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***Broadband acoustic measurement of the agar-based tissue mimicking material – a longitudinal study*.**

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ABSTRACT

Commercially available ultrasound quality assurance test phantoms rely upon the long-term acoustic stability of tissue-mimicking-materials (TMMs). The measurement of the acoustic properties can be technically challenging and it is important to ensure its stability. The standard technique is to film-wrap samples of TMM and to measure the acoustic properties in a water bath. In this study, a modified technique is proposed whereby the samples of TMM are measured in a preserving fluid that is intended to maintain their characteristics. The acoustic properties were evaluated using a broadband pulse-echo substitution technique over the frequency range of 4.5 – 50 MHz at 0, 6 and 12 months using both techniques. For both techniques, the measured mean values for the speed of sound and the attenuation were very similar and within the IEC recommended value. However, the results obtained using the proposed modified technique demonstrated greater stability over the 1-year period when compared with the results acquired using the standard technique.

Key words: ultrasound, high frequency, tissue mimicking material, speed of sound, attenuation coefficient, long-term.

INTRODUCTION

Commercially available quality assurance (QA) test phantoms are widely used to test the performance of clinical ultrasound scanners. These phantoms, are manufactured from tissue-mimicking-material (TMM) which is designed to closely match the acoustical properties of the speed of sound (SoS) and the attenuation coefficient from soft tissue. The aim of these phantoms is to provide a reproducible method to assess the performance of ultrasound scanners. However, these phantoms are intended for use with clinical ultrasound scanners at frequencies up to 20 MHz. To the best of our knowledge, there are no commercially available test phantoms to assess the performance of ultrasound scanners employing ultrasound frequencies above 20 MHz.

A variety of TMM materials are currently produced both commercially and within laboratories. These include: agar-based TMMs (Teirlinck et al., 1998), condensed milk TMMs (Madsen et al., 1998), gelatine TMMs (Culjat et al., 2010), konjac-carrageenan TMMs (Kenwright et al., 2014; Meagher et al., 2007), urethane rubber TMMs (Culjat et al., 2010), Poly (vinyl alcohol) Cryo-gel (PVA-C) TMMs (Cournane et al., 2010; Culjat et al., 2010; King et al., 2011) and Zerdine™ TMMs (CIRS, Inc. Norfolk, VA). The International Electrotechnical Commission (IEC) agar-based TMM has become widely used and popular for clinical and preclinical test objects (Brewin et al., 2008; Browne et al., 2003; Cannon et al., 2011; Culjat et al., 2010; Inglis et al., 2006; Moran et al., 2011; Rajagopal et al., 2014; Sun et al., 2012; Yang et al., 2013). The acoustical properties of this agar-based TMM are designed to comply with the ultrasound acoustical parameters provided by the IEC (IEC, 2001) with the recommended SoS and attenuation over the frequency range 2 – 10 MHz being 1540 ± 15 ms^{-1} and 0.5 ± 0.05 dB cm^{-1} respectively.

Moderately high-frequency ultrasound scanners (up to 20 MHz) have been manufactured for many years and have been utilised clinically in the assessment of skin (Machet et al., 2009), vascular structures (Rhee, 2007) and retinal imaging*. In recent years, reliable high (20 – 50 MHz) and very high (>40 MHz) ultrasound scanners have become mainstream technology for the imaging of*

superficial structures in clinical imaging and for preclinical imaging applications due to improvements in transducer engineering and software technology (Banchhor et al., 2016; Schmitt et al., 2010; Sundholm et al., 2015; Xu et al., 2012).

With the increase of high-frequency ultrasound imaging applications, there is a need to develop and to acoustically characterise TMMs suitable for high-frequency ultrasound QA and training phantoms. It has been shown that above 10 MHz the TMMs in the commercial test phantoms, do not have optimum acoustic properties as the attenuation starts to exhibit a nonlinear response with increasing frequency (Browne et al., 2003), whereas the IEC guidelines for TMM properties recommend a linear relationship between attenuation and frequency up to 10 MHz.

The agar-based TMM developed under the IEC guidelines and used in this study, has previously been found to have a non-linear response when acoustically investigated at frequencies up to 23 MHz by Brewin et al., *(2008)*, in our own lab and in the National Physical Laboratory at frequencies up to 47 MHz and 60 MHz respectively (Sun et al., 2012 and Rajagopal et al., 2014). In these studies, the use of test cells or TMM samples wrapped with film material (Saran Wrap® or Mylar®) was employed to preserve the samples during acoustic characterisation when degassed, deionised water was used as the reference medium. Moreover, thin slices of TMM ranging in thickness from 2.5 – 30mm were used, enabling higher ultrasound frequencies to propagate through the TMM slices (Brewin et al., 2008; Rajagopal et al., 2014; Sun et al., 2012). *The encasing of the TMM in film is important as, without the film, the TMM will degrade rapidly. This degradation is due to leaching of the glycerol from the TMM into the water reference medium, thus altering the acoustic properties of the TMM (Brewin et al., 2008). A reference water test cell, also encapsulated in Saran Wrap® or Mylar® film, was used in the reference measurement in order to account for the effect of the film on measurements (Rajagopal et al., 2014; Sun et al., 2012).* However, the production of both the TMM slices wrapped in film and water test cells is time-consuming and technically challenging, especially for thin TMM samples. *Therefore, the aim of this study was to compare this well-*

established technique for the measurement and preservation of an IEC agar-TMM to a technique where TMM is characterised and preserved in a preserving fluid. Furthermore, this method was evaluated over a 1-year period, to determine the longitudinal stability of the acoustic properties.

MATERIALS AND METHODS

Acoustical measurements

Data was captured using two different acoustical systems, described briefly here and elsewhere (Sun et al., 2012). Firstly, a Vevo 770® preclinical ultrasound scanner (Visualsonics Inc., Toronto, Canada) was used at the University of Edinburgh and secondly, a Scanning Acoustic Macroscope (SAM) system developed in-house at the Dublin Institute of Technology (Cannon et al., 2011). The SAM system was used to provide additional acoustic data and to extend the lower limit of the bandwidth of the measurements to 4.5 MHz.

Manufacture of UTMMs and FTMMs samples.

