A Lateral Mode Flow-through PMMA Ultrasonic Separator

Nick Harris [1], **Martyn Hill**[2], **Adrian Keating** [3], and **Pierre Baylac-Choulet** [4],

ABSTRACT

A flow-through lateral-mode ultrasonic standing wave separator constructed entirely from PMMA coupled to a piezoceramic transducer has been demonstrated for the first time. Use of PMMA allows for simple, low cost construction, with channels formed either by embossing at relatively low temperatures (120°C) or by direct machining. A simple room-temperature solvent bonding technique is also reported suitable for closing the channel. Initial results using milk show that both construction techniques allow lateral modes to be established, with visual confirmation of separation of lipids within the milk, offering the promise of disposable continuous concentration devices.

Keywords: microfluidics, ultrasonics, separation

1. INTRODUCTION

There is significant interest in separating the different constituents of complex biological fluids continuously, for example, lipid separation from blood, or milk. One of the motivations for this work is to develop an instrument for rapid energy analysis for human milk to allow pre-term babies to be breast fed, but still have their nutritional needs met by augmentation with correctly tailored supplements. The device described here is a component that will be necessary to realise this system in the future. The use of acoustic radiation forces, generated by ultrasonic standing waves has been demonstrated to allow lipids to be manipulated[1], but this has generally been achieved by complex construction using acoustically hard materials such as glass and silicon.

Ultrasonic standing waves have been used previously to allow particle concentrators and separators to be realised. Such devices can either be lateral or planar in their operating mode, with planar devices being more suitable for concentrating particles onto a surface [2], and lateral devices being more suitable for continuous separation [1]. Previous work on multilayer resonant structures has demonstrated that the acoustic conditions can be modelled reasonably accurately [3] but that the necessary acoustic conditions are very sensitive to layer thicknesses. More recent work at the University of Southampton [4] has demonstrated that particles can be driven to a surface by using sub-wavelength reflectors, and that such systems are much less susceptible to thickness variations. In addition, as the acoustic paths are short, it is possible to use acoustically lossy plastics as the construction material. Several other particle manipulation devices have been described in which a polymer forms an element in the structure of an ultrasonic resonator. Hultström et al. [5] use a PDMS spacer within a glass sandwich, but in this case the polymer is not in the acoustic path. Shi et al [6, 7] describe innovative devices in which a PDMS fluid channel is ultrasonically excited by standing surface acoustic waves from a lithium niobate substrate, with the ultrasonic energy coupling directly into the channel through the substrate and coupled wall movement. Dron et al. [9] used a planar microchannel comprising PMMA sheets and a mylar spacer to examine the use of acoustic forces to aid PIV (particle image velocimetry) measurements. A recent paper from Gonzalez et al. [8] demonstrated the use of PMMA and SU-8 to form a planar ultrasonic resonator. The authors demonstrate a high separation efficiency in this device despite the acoustically lossy materials.

The current paper demonstrates that PMMA can also be used to create lateral resonators with channels created by direct machining. Recent work [10] at The University of Western Australia has demonstrated the construction of PMMA channels by embossing, using a stamp created by PCB techniques, potentially offering a rapid and cost-effective way to produce microfluidic systems. In this paper we investigate a variation of this embossing technique to create a channel, using a spray etched master, and compare it against manufacture by milling. Both techniques proved adequate for creating channels that could support lateral standing waves and demonstrate for the first time a flow-through lateral mode separator constructed entirely from PMMA. This offers the potential for easily constructed and disposable flow-through separators for lab-on-chip style systems with good optical accessibility.

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[1] N. R. Harris is with the School of Electronics and Computer Science, University of Southampton, Hampshire, SO18 3LZ, UK, E-mail: nrh@ecs.soton.ac.uk

[2] M. Hill is with the School of Engineering Sciences, University of Southampton, Hampshire, SO18 3LZ, UK , E-mail: M.Hill@soton.ac.uk

[3] A. Keating is with the School of Electrical, Electronic and Computer Engineering, The University of Western Australia, Crawley, WA, Australia, E-mail: A.Keating@uwa.edu.au

[4] P.Baylac-Choulet is a student at LÉcoles dÓfficiers de lÁrmee de lÁir 13361 Salon de Provence, France

