkling artifact (TA) that occurs during Doppler ultrasound imaging of kidney stones was investigated. The TA expresses itself in Doppler images as time-varying color. To define the TA quantitatively, beam-forming and Doppler processing were performed on raw per channel radio-frequency data collected when imaging human kidney stones in vitro. Suppression of twinkling by an ensemble of computer-generated replicas of a single radio frequency signal demonstrated that the TA arises from variability among the acoustic signals and not from electronic signal capture or processing. This variability was found to be random, and its suppression by elevated static pressure and return when the pressure was released suggest that the presence of bubbles on the stone surface is the mechanism that gives rise to the TA. (E-mail: olegs@apl.washington.edu) Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology.

Key Words: Ultrasound imaging, Twinkling artifact, Kidney stones, Doppler processing, Overpressure, Microbubbles.

INTRODUCTION

The twinkling artifact (TA; Rahmouni et al. 1996) appears as a dynamic color mosaic on the image of hard objects in a color-Doppler ultrasound display. Recent studies (Aytac and Ozcan 1999; Gromov and Zykin 2002; Kim et al. 2010; Lee et al. 2001; Mitterberger et al. 2009; Shabana et al. 2009; Trillaud et al. 2001; Turrin et al. 2007; Vasiliev and Gromov 1997; Winkel et al. 2012) have reported that the TA as a Doppler ultrasound artifact has a great potential to improve kidney stone detection; however, this artifact is inconsistent precisely because its origins are unknown. Its manifestation depends on the specific ultrasound imager, the sonographer’s skills, the machine parameter settings and the type of stone. Our goal is to improve the understanding of the mechanisms that give rise to the TA as a step toward making the TA a reliable clinical tool.

Several studies have investigated the mechanism of the TA displayed by kidney stones. The sources of the artifact were attributed to either the acoustics or the machine—that is, to peculiarities either of ultrasound scattering from the stone or in the system processing of the unique scattered signal. In one study of the acoustics, the TA was explained to be a result of the random scattering of the ultrasound beam at multiple reflectors associated with the rough interface typical for the stones (Rahmouni et al. 1996). A study of the TA from different types of stones showed that the intensity and character of the TA might depend on the morphology and biochemical content of the stones (Chelfouh et al. 1998). A more recent study showed that the strength of the twinkling depends on the color-Doppler carrier frequency (Gao et al. 2012). Other investigators believe that the appearance of the TA is determined by the ultrasound machine or machine settings, such as the scan type, technical parameters, gain and scan settings (Aytac and Ozcan 1999; Lelyuk et al. 2003; Rubaltelli et al. 2000). One of the more recent mechanism studies has suggested that the cause for the artifact is narrow-band internal noise owing to “phase jitter,” and the irregular stone surface is only secondary and serves only to broaden the spectrum (Kamaya et al. 2003); however, they also concluded that “experiments were limited by the inability to control all machine settings separately,” which leaves many steps in their outline unexplained.

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For most of the studies mentioned here, the conclusions were based on analysis of the Doppler images and Doppler spectrum that were generated by commercial ultrasound machines. Those images can vary between machines, depending on the imaging processing methods used and the different machine settings. The commercial ultrasound machines are "black boxes," in that it is difficult to separate the acoustic effects (e.g., scattering from a rough surface, stone reverberation) from the effects of the machine (e.g., phase jitter, machine settings and signal processing). In addition, clinical ultrasound machines rarely provide users with access to the raw radio-frequency (RF) data that are more fundamental than the images, because the raw per-channel RF signals originate directly from the ultrasound array elements—that is, they are not distorted by any post-processing from the machine.

In this study, the raw per-channel RF data immediately following the analog-to-digital converter (ADC) were used. According to the conventional color Doppler imaging algorithm (Evans and McDicken 2000), the color pixels shown on the image are always encoded based on the variability within the Doppler ensemble that corresponds to strong Doppler power. With that in mind, the Doppler power was used as the criterion of the TA. Based on the RF data analysis, the dominant reason for the occurrence of the TA was investigated by estimating whether the variability within the Doppler ensemble is introduced from the acoustic field or from the machine. In addition, a high static pressure study and other studies were performed to further investigate the mechanisms for the generation of the TA.