A batch of the IEC agar-based TMM was manufactured following a widely used standard recipe and method (Brewin et al., 2008; Browne et al., 2003; Cannon et al., 2001; Ramnarine et al., 2001; Teirlinck et al., 1998). This mixture was poured at 42°C onto a pre-warmed metal plate. The plate was pre-warmed to ensure that the mixture spread uniformly. The TMM mixture was then left to cool to room temperature. *From this batch of TMM, 22 cylindrical slices of TMM of diameter 5.5cm were cut using a thin-walled plastic tube. Due to the short focal lengths associated with high frequency transducers (Table 1), the thickness of the TMM slices was constrained to less than 3.2mm and ranged in value from 1.8mm – 3.2 mm.*

After being cut, eleven of these cylindrical TMM samples were left uncovered, and placed in a sealed container with TMM preserving fluid. This TMM preserving fluid was manufactured in-house (Brewin et al., 2008; Cannon et al., 2011; Inglis et al., 2006). These samples will be referred to as unwrapped-TMM (UTMM) (Figure 1a).

The remaining eleven TMM samples were used to manufacture the samples which were subsequently covered with clear film in the following manner. Initially, a layer (0.015mm thick) of Saran Wrap® film (SC Johnson Inc., Racine, USA) was stretched over an embroidery ring, of 10cm

106 diameter. A fast-hardening epoxy (Araldite Rapid; Huntsman Advanced Materials, Basel, Switzerland)
107 was then applied to one side of a PVC ring (2mm thick, 5.8mm outer diameter) and the stretched
108 Saran Wrap® was lowered onto the PVC ring. This was left to set overnight. The TMM was then
109 manufactured as described above. After setting and cutting, the eleven samples were placed into
110 the PVC rings. Five drops of TMM preserving fluid were added to the surface of the TMM to ensure
111 good acoustic coupling between the film and the TMM, then a second layer of Saran Wrap® was
112 glued to the other side of the ring, similar to that described above, such that the TMM slices were
113 "sandwiched" between the two films and thereby, film-wrapped-TMM (FTMMs). These final film-
114 wrapped samples were left to set overnight. Finally, epoxy was used to seal the edges of the film–
115 ring–film to ensure the FTMMs did not leak. This was re-enforced with insulating tape to ensure that
116 the film would not peel off during the 1-year period of investigation (Figure 1b). These FTMMs were
117 preserved in a box with tissue paper moistened with TMM preserving fluid to create a humid TMM
118 preserving fluid saturated environment. In a similar manner, a water test cell was manufactured
119 whereby the TMM was replaced by degassed deionized water in the manufacturing process.

120 *Experimental set-up of Vevo 770® preclinical ultrasound scanner.*

121 In this study, the radio frequency (RF) data was collected and analysed from 11 FTMMs and
122 11 UTMMs. To measure the acoustic properties, the FTMMs were submerged in a tank filled with
123 degassed, deionised water as the reference medium, while for the UTMM measurements, the tank
124 was filled with TMM preserving fluid. For both measurements, a Polymethylpentene (TPX, Boedeker
125 Plastics, Texas, USA reflector) of 2.5cm diameter and 5mm thickness was located beneath the
126 samples at the focal position of each transducer as illustrated in Figure 2.

127 Measurements were made using 4 transducers (Table 1) at 10% output power, using a high-
128 frequency ultrasound scanner Vevo 770®. This power output was considered sufficient signal
129 magnitude to obtain good signal-to-noise data without the generation of significant nonlinear
130 effects (Sun et al., 2012). The regions of interest (ROI) were located at the upper surface of the TPX

reflector with and without the sample in place and from the lower and upper surfaces *of each sample. For each measurement the RF data was collected from 10 scan-lines within these pre-selected ROIs at 4 different positions on the FTMMs or UTMMs. The data was* analysed off-line using a MatLab script (MatLab R2013a MathWorks, Inc). The calculated angular separation between the RF acquisition lines is approximately 0.15°, so the lines were considered parallel and perpendicular to the TPX reflector. For the FTMMs, identical acoustic measurements were also taken through the water test cell to take into account potential reflections from the Saran Wrap® interfaces and to obtain absolute values of attenuation (Cannon et al., 2011; Sun et al., 2012).

The performance of both FTMMs and UTMMs were assessed over a period of one year at *approximately* 0, 6 and 12 month time points.

The 3dB bandwidth of each transducer (Table 1) was measured from the frequency spectra taken from the TPX reflector in a degassed, deionized water tank without the sample in place, where the reflector was placed at the focal length of the transducer.

Analysis of speed of sound, thickness and attenuation data of FTMMs and UTMMs samples.

The analysis of the FTMMs was performed based on a broadband pulse-echo substitution technique (AIUM, 2014). The pulse-echo return times from the front and rear surfaces of the FTMMs and from the front surface of the TPX reflector were used to determine the thickness and SoS of the samples. The magnitude of these pulse-echoes were used to calculate the attenuation. In addition, the echoes from the TPX reflector with the water test cell was acquired. This data was then used to calculate the SoS, thickness and attenuation of the FTMMs samples in a manner similar to that in Sun et al., (2012).

For the SoS and thickness of the UTMMs, the measurement technique and analysis was carried out in a similar manner to the FTMMs. However, since the UTMMs were not wrapped in Saran Wrap®, no water test cell was required and the reference measurements from the TPX were

taken through the bath of TMM preserving fluid. *The absence of Saran Wrap® meant that the magnitude of the echoes from the boundaries of the TMM was reduced. This, necessitated manual selection of the position of the boundaries from the raw RF signals. This was performed by selection of the largest pulse-echo at each interface of the UTMMs. The criterion for this was that the peak selected was at least 100% greater than the magnitude of the previous peak within 2 µs time-window. This was carried out for each of the 10 lines of the raw RF signal inside the ROI of the UTMMs, enabling the thickness of the sample to be determined at each of these positions. In addition, the thickness values of the UTMMs were measured manually in five different positions on each UTMM with a digital calliper (DURATOOL, TM Taiwan, 0 – 150mm). Each sample was placed between 2 plastic plates of known thickness in order to avoid excessive compression of the UTMMs (Brewin et al., 2008; Rajagopal et al., 2014). This was performed at the initial time-point before the acoustic measurements commenced.*

The SoS in the UTMMs was calculated using Equation (1).

$$SoS_{TMM} = \left(1 + \frac{T_R - T_{TMMR}}{T_{TMMLw} - T_{TMMUp}}\right) SoS_{TMMfluid} \quad (1)$$

Where T_R is the time from the transducer to the TPX in the tank with no sample in the acoustical path, T_{TMMR} is the time from the transducer to the TPX reflector through the sample, T_{TMMLo} and T_{TMMUp} are the time from the transducer to the lower and upper surface respectively of the sample and $SoS_{TMMfluid}$ is the SoS of the TMM preserving fluid in which the samples were immersed.