2. THEORY

Acoustic radiation forces are a second order effect, and are due to discontinuities in the sound field. The discontinuity (particle) will experience a force due to the scattering and will move in the direction of the force. A simple 1-D model can be used to give an indication of the magnitude and direction of the force, allowing a working design to be produced, although the situation within the acoustic field is more complex [11]. Theory developed in [12] for compressible material in a fluid shows that the time-averaged acoustic radiation force on a spherical particle of radius a, at position x within a one dimensional standing wave of acoustic energy density ε is given by:

$$F\left(x\right) = 4\pi k\varepsilon a^{3}\Phi(\beta,\rho)sin(2kx) \qquad (1)$$

$$\Phi(\beta,\rho) = \frac{\rho_p + \frac{2}{3}(\rho_p - \rho_f)}{2\rho_p + \rho_f} - \frac{\beta_p}{3\beta_f} \qquad (2)$$

where β and ρ are the compressibility and the mass density of the fluid and the particle, indicated by subscripts f and p respectively. The wave number, k is equal to $2\pi/\lambda$ where λ is the wavelength, and the compressibility is related to the speed of sound, c, by $\beta = 1/\rho c^2$.

The practical result of this is that particles will move to either the node or antinode of the standing wave, dependent of the value of Φ. In practical systems, this means that lipids will move towards antinodes and other 'harder' materials such as latex microbeads, commonly used in assays, will move to the nodes.

Although 1-D modelling allows the prediction of planar solutions where the dimension of interest is an integer number of half-wavelengths, in practice 2-D effects are noticed, resulting in irregularities within the acoustic field [11]. In effect, the chamber can be viewed as a resonant box, with Eigen solutions in all three dimensions. The relative strength of these modes is of interest and by trying to enhance the lateral acoustic modes, it is possible to create separators that allow lateral modes to be dominant, resulting in separation effects orthogonal to the direction of the applied field. For the purposes of a separator, we require particles to be held away from the walls in both the x and y directions with flow in the z direction, as we wish to avoid potential clogging issues with lipid particles sticking to the walls. Thus as a first approximation we solve a thin layer multilayer resonator in the y direction to allow lipids to be held away from the top and bottom surfaces, and then calculate the width (x direction) to be a multiple number of half wavelengths (i.e. a simple cavity resonator) to emphasise lateral modes. As lipids move to pressure antinodes, a device with a width of a single half wavelength would result in the lipids being driven only to the sidewalls. However a device with a width of one wavelength allows the potential for concentration within the centre of the channel, as well as

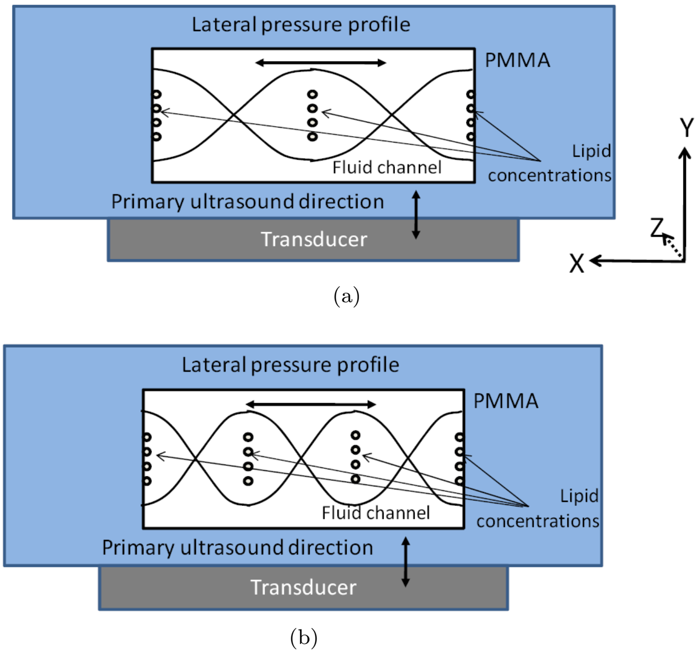

Fig.1:: Generation of lateral pressure profile within a) a 1 wavelength device, and b) a 1.5 wavelength device, orthogonal to primary acoustic direction, showing the position of lateral pressure nodes and antinodes.

at the walls (fig 1). Driving lipids to the walls is usually undesirable as the lipids will then stick and cause clogging. Previous researchers have overcome this by using hydrodynamic focussing to pre-concentrate the subject fluid into a central area, thus not giving the opportunity for the lipids to be affected by the outer nodes[1]. For this study we are interested in confirming the suitability of PMMA and were therefore expecting lipid accumulation at the side wall. However, as reported later and illustrated in figure 12, it is possible to concentrate lipids only in the channel centre and not at the walls. This will be investigated further in future studies.

