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D4.1
Selected components for the innovative LIPUS stimulation set-ups

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2	23/09/2019	Andrea Cafarelli, Francesco Fontana SSSA	Contribution to the Deliverable from SSSA
3	25/09/2019	Erik Dumont IGT	Contribution to the Deliverable from IGT
4	26/09/2019	Irene Bernardeschi SSSA	First fully integrated version of the Deliverable
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6	30/09/2019	Leonardo Ricotti SSSA	Final version of the Deliverable and submission

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1 Executive summary

In this Deliverable, a complete description of the components selected for building the highly controlled in vitro LIPUS stimulation systems is provided. These systems will be used in the project to identify the optimal therapeutic US stimulation conditions and to study the underlying effects before the in vivo translation. Two different LIPUS setups were designed. The first one (SYSTEM 1) will ensure control of the US dose at the target for identifying (off-line) the optimum US parameters able to trigger beneficial bioeffects in terms of cartilage regeneration. The second set-up (SYSTEM 2) will also allow to investigate intracellular phenomena occurring during LIPUS, in real-time.
This document does not pretend to describe the assembled systems and their validation (expected at later stages of the project), but aims at providing a complete description of all the selected constituent parts.

2 LIPUS stimulation setups: systems description

Two systems for a controlled in vitro LIPUS cell stimulation will be used within the project. Both systems will guarantee a highly precise ultrasound stimulation of the target.
The systems will be designed in such a way to be inter-changeable and adaptable to several characterized transducers and to enable a controlled US cell stimulation at predefined distances with respect to US transducers. As outlined in Deliverable 2.1, the main goal is to enable a controlled LIPUS stimulation by exploring various US stimulation parameters in terms of:

- intensity (**up to 2000 mW/cm^2**);
- therapy duration (**from few seconds to several minutes**);
- duty cycle (**0% – 100%**);
- frequency (**40 kHz – 5 MHz**).

These parameters were selected after a careful study of the research field.[1] The doses in the identified range do not induce lethal effects on cells.
The LIPUS systems were designed in order to minimize the typical exposure errors of traditional in vitro US cell stimulation systems.
To this purpose, two different LIPUS setups were designed. The first one (SYSTEM 1) will ensure control of the US dose at the target for identifying (off-line) the optimum US parameters able to trigger beneficial bioeffects in terms of cartilage regeneration. The second one (SYSTEM 2) will also allow to investigate intracellular phenomena occurring during LIPUS, in real-time. Both systems are depicted in Figure 1.

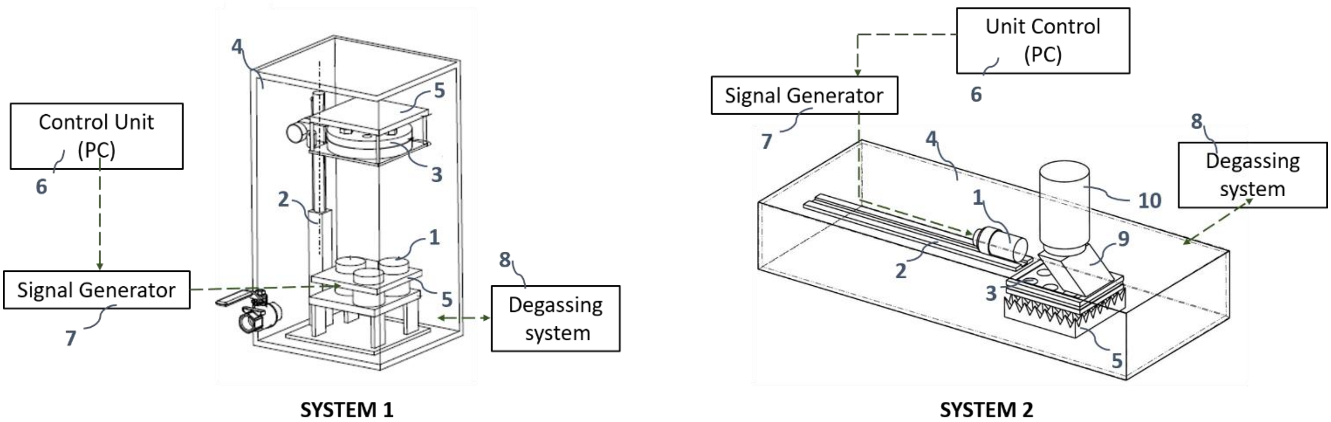

Figure 1. Design of the two innovative LIPUS stimulation systems; SYSTEM 1 (left) allows a multiple controlled ultrasound stimulation without reflections/attenuations; SYSTEM 2 (right) allows a real-time optical visualization of the biological phenomena occurring in vitro. The depicted key elements are: 1) Ultrasound transducers; 2) Support frames; 3) Biological sample holder; 4) Tank filled with deionized and degassed water; 5) Acoustic absorber; 6) Control unit; 7) Signal generator; 8) Degassing system; 9) Opto-transparent / Acousto-reflective element; 10) Optical monitoring device.

[1] Jiang, Xiaoxue, et al. "A Review of Low-Intensity Pulsed Ultrasound for Therapeutic Applications." IEEE Transactions on Biomedical Engineering (2018).

2.1 System 1: selected components and their technical features

Two different LIPUS systems were designed for covering a broad frequency spectrum (40 kHz – 5 MHz), as indicated in Deliverable 2.1. More precisely, as depicted in Figure 2, a **high frequency setup** will be used to test ultrasound exposure conditions in an approximate range of 300 kHz – 5 MHz, while **the low frequency** setup will explore lower frequencies (around 40 kHz). All the key elements are the same for both systems, with only few minor differences, which will be described below. Each system will be fabricated in duplicate, thus to perform LIPUS experiments in parallel in two different ADMAIORA research centres (i.e., SSSA and IOR).

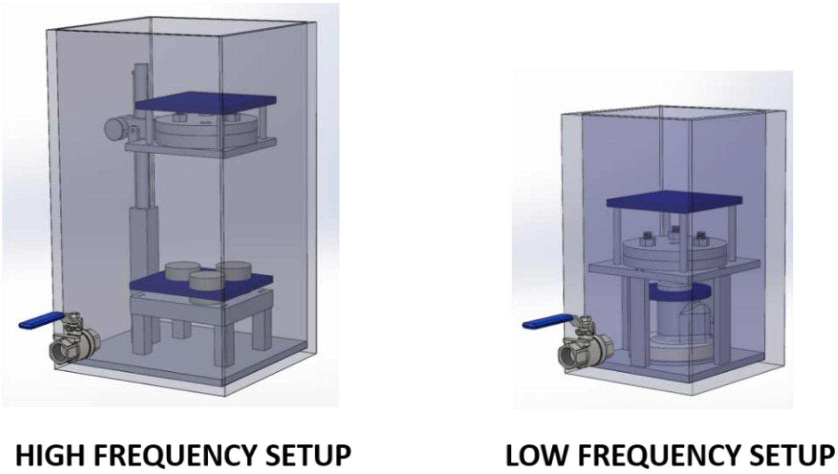

Figure 2. High Frequency setup (left) and low frequency setup (right). The high frequency setup allows a multiple parallel stimulation (i.e., 3 samples stimulated at the same time), while the low frequency setup, due to geometrical constraints, allows the positioning of one transducer and one sample at the time.

2.1.1 Ultrasound transducers

2.1.1.1 High Frequency Ultrasound Transducers

The following high frequency US transducers have been identified and purchased from Precision Acoustics Ltd (Dorchester, UK, Figure 3). Five central frequencies were selected to cover a broad frequency range; for each selected frequency (0,60 MHz, 1 MHz, 2 MHz, 3 MHz, 4 MHZ) 6 different transducers were purchased.

Figure 3. Examples of high frequency ultrasound transducers purchased from Precision Acoustics: a) 44 mm, 0.60 MHz central frequency PA1202, b) 23 mm, 1 MHz central frequency PA1211, c) 23 mm, 2 MHz central frequency PA1218, d) 15 mm, 3 MHZ central frequency PA1224, e) 15 mm, 4 MHz central frequency PA1230.

Table 1. Main features of the high frequency transducers purchased from Precision Acoustics.

Nominal Centre Frequency	0.60 MHz	1.0 MHz	2.0 MHz	3.0 MHz	4.0 MHz
Model Number	1. PA1202 2. PA1203 3. PA1204 4. PA1205 5. PA1206 6. PA1207	1. PA1211 2. PA1212 3. PA1213 4. PA1214 5. PA1215 6. PA1216	1. PA1218 2. PA1219 3. PA1220 4. PA1221 5. PA1222 6. PA1223	1. PA1224 2. PA1225 3. PA1226 4. PA1227 5. PA1228 6. PA1229	1. PA1230 2. PA1231 3. PA1232 4. PA1233 5. PA1234 6. PA1235
Transducer Type	PZT - Unfocused	PZT - Unfocused	PZT - Unfocused	PZT - Unfocused	PZT - Unfocused
Transducer Diameter	44.0 mm	23.0 mm	23.0 mm	15.0 mm	15.0 mm
Frequencies Used	500 kHz 750 kHz	1 MHz	2 MHz	3 MHz	4 MHz 5 MHz

2.1.2 Low Frequency Ultrasound Transducers

For the low frequency ultrasound stimulation system, a flat surface transducer (Figure 4) produced by BAC Technology srl was selected.

Figure 4. P38F, 38 kHz central frequency, flat transducer purchased from BAC Technology.

The main characteristics of the transducer are summarized below.

Table 2. Main features of the low frequency transducer provided by BAC Technology.

Nominal Centre Frequency	Model	Transducer Type	Transducer Diameter	Frequency used
38 kHz	P38F	Flat	50 mm	38 kHz

2.1.3 Biological sample holder

A water-proof and US-transparent culture well has been designed to be used as biological sample holder. Such a design generated an Italian patent that has been recently filed[2]. The main components and technical features of the biological sample holder are:

- a polycarbonate disc, fabricated by CNC machining (145 mm diameter, 18 mm thickness);
- elastomeric O-rings (internal diameters: 14 mm, 20 mm and 126 mm; external diameters: 18.6 mm, 25 mm and 136.6 mm);
- screws (VIBO, carbon steel class 8.8, M12, DIN 961 UNI 5740 ISO 8676);
- polystyrene film (Goodfellow, ST311029, Thickness: 0.029 mm);
- CellCrowns (Scaffdex, CellCrown™24NX inserts C00006S, Outer diameter of the combination of the two parts: 13.7 mm, inner open diameter: 8.6 mm, inner open surface area: 0.58 cm^2);

While the biological sample holder used in the high frequency stimulation system can host up to three samples, thus enabling an ultrasonic stimulation of three biological samples at the same time, in the low frequency stimulation system the biological sample holder is designed to host only one sample at a time, due to geometrical constraints.