Calculation of the attenuation of the UTMMs was carried out in a similar manner to the FTMMs, but without the use of the water test cell, to compensate for the interfacial attenuation loss.

Acoustical properties of TMM preserving fluid and degassed, deionized water.

The acoustic properties of the TMM preserving fluid were measured by the National Physical Laboratory (Teddington, UK). The SoS was found to be 1538.15 ± 0.22 ms^{-1} at 19.3 ± 0.1°C and a 2nd degree polynomial function was fitted to the attenuation data (α [dB cm^{-1}]) as a function of frequency (f (MHz)): as $\alpha_{TMMfluid} = 0.00309f^2 - 0.004996f$ (R^2=0.99) over the frequency range of 1 – 60 MHz.

The acoustic properties of the degassed deionized water have previously been measured and found to have an attenuation proportional to f^2 over the range of 7.5 – 67.5 MHz (Duck, 1990; Pinkerton, 1949). Furthermore, the SoS of degassed deionized water varies with temperature (Bilaniuk et al., 1992; Del Grosso et al., 1972). In this study all measurements were undertaken at 22.2 ± 0.5°C with a SoS of 1488.88 ms^{-1}.

Experimental set-up and data analysis using the SAM system (Dublin Institute of Technology).

The SAM system uses broadband transducers which work as both transmitter and receiver (Olympus NDT Inc., Waltham, MA, USA). The experimental setup was similar to that used with the Vevo 770® (Figure 2). Three different transducers were used (Table 1) where the 3dB bandwidth was measured in a similar manner to that of the transducers of the Vevo 770®.

A pulser-receiver (Model 5052PR; Panametrics, Waltham, MA, USA) was used to transmit and receive the pulses. The received reflected pulse was digitised and captured using a data acquisition card (PCI-5144: National Instruments, Austin, TX, USA) with the data acquisition controlled by a LabView (National Instruments Corporation, TX, USA) program.

The SAM system displayed the RF data in real time during the measurements. Ten lines of data were acquired from each of four different positions on the reflector with and without the sample (FTMMs or UTMMs) in place.

The calculation of the SoS and the attenuation coefficient was also based on the broadband pulse-echo substitution technique. However, here the thickness of the sample was inputted into the calculation of SoS and attenuation coefficient. The thickness value input for each FTMMs and UTMMs was the mean of the 10 different measurements at each of 8 locations calculated using the 4 transducers of the Vevo 770® ultrasound scanner.

At all-time points, measurements taken with the Vevo 770® were performed before measurements with the SAM system.

Unpreserved samples, batch to batch variation, and repeatability of the UTMMs.

The acoustic properties of two uncovered TMM samples (UTMM) were measured in an identical manner to the UTMMs described previously using the RMV704 transducer (centre frequency 40 MHz, Table 1). Measurements were undertaken initially and then approximately once every 24 hours over a 96 hour period. However, in between measurements, the samples were left exposed to the air. These samples will be referred to unpreserved samples.

An indication of TMM batch-to-batch variability was assessed by measuring the acoustical properties of 6 UTMMs manufactured from a different batch of TMM. These samples will be referred to as UTMM2. These samples had a mean thickness of 2.01 ± 0.05 mm as measured using the Vevo 770® scanner. The acoustical properties were measured with the Vevo 770® and with the SAM system at the 6 and at 12 month time points.

Data analysis was performed in an identical manner to that with the 11 UTMMs described above.

To assess the repeatability of the measurement system, the acoustic properties of the 11 UTMMs were measured with one transducer RMV710 with the Vevo 770® at 5 different times over 1 month period. The reference medium was TMM preserving fluid. The temperature for these measurements was 22.0 ± 0.4°C.

RESULTS

The mean thickness of the FTMMs and UTMMs calculated using the RF ultrasound signals measured with the Vevo 770® over all the time points showed a maximum variation of 0.25mm in the case of FTMMs and up to 0.08mm for the UTMMs. The thicknesses measured by the digital *calliper* from 11 UTMMs showed a maximum variation of 0.03mm.

Table 2 shows the mean SoS of FTMMs and UTMMs at each time point. It can be seen that the SoS of the FTMMs exhibited larger variability than the SoS of the UTMMs. Using a Student's t-test it was shown that the mean SoS value of FTMMs and UTMMs samples were not statistically different ($p>0.5$) at 0 time point, but displayed a significant difference ($p<0.05$) at the rest of the time points. The results after 1 year showed that the SoS of the FTMMs decreased 22.1 ms^{-1} when compared with the first measurement at 0 months, whereas the SoS of the UTMMs samples decreased 4.1 ms^{-1} over the same 12 month period. The SoS of UTMM2 samples calculated over a 6 month time period showed a decrease from 1558.1 ± 5.3 ms^{-1} to 1544.8 ± 3.3 ms^{-1}, with a difference of 13.3 ms^{-1}.

Table 3 shows the mean SoS averaged all time-points for each of the measurement systems. It was found that the SoS measurements using the SAM system showed smaller variability than the SoS measurements using the Vevo 770® for the FTMMs and UTMMs. The mean values over all the samples over all time points, using both measurement systems, were found to be 1538.2 ± 14.5 ms^{-1} for the FTMMs and 1544.0 ± 3.5 ms^{-1} for the UTMMs (Table 4). The mean SoS for the UTMM2 was found to be 1551.4 ± 6.2 ms^{-1}. Table 4 also shows the SoS results in comparison with those values published for IEC agar TMM (Brewin et al., 2008; Browne et al., 2003; IEC, 2001; Rajagopal et al., 2014; Sun et al., 2012). The mean SoS of FTMMs and UTMMs are within the values specified by the IEC (IEC, 2001).

Figure 3 and Figure 4 show the attenuation as a function of frequency for the FTMMs and the UTMMs respectively at 0, 6 and at 12 month time points measured using the seven different transducers. It can be seen that the variation in mean attenuation values for the UTMMs is small in comparison to the variation observed in mean attenuation values measured from the FTMMs.

Figure 5 and Figure 6 shows the mean attenuation data over the frequency range 4.5 – 50 MHz, averaged over all 11 FTMMs and over all 11 UTMMs and time points, respectively.