3. MODELLING

Use was made of a 1-D model previously developed at the University of Southampton [13]. This treats the separator as a multilayer model and allows the prediction of the nodes and antinodes. There are several layers in the structure whose parameters need to be defined, these being the carrier layer, the fluid layer and the reflector layer. It is assumed that the reflector layer is air backed. In this case, the material is defined as PMMA and so the problem reduces to the selection of suitable layer thicknesses.

From a practical viewpoint, transducers with a nominal thickness frequency of 2MHz were available (PZT5H plates), and thin PMMA (Poly(methyl methacrylate)) sheets of 125, 250 and 375 μm were available. Other frequencies could be used such as those that have been used in [23] if practicalities dic-

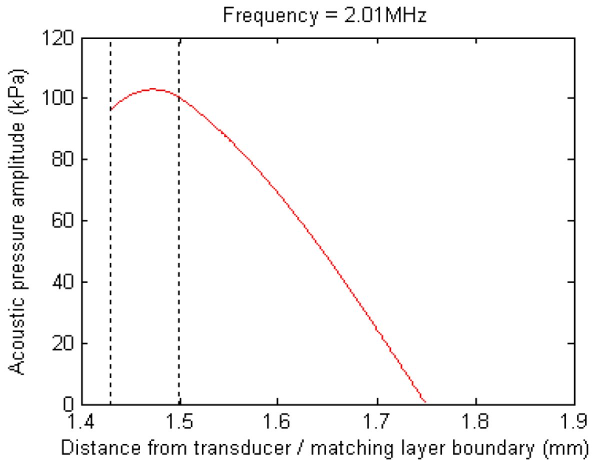

Fig.2:: Modelled pressure profile through the multilayer structure. The air boundary is at 1.75 mm. The fluid layer is between the two dotted lines. The antinode is clearly seen between these lines.

tate. Modelling was done using these constraints and it was found that the thickness of the carrier layer was relatively unimportant, but that a good solution was achieved for a fluid layer of 70μm with a reflector of 250μm. However, it is likely that better solutions are available if the thickness of the reflector layer is not constrained.

The device was thus defined to have a reflector layer of 250 μm, a fluid layer of 70 μm and a carrier layer of 1430 μm, which was defined as a 1.5 mm plate of PMMA with the 70 μm channel removed from it. Figure 2 shows the pressure profile through the fluid and reflector layers and an antinode can be seen within the fluid layer, indicating that lipids will be suspended away from the walls. Dynamic modelling [14],[15] of particle paths also shows that we expect forces to be of a magnitude that will move lipids to an equilibrium position with reasonable flow rates, although due to uncertainties of lipid parameters such as size and relative densities, such values as shown are indicative only. Dynamic modelling of the paths of lipids is shown in figure 3, where only the fluid layer is shown where it can be seen that particles migrate to the antinodal plane as they progress down the channel.

As previously mentioned, the lateral dimensions are limited to be an integer number of half-wavelengths and so the width of the channel is defined by the speed of sound in the fluid and the frequency of operation. For a nominal frequency of 2 MHz and a fluid based on water with a sonic velocity of 1500 m/s, one wavelength is 750μm. One and a half wavelengths is therefore 1125μm.

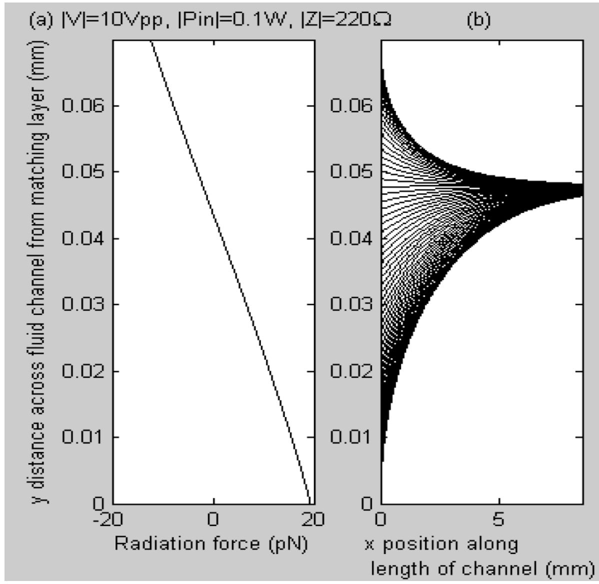

Fig.3:: Dynamic modelling of lipid paths. The traces show the estimated trajectory of lipids as they progress down the first 8 mm of the fluid channel, at flow velocities of 5 mm/s.