MATERIALS AND METHODS

Rationale for the chosen materials and methods

The experiments were preceded by many unreported observations of the distinct features of the TA in vivo and in vitro. Although the TA is seen frequently when imaging stones in patients, it was important for the mechanistic study to mimic the clinical situation in vitro, for which the stone position and medium around it could be controlled. To ensure that the in vitro conditions would be close to the in vivo ones and no patterns of difference between the raw signals from both were detected, real human kidney stones were used as imaged objects. The stones were either embedded in a degassed gel block or held fixed in degassed water. Several stones under study that showed twinkling with the Verasonics Ultrasound Engine (VUE) used in the studies reported here were also imaged by other ultrasound machines, namely ATL-Philips HDI 5000 (Philips Medical Systems, Bothell, WA) and Ultrasonix RP (Ultrasonix, Richmond, BC, CA), which also showed the TA. Although the details of the stone images were not identical, there are also undefined differences in signal processing and image formation in different ultrasound machines. The fact that the VUE showed the TA to be similar to that produced in other machines supported the choice of the VUE as the main experimental tool (an open architecture ultrasound imager). To clarify the rationale for the performed experiments, we mention in this section some results in advance. The first fundamental need was to determine the primary cause of the TA—specifically, the acoustics (i.e., the ultrasound propagation and scattering) or the machine (i.e., the electronics of the transmitter or receiver and the Doppler signal processing). By examining the machine aspect with a stable signal from a function generator that mimicked the signals originated from ultrasound scattering, we determined that it was the acoustics, thus removing the potential pulse-to-pulse variability. The next logical step was to investigate the acoustic origin of the TA. It was determined that the signal variability was uniformly distributed within the Doppler pulse ensemble. Because the origin of the TA was in the acoustics, bubbles (as random scatterers) were a reasonable mechanism to investigate. Of course, other causes could also be involved (e.g., speckle noise due to the rough surface of a stone). To test the bubble hypothesis, a special chamber was built that allowed imaging of the stones under elevated static pressure. The application of sufficient overpressure should collapse any bubbles present on the stone surface and suppress the TA, which it did. To confirm this rather surprising observation, scratches, which would harbor bubbles, were created in smooth plastic stone models. These scratched models displayed the TA, whereas their smooth predecessors did not. It was also discovered that immersing the stones in ethanol, which would modify the bubble-stabilization mechanism and thus reduce the size and number of surface bubbles (Crum 1979, 1982), also suppressed the TA. Therefore, the experiments of the current study consisted of multiple steps, and different physical and signal processing tools were used and are described in more detail below.

Platform

A Verasonics Ultrasound Engine (VUE, Verasonics, Redmond, WA) and a 128-element linear ultrasound array with a 5-MHz central frequency (a clinical probe ATL/Philips HDI L7-4; Bothell, WA, US) were used for all experiments. The pressure waveform radiated from the transducer was measured by a broadband calibrated hydrophone (HGL0085, ONDA Corp., Sunnyvale, CA) having a sensitivity of 48 nV/Pa at 5 MHz. The acoustic pulse was similar to the transducer voltage; it had the form of a three-cycle tone burst with the central frequency of 5 MHz. At 4 cm away from the transducer in water the, measured peak positive and negative pressures were
P_+ \approx 2 \text{ MPa} \text{ and } P_- \approx -1 \text{ MPa}, \text{ respectively. Knowledge of these values was important for choosing a sufficiently high level of the static pressure in the overpressure test (discussed later). The imaging was performed in flash transmitting mode, when all the array elements were excited simultaneously to emit a quasi-plane wave in the direction orthogonal to the radiating surface (0° incident angle). This mode simplified the analysis of the received signals without limiting the possibility of stone imaging. Both B-mode and Doppler mode were used. In Doppler mode, which showed the TA, the array elements were excited by a series of 14 identical pulses emitted continuously with a 3-kHz pulse-repetition frequency (PRF), which is typical for Doppler regimes with conventional ultrasound machines. The PRF could be changed; the value of 3 kHz was used because it was a default setting for the machine. Each pulse in the aforementioned 14-pulse Doppler ensemble was a tone burst consisting of three cycles at the central frequency of 5 MHz. As with conventional ultrasound imagers, the scattered acoustic signals were received by the same array. The corresponding electrical signals of the array elements went through an anti-aliasing band-pass filter with a bandwidth of 0.7–17 MHz, an amplifier with the time-gain compensating feature and a clipping diode (to limit excessive signals); finally, they were sampled at a frequency of 20 MHz by a 12-bit. The digitized signals were then processed in MATLAB (MathWorks, Nattick, MA, USA) either by the VUE software or by a separate in-house code written in MATLAB. The signals could be either processed in real time or stored in a buffer and post-processed later. The saved signals were RF data from the output of ADCs of each channel. Access to these RF data provides a possibility to study the raw ultrasound signals associated with the TA.

To test the response of the receiving electronic tract and signal processing of typical Doppler signals without using the ultrasound probe, a special break-out board was connected to the entrance of the electronic tract, which enabled the transmission of an electric signal from an external source to any selected receiving channel. In particular, such a source could provide an electrical signal similar to that appearing at the array element when receiving an ultrasound pulse scattered from a stone. This replacement of an acoustically originated signal by a controlled-source signal allowed us to determine whether the TA originated in the machine (i.e., in the electronic tract and signal-processing box) independently or in the acoustics (i.e., from fluctuations during acoustic scattering and propagation). As an external voltage source, a function generator (AFG 3022B; Tektronix, Beaverton, OR, USA) was used. The generator signal was programmed to be identical to the acoustically originated signal.