The batch to batch variation of TMM was assessed by the UTMM2s (2.01 ± 0.05 mm thickness). These samples were measured with both the Vevo 770® and the SAM system (at 6 and 12 month time points). The mean SoS of UTMM2s was found to be 0.44% higher (7.4 ms^{-1}) than the mean SoS of the 11 UTMMs. For the attenuation, the UTMM2s were found to have a maximum difference of ± 1dB cm^{-1} in mean attenuation across the frequency range shown in Figure 7.

For the unpreserved samples, after 96 hours of exposure to air, the samples were visibly dehydrated. The mean thickness of the two samples had decreased by 1.22mm and the diameter was decreased by 1.5cm. Additionally, the SoS was shown to increase by 140 ms^{-1} for sample 1 and 180 ms^{-1} for sample 2 over the total period of time. The attenuation was found to increase by approximately 10 $dBcm^{-1}$ per day.

In the assessment of repeatability, the mean SoS over the five measurements taken from the 11 UTMMs was calculated to be 1543.0 ms^{-1} with a range in SoS of ± 11.0 ms^{-1}. The mean SoS was found to be smaller by 1 ms^{-1} when compared to the mean SoS calculated using all the transducers at all time points (Table 4) of the UTMMs. The variation in attenuation as a function of frequency was ±1 $dBcm^{-1}$ over the frequency range of the Vevo770® RMV710B probe.

Polynomial functions were calculated for the attenuation as a function of frequency at each time point for the FTMMs and for the UTMMs. The best fit polynomial function was determined over all the attenuation versus frequency data in the range 4.5 – 50 MHz as a combination of Vevo 770®

and SAM system data over all time points (Figure 8). The polynomial fit found for FTMM was $0.4649f + 0.007363f^2$ (R^2=0.80) and $0.4897f + 0.008366f^2$ (R^2=0.99) for UTMM. The goodness of fit (R^2) of the three polynomial fits at each of the three time points for the FTMMs ranged between 0.78 – 0.92 whereas for the UTMMs this value ranged between of 0.96-0.99. In addition, in Figure 8, for comparison, the attenuation data of the IEC agar TMM from studies already published is included.

DISCUSSION

This aim of this study was to develop a robust and easy-to-use technique for the characterisation and preservation of the IEC agar TMM and to compare the acoustic properties obtained using this modified technique with the standard technique over a period of one year.

The thickness used in the calculation of SoS from the SAM system was a mean thickness measured by the Vevo 770® ultrasound scanner at eight different locations on the UTMMs (10 lines at each position). Although this mean thickness was used, the SD of the SoS values from the SAM system were smaller than the SD variation calculated using the Vevo 770® (Table 4) which would suggest that the use of this mean thickness in the SAM system measurements did not contribute significantly to the experimental error.

The acoustic properties of the UTMMs were measured in TMM preserving fluid whose acoustical properties were assessed by NPL at a 19.3 ± 0.1°C, whereas the UTMMs in this study were measured at 22.2 ± 0.5°C. The TMM preserving fluid is composed of the same fluid as used in the TMM manufacture process and Brewin et al (2008) has previously shown a TMM SoS temperature dependence of 2.1 ms^{-1} $°C^{-1}$. Consequently, there is likely to be a maximum variation of 6 ms^{-1} in the SoS of the TMM preserving fluid which could be attributable to the temperature change. Such a change would result in a potential error of less than 7 ms^{-1} in the measured SoS of the UTMMs. Nevertheless, the SoS values of the UTMM were found to be in good agreement with Rajagopal et al., (2014); Sun et al., (2012) and Brewin et al., (2008). *Furthermore, the SoS of the UTMMs was found to decrease by 4.1 ms^{-1} over a 12 month period compared with FTMMs which showered a decrease in the mean SoS of 22.1 ms^{-1} over the same period of time. Additionally the standard deviation* of the mean SoS values for FTMM samples was larger than that for UTMMs at all time-points. This increased variation in SoS values for the FTMMs in comparison to UTMMs may be attributable to a number of reasons. Firstly, although a visual inspection was performed on each of the FTMMs before each acoustical measurement and no evidence of leakage was observed,

nevertheless, in several of the samples, the epoxy securing the film to the rings showed signs of ageing and the Saran Wrap® film appeared to become less taut over the 1 year period. This could have potentially increased the permeability of the film allowing the glycerol from the TMM to leach into the water medium resulting in a decrease in the measured SoS properties of the FTMM. However, although a decrease in SoS in FTMMs was measured between 0 and 12 months, this did not decrease continuously over the 1-year period which would suggest that the measured variation is not likely to be attributable to glycerol leakage. Secondly, for the FTMMs, the position of the water-film interfaces was selected using Matlab code based on the identification of the position of the maximum rectified RF signal and it was assumed that this signal also marked the TMM interface. Although this is a reasonable assumption, if any of the FTMM samples were subject to shrinkage (by drying out) over the 1-year period, this would represent a potential source of error.

The SoS results of both FTMMs and UTMMs in this study were compared with previously published work (Table 4). *It can be seen that the UTMM mean SoS values are in good agreement with those published, whereas the mean SoS of FTMM were found to be 5.8 ms^{-1} smaller when compared with Rajagopal et al., 2014 and up to 9.6 ms^{-1} smaller when compared with Sun et al., 2012*. In Rajagopal et al., (2014) the manufacture of FTMM was achieved by sandwiching the TMM slice between 2 sheets of Mylar® (~12µm thickness) affixed into Perspex frames, whereas in Sun et al., (2012) the manufacture process of the FTMMs was similar to the method used in this study (referred to as TMM test cells in that study). However, in Rajagopal even though the acoustic measurement was completed relatively quickly (within seconds) the edges of the TMM were not covered, which is likely to have led to some undefined glycerol leakage and potentially affect the measured acoustical properties. Furthermore, in Brewin et al., (2008) the acoustical properties of 2 different batches of TMM were measured over a 3 year period with a thickness range from 3mm to 12.7mm. In Brewin, the first batch consisted of TMM samples which were not protected by a film and were measured in double degassed, deionized water. As a result of glycerol leaching from the samples in this batch, the SoS was found to decrease by 2.1 ms^{-1} $°C^{-1}$. The second batch consisted of

TMM samples protected by Saran Wrap® and were also measured in water. Using this method thinner samples (3mm) displayed the largest SoS variation of 13.4 ms^{-1}. This value is comparable to the SD found in this study for the FTMMs (Table 6) but considerably higher than that measured for the UTMMs.