4. CONSTRUCTION

A device with a nominal channel width of one wavelength was designed, as shown in figure 4. The device was fed by a single inlet and had two outlets as shown, to allow the potential to concentrate material in the centre and extract it through the centre port. The target width was 750 μm, corresponding to a drive frequency of 2MHz. As per the modelling, the target channel height was 70 μm. In addition, a device with a nominal width of 1125 μm (1.5 wavelengths) was designed, to investigate the potential for multiple nodes [1].

4.1 Channel Formation

Two techniques were used to form the channels in the carrier layer, and these were embossing and milling. Milling is a direct approach which allows good definition of features such as side walls. Intuitively, vertical and parallel sides walls offer the best chance of being able to generate strong lateral resonances, however, other workers have indicated that this need not be the case[17]. Milling offers a good starting point though and devices were milled to a depth of 70 μm with a nominal width of 1125 μm.

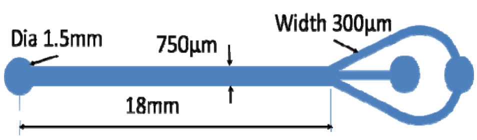

Fig.4:: Planar separator design showing channel arrangement (inlet on left).

CNC milling of 1.5 mm thick blank PMMA samples was done with a Datron micromill. However, measurements showed that the actual width achieved was 1036 μm. After drilling of fluidic access ports, the PMMA samples were annealed at 85 ° Centigrade for 4 hours to relieve any inherent stress caused by the machining. If this step is not carried out, there is the potential for the PMMA to develop propagating cracks. This problem is removed if annealing is carried out.

The disadvantage of milling is that it is time intensive. An alternative approach that lends itself to batch processing is that of embossing. Embossing is a well-established technique for polymers with many options for constructing the stamp. For this study a positive mould was produced by spray etching a stainless steel plate, as shown in figure 5. This produced a stamp of the required dimensions, but the walls were not sharply vertical, which was expected to cause problems in establishing the ultrasonic standing wave. However, this turned out to be much less of a problem than anticipated. In addition the results also showed that in this case the target dimension were not quite achieved, with a width of about 850 μm for the 750 μm target device, and about 1220 μm for the 1125 μm target device. Having achieved a mould, it is now necessary to emboss the carrier layer. The channel was embossed by using a hydraulic force-controlled hot press. The PMMA blank was placed on the mould, and these were sandwiched between two flat steel plates before being placed in the press. A stepped force profile was used. Initially a force of 3000 N was applied whilst heating to 70° Centigrade to allow thermal equalisation to occur. The force was then increased slightly to 3500 N whilst the temperature was raised to 12°C. Once 120°C was achieved, the force was increased to 15000 N and maintained at a temperature of 120°Centigrade for 30 minutes. The force was then reduced to 3000 N and the rig allowed to cool to room temperature before the press was released.

4.2 Bonding

To close the channel, a sheet of 250 μm PMMA was solvent bonded onto the machined/embossed PMMA. Bonding of PMMA has been achieved by several methods, including direct thermal/pressure bonding, surface activated bonding, and solvent bonding. However, bonding of PMMA still remains a non-trivial exercise. Solvent bonding using ethanol for example was reported in [18] but still required 90 minutes at an elevated temperature for success. A recent review article on the bonding of thermoplastic polymers [20] gives a comprehensive review of common techniques including room temperature methods but it is apparent that there is still a requirement for a simple and reliable bonding method that does not require accurate control of temperature and pressure. For example, the method reported by Brown et al. [19] offers a low temperature solution but still

Fig.5:: Picture of an etched steel embossing mould.