2.1.4 Support frames

2.1.4.1 High Frequency Setup

The support frames assembly for the high frequency stimulation system is represented in Figure 5. The single components are listed and described below.

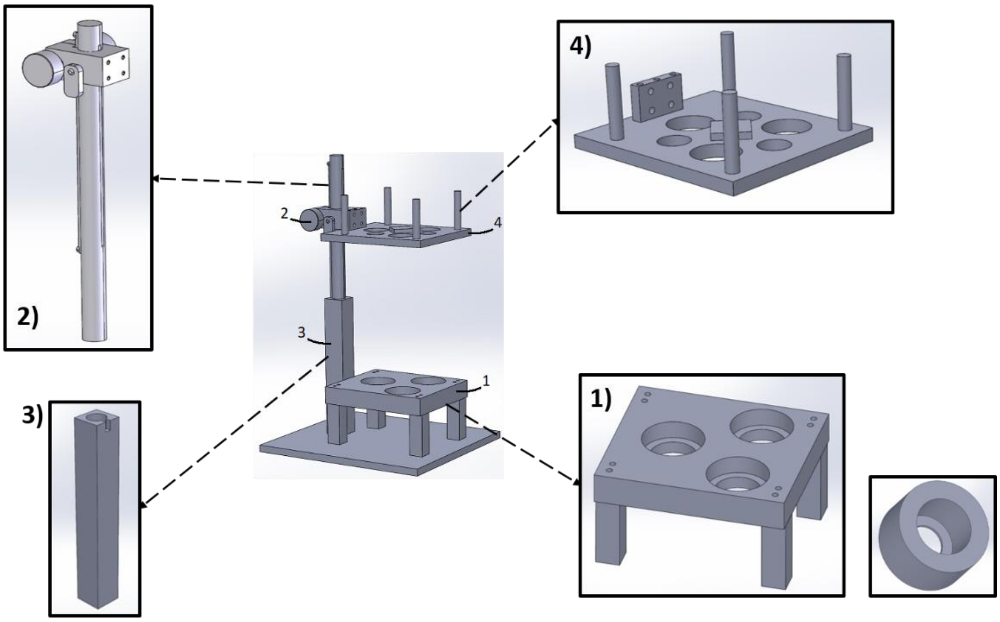

Figure 5. Support frames assembly for the high frequency stimulation system; 1) Transducers support, 2) Rail, 3) Rail support, 4) Biological holder support.

1. **Transducers support**. A mechanical support is used to hold the transducers, thus coaxially positioning them with respect to the biological targets. The frame is elevated

[2] F. Fontana et al. "*Supporto per colture cellulari per stimolazione ultrasonica controllata*". Italian patent. Filed on 25/07/2019.

from the ground in order to guarantee enough space for the connection of electric cables. The upper part of the support is made of Delrin (polyoxymethylene), while the lower pillars are made of aluminum. Transducers adapters, made of Delrin, are used to keep in position transducers of different sizes.

2. **Rail**. A steel tool is used to translate the biological sample holder in the orthogonal direction with respect to the upper surface of the transducers, thus positioning the biological target at a precise distance from the transducers, thus to exploit the desired pressure fields.
3. **Rail support**. An aluminum support is used to block the rail, thus precisely positioning it with respect to the transducers support.
4. **Biological holder support**. An aluminum support, mechanically connected to the rail, is able to fix the biological sample holder and position it concentrically to the transducers. The tool shows four pillars that support the upper acoustic absorber material.

2.1.4.2 Low Frequency Setup

Few modifications with respect to the high frequency system were made in order to adapt the low frequency transducer and the slightly different biological holder used in this system. The main changes are the following: only one sample, instead of three, is stimulated at the same time, and no rail is used to make the biological sample holder translate with respect to the transducer (the sample is fixed at a certain distance from the ultrasound source, equal to 25 mm). The modified support is shown in Figure 6.

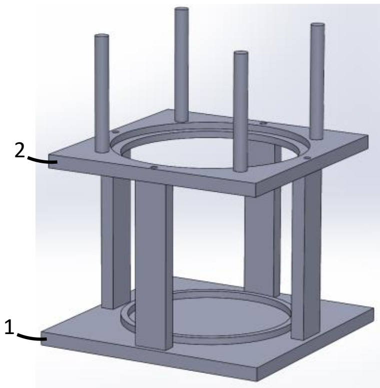

Figure 6. Support frames assembly for the low frequency stimulation system.

The Transducer support (1) is made of Delrin and has the function of hosting and maintain in its position the low frequency transducer. An aluminum support (2) mechanically connected to the lower transducer support is able to fix the biological sample holder and position it concentrically to the transducer. The tool shows four pillars, to support the upper acoustic absorber material.

2.1.5 Tank filled with deionized and degassed water

Cast acrylic tanks (Figure 7) will be used for the in vitro LIPUS experiments. The tanks have the function to host the transducers, the sample holder and the support frames in a liquid environment. The tanks will be filled with deionized and degassed water in order to guarantee a good acoustic coupling for the ultrasound wave generated by ultrasound transducers.

The dimensions of the high frequency system tank are: 265 mm x 245 mm base, 490 mm height, 10 mm thickness, 30 Lt volume. The dimensions of the low frequency system tank are: 200 mm x 200 mm base, 350 mm height, 10 mm thickness, 14 Lt volume.

Figure 7. Cast acrylic tanks.

2.1.6 Acoustic absorbers

Three different types of acoustic absorbers (Figure 8) will be used in order to minimize ultrasound reflections within the setups. All the acoustic absorbers were purchased from Precision Acoustics.

Figure 8. a) Aptflex F28P; b) Aptflex F28; c) Aptflex F48.

- **Aptflex F28P** (Figure 8a): longitudinal wave speed (1-10 MHz) ≈ 1500 m/s, acoustic impedance: 1.5 MRayls, echo-reduction: greater than 20 dB at 1 MHz, to greater than 40 dB above 6 MHz; pyramidal shape.
- **Aptflex F28** (Figure 8b): longitudinal wave speed (1-10 MHz) ≈ 1500 m/s, acoustic impedance: 1.5 MRayls, echo-reduction: > 22 dB at 1 MHz, degrading to < 13 dB at 10 MHz, > 18 dB over frequency range 1-3 MHz.
- **Aptflex F48** (Figure 8c): average wave speed (50-200 KHz): 990 ± 30 m/s, acoustic impedance: 1.89 MRayls, high echo-reduction in a frequency range 30 kHz - 100 KHz.

For the high frequency system, Aptflex F28P and Aptflex F28 will be used, while in the low frequency system, Aptflex F28P and Aptflex F48 will be integrated within the system.

2.1.7 Control Unit

The multi channels, high power signal generator designed to drive the LIPUS transducers is controlled via an Ethernet connection from a PC with a dedicated software based on an easy-to-use Graphical User Interface (GUI). The control software enables full control of the phase, frequency and amplitude of the signal generated on each individual channel (Figure 10).

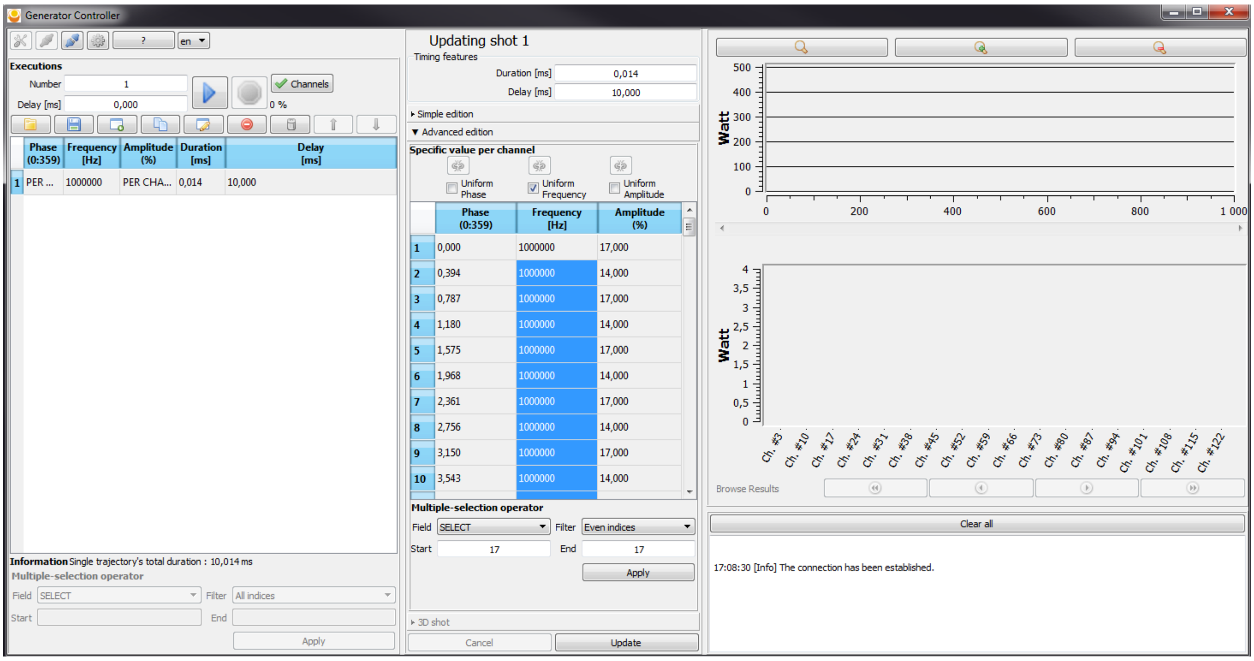

Figure 9. GUI to control the multi-channels signal generator with individual control of each channel. The system also provides power output measurements for each channel and every pulse (displayed on the right in the GUI).

The pulse sequence is built by adding pulses of adequate duration followed by delays (Figure 10). The multi channels system is also phase synchronized so that it can be used as a phased array generator.