### Signal processing

B-mode images combined with Doppler-mode images were prepared by using the signal processing internal algorithms provided by the VUE (Daigle 2011). In doing so, the Doppler threshold, which accepts the color information for a certain sample volume based on the comparison of the corresponding Doppler power of the sample volume to a certain percentage of the maximum Doppler power, was decreased to the minimum possible level, just above the level of the appearance of the background noise. The color-write priority was set to the highest level that the color information rather than the B-mode information was always plotted on the screen. In the experiments where the raw signals were analyzed to reveal the origin of the TA, the signal processing was performed using in-house MATLAB codes. In particular, such characteristics as the Doppler residual and Doppler power were calculated to correlate them with the TA. Because those parameters will be used in the consequent sections, they are described here in some detail.

Consider \( N \) pulses \( U_n(t), \ n = 1, 2, \ldots, N \), which represent an ensemble of signals received from one of the elements of the ultrasound probe in the course of Doppler imaging. The pulses follow each other with the PRF. In the VUE, the default Doppler ensemble consists of 14 pulses. However, the first two pulses are omitted to avoid possible unrepeatable tissue reverberation; therefore, \( N = 12 \) is used. The Doppler shift can be measured by comparing signals \( U_n(t - nT) \) for different \( n \), where \( T = 1/\text{PRF} \), is the ensemble period. When those signals are identical, no Doppler shift appears. Any differences between the signals would signify that the scatterer is changing from pulse to pulse. For example, the signals \( U_n(t - nT) \) for different \( n \) would arrive at different times if such a scatterer moved with a certain velocity. The velocity can be calculated from the time shift, which is one of the methods for the velocity estimation in the Doppler mode. If the signals \( U_n(t) \) are not only delayed or advanced but fluctuate in a more general manner, then the Doppler processing would also provide some velocity, but it will not necessarily be related to true scatterer movement. The corresponding Doppler signal would be an artifact, and the TA is one such signal.

Although Doppler processing is a well-known procedure, it can be used in several alternative ways. To clarify the TA analysis of the current study, below the Doppler processing is described as it was implemented in the in-house algorithm. After ADC, the signals \( U_n(t) \) are transformed to a digital form:

\[
U_{\text{num}} = U_n(t_m = m \Delta t)
\]

where \( \Delta t \) is the signal sampling step (50 ns for our 20-MHz sampling frequency), \( m = 1, 2, \ldots, M \), and \( M \) is
the total number of samples recorded in one period of the Doppler ensemble \((M = 1024)\). In addition to analysis of received signals from the individual elements of the array, beam-formed signals were calculated and processed for each channel. Such signals were formed by the conventional “delay-and-sum” beam-forming method. Let \(U_{nm}\) be the digitized signal for either non–beam-formed or beam-formed single-channel data. The first step in the signal processing of the channel data was the calculation of the quadrature components of the signals \(U_{nm}\) using the Hilbert transform. The transform was made in MATLAB by the corresponding tool \(V = \text{hilbert}(U)\). As a result, for each \(n\), a complex (analytic) signal

\[
V_{nm} = U_{nm} + iQ_{nm}
\]  
(2)

was calculated, where the quadrature signal \(Q_{nm}\) is the Hilbert transform of \(U_{nm}\) and \(i\) is the imaginary unit. The next step was “wall filtering” that reduced the signal fluctuations resulting from possible slow-moving scatterers. The first-order regression filter was applied to the analytic signal \(V_{nm}\). Specifically, for each fast time-moment \(m\), the corresponding signals from different pulses within the Doppler ensemble were considered as a function of the pulse number \(n\), and a linear fit

\[
\bar{V}_{nm} = a_m + n b_m
\]  
(3)

was found for \(\bar{V}_{nm}\) using the least squares estimation \((a_m\) and \(b_m\) are coefficients that do not depend on the pulse number \(n\)). Next, the residual signal

\[
\tilde{V}_{nm} = V_{nm} - \bar{V}_{nm}
\]  
(4)

is a wall-filtered signal. The absolute value of that signal, the residual amplitude

\[
A_{nm} = |\tilde{V}_{nm}|
\]  
(5)

can be used to represent the signal fluctuations within the Doppler ensemble. The average power

\[
W_m = \frac{1}{N} \sum_{n=1}^{N} |\tilde{V}_{nm}|^2
\]  
(6)

provides the strength of the fluctuations at the moment of time \(t_m = m\Delta t\) and is usually called the Doppler power.

Because the TA is a fluctuating (i.e., random) phenomenon, additional averaging both in time and space would give a more representative description for the effect. This averaging can be done in a way to mimic what the sonographer does when observing the twinkling color around the stone image. The following procedure was developed for such a characterization. First, the RF data were used to calculate the beam-formed channel data using a conventional sum-and-delay algorithm. Second, one pulse of the Doppler ensemble of each channel was used to build the B-mode image of the stone. The stone contour was defined based on the brightness of the image, and the total number of pixels \((N_{\text{total}})\) within this contour was counted. Third, the Doppler power traces \(W_m\) were calculated for all the beam-formed channels, so that the Doppler power was found for each pixel of the B-mode image. Fourth, the background noise level was defined as the average of the Doppler powers over all pixels in the entire image. Fifth, if the Doppler power exceeded the background noise level by more than 6 dB, the corresponding pixel was counted as a color pixel. Last, the number of color pixels \((N_{\text{color}})\) was counted within the stone contour, and the color percentage was calculated for the twinkling image of the stone under study:

\[
C = \frac{N_{\text{color}}}{N_{\text{total}}} \times 100\%
\]  
(7)