requires significant pressure, ideally in a controlled press. It is the authors experience that methods such as this require the large pressures to be reliable. Here we report a room temperature solvent bonding technique that is relatively insensitive to pressure which allows simple yet effective bonding with the minimum of equipment. The main active ingredient of the bonding solution is acetone, as this dissolves PMMA. However, in concentrated form this is too aggressive, as reported by Tsao and DeVoe [20]. It was found that mixing the acetone with a solvent such as ethanol or methanol reduces the aggressiveness of the solvent and allows bonding to occur. A mixture of 33% acetone to 67% ethanol was found to give excellent results with bonding occurring within 15 minutes at room temperature, giving ample working time. The solvent mixture was simply pipetted onto one PMMA surface, and the other surface brought into contact with it. Excess solvent was removed with a tissue, and a 1 kg mass placed on the PMMA to provide pressure. After 15 minutes, as the solvent evaporates, the bond starts to form, and strengthens with time. After about an hour, the bond is such that the layers cannot be separated without physical damage. As there are channels within the PMMA which potentially are filled with solvent, care needs to be taken to ensure that excess solvent is removed from these channels, as otherwise the solvent can cause the channel walls to collapse or disappear. Excess solvent was removed by gently passing air through the channels by using a small syringe. Air can be blown through the fluidic access ports which will drive excess solvent out through the opposite port. This can then be removed by a tissue. If areas are found to be not well bonded, which can happen around the channel boundaries, it is possible to pipette more solvent into the channels which then fills the gap by capillary action. Again ex-

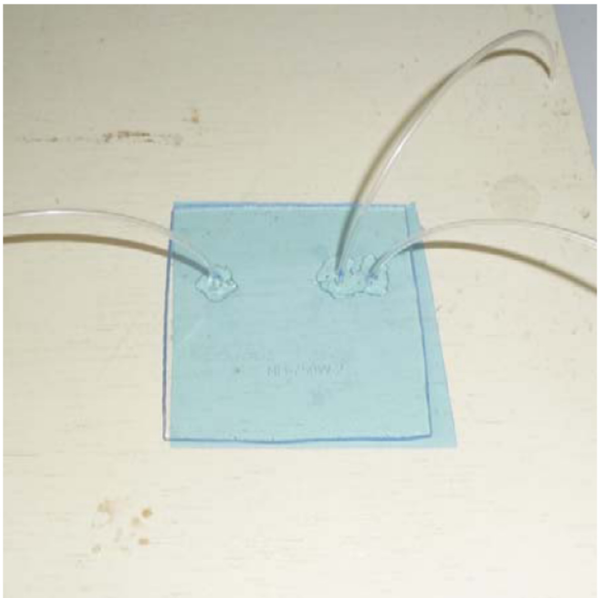

Fig.6:: The completed fluidic device, showing attached tubes for fluid access, bonded by epoxy adhesive (Araldite), prior to attachment of the ultrasonic transducer.

cess solvent can be expelled by passing air though the channel, and the mass reapplied to bring the surfaces into contact again. This technique has been tried on several sources of PMMA and has been found to be robust and easy to use, with the advantage of not requiring elevated temperatures or accurate pressure application. For rapid production of prototypes with the minimum of equipment, we have found this to be more than adequate.

4.3 Final Construction

After this stage, the fluidic channel is complete, and it is necessary to add fluidic connectors or tubes to allow fluid to enter the device. For our test devices we used Araldite epoxy to glue nylon tube in to the entry ports. Figure 6 shows a completed device. In order to introduce ultrasound into the device, a transducer is required. For this study we used a PZT5H plate, resonant in the thickness mode at a nominal 2 MHz. This transducer is coupled to the fluidic device by a coupling component, which allows good transference of the ultrasound to the plastic. In our case we achieved good results with heatsink compound. This material is usually used to give good thermal contact between electronic devices and a substrate, but it works well for acoustic coupling as well. In addition it offers the opportunity to remove the transducer without damage, if necessary, as it doesn't set hard. The transducer requires a signal generator to drive it. In our setup we also made use of a custom amplifier, allowing up to 50 V peak to peak to be applied to our transducer at a nominal 2MHz, although for the experiments described here, 25 V was the maximum used. Fluids were introduced into the device either by hand by a syringe, or by using a syringe driver, and a microscope with attached camera was used for visualisation.