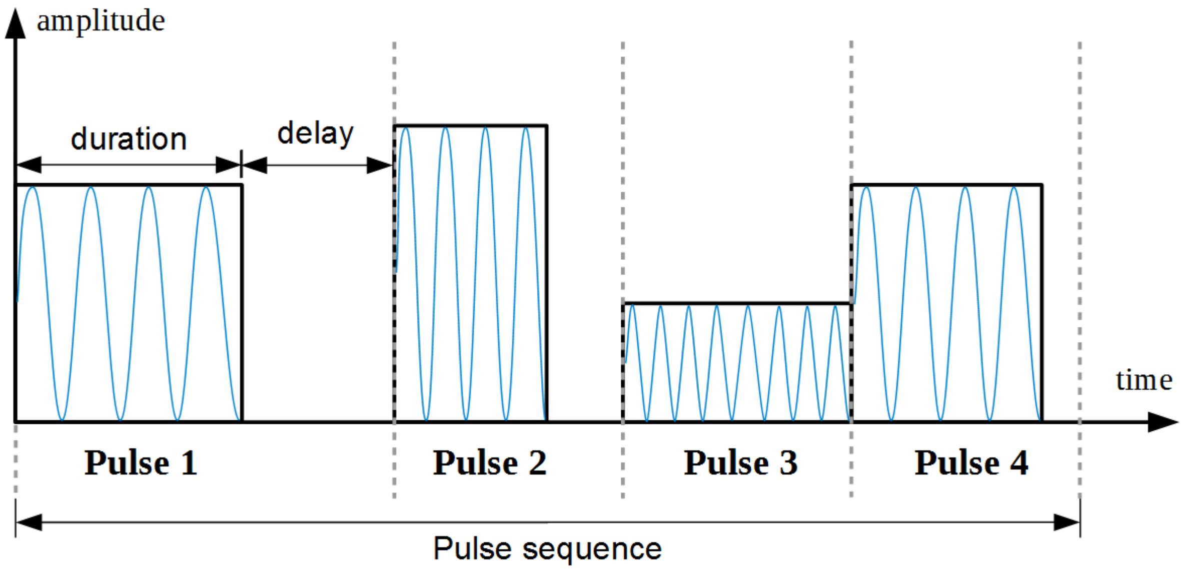

Figure 10. Pulse sequence definition with each pulse having its own frequency, phase and amplitude.

2.1.8 Signal Generator

2.1.8.1 High Frequency Signal Generator

The multi-channels, high power signal generator is based on a high performance Direct Digital Synthetiser (DDS) that provides a pure sinusoidal signal with a frequency of 1 Hz. The amplitude is also controlled by a 10 bits Digital to Analog Converter (DAC), providing a fine control of the output signal. The synthetized sinusoidal signal is then amplified by a class AB linear power amplifier for low harmonic distortion (less than 3% at 75% amplitude). The synthesizers and amplifiers are broadband and guarantee an operational bandwidth of 5 MHz (0.5 MHz to 5.5 MHz). The generator provides also individual measurements of the forward and reflected signals for output power monitoring. A picture of the multi-channels signal generator is shown in Figure 11.

Figure 11. Multi-channel, high power, broadband sinusoidal signal generator with individual control for phase, frequency and amplitude per channel.

Generator specifications are:

- Amplifier technology: class A/B linear amplifier;
- Pure sinusoids signal generator;
- Output Power per channel: 20 W;
- Usable bandwidth: 500 kHz – 5.5 MHz;

- Control per channel: phase, frequency, amplitude;
- Number of channels: 4;
- Duty Cycle at 100% power: 100% for 10 min;
- Measurement per channel: Vrms forward, Vrms reflected, signal phase;
- Harmonic distortion: <4% at 75% amplitude.

2.1.8.2 Low Frequency Signal Generator

The low frequency transducers will be electrically driven by a SIRIO portable system (Figure 12), provided by BAC Technology.

The system generates a sine wave at an acoustic working frequency of 38 kHz, and allows to select the transducer the duration of the treatment and the output power, expressed in percentage, between 10% and 100%. After an initial check of the functionality of the system, the selection of a specific output power percentage leads the control unit to modulate the voltage amplitude delivered to the transducer. Both the duration and the output percentage can be changed during the treatment.

Figure 12. SIRIO portable system provided by BAC Technology.

2.1.9 Degassing System

An online degassing system (Figure 13) was developed to ensure rapid and sustained degassing of water in the tanks, in order to prevent bubble formation or cavitation, which would interfere with the experiments, by distorting the acoustic field and by introducing possible undesired mechanical effects. The degassing system consists of a high flow pump which drives the water through a small particles filter and a micro-porous membrane connected to a vacuum pump for rapid degassing of the flowing water.

The specifications of the degassing system are:

- Pump flow rate: 3 L/min;
- Degassing rate (time to divide amount of dissolved gas by 2 in 1 liter): 40 s/L.

Figure 13. Online degassing system including a particles filter, a vacuum pump, a flow pump and a degassing filter.

2.2 System 2: selected components and their technical features

System 2 enables a controlled US cell stimulation with a simultaneous optical monitoring of the target. SSSA owns a patent[3] on a multi-frequency in vitro LIPUS stimulation system, allowing to acquire optical signals exactly from the stimulated region, for real-time investigation of US-triggered phenomena.
The vast majority of the elements described for System 1 (Ultrasound transducers, signal generators, biological sample holder, acoustic absorbers, etc.) will be used also for System 2. However, two key additional elements (i.e. the opto-transparent/acousto-reflective element and the optical monitoring device), will be added in System 2.

2.2.9 Opto-transparent / Acousto-reflective element

An acoustically-reflective/optically transparent element (Figure 14) will be positioned in the US path and between the objective of the monitoring system and the target (i.e., the cells to be invested by US). The ultrasound transducers will be positioned at 90° respect to the optical imaging system (e.g., a microscope objective), used to optically monitor in real-time the cell processes occurring during US stimulation.
This element must guarantee good transparency to the light and a complete reflection to acoustic waves. Preliminary tests will be performed with a structure made of a thin shell of plastic material (e.g., plexiglass - poly(methyl methacrylate) - with a thickness of 40 μm) with a gaseous substance inside (e.g., air).

[3] Cafarelli *et al*. Italian Patent No. 102016000052583, Filing date: 23/05/2016

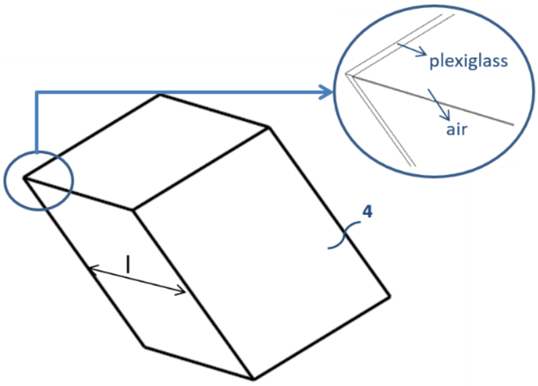

Figure 14. Acoustically-reflective/optically transparent element made of a shell of plexiglass and air inside.

2.2.10 Optical monitoring device

Even if the optical monitoring system will be not a part of the system, it is worth mentioning that System 2 will be tested with different standard monitoring systems usually available in biological labs (e.g., standard cameras, stereoscopic microscope, digital microscope and long working distance confocal microscope), in order to check if the distance between the target and the objective falls within the working distance of the instrument objective and if the optical path is transparent enough to ensure a good visualization of cells.

3 Conclusion

One of the ADMAIORA goals is to enable the use of several LIPUS transducers within *ad hoc* US stimulation setups for a controlled in vitro US cell stimulation.
Two different systems (System 1 and System 2) are under development in the project to investigate the optimal ultrasound therapeutic conditions and the underlying effects before the in vivo translation.
In this deliverable, the components selected and that will be integrated in the *in vitro* setups are described. Below, the technical datasheets of the high-frequency transducers are reported.

4 Transducers datasheets

4.1 600 kHz

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UNITED KINGDOM

TRANSDUCER TEST CERTIFICATE

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Device Identification

Transducer S/N	PA1203
Transducer Type	PZT - Unfocussed
Transducer Diameter	44.0 mm
Nominal Centre Frequency	600.00 kHz

Calibration Conditions

Calibration date	28/06/2019
Water temperature	22.0 °C
Water treatment	De-gassed, De-ionised, filtered
Acoustic path length	196 mm
Source signal type	Sine wave Toneburst
Peak to peak source signal amplitude	64 V
Electrical impedance	50 Ohms
Cable type, length	RG58, 1.5 m
Measurement type	Hydrophone measurement

Test Equipment used

Signal Generator	Keysight Technologies 33519B
Signal Amplifier	E&I A150
DAQ Device / Analyser	Agilent Technologies DSO-X 3024A