Figure 3 and Figure 4 show the attenuation of FTMMs and UTMMs respectively, at 0, 6 and 12 month time points. It can be observed, that there is a much larger variation in the *attenuation* measurements obtained from the FTMMs compared to the UTMMs*. At both the lower (4.5 – 9MHz) and the higher (40 – 50MHz) frequency ranges, the data displayed is obtained from a single transducer. Nevertheless, the attenuation versus frequency for the FTMMs would suggest, that with increasing frequency there is an increasing difference in measured mean attenuation values between the 6 month data and 0 and 12 month data. The maximum difference was of 7 dB cm^{-1}, ocurring over the frequency range from 30 – 42MHz and a minimum difference at 15 – 19MHz. For the UTMMs, there is less variation, even in the lower and upper frequency ranges. A maximum variation of 2 $dBcm^{-1}$ was observed across the time points, at a frequency of 47MHz and a minimujm variation from 37 to 47MHz.*

Moreover, the difference in mean attenuation values between the UTMMs and FTMMs would suggest that, despite compensation for the effects of the Saran Wrap®, some additional acoustic effects are introduced which are not fully compensated using the Saran-wrapped reference water test-cell. *However, these effects are unlikely to be due to the difference in non- linear effects between water and TMM preserving fluid. It has previously been shown (Sun, 2012) that even in water, at these output powers, the second harmonic component of the ultrasound beam is at least 30dB smaller in magnitude than the first harmonic. Since non-linear effects are easier to generate in water than in the TMM preserving fluid, it is unlike that nonlinearities are significantly greater than the experimental errors identified.*

Figure 5 and Figure 6 show the mean attenuation of the 11 FTMMs and the 11 UTMMs across the 7 different transducers and measured 3 times during the time period of 1 year. The FTMMs (Figure 5) showed larger variability (~15dB cm^{-1}) across samples and transducers. This may be due to inadequate acoustical correction for the interface layers when using the reference water test cell, leading to an increased uncertainty in the attenuation measurements in addition to the factors previously described. The UTMMs (Figure 6) showed good consistency and little variability in the attenuation over the frequency range of 4.5 – 50 MHz.

Polynomial fits from FTMM and UTMMs were in good agreement with previous studies in the frequency range of 17 – 23 MHz (Brewin et al., 2008), 10 – 47 MHz (Sun et al., 2012) and 1 – 60 MHz (Rajagopal et al., 2014). The polynomial fits were also in good agreement at lower frequencies 4.5 to 10 MHz as reported by IEC (IEC, 2001) and in other studies (Brewin et al .,2008; Inglis et al., 2006) as can be seen in Figure 8. The attenuation from FTMMs and UTMMs does not increase linearly with frequency as shown by the quadratic terms of the polynomial fit. This quadratic term was found to be 0.0073 for FTMMs and 0.0083 for UTMMs which is in good agreement with 0.0076 reported by Sun et al., 2012 and with 0.0081 reported by Rajagopal et al., 2014.

Finally the unpreserved samples displayed significant visual degradation and changes in SoS and attenuation, over the 96 hours. These results are consistent with those of Brewin et al., (2008) who also reported shrinking and hardening of TMM samples which were not preserved.

CONCLUSIONS

In this study, two different measurement techniques were evaluated for assessing the temporal stability of the acoustic properties of the IEC agar TMM over the frequency range 4.5 – 50 MHz. In the first technique thin slices were wrapped and stored in Saran Wrap® and measured in degassed, deionised water. In the second modified technique, thin slices of TMM were preserved and measured in TMM preserving fluid. Measurements were undertaken, over the period of 1 year. The measured SoS of an IEC agar TMM calculated by the Vevo 770® and SAM system was found to be 1538.2 ± 14.5 ms-1 for the FTMMs and 1544.0 ± 3.5 ms^{-1} for the UTMM. For FTMMs the SoS results were less than 10 ms^{-1} lower when compared with those published. The acoustic properties of UTMMs (SoS and attenuation values) were found to be in good agreement with results in earlier studies by Brewin et al., (2008) over the range of 17 – 23 MHz, Sun et al., (2012) over the range of 10 – 47 MHz and Rajagopal et al., (2014) over the range of 1 – 60 MHz. Nevertheless, the results for both FTMMs and UTMMs were consistent at low frequencies (Browne et al., 2003; Inglis et al., 2006) and within the range provided by the IEC (IEC, 2001). However, the attenuation coefficient was shown to be nonlinear as a function of frequency. The attenuation was found to increase as $0.4649f + 0.007363f^2$ for FTMMs and as $0.4897f + 0.008366f^2$ for UTMMs with increasing frequency. This second degree polynomial fit was derived based on the data generated in this study using two different measurements systems and was shown to be able to estimate the attenuation of this IEC agar TMM in the frequency range of 4.5 – 50 MHz. The quadratic term was also found to be in good agreement with previous studies.

Finally, this study has demonstrated that using unwrapped TMM slices (UTMM), maintained and measured in TMM preserving fluid, results in approximately 4 times smaller SD values for the SOS and up to 5 times smaller variation for the attenuation values when compared with the common method of film-wrapped TMM samples (FTMM) measured in degassed, deionised water. This suggests that, despite compensation within the calculation of the attenuation effects of the

393 Saran Wrap®, additional acoustic effects are introduced which are not fully compensated using the
394 standard technique (FTMMs). Moreover, this study has also brought into question, the validity and
395 subsequent stability of encasing gel TMM QA phantoms in a sealed film-dry environment.