5. EXPERIMENT

Verification of the 1-D modelling was achieved by filling the device with a mixture of vegetable oil and water, with a small amount of detergent to aid the formation of globules within the water. Under static conditions, the ability to manipulate the position of the oil globules was checked with the application of ultrasound [21]. With no ultrasound, oil globules rose to the fluid/reflector layer interface. When ultrasound was applied, the globules moved away from this interface and were suspended about a third of the way through the cavity, as identified by adjusting the focal point of the microscope. To investigate the flow-through performance of the devices, homogenised cow's milk was injected via a syringe and ultrasound applied. Flow velocities were typically of the order of 5 mm/second which equates to about 150 to 200 μl/min. Milk was driven via a syringe into the device, and the acoustic field applied with the transducer drive being up to 25 V pk-to-pk at a nominal 2MHz, although this frequency was adjusted while a visual observation was being made until a maximum response was observed.

With these devices, good responses have been observed at frequencies between 1.85 MHz and 2.12 MHz. A common figure given for the speed of sound in milk is 1485 m/s [16] (which is slightly different for the notional design figure used of 1500 m/s) and given the differences in the actual fabricated devices from the design it is not surprising that there are these differences in frequency. However, the fact that significant responses are seen at these different frequencies shows that the fundamental design principles are robust. Both embossed and milled devices gave good responses, although with the embossed devices, with the larger dimensions, significant results were achieved at 2.12 MHz where the lateral dimension is actually closer to 2 lamda. This gives an extra node across the width. When a mix of 3 μm red latex beads and milk were placed in this device and the ultrasound applied under static conditions, the nature of the 1-D approximations are seen, with the red beads moving to pressure nodes and the lipids in the milk moving to the antinodes. The spacings of the nodes and antinodes across the device are clearly seen, and the acoustic hotspots are very apparent.

These hotspots allow an averaging effect when flow is introduced leading to the response shown in figure 8, showing how a flow-through concentrator may be achieved. The milled channel tends to give a more regular structure, as shown in figure 9.

Figure 9 shows the results for the nominal 1125 μm milled channel, again filled with red latex beads and milk. In this figure the frequency is 1.85 MHz (due to the smaller width dimension of 1036 μm compared with 1220 μm for the embossed device) and

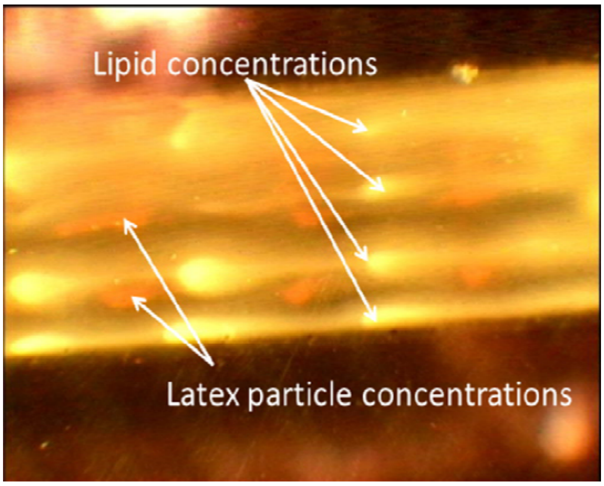

Fig.7:: A nominal 1175 μm embossed device filled with a mixture of 3 μm latex beads and milk, with no flow. Nodal spacings in 2 dimensions are clearly seen.

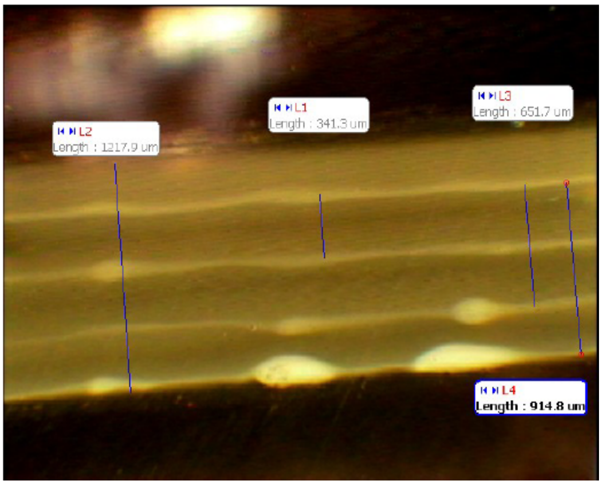

Fig.8:: The same device as shown in figure 8 filled with milk and flow applied. Streams of lipids are clearly seen.