Calibrated by;	Checked by;
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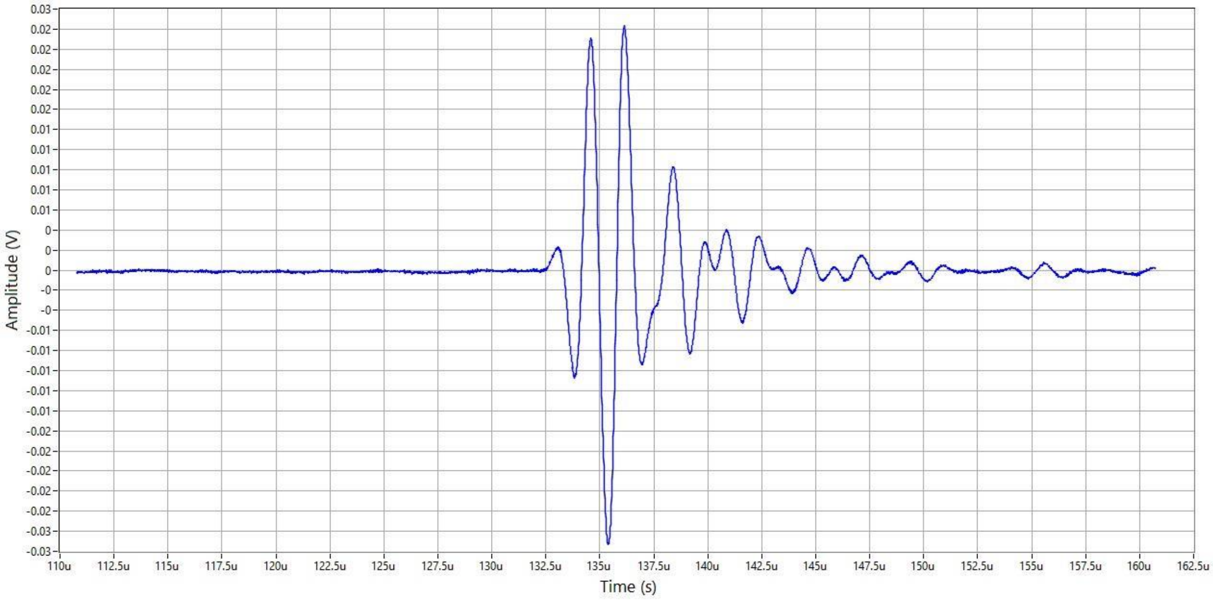
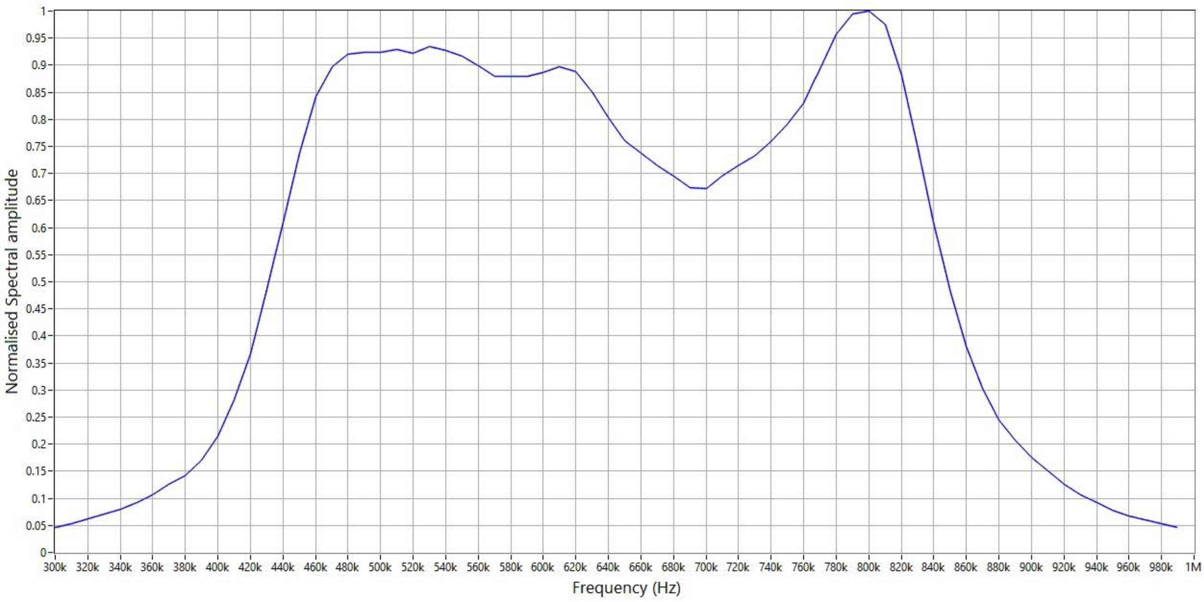
Megan Jenkinson	Thomas Kelley

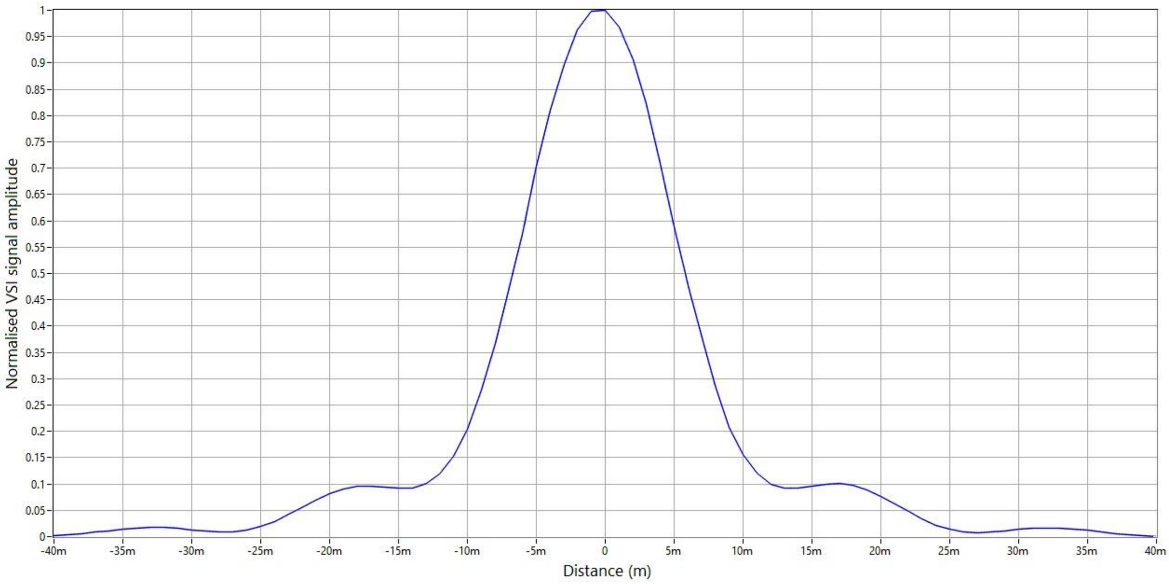


Time domain response to a single cycle sine wave at transducer's nominal centre frequency, PA1203

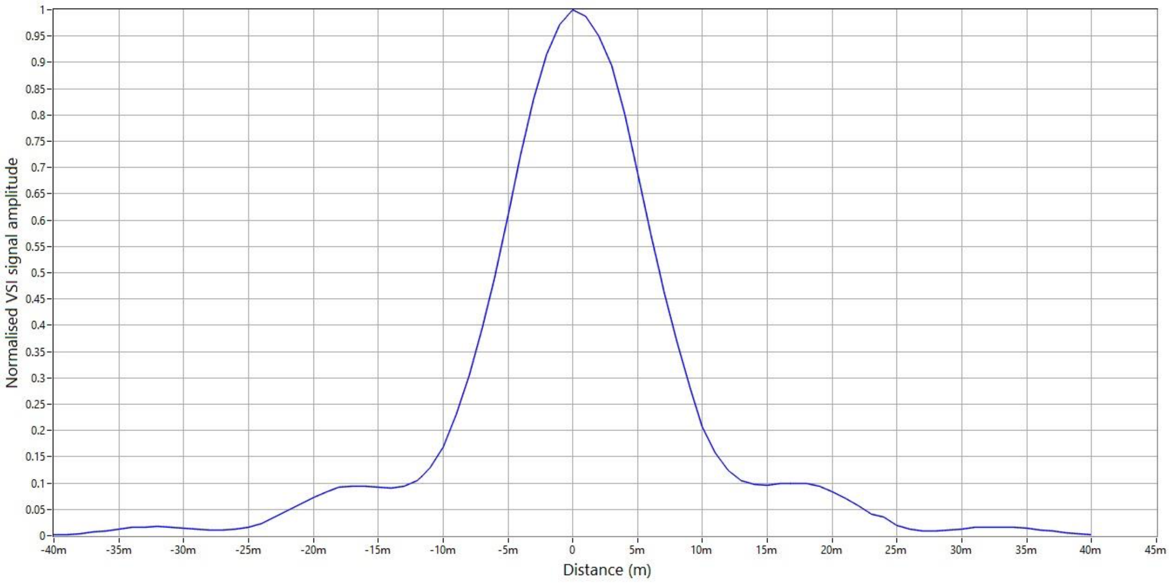


Swept frequency profile for transducer PA1203

Peak Frequency (MHz): 0.80	-3.0 dB	-6.0 dB	-12.0 dB	-20.0 dB
Lower limit (MHz)	0.45	0.43	0.41	0.36
Centre frequency (MHz)	0.64	0.64	0.64	0.65
Upper limit (MHz)	0.83	0.85	0.88	0.93
Bandwidth (MHz)	0.39	0.42	0.47	0.58
Bandwidth (%)	60.21	65.25	73.76	89.80



Transverse X Profile for PA1203



Transverse Y Profile for PA1203

	-3.0 dB	-6.0 dB	-12.0 dB	-20.0 dB
X-profile beamwidth (mm)	12.48	17.84	42.46	62.22
Y-profile beamwidth (mm)	12.65	18.16	42.40	62.77

4.2 1 MHz

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Device Identification

Transducer S/N	PA1211
Transducer Type	PZT - Unfocussed
Transducer Diameter	23.0 mm
Nominal Centre Frequency	1.00 MHz

Calibration Conditions

Calibration date	25/06/2019
Water temperature	22.0 °C
Water treatment	De-gassed, De-ionised, filtered
Acoustic path length	88 mm
Source signal type	Sine wave Toneburst
Peak to peak source signal amplitude	57 V
Electrical impedance	50 Ohms
Cable type, length	RG58, 1.5 m
Measurement type	Hydrophone measurement

Test Equipment used

Signal Generator	Keysight Technologies 33519B
Signal Amplifier	E&I A150
DAQ Device / Analyser	Agilent Technologies DSO-X 3024A