The value of \(N_{\text{total}}\) for each imaged stone was a constant number, but the \(N_{\text{color}}\) varied from frame to frame; therefore, the color percentage \(C\) was also fluctuating from frame to frame. To analyze the differences for two cases (e.g., images of the same stone under different static pressures), the mean value of \(C\) was calculated by averaging more than 100 frames, and the comparison was made using Student’s \(t\)-test, with the \(p < 0.05\) criterion for the decision whether the two mean values were statistically different.

**Experimental targets**

Human kidney stones from a stone laboratory (Beck labs, Indianapolis, IN, USA) that consisted of more than 90% calcium oxalate monohydrate were used for the majority of the experiments. The stones were 5–12 mm in diameter. In total, 18 stones were used. Half of the stones (9) were imaged while embedded in a tissue-mimicking gel; the other 9 stones were held on a metal needle and imaged directly in degassed water. Gel was used to better mimic the stone surroundings in vivo. The ultrasound absorption coefficient of gel was similar to tissue (discussed later). However, no obvious differences were observed in the TA manifestation between the stones in gel and in water; therefore, several experiments (e.g., the overpressure tests) were made directly in water. In those cases, the stones were immersed in the degassed water for more than 48 h before imaging.

The tissue-mimicking gel used in a part of the experiments was a polyacrylamide hydrogel (Sigma-Aldrich, St. Louis, MO, USA). The liquid mixture was first degassed for at least 1 h in a desiccant chamber and then poured into a plastic container (measuring \(10 \times 10 \times 13\) cm) to the 6-cm mark, and a polymerization agent was added. When the agent was set, the studied kidney stones, which were previously immersed in
degassed water for 48 h, were placed on the concretionary gel surface, and another portion of degassed liquid mixture was poured in the container with polymerization agent to the 10-cm mark. The end result was gel with stones suspended in the middle of the container at a depth of 4 cm. The empty top part of the container was later filled with degassed water, and the imaging probe was immersed in the water layer facing the stone. The attenuation coefficient, sound speed and impedance of the gel were 0.08 dB/cm/MHz, 1546 m/s and 1.58 MRayl, respectively (Prokop et al. 2003).

In addition to the stones from the stone laboratory, kidney stones recently removed from patients by percutaneous nephrostomy were used. Because there is the possibility of bubbles being created on stones that have been exposed to air, it was desirable to test stones that were freshly removed from the in vivo environment. Those stones were placed in degassed saline immediately after removal and tested within 12 h. Only two stones were tested because the possibility of getting such stones was limited. The two stones were 6 and 7 mm in diameter.

Besides natural kidney stones, stone-mimicking plastic spheres were also examined. Acrylic spheres (Small Parts Inc., Logansport, IN, USA) with two different diameters (9.5 and 6.4 mm) were used in the tests. Those spheres originally had a smooth surface and did not show any TA. The TA appeared after their surface was scratched. The corresponding rough spots were created by making multiple cuts with a knife with a 0.3-mm-thick blade. The acoustic parameters of acrylic are as follows: longitudinal sound speed of 2750 m/s and density measuring 1200 kg/m$^3$ (Selfridge 1985). An acrylic scatterer is easy to image by ultrasound, although it has lower acoustic contrast to water compared with a natural kidney stone (e.g., calcium oxalate monohydrate stones have a longitudinal sound speed of 4530 m/s and a density of 2040 kg/m$^3$, Heimbach et al. 2000).

**Experimental design and methods**

**Test 1: simulated acoustic source experiment.** The experimental arrangement and procedure are shown in Figure 1. The kidney stones within the tissue-mimicking gel phantoms were placed in front of the center of the ultrasound probe at 4 cm and imaged in color Doppler mode. During the experiment, the time-gain-compensation was set to the minimum level for RF data acquisition to avoid signal saturation. The cases that showed the TA were chosen. For those cases, the RF data were acquired at the output of the ADC of the VUE. The Doppler power was calculated for all the channels, and the channel with the highest level of Doppler power was selected; typically, it corresponded to one of the elements close to the center of the probe (e.g., no. 64). To prepare an artificial signal that mimicked the scattered signal for that channel, one of the pulses of the Doppler ensemble was picked. (Because all the pulses were almost identical, this could be any pulse of the 14-pulse sequence.) The chosen pulse (no. 7) was interpolated using the MATLAB function “interpft” to resample with the higher sampling frequency of 200 MHz and thus smooth the waveform. Next, this waveform was repeated 14 times to build a periodic sequence mimicking the Doppler ensemble, which was programmed into the function generator. Next, the ultrasound probe was disconnected from the VUE, and the function generator was connected to the chosen channel in place of the probe. The generator was triggered directly from the VUE, so that the machine received the generator signal in the same way as it would in real imaging. The function generator amplitude level was adjusted so that the RF signal measured at the output of the ADC was of the same level as that measured in the original imaging experiment. Afterward, the Doppler power of the generator-born ensemble was calculated using the same algorithms as in the actual ultrasound imaging.