a much more linear banding down the length of the device (from right to left in the picture) is apparent. Running the embossed channel at a lower frequency (in this case 1.85 MHz) also results in strong lateral modes. Figure 10 is a still taken from a video sequence with flow running from bottom to top, clearly showing lipids separating out into tight streams. Inspection of the picture shows that the lateral distance of the streams from each other is not regular. This may be due to imperfections in the embossing, or 2-D effects causing differences in the flow averaging. However, video evidence shows that this separation pattern is very stable.

For the 750 μm embossed device, when tested with red latex beads, the two expected antinodes are very obvious as shown in figure 11. Interestingly, although the spacing of these nodes corresponds to a fluid

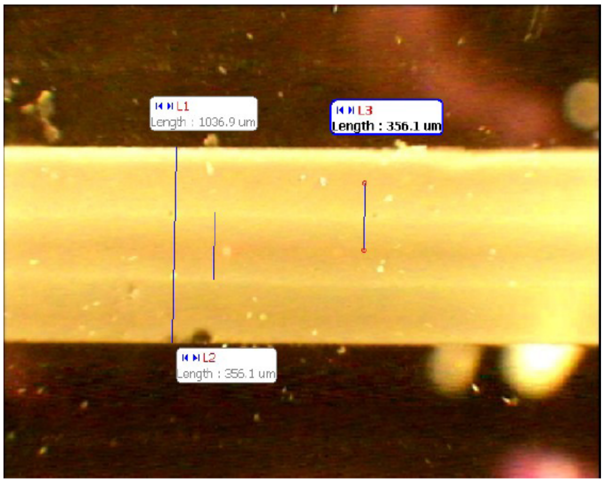

Fig.9:: The milled device with latex particles and milk, with flow. The lines of concentrated material are more regular.

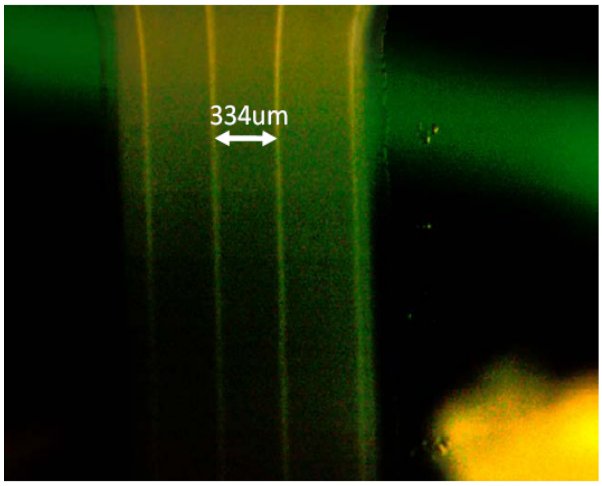

Fig.10:: An embossed device, operating at 1.85 MHz showing tightly focussed lipid streams.

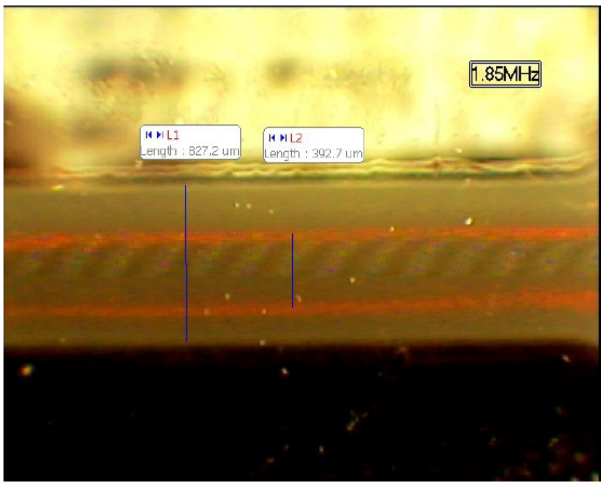

Fig.11:: A 750 μm device filled with 3 μm latex beads, showing the formation of two bands of particles in the pressure nodes, at a frequency of 1.85 MHz.