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494 LIST OF FIGURES

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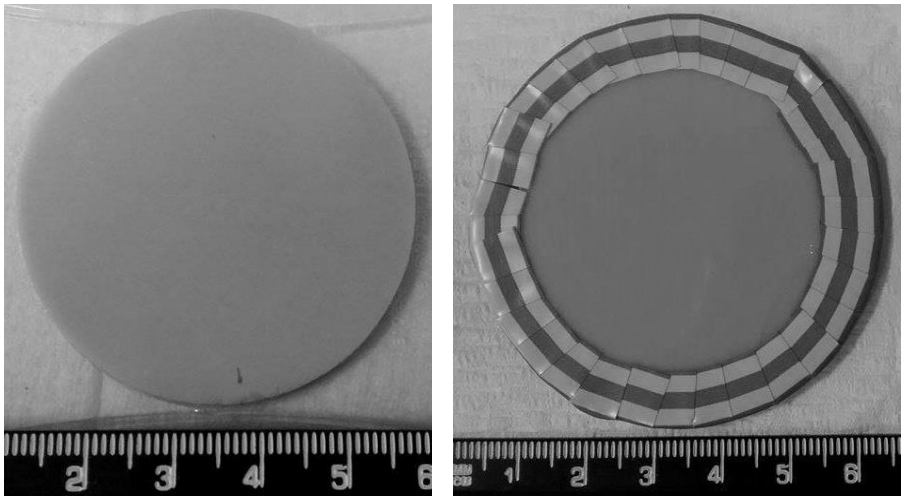

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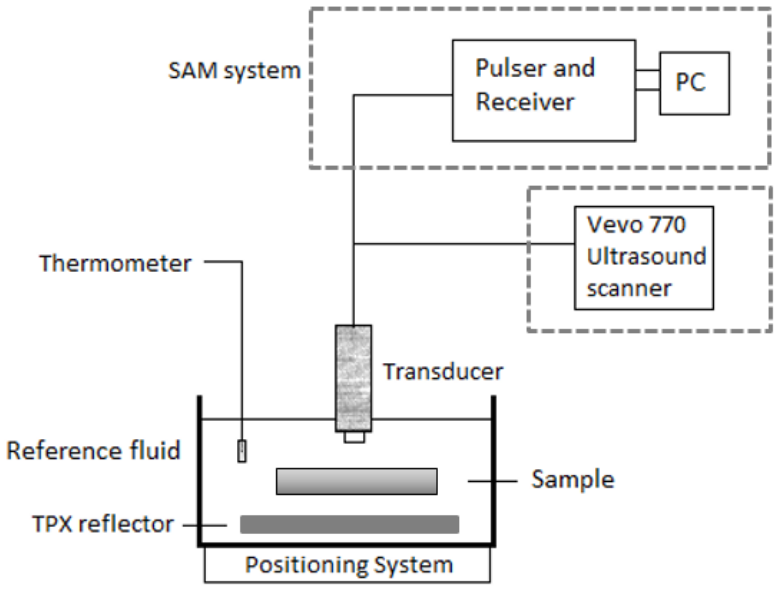
497 Figure 1. a) Non-wrapped TMM slices (UTMMs), arrow indicates the identification mark on the

498 sample and b) wrapped TMM slices (FTMMs).



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500 Figure 2. Experimental set-up using a high frequency ultrasound scanner Vevo 770® used at the

501 University of Edinburgh and a SAM system used at the Dublin Institute of Technology.

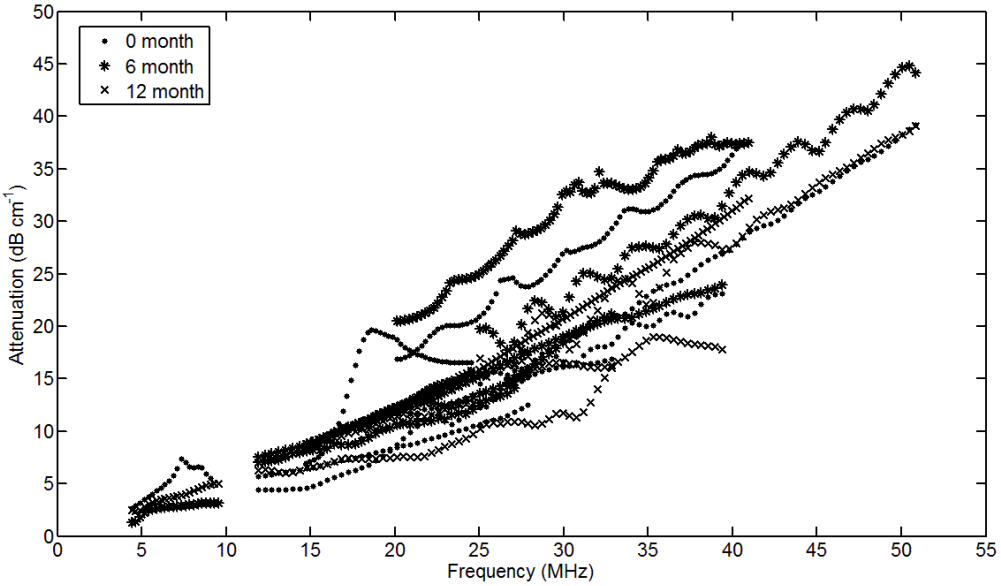


Figure 3. Measured attenuation with the Vevo 770® and SAM system of 11 FTMM at 0, 6 and 12 month time points as a function of frequency. Each line represents one of the seven different transducers used at each time point [RMV704 (18 – 40 MHz), RMV707B (12 – 32 MHz), RMV710B (12 – 28 MHz), RMV711 (25 – 50 MHz), V320 (4.5 – 9 MHz), V317 (14 – 25 MHz), V390 (20 – 40 MHz)].