**Test 2: overpressure experiment.** An overpressure test (Bailey et al. 2000; Sapozhnikov et al. 2002) was used to test for the possible role of bubbles. The overpressure system and experimental design are shown in Figure 2. The chamber had a cylindrical shape with an inner diameter of 11.2 cm and a height of 7 cm. The walls, base and lid were made of aluminum. The walls were 4.5 cm thick, and the base and lid were 3.6 cm thick to sustain high pressures. A rubber acoustic absorber (1 cm thick) was placed on the bottom of the chamber.
to dampen the possible reverberations during the stone imaging. The stone being studied was fixed on the tip of a brass needle (1.6 mm in diameter) that was rigidly attached to the chamber wall. A polystyrene puck was fixed in the middle of the lid to serve as an acoustic window for better ultrasound transmission; it had a diameter of 5.3 cm and was 2.16 cm thick. The window material parameters were the following: longitudinal sound speed, 2400 m/s; shear wave speed, 1150 m/s; density, 1050 kg/m³; acoustical impedance, 2.52 MRayl; absorption coefficient, 1.8 dB/cm at 5 MHz (Selfridge 1985).

The ultrasound refraction in the acoustic window owing to different sound speed from that of water was later taken into account in the beam-forming in-house algorithm. On the top of the lid, a plastic cylinder measuring 8.8 cm in diameter and 5.1 cm tall was attached to form an external water tank where the imaging transducer was immersed. The transducer was fixed on the positioning system with its axis oriented perpendicularly to the chamber lid. The surface of the transducer was close (<1 mm) but did not touch the acoustic window during the experiment; this was done to avoid transducer displacement that could be caused by the acoustic window bending under high pressure. The transverse position of the transducer was adjusted by the positioning system to find the best twinkling location on the stone.

High static pressure was generated inside the chamber by a piston screw pump (model 37-6-30; High Pressure Equipment Co., Erie, PA, USA). This pump was capable of producing pressure up to 200 MPa, although lower pressures (<9 MPa) were used in the experiment because it was sufficient to exceed several times the peak negative pressure of the ultrasound pulses (P₂ ≈ −1 MPa). A gauge of maximum reading of 13.8 MPa was used for determining pressure in the chamber (Ashcroft 1008, Huntington Beach, CA, USA).

The stone being studied was glued to a brass needle using 5-min epoxy (McMaster, CA) and fixed in the chamber. The open chamber was completely immersed in degassed water in an auxiliary plastic tank, and the lid was closed and screwed to the chamber. Next, the chamber was removed from the water tank and placed on a bench for the consequent experiment. The pressure was changed by winding the wheel of the pump. Three different pressure conditions were used: (a) before applying excess pressure, (b) under high pressure and (c) after pressure was released to the normal value. For the high-pressure condition, the stone was left under 8.5 MPa of pressure for at least 4 h. This pressure level was chosen because it exceeded several times the absolute value of the peak negative pressure of the original ultrasound pulse (P₂ ≈ −1 MPa)—that is, a negative pressure (tension) could not appear during the stone imaging, and the possible ultrasound-induced bubble growth was suppressed. For each pressure condition, the image on the ultrasound machine display was recorded on video for 1 h to obtain the whole twinkling pattern. The corresponding RF data were collected periodically. All videos were compared for different pressure conditions, and the corresponding RF data were analyzed for the quantitative description of the TA. The overpressure tests were applied to laboratory stones, fresh stones and acrylic spheres with rough surfaces. For the overpressure test with fresh (recently removed from patients) stones and to keep the stone contact with air minimal, the preparation of the experiment was performed in degassed water, including the stone gluing to the holder.

Test 3: imaging of acrylic spheres in liquids with different surface tension. In addition to water, ethanol was used to improve wetting of the acrylic spheres,
reducing or eliminating bubbles stabilized on the stone surface and thus suppressing bubble effects. The surface tension of ethanol (25 mN/m; Vazquez et al. 1995) is several times lower than that of water (72 mN/m; Vargaftik et al. 1983). Five acrylic spheres were used in this study. The transducer was fixed in the positioning system, and the sphere was placed 4 cm from the transducer in front of its center, as in the experiments with natural kidney stones. The rough side of the sphere faced the transducer. Both the transducer and sphere were placed in a tank filled with liquid. The TA was filmed, and RF data were collected first in degassed water and then water was replaced with ethanol of 70% volume concentration. Next, ethanol was again replaced with degassed water. Every time that a new liquid was filled, a plastic pipette was used to create agitation around the sphere to remove possible bubbles and clean the surface from the remnants of the previous liquid.