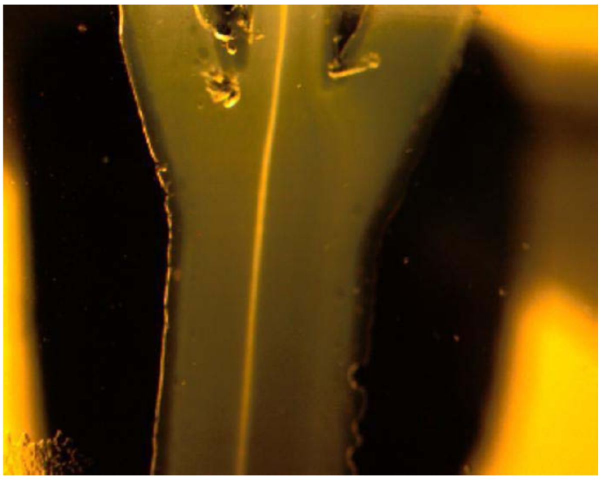

Fig.12:: Outlet of 750 μm channel showing concentration of lipids primarily concentrated into the centre laterally and moving through the centre outlet. Flow is from bottom to top.

sound velocity of 1450 m/s, the bands are not central in the channel. The reason for this is unclear at this time and will be investigated further. On filling this device with milk it was more difficult to get defined nodes, but it was possible to achieve a very strong antinode in the centre of the channel (fig 12), where lipids can be seen concentrated into a single antinode, with very little evidence of antinodes at the channel edges, where they would be expected. This fortunate effect is probably linked to flow geometry variations downstream and will be the subject of future investigations, as it offers the potential to allow lipid separation without the need for sheath flow, thus simplifying the required fluidic system tremendously.

6. CONCLUSIONS

This work has reported on a study into the potential use of plastic to create ultrasonic lateral mode particle manipulators, with a view to allowing the construction of a lateral mode lipid separator. A thin layer resonator structure was utilised, and channels created in PMMA by two methodsmilling and embossing. Further, a robust and simple room temperature PMMA solvent bonding procedure has been reported. The resulting fluidic devices are simple to make, and offer the potential for disposable elements. The performance of the devices with ultrasound has been demonstrated, showing that for thin layer devices, lateral mode manipulators are entirely feasible and are robust in their dimensional stability, and further, their potential as use as flow though concentrators has been verified. Future work will develop further refinements by using milled embossing tools with better dimensional accuracy, together with quantitative measurements on the separation performance, for example, measuring the acoustic power of the transducer [22]. In addition, the reason for the predominantly single mode operation in the 750 μm device will be investigated in more detail, as this offers the promise of devices with much reduced fluidic complexity, as sheath flow preconcentrators can be removed.

7. ACKNOWLEDGEMENT

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Nick Harris is a senior lecturer in the School of Electronics and Computer Science at the University of Southampton, Southampton, UK, and he has more than 100 publications in the fields of wireless sensor networks, biosensors, microfluidic systems, and processing. He was awarded a Ph.D. degree in 1997 by the University of Southampton, Department of Mechanical Engineering. Current research areas include self-powered health and usage monitors and embedded condition-monitoring microsystems, and wireless sensor networks. He is a member of the IET and a Chartered Engineer, and a co-founder of Perpetuum Ltd, the worlds leading vibration energy harvesting company.

Martyn Hill is a Professor in Electromechanical Systems in the School of Engineering Sciences, Southampton University. He graduated in 1985 from the Institute of Sound and Vibration Research, was appointed lecturer in 1990 and has research interests in acoustics (particularly ultrasonics) and in the measurement and characterisation of surfaces. His work has led to more than 150 technical publications. He is a chartered engineer, a member of the Institute of Measurement and Control and a member of the Institute of Acoustics.

Adrian Keating is an Associate Professor at the University of Western Australia, Perth, WA. He is head of the Sensors and Advanced Instrumentation Laboratory, with research interests in microfluidics, porous silicon, and infra red spectrometers. He won the Lynsey Ann Welsh Award for Innovation in NIR Science in 2004 and received the DSTO Eureka Prize for Outstanding Science in Support of Defence or National Security in 2008 for his contributions to the development of the MEMS microspectometer. He has more than 65 publications and 7 patents.

Pierre Baylac-Choulet Choulet is a student at the École d'officiers de l'Arme de l'air, Salon de Provence, France, with research interests in ultrasound and microfluidics.