Calibrated by;	Checked by;
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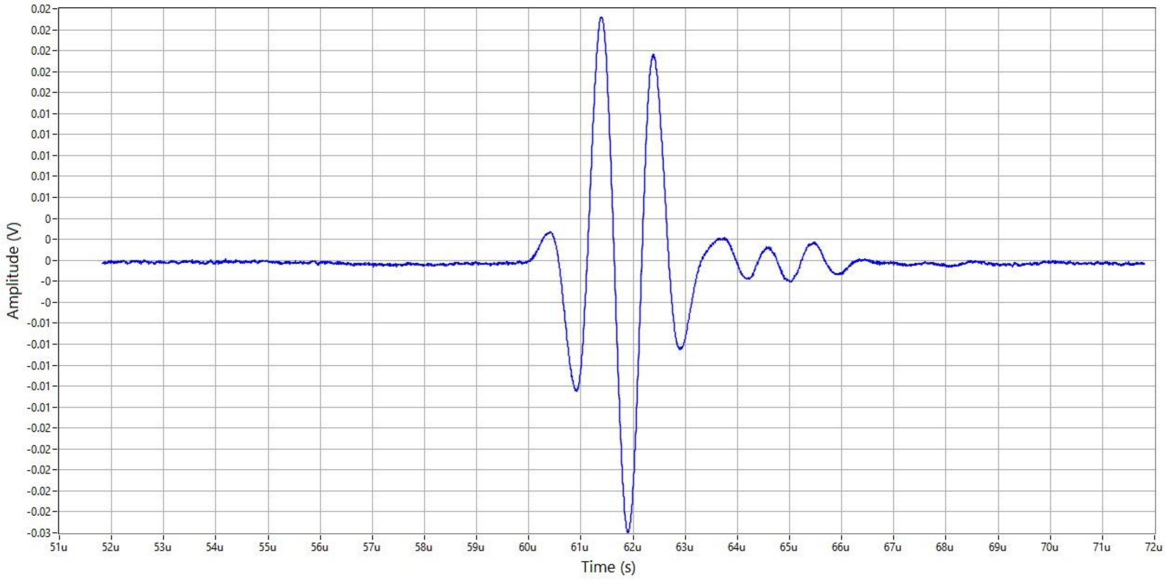
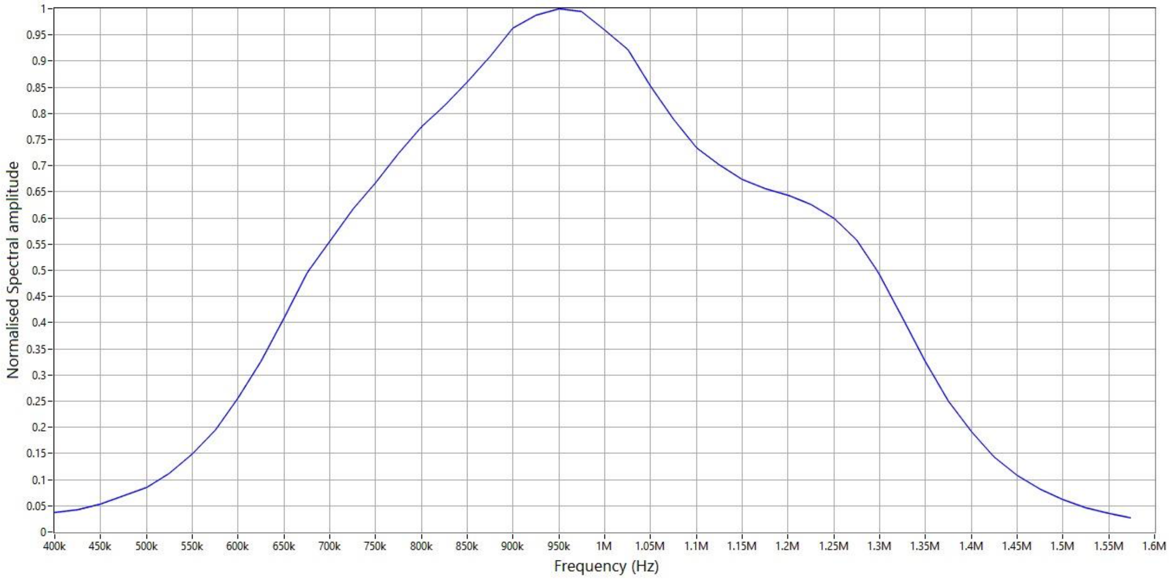
Megan Jenkinson David Bell

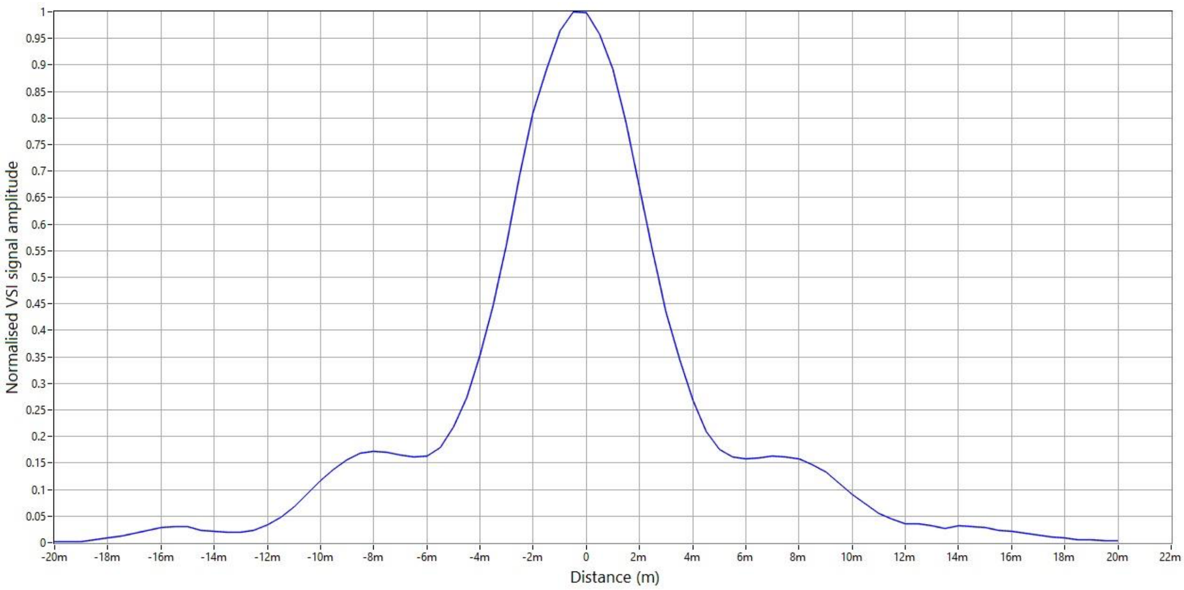


Time domain response to a single cycle sine wave at transducer's nominal centre frequency, PA1211

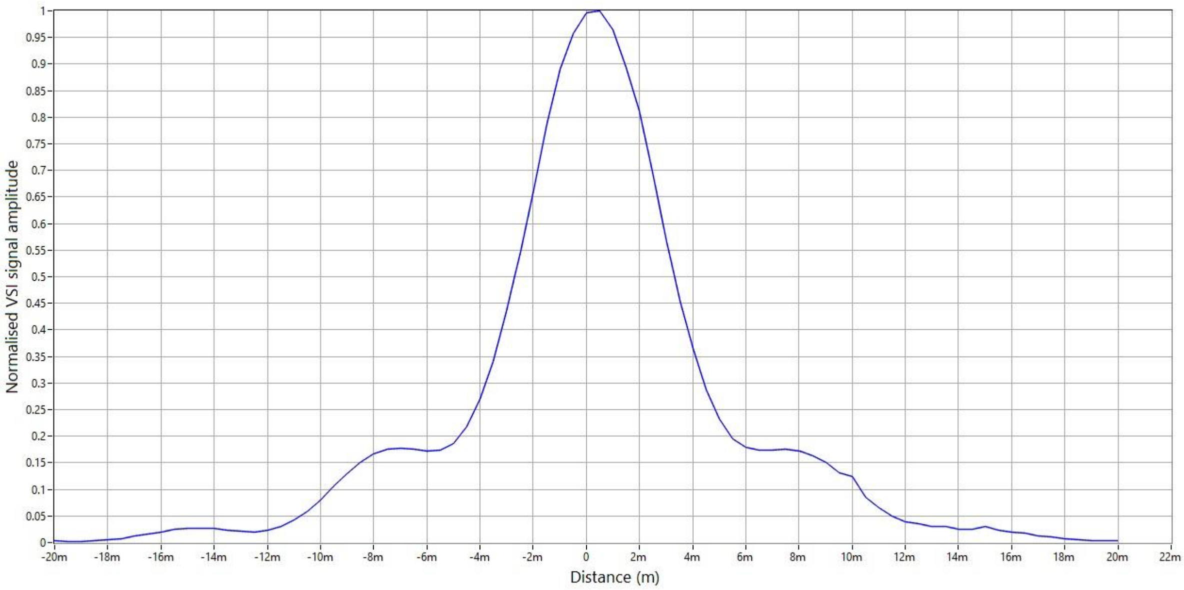


Swept frequency profile for transducer PA1211

Peak Frequency (MHz): 0.95	**-3.0 dB**	**-6.0 dB**	**-12.0 dB**	**-20.0 dB**
Lower limit (MHz)	0.77	0.68	0.60	0.51
Centre frequency (MHz)	0.94	0.99	0.99	0.99
Upper limit (MHz)	1.12	1.30	1.38	1.46
Bandwidth (MHz)	0.35	0.62	0.78	0.94
Bandwidth (%)	37.34	62.70	78.82	95.76



Transverse X Profile for PA1211



Transverse Y Profile for PA1211

	-3.0 dB	**-6.0 dB**	**-12.0 dB**	**-20.0 dB**
X-profile beamwidth (mm)	5.99	8.86	21.93	35.48
Y-profile beamwidth (mm)	6.01	9.03	21.51	34.66

4.3 2 MHz

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Device Identification

Transducer S/N	PA1218
Transducer Type	PZT - Unfocussed
Transducer Diameter	23.0 mm
Nominal Centre Frequency	2.00 MHz

Calibration Conditions

Calibration date	04/07/2019
Water temperature	22.0 °C
Water treatment	De-gassed, De-ionised, filtered
Acoustic path length	178 mm
Source signal type	Sine wave Toneburst
Peak to peak source signal amplitude	61 V
Electrical impedance	50 Ohms
Cable type, length	RG58, 1.5 m
Measurement type	Hydrophone measurement

Test Equipment used

Signal Generator	Keysight Technologies 33519B
Signal Amplifier	E&I A150
DAQ Device / Analyser	Agilent Technologies DSO-X 3024A