**RESULTS**

Figure 3 shows typical signals that were analyzed to reveal the TA features. The plots in Figure 3 (a–c) describe data for imaging of a natural kidney stone from the stone laboratory, which was placed in gel. The plots in Figure 3a and b overlay 12 successive waveforms of the Doppler ensemble ultrasound pulses scattered from the stone recorded at the central element of the array. The imaging depth d corresponds to the time delay of the scattered signal t in accordance with the formula

\[
d = \frac{ct}{2},
\]

where \( c = 1540 \text{ m/s} \). The waveforms that are plotted on top of each other are barely distinguishable because the corresponding changes are small. To reveal the differences between them in more detail, the Doppler power calculated from those waveforms is shown in Figure 3c. The Doppler power is shown in decibel scale, relative to the background noise level. An obvious spike occurs 2–3 \( \mu \text{s} \) after the arrival of the front of the stone-scattered pulse, which indicates that the corresponding part of the signal within the Doppler ensemble is fluctuating from pulse to pulse. Such a spike was observed for all studied stones and corresponded to the twinkling part of the color Doppler image. In Figure 3d–f, results obtained for the simulated acoustic source experiment test are shown (test no. 1, described earlier). The waveforms of the simulated Doppler ensemble pulses are shown in Figure 3d and e, also with 12 waveforms on top of each other. The waveforms of Figure 3b and e are visually identical, which indicates the high quality of the mimicking procedure. As described earlier, the artificial Doppler ensemble was sent through the same signal path inside the machine as the original Doppler sequence. Figure 3f represents the result for the Doppler power calculated from the waveforms shown above; no obvious spike is seen. Again, this test was conducted for six stones, and the result was repeatable. This repeatability is an important observation, because it strongly suggests that the origin of the TA is not related to the machine. Therefore, the TA appearance must result from some effect, which we assume is acoustics.

To understand the properties of the scattered signals during the TA manifestation, the residual amplitude \( A_{\text{res}} \) was calculated for each Doppler pulse within the ensemble. To make the data processing closer to that used in the color Doppler imaging, beam-formed channel data were analyzed. A typical result is shown in Figure 4a; it shows that the residual amplitudes vary from pulse to pulse, which indicates that the scattered pulses have different waveforms. Some of the pulses show spikes in the residual amplitude, which occur at the same depth as the spike of the corresponding Doppler power \( W_m \), in accordance with the definition of the Doppler power (see eqn [6]). It is useful to determine whether these spikes in the residual amplitude occur only at specific pulses within the Doppler ensemble, or if they are randomly distributed within the ensemble. To perform such a test, we considered the appearance of a spike of the Doppler residual amplitude as “an abnormal event” in the given pulse of the ensemble when the waveform is noticeably distorted. The following criterion was used: for the abnormal event to occur, the maximum residual amplitude for the given pulse should exceed 3 dB the averaged maximum residual amplitude for that ensemble. The distribution of the abnormal events within the 12 pulses of the Doppler ensemble is shown on Figure 4b. The data were collected for three stones by repeatedly detecting the appearance of abnormal events during 800 imaging frames for each stone. The diagram shows the net result. The probability for the event to happen appeared to be uniform within the ensemble. This result suggests that the origins of the TA are random scatterers.

Common candidates for random ultrasound scatterers are bubbles. Indeed, the gas bubbles can interact stochastically with ultrasound (Leighton 1994). The bubbles may be present in the bulk of propagation medium or rest on the stone surface, especially if there are microscopic cracks and crevices. The possible presence of bubbles was studied using test no. 2 described earlier. Figure 5 shows a color Doppler image of a stone positioned in the overpressure chamber under varied static pressure. The images were recorded directly from the VUE display. There are obvious TAs in Figure 5a and c that correspond to ambient pressure conditions.
Figure 5b shows the stone image when high pressure was applied. The TA has completely disappeared. After the pressure was released, the twinkling reappeared almost immediately. Nine stones were tested, and the results similar to those shown in Figure 5 were observed (i.e., the TA suppression by high static pressure was repeatable). In addition to the measurements at 8.5 MPa of static pressure, the TA behavior was monitored during varying static pressure to find the TA disappearance threshold. This threshold varied by stone and lay within the range of 0.34–1.38 MPa. Interestingly, this pressure level is of the same order as the peak negative pressure of the imaging pulse measured at the stone location ($P = -1$ MPa).

![Fig. 3. Results of natural kidney stone imaging and simulated acoustic source experiments.](image)

Figure 6a shows the color percentage $C$ (see eqn [7]) calculated for the three pressure conditions (1, 8.5 and 1 MPa) for one of the nine stones tested. The parameter $C$ was 20.6%, 2.1% and 19.1% for the cases before increasing pressure, under high pressure and after pressure was released, respectively. The statistical analysis showed that the color percentage $C$ in the first and last cases within the criterion $p > 0.05$ were statistically identical ($p = 0.3$), but the high-pressure case showed a significant drop in the value of $C$ ($\rho = 5.8 \times 10^{-12}$ and $1.6 \times 10^{-15}$, when compared with $C$ for before increasing pressure and after pressure was released, respectively). These results confirmed more quantitatively the observations shown in Figure 5. A comparison was made for other stones (nine in total), and the results were similar.