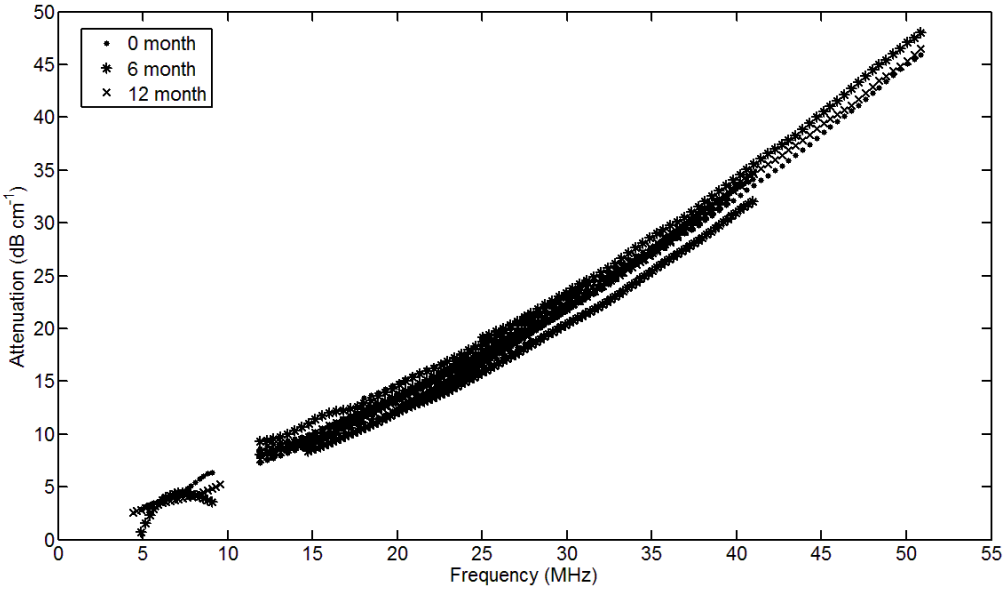
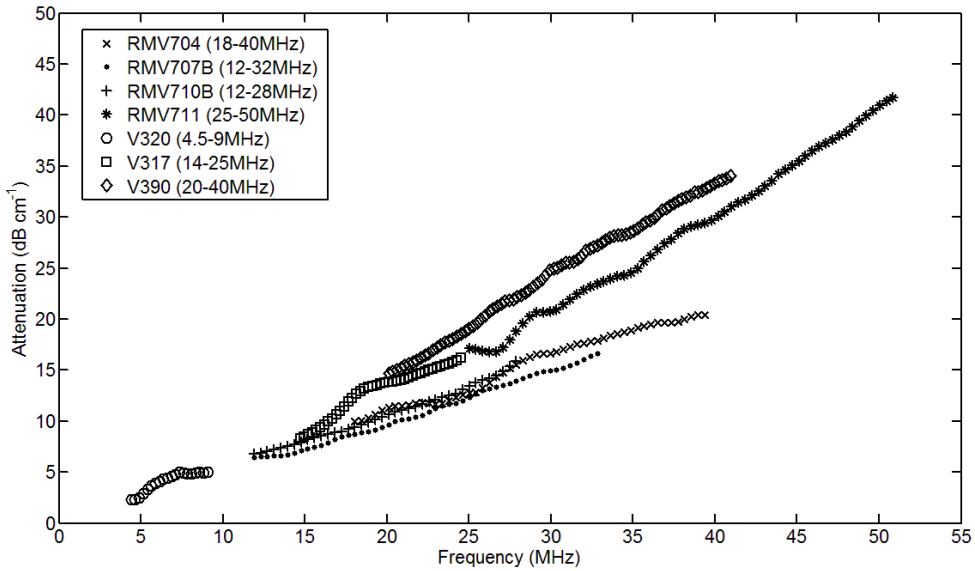


Figure 4. Measured attenuation with the Vevo 770® and SAM system of 11 UTMM at 0, 6 and 12 month time points as a function of frequency. Each line represents one of the seven different transducers used at each time point [RMV704 (18 – 40 MHz), RMV707B (12 – 32 MHz), RMV710B (12 – 28 MHz), RMV711 (25 – 50 MHz), V320 (4.5 – 9 MHz), V317 (14 – 25 MHz), V390 (20 – 40 MHz)].



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519 Figure 5. Attenuation data as a function of frequency averaged over all time points (data set: 11
520 FTMM measured in degassed deionized water by the Vevo 770® and SAM system in the
521 frequency range of 4.5 – 50 MHz).

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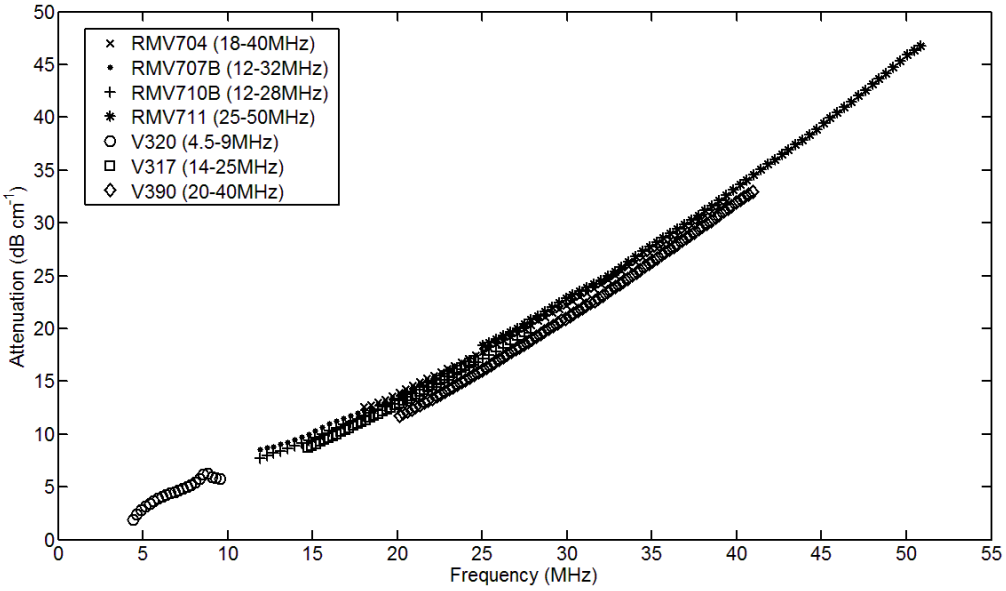

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527 Figure 6. Attenuation data as a function of frequency averaged over all time points (data set: 11
528 UTMM preserved and measured in TMM preserving fluid by the Vevo 770® and SAM system in
529 the frequency range of 4.5 – 50 MHz).

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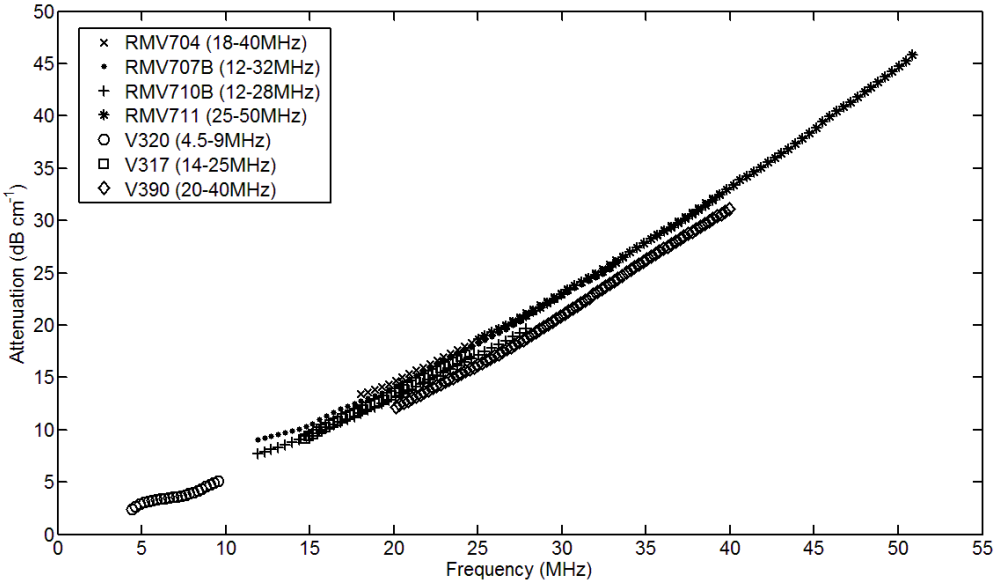