Calibrated by;	Checked by;
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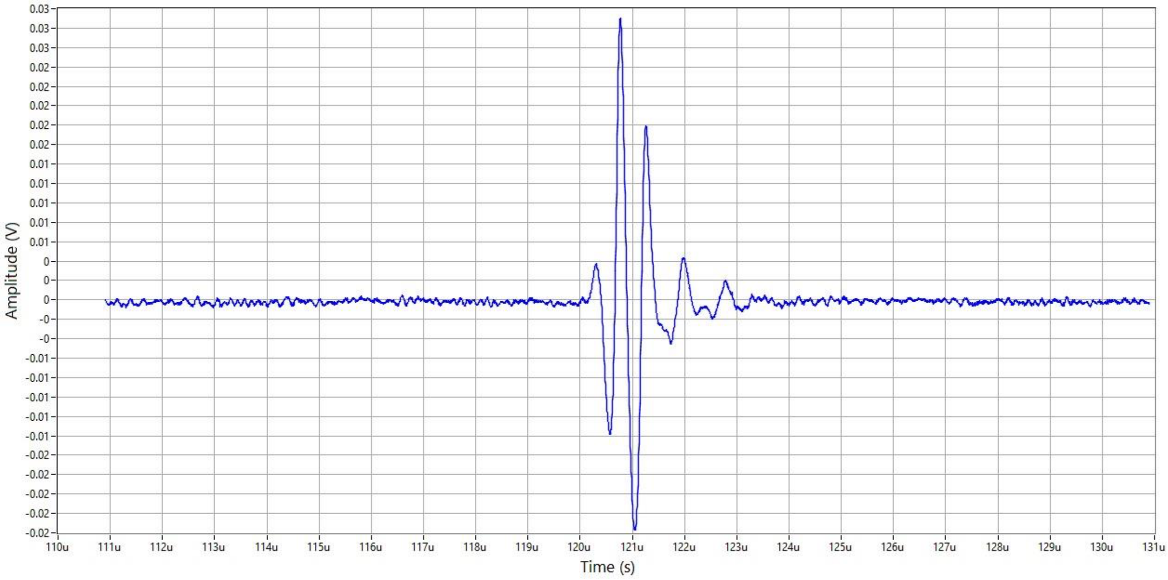
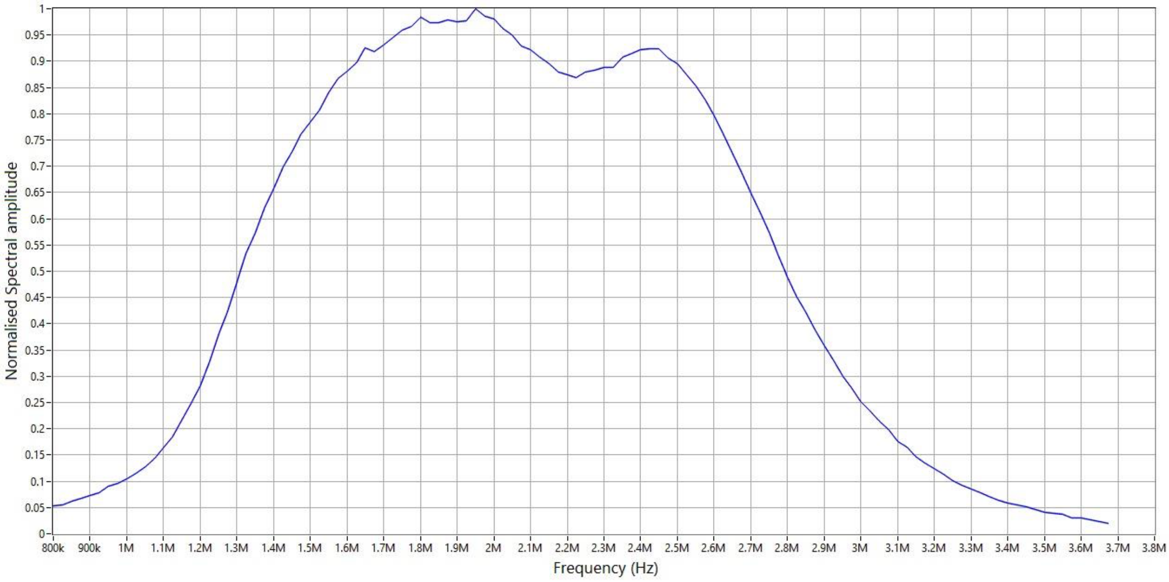
Megan Jenkinson	Thomas Kelley

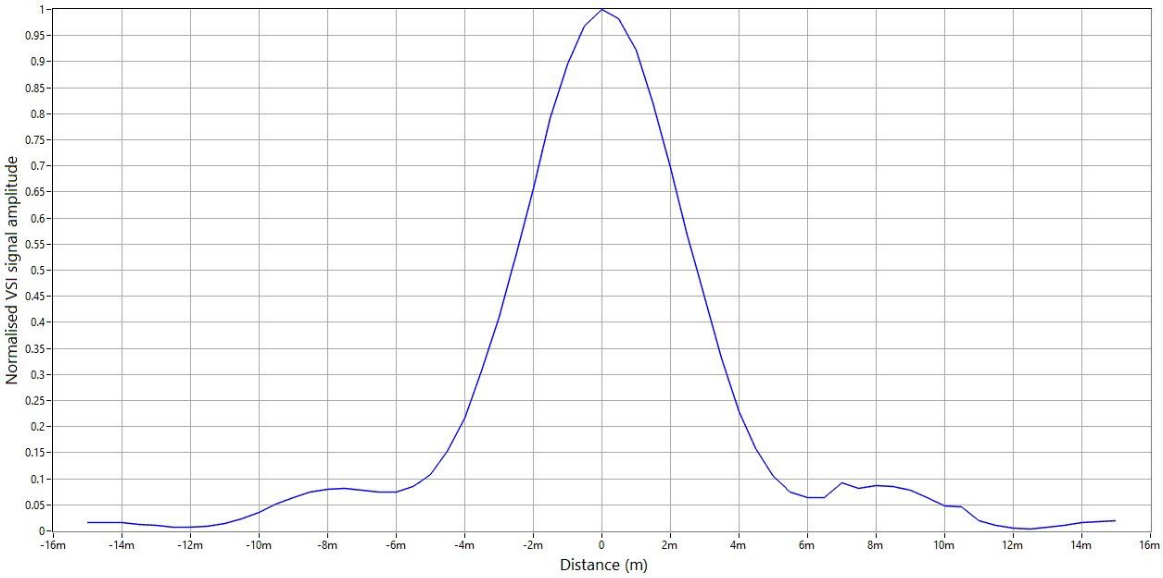


Time domain response to a single cycle sine wave at transducer's nominal centre frequency, PA1218

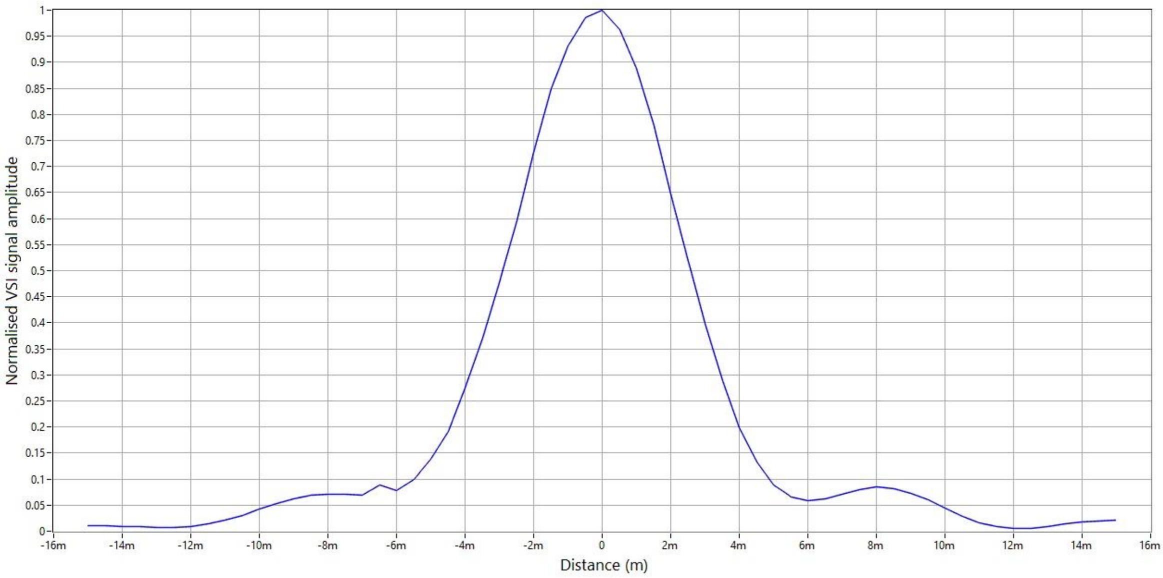


Swept frequency profile for transducer PA1218

Peak Frequency (MHz): 1.95	**-3.0 dB**	**-6.0 dB**	**-12.0 dB**	**-20.0 dB**
Lower limit (MHz)	1.43	1.31	1.18	0.99
Centre frequency (MHz)	2.05	2.05	2.09	2.12
Upper limit (MHz)	2.66	2.79	3.00	3.25
Bandwidth (MHz)	1.23	1.48	1.83	2.27
Bandwidth (%)	60.10	72.30	87.39	106.86



Transverse X Profile for PA1218



Transverse Y Profile for PA1218

	-3.0 dB	-6.0 dB	-12.0 dB	-20.0 dB
X-profile beamwidth (mm)	5.39	7.71	18.63	26.50
Y-profile beamwidth (mm)	5.48	7.86	14.67	26.44

4.4 3 MHz

PRECISION ACOUSTICS LTD

Hampton Farm Business Park
Dorchester, DT2 8QH
UNITED KINGDOM

TRANSDUCER TEST CERTIFICATE

This certificate provides traceability of measurement to recognised national standards and to units of measurement realised at the National Physical Laboratory or other recognised national standards laboratories. Precision Acoustics Ltd is certified to the ISO 9001 standard.