The overpressure test was performed for the two stones recently removed from patients. Again, similar results were obtained. The pressure threshold of the TA disappearance was found to be 0.34 and 1.72 MPa for those stones. Figure 6b shows the color percentage $C$ change following the change in pressure from 1 to 8.5 and back to 1 MPa for the first stone. Similar to the laboratory stone test, the TA was statistically identical at normal pressure before and after the overpressure test, and it was statistically different compared with the high-pressure case.

The aforementioned results show that the random scatterers that cause the TA are suppressed by overpressure, which is a strong indication that those scatterers are gas bubbles. The application of external pressure shrinks the bubbles that are presumably stabilized in cracks and crevices on the stone surface, and they then return with the release of pressure. However, the natural kidney stones can contain internal cracks that could also behave similarly to bubbles; namely, they could scatter the sound randomly and be sensitive to static

![Fig. 6a, 6b.](image)
levels, similar to Figure 5. The results of this observation, as well as the results of the color percentage C analysis, demonstrated that overpressure suppressed the TA, as in case of natural kidney stones.

To provide additional evidence for the vital role of crevice bubbles in the TA appearance, an alternative bubble-influencing factor was used, namely that of surface tension. The corresponding test no. 3 was performed (see Materials and Methods). The TA for the acrylic spheres was compared in water and ethanol, which wet the surface differently and presumably affect the bubble stabilization mechanisms. The results are shown in Figure 8 (Supplemental video 2). There was obvious twinkling when the sphere was immersed in water, whereas the TA almost disappeared when water was replaced by ethanol. When the ethanol was replaced back with degassed water, the twinkling returned to its original level. Note that Figure 8 is similar in appearance to Figure 7, which suggests that static pressure and a low surface tension fluid have similar effects—namely, that they reduce the effect of surface bubbles.

**DISCUSSION**

Interaction of gas bubbles with a pressure field is an important subject in the acoustics of liquids. The corresponding phenomenon (acoustic cavitation) is initiated when the ultrasound intensity is high and the frequency is low (Leighton 1994). Bubbles are activated by the tension created during the negative pressure phase of the acoustic wave. The onset of cavitation depends on the acoustic pressure and on whether there are impurities in the liquid (cavitation nuclei), where the bubbles appear and grow. Cavitation can happen in biological tissue as well. This subject is important in medical ultrasound, especially in relation to ultrasound therapy and safety of ultrasound imaging (Bailey et al. 2003). To characterize a possible appearance of cavitation in tissue during ultrasound imaging, the original mechanical index was introduced by Apfel and Holland (1991):

$$MI = |P_-|/\sqrt{f},$$  \hspace{1cm} (9)

where $P_-$ is negative pressure in megapascals, and $f$ is ultrasound frequency in megahertz. The condition $MI > 1$ is usually considered to be an indication of possible cavitation damage. In the current study $|P_-| = 1$ MPa and $F = 5$ MHz, which gives $MI = 0.45$ (i.e., the pressure is less than the critical level, although close to it). However, the concept of mechanical index was proposed in relation to cavitation in soft tissue, not kidney stones or other concretions that are exposed to ultrasound. In the presence of such inclusions, the cavitation threshold can be significantly reduced and pressures might increase.
by superposition with reflections. The reason is that the surface of the solid objects may be weaker to tension, such as when the liquid does not fully wet the surface. The bubbles can be activated easily if there are pre-existing gas pockets on the surface. These pockets are associated with microscopic cracks and crevices or other irregularities of the surface. The role of crevices in the strength of liquids against tension has been studied for many years (Strasberg 1959), specifically in relation to acoustic cavitation (Apfel 1970; Crum 1979, 1982). Note that the crevices and the corresponding bubbles are frequently of micrometer or submicrometer size; therefore, they are invisible to the naked eye and to many microscopy techniques. As result, they are usually detected only indirectly from the effects that they produce. It is likely that any bubbles that have been stabilized on a stone will be small and will not grow to visible sizes, because the acoustic pulse length is not long. It is also likely that there will be some rectified diffusion (Crum 1980), but perhaps just enough to change the scattering coefficient and possibly the phase of the reflected signal.

Although the overpressure experiments provide strong evidence for the important role of bubbles, we have also considered other factors that might influence the scattering and thus modify the TA. One factor is the change in the speed of sound in the liquid under overpressure. Increase in speed of sound $\Delta c$ owing to excess pressure $\Delta p$ can be calculated as:

$$\Delta c = \beta - \frac{1}{\rho_0 c_0} \Delta p,$$

where $c_0$ and $\rho_0$ are the ambient speed of sound and density and $\beta$ is the parameter of acoustic non-linearity (Hamilton and Blackstock 1997). For water, $c_0 = 1500 \text{ m/s}$, $\rho_0 = 1000 \text{ kg/m}^3$ and $\beta = 3.52$, which gives $\Delta c = 12.6 \text{ m/s}$ for the excess pressure $\Delta p = 7.5 \text{ MPa}$ used in the reported experiments. It is noted that this sound speed change is rather small; it is even smaller than the difference between speed of sound in water and in soft tissue (1540 m/s). In the Verasonics imager, the speed of sound can be set manually. No difference in the TA appearance was seen between the cases of $c = 1500 \text{ m/s}$ and $c = 1512.6 \text{ m/s}$.