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535 Figure 7. Attenuation data as a function of frequency averaged over all time points from the 6
536 UTMM2 (data set: 6 UTMM2 preserved and measured in TMM preserving fluid by the Vevo
537 770® and SAM system at 6 and 12 month time points in the frequency range of 4.5-50 MHz).

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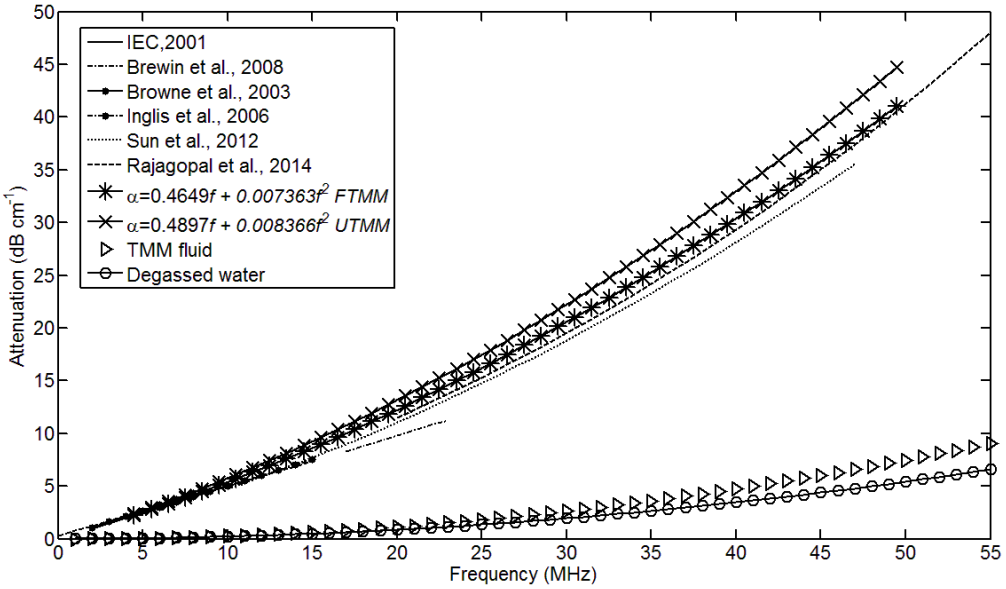

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540 Figure 8. Polynomial curve-fit of all the attenuation data as a function of frequency and the
541 attenuation (compensated for the attenuation in water) of TMM in 2 – 10 MHz (IEC, 2001), 17

– 23 MHz (Brewin et al., 2008), 2.25 – 15 MHz (Browne et al., 2003), 6 – 15 MHz (Inglis et al., 2006), 10 – 47 MHz (Sun et al., 2012) and 1 – 60 MHz (Rajagopal et al., 2014). Also the attenuation as a function of frequency for the TMM preserving fluid and degassed deionised water.

LIST OF TABLES

Transducer model and measurement system		Central Frequency (MHz)	Focal Length (mm)	Measured 3dB bandwidth (MHz)	Peak negative pressure (MPa)	Beam width (cm)
704	Vevo 770®	40	6	18 – 40	0.52	0.008
707B		30	12.7	12 – 32	1.05	0.0115
710B		25	15	12 – 28	1.06	0.014
711		55	6	25 – 50	0.23	0.009
V320	SAM system	7.5	95	4.5 – 9	0.05	1.27
V317		20	65	14 – 25	0.021	0.63
V390		50	12	20 – 40	0.022	0.63

Table 1. Characteristics of the Vevo 770® and SAM system transducers. The central frequency and focal length are parameters provided by the manufacturer from Vevo 770® (VisualSonics, Inc., Toronto, Canada) and SAM system (Olympus Panametrics NDT), the 3dB bandwidth from measurements and the peak pressure from (Sun, 2012).

SoS ± SD (ms^{-1})	0 months	6 months	12 months
FTMM	1547.4 ± 19.2	1547.2 ± 21.5	1525.5 ± 16.5
UTMM	1545.9 ± 10.4	1544.2 ± 11.0	1541.8 ± 1.6

Table 2. The mean and SD of SoS (ms-1) measured with the Vevo 770® and SAM system at each time point for the FTMMs and UTMMs samples.

SoS ± SD (ms^{-1})	Vevo 770®	SAM system
FTMM	1539.6 ± 17.1	1536.3 ± 10.3
UTMM	1542.9 ± 3.6	1545.3 ± 3.0

560 Table 3. The mean and SD of SoS (ms-1) over all time points of 11 FTMM and 11 UTMM measured by

561 the four transducers of the Vevo 770® and by the three transducers of the SAM system.

562

Sources	Type of samples (covered with film or uncovered)	Mean SoS ± SD (ms^{-1})	Frequency range (MHz)
IEC, 2001		1540 ± 15	2 – 10
Browne et al., 2003	TMM uncovered measured in degassed water	1546.5 ± 3	2.25 – 15
Brewin et al., 2008	TMM uncovered measured in degassed water	1537 ± 2.6	17 – 23
	TMM covered	1540.9 ± 8.7	
Sun et al., 2012	TMM covered	1547.8 ± 3.7	10 – 47
Rajagopal et al., 2014	TMM covered	1544 ± 3.1	1 – 60
This study	TMM covered (FTMM)	1538.2 ± 14.5	4.5 – 50
	TMM uncovered (UTMM) measured in TMM fluid	1544.0 ± 3.5	

563 Table 4. Values of SoS (ms-1 ± SD) measured in this study using 2 different methods, compared with

564 the published data.