Device Identification

Transducer S/N	PA1224
Transducer Type	PZT - Unfocussed
Transducer Diameter	15.0 mm
Nominal Centre Frequency	3.00 MHz

Calibration Conditions

Calibration date	02/07/2019
Water temperature	22.0 °C
Water treatment	De-gassed, De-ionised, filtered
Acoustic path length	114 mm
Source signal type	Sine wave Toneburst
Peak to peak source signal amplitude	59 V
Electrical impedance	50 Ohms
Cable type, length	RG58, 1.5 m
Measurement type	Hydrophone measurement

Test Equipment used

Signal Generator	Keysight Technologies 33519B
Signal Amplifier	E&I A150
DAQ Device / Analyser	Agilent Technologies DSO-X 3024A

Calibrated by;	Checked by;
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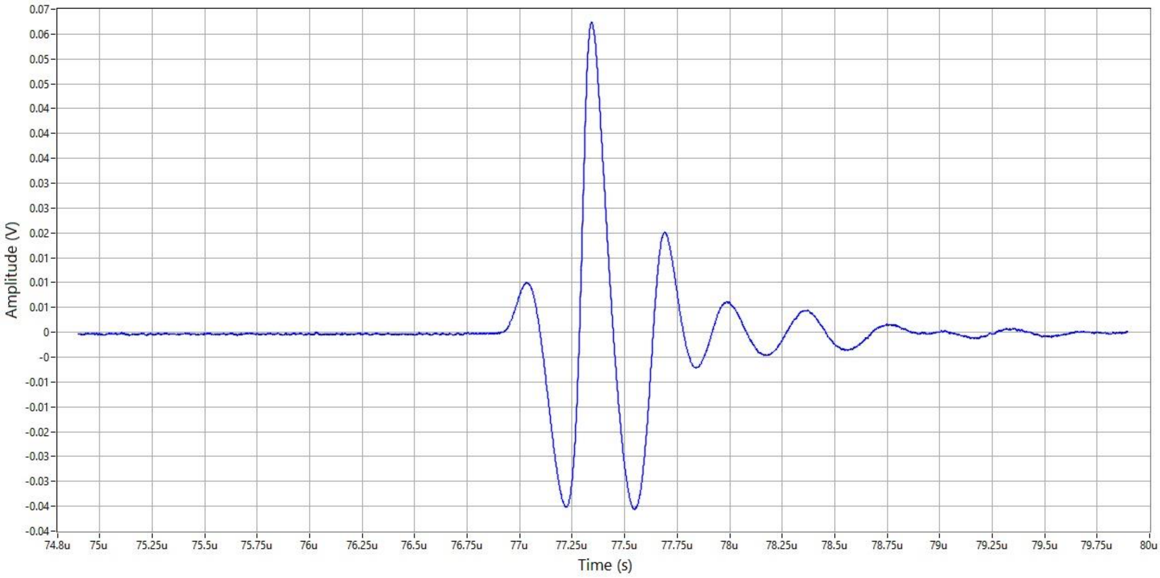
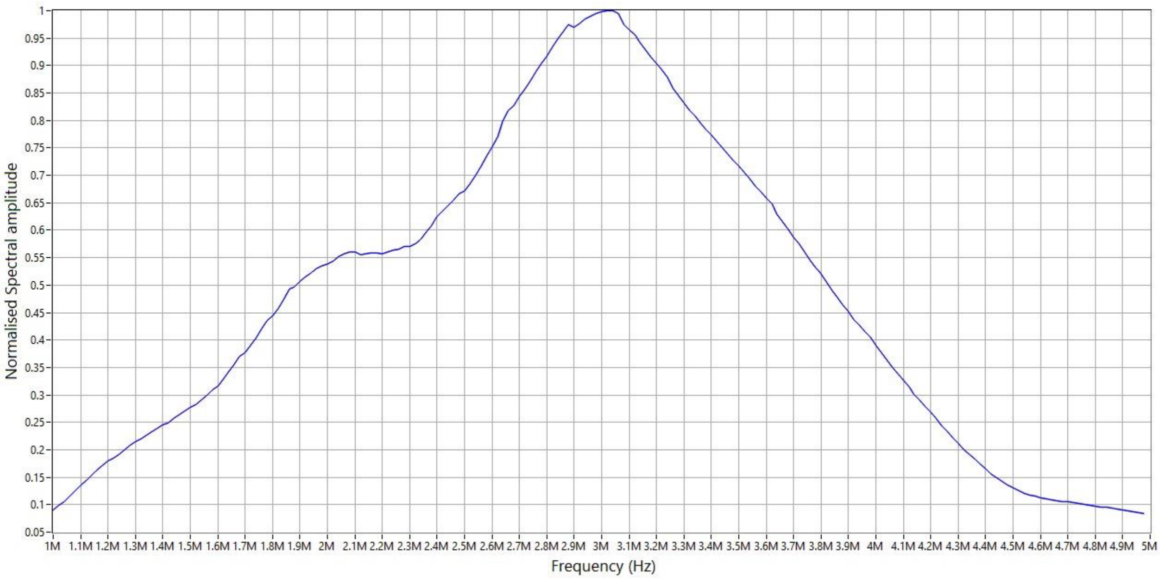
Lucy Buckwell　　Thomas Kelley

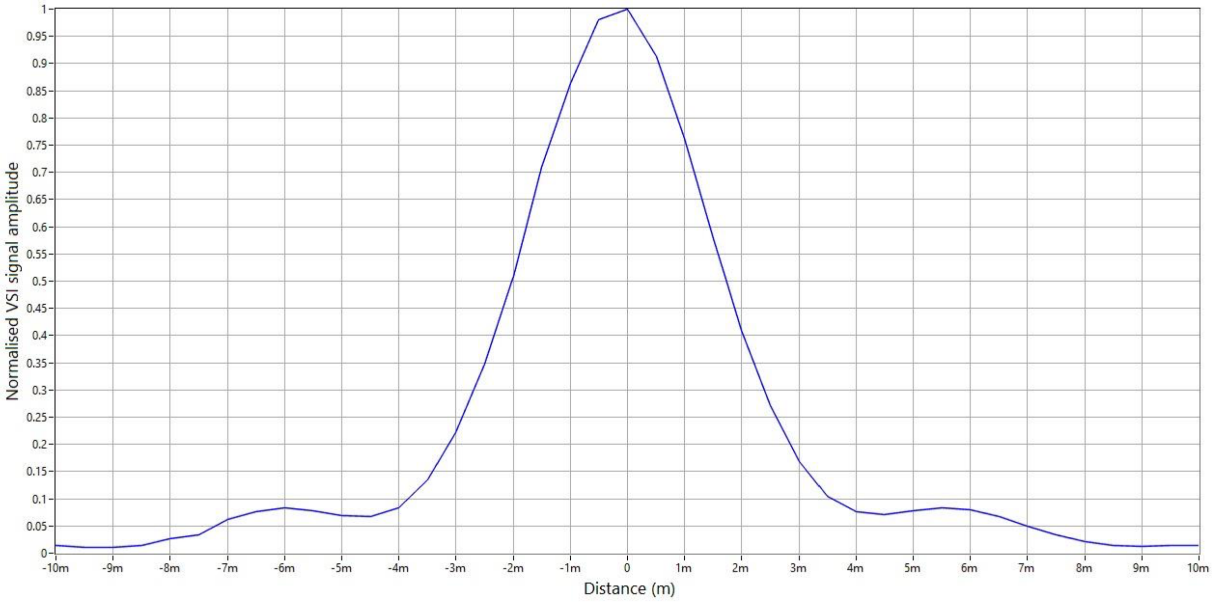


Time domain response to a single cycle sine wave at transducer's nominal centre frequency, PA1224

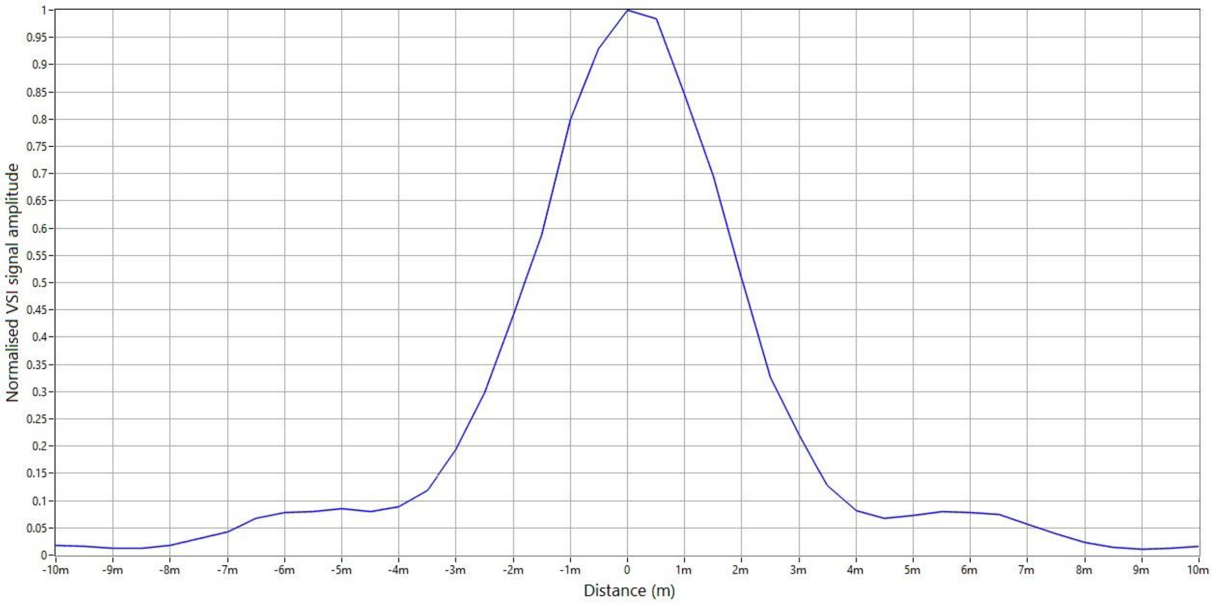


Swept frequency profile for transducer PA1224

Peak Frequency (MHz): 3.02	**-3.0 dB**	**-6.0 dB**	**-12.0 dB**	**-20.0 dB**
Lower limit (MHz)	2.55	1.89	1.42	1.02
Centre frequency (MHz)	3.03	2.86	2.83	2.90
Upper limit (MHz)	3.52	3.83	4.23	4.77
Bandwidth (MHz)	0.97	1.94	2.81	3.75
Bandwidth (%)	31.90	67.85	99.39	129.34



Transverse X Profile for PA1224



Transverse Y Profile for PA1224

	-3.0 dB	-6.0 dB	-12.0 dB	-20.0 dB
X-profile beamwidth (mm)	3.76	5.49	13.60	NaN
Y-profile beamwidth (mm)	3.83	5.59	13.43	NaN

4.5 4 MHz

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Device Identification

Transducer S/N	PA1230
Transducer Type	PZT - Unfocussed
Transducer Diameter	15.0 mm
Nominal Centre Frequency	4.00 MHz

Calibration Conditions

Calibration date	03/07/2019
Water temperature	22.0 °C
Water treatment	De-gassed, De-ionised, filtered
Acoustic path length	152 mm
Source signal type	Sine wave Toneburst
Peak to peak source signal amplitude	60 V
Electrical impedance	50 Ohms
Cable type, length	RG58, 1.5 m
Measurement type	Hydrophone measurement

Test Equipment used

Signal Generator	Keysight Technologies 33519B
Signal Amplifier	E&I A150
DAQ Device / Analyser	Agilent Technologies DSO-X 3024A