Another potential effect is the shift of the plastic acoustic window of the chamber (Fig. 2) when the water...
is pressurized. The corresponding displacement was measured based on the delay in the RF signal and appeared to be less than 0.2 mm. This shift was not significant either, which was determined by displacing the ultrasound probe within several millimeters. The TA was not sensitive to this change. Note that the transducer was always distant from the acoustic window (>0.2 mm but <1 mm), meaning that it never touched the acoustic window.

Analysis of the RF signals has shown that the spike in the Doppler power occurred not at the front of the imaging pulses but a little later (~2–3 μs; Fig. 3). This delay was consistent for different stones. A possible explanation for the delay could be the time needed for a bubble to grow or to leave the crevice to become an efficient scatterer. The delay could also be caused by bulk and surface waves in the stone and how they are affected by the variable loading of changing surface bubbles. More studies are needed to clarify this peculiarity. An important feature shown in Figures 5–7 is the almost complete recovery of the TA to its initial status after the high pressure was released. This recovery was an indication that the bubbles were compressed but not dissolved during the overpressure stage, which is a typical behavior of bubbles in the crevice (Apfel 1970). Some visible reduction in the color percentage C after the pressure was released compared with its initial value (Fig. 6) could be explained because some of the bubbles were not associated with the crevices and were completely dissolved by overpressure. Note, however, that this change was not statistically significant. In addition, the TA is weaker in the fresh stones, suggesting that fresh stones have less contact with air and therefore fewer bubbles.

Our hypothesis that stabilized surface bubbles are the mechanism for the appearance of the TA does not contradict many of the existing observations reported in previous publications on this subject. For example, the evidence that roughness of the stone surface enhanced the TA agrees well with the fact that a rough surface could harbor more bubbles (Kamaya et al. 2003; Louvet 2006; Rahmouni et al. 1996). Observations that the biochemical content of the stones correlates with the TA efficiency also can be explained by the bubble hypothesis, because surface wetting is sensitive to the composition of the stone (Chelfouh et al. 1998). A recent study of the effect of ultrasound frequency on the TA indicated that the artifact is pronounced at low frequencies (Gao et al. 2012), which is a typical feature of acoustic cavitation and supports the bubble hypothesis.

An adequate depiction of how the bubbles are formed on the stone surface is not simple and is beyond the scope of this study (see Crum 1982 for more details on this phenomenon). What is lacking for this specific case is a fuller understanding of kidney stone biochemistry and surface microstructure. A typical stone consists

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**Fig. 7.** Overpressure experimental results of acrylic spheres with rough surfaces: (a) before increasing pressure, (b) under high-pressure and (c) after high-pressure was released. The results are similar to those for natural kidney stone overpressure experiments. The TA was suppressed under high pressure.

**Fig. 8.** Results of the TA on acrylic spheres with rough surfaces immersed in different liquids. The TA on the sphere immersed in (a) water, (b) ethanol and (c) water again. The results are similar to results shown in Figure 7. The TA was suppressed under ethanol.
of proteins and crystals. Some hydrophobic regions on the stone surface (e.g., proteins) can trap gas and form crevice bubbles. The crevice bubbles are probably extremely small (submicron or even nanometer size) and might not be seen even with X-ray microtomography (μCT) and other imaging technologies. For example, we have been unsuccessful in detecting these bubbles via diagnostic ultrasound. Nevertheless, considerable evidence has been presented here that the TA is caused by bubbles on the surface of stones, even those taken freshly from patients. This observation raises the question that if in vivo stones or other calcifications in the body twinkle, does any object that twinkles has bubbles on or in it? And if so, where do these bubbles come from and what role do they play in body chemistry?

CONCLUSIONS

This study provides several important experimental facts concerning the TA: (i) the TA is caused by acoustic effects, not by an abnormal response of the machine electronic circuits or improper signal processing; (ii) the acoustic scatterers that cause the artifact respond to the imaging Doppler pulses randomly; and (iii) the TA is suppressed by overpressure and by more efficient wetting of the stone surface. These results provide strong evidence that the TA is caused by small bubbles that are trapped and stabilized in cracks and crevices on the stone surface. Such bubble stabilization mechanisms have been known as the principal nucleation sites for cavitation (Apfel 1970; Crum 1979, 1982). Other possible sources of the TA (e.g., radiation force, phase jitter of the machine, scattering from the rough surface) would not be sensitive to overpressure or the type of liquid in which the stone is immersed.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ultrasmedbio.2013.01.011.

REFERENCES