Calibrated by;	Checked by;
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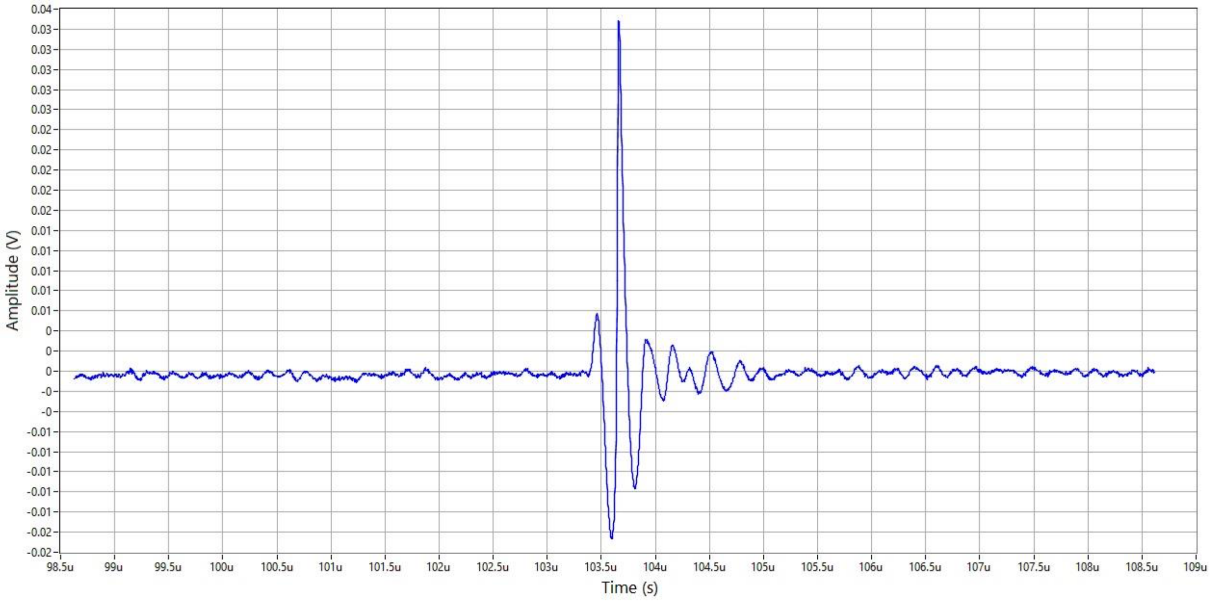
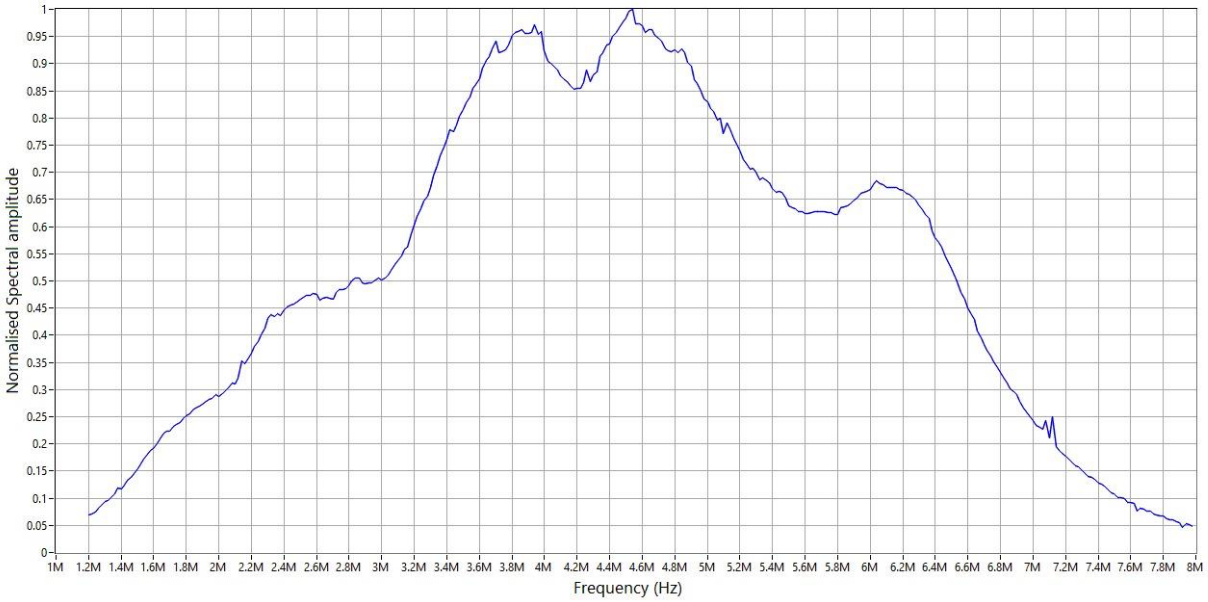
Megan Jenkinson Thomas Kelley

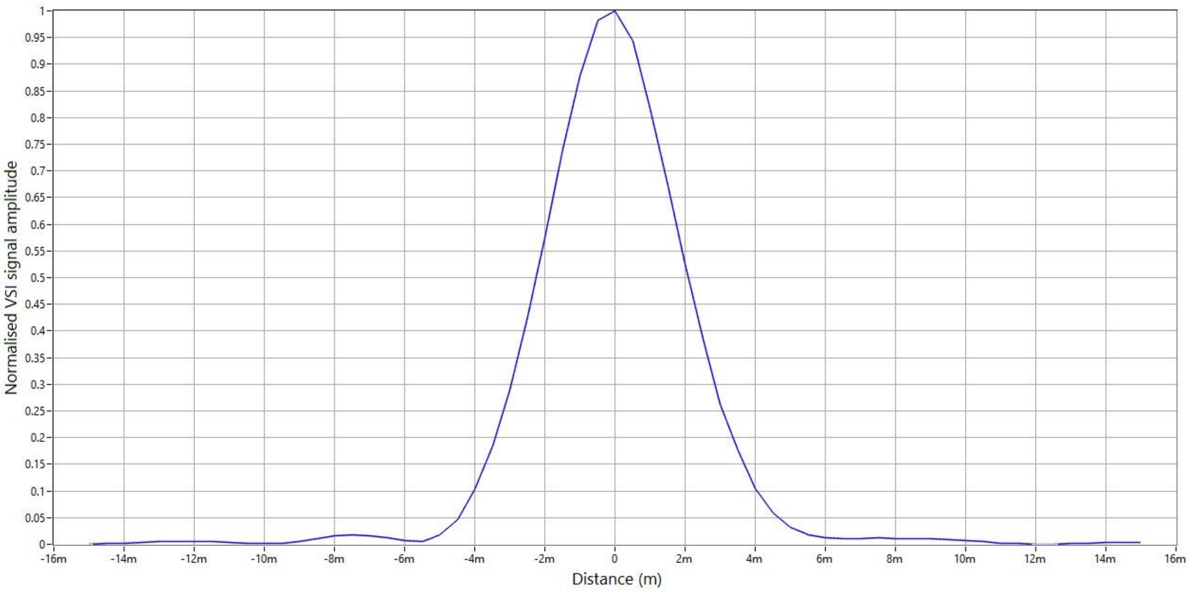


Time domain response to a single cycle sine wave at transducer's nominal centre frequency, PA1230

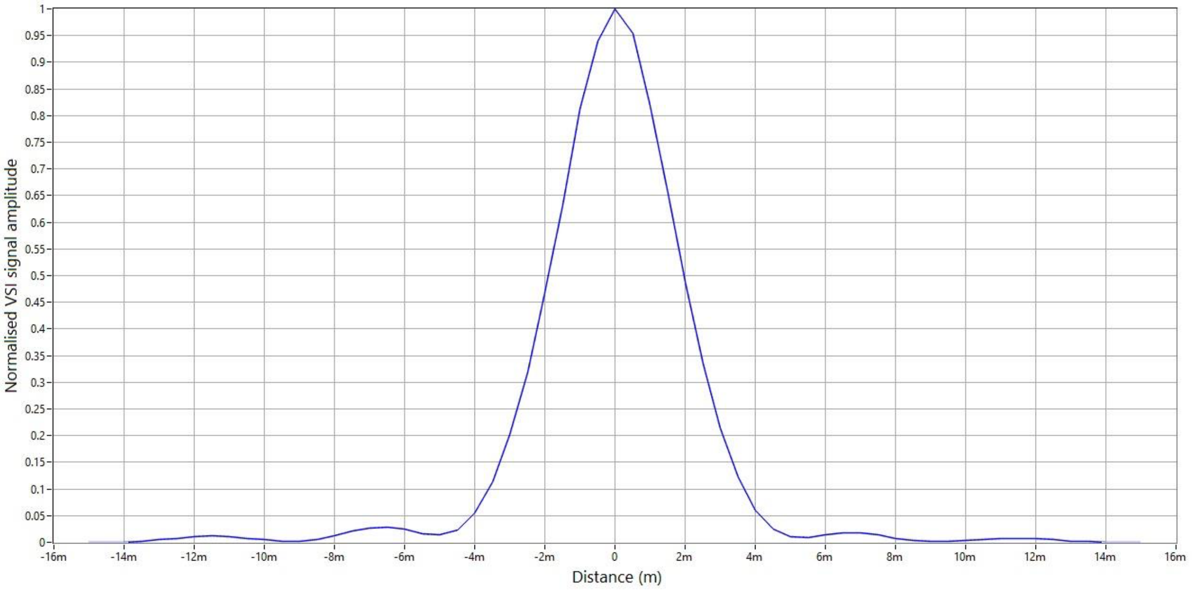


Swept frequency profile for transducer PA1230

Peak Frequency (MHz): 4.54	**-3.0 dB**	**-6.0 dB**	**-12.0 dB**	**-20.0 dB**
Lower limit (MHz)	3.33	2.82	1.79	1.33
Centre frequency (MHz)	4.30	4.67	4.39	4.44
Upper limit (MHz)	5.26	6.53	6.98	7.55
Bandwidth (MHz)	1.92	3.71	5.19	6.22
Bandwidth (%)	44.76	79.48	118.34	139.98



Transverse X Profile for PA1230



Transverse Y Profile for PA1230

	-3.0 dB	-6.0 dB	-12.0 dB	-20.0 dB
X-profile beamwidth (mm)	4.34	6.26	8.81	17.49
Y-profile beamwidth (mm)	3.87	5.65	7.91	17